

Synthesis of a series of novel 2-aminopyrimidine derivatives and their biological evaluation as adenosine receptor antagonists

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Preface

This thesis is submitted in article format consisting of four original research articles. One of the aforementioned articles has been published in the *European Journal of Medicinal Chemistry*, one article has been submitted to *Bioorganic and Medicinal Chemistry Letters* and the last two are awaiting submission. The author guidelines for the submitted articles have also been included. All scientific research (synthesis, biological assays and writing of thesis as well as articles) for the purpose of this thesis was conducted by Mr S.J. Robinson at the North-West University, Potchefstroom campus.

Letters of agreement from the co-authors of the research articles and the publishing agreements from the editors of the stated journals are included.

Declaration

This thesis is submitted in fulfilment of the requirements for the degree of Philosophiae Doctor in Pharmaceutical Chemistry, at the School of Pharmacy, North West University.

I, Sarel Johannes Robinson hereby declare that the thesis with the title: **Synthesis of a series of novel 2-aminopyrimidine derivatives and their biological evaluation as adenosine receptor antagonists** is my own work and has not been submitted at any other university either whole or in part.

SJ Robinson

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Letter of agreement



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To whom it may concern,

Dear Sir/Madam

CO-AUTHORSHIP ON RESEACH PAPERS

The undersigned as co-authors of the research articles listed below, hereby give permission to Mr S.J. Robinson to submit these articles as part of the degree PhD in Pharmaceutical Chemistry at the North-West University, Potchefstroom campus.

- 2-Aminopyrimidines as dual adenosine A_1/A_{2A} antagonists;
Published in European Journal of Medicinal Chemistry. (2015, 104:177-188).
- Amide substituted 2-amino-4,6-diphenylpyrimidines as adenosine receptor antagonists;
Awaiting submission
- Carbamate substituted 2-amino-4,6-diphenyl-aminopyrimidines as adenosine receptor antagonists;
Submitted to Bioorganic and Medicinal Chemistry Letters.
- Synthesis and evaluation of novel ether derivatives of 2-amino-4,6-diphenylpyrimidine as potential dual adenosine A_1 and A_{2A} antagonists
Awaiting submission

Yours sincerely,

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ABSTRACT

Parkinson's disease (PD) is a progressive, neurodegenerative movement disorder caused by a substantial loss of dopamine in the striatum. This deficiency of dopamine in the brain results in the typical motor symptoms such as muscle rigidity, dyskinesia, tremor and impairment of postural balance. PD patients not only have to deal with the life altering motor deficits, but usually suffer from non-motor symptoms like depression and dementia. Current treatments, which mainly involve dopamine replacement therapies, are symptomatic and do not prevent the progression of PD. These treatments are also associated with numerous side effects that further complicate the lives of patients. These shortcomings have spurred a search for novel, alternative non-dopaminergic therapies.

Dual antagonism of adenosine A₁ and A_{2A} receptors is a potential, promising non-dopaminergic alternative. Reports indicate that dual antagonism of A₁ and A_{2A} receptors will act synergistically to reverse the motor deficiencies of PD. Non-motor symptoms may also be addressed by dual antagonism, as adenosine A₁ receptor antagonism is linked to increased cognition, whereas antagonism of the A_{2A} receptor may improve depression symptoms. Neuroprotection, which remains the single, most elusive problem in PD, may also possibly be attained by A_{2A} receptor antagonism in particular. The benefits of dual adenosine A₁ and A_{2A} antagonism therefore extend further than the mere symptomatic treatment of the disease and these agents have the potential to influence the progression of PD.

The 2-aminopyrimidine chemotype is a privileged scaffold for antagonism of adenosine receptors as this motif frequently occurs in compounds that exhibit potent adenosine A_{2A} and/or adenosine A₁ affinity. Selected compounds from a series of 2-aminopyrimidine derivatives designed and synthesised in a previous study exhibited potent adenosine A_{2A} affinities as well as *in vivo* activity in the haloperidol induced catalepsy assay in rats. The first aim of this PhD study was therefore to determine the adenosine A₁ affinities of these compounds and to evaluate their potential cytotoxicity. After identification of 2-amino-4,6-diphenylpyrimidine as a feasible scaffold for the design of dual adenosine A₁ and A_{2A} antagonists, the second aim of this study was to further explore the structure-activity relationships of these aminopyrimidines with regards to their potential as dual adenosine A₁ and A_{2A} antagonists.

The adenosine A₁ receptor affinities of the 2-aminopyrimidines synthesised in the preceding study were determined using radioligand binding studies. 1,3-[³H]-Dipropyl-8-cyclopentylxanthine ([³H]DPCPX) was used as a radioligand to determine binding to the A₁ receptors. Whole brains obtained from male Sprague-Dawley rats (NWU-0035-10-A5) were used as receptor source. These 2-aminopyrimidines illustrated moderate to good A₁ receptor affinities with K_i values ranging from 9.54 nM – 650.1 nM. These compounds are therefore promising dual adenosine A₁ and A_{2A} antagonists since potent A_{2A}

receptor affinities have been illustrated in the preceding study. These compounds were also not toxic in a preliminary cytotoxicity assay as cell viability was generally still above 70% at a concentration of 10 μ M, which is almost 1000 fold higher than the reported K_i values.

Three novel series of amide, carbamate and ether substituted 2-amino-4,6-diphenylpyrimidines were further synthesised. The synthesis of the ether and carbamate derivatives involved firstly, the reaction of acetophenone and 3-hydroxybenzaldehyde under basic conditions to yield (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one, as precursor. Similarly, 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid, the precursor for the amide series, was synthesised by reacting 3-formylbenzoic acid and acetophenone under basic conditions. Different carbamoyl chlorides, alkyl chlorides and amines were coupled to the respective precursors to yield chalcone intermediates for the carbamate, ether and amide series, respectively. Cyclisation of these intermediates with guanidine hydrochloride and sodium hydride in DMF afforded the desired 2-aminopyrimidines. Structures were confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry.

The adenosine A_1 and A_{2A} receptor affinities of the newly synthesised 2-amino-4,6-diphenylpyrimidines were determined using radioligand binding studies. The non-selective radioligand, [3 H]5'-*N*-ethylcarboxamide-adenosine ([3 H]NECA) was utilised in the presence of *N*⁶-cyclopentyladenosine (CPA) to assess binding to the adenosine A_{2A} receptor. Striata dissected from male Sprague-Dawley rats (NWU-0035-10-A5) served as receptor source. Evaluation of A_1 affinities were done as described above. Compounds from both the amide and carbamate series showed moderate to potent dual adenosine A_1 and A_{2A} affinities, while compounds from the ether series were more selective towards the A_1 receptor. K_i values for the A_1 receptor ranged from 5.42 - 25.2 nM, 0.175 - 10.7 nM and 5.66 - 48.8 nM for the amide, carbamate and ether series, respectively. Moderate $A_{2A}K_i$ values of 47.0 - 351 nM were observed for the ethers, whereas the amides and carbamates had superior affinities ranging from 3.37 - 106.5 nM and 1.58 - 451 nM, respectively.

Molecular docking studies (C-Docker, Discovery studio 3.1), using the crystal structure of the adenosine A_{2A} receptor (PDB 3EML) were further performed in an attempt to rationalise the results obtained in radioligand binding assays. Unfortunately, the crystal structure of the adenosine A_1 receptor is not yet available. Important anchoring interactions, such as those of the exocyclic amino group and Glu169 as well as hydrophobic interactions between the tricyclic ring system and Phe168, were observed for most compounds. The amide and carbamate derivatives, however showed additional interactions between the side chain carbonyl and either Glu169 or Tyr271, located in the binding site, suggesting that this interaction is important for A_{2A} affinity. It is postulated that the decrease in A_{2A} affinity observed for the ether series is a result of the absence of this carbonyl group in the side chain, as this interaction is no longer possible.

The compounds with the most promising dual affinities were selected for *in vivo* screening using the haloperidol induced catalepsy assay. This assay is often used as an indication of A_{2A} receptor antagonism, as administration of known antagonists results in reversal of haloperidol induced catalepsy. At the same time, this assay gives a preliminary indication of bioavailability. The following compounds, [3-(2-amino-6-phenylpyrimidin-4-yl)phenyl-4-methylpiperazine-1-carboxylate, 3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate and 3-(2-amino-6-phenylpyrimidin-4-yl)-N-[3-(morpholin-4-yl)propyl]benzamide] were selected and illustrated *in vivo* activity as catalepsy was attenuated to a significant degree when compared to the control groups. One carbamate compound, 3-(2-amino-6-phenylpyrimidin-4-yl)phenyl-4-methylpiperazine-1-carboxylate had no *in vivo* activity. Determination of both Log D and water solubility values for this compound indicated that this derivative is highly lipophilic (Log D = 4.03), with low water solubility and it is postulated that these unfavourable physicochemical properties are responsible for the lack of *in vivo* activity.

All objectives as set out, were met successfully as 26 novel 2-amino-4,6-diphenylpyrimidines were synthesised and evaluated as dual adenosine A₁ and A_{2A} antagonists. Promising dual adenosine A₁ and A_{2A} affinities and good *in vivo* results were obtained for the newly synthesised derivatives, clearly illustrating the promise of the 2-aminopyrimidines in the potential treatment of PD.

Keywords

Parkinson's disease, dual adenosine antagonists, 2-aminopyrimidines, A_{2A} antagonists, A₁ antagonists

Uittreksel

Parkinson se siekte (PS) is 'n progressiewe, neurodegeneratiewe bewegingsiekte wat veroorsaak word deur 'n verlaging in die dopamienkonsentrasie in die striatum. Hierdie verlies van dopamien in die brein veroorsaak die tipiese motorsimptome soos spierstyfheid, diskinesie, tremor en versteuring van posturale balans. Pasiënte met PS moet nie net saamleef met motoriese inkorting nie, maar ondervind ook dikwels nie-motoriese simptome soos depressie en demensie. Huidige behandeling, wat hoofsaaklik dopamienvervangingsterapie behels, is simptome en verhoed nie die progressiewe verloop van PS nie. Huidige behandeling word verder geassosieer met verskeie nuwe-effekte wat die lewens van pasiënte verder kompliseer. Hierdie tekortkominge het die soeke na nuwe, alternatiewe nie-dopaminergiese terapieë aangevuur.

Dualistiese antagonisme van adenosien A_1 - en A_{2A} -reseptore is 'n potensiële nie-dopaminergiese alternatief. Daar is vermeld dat dualistiese antagonisme van A_1 - en A_{2A} -reseptore sinergisties sal optree om motoriese simptome te verhoed. Nie-motoriese simptome mag ook moontlik aangespreek word deur dualistiese antagonisme, aangesien antagonisme van die A_1 reseptor geassosieer word met 'n toename in kognisie, terwyl antagonisme van die A_{2A} reseptor depressiewe simptome mag verbeter. Neurobeskerming, wat die belangrikste doel is in die behandeling van PS, mag ook deur veral A_{2A} antagonisme 'n werklikheid word. Die voordele van dualistiese adenosien A_1 - en A_{2A} -antagonisme strek dus verder as die blote simptome behandeling van die siekte en hierdie middels mag moontlik ook die verloop van PS beïnvloed.

Die 2-aminopirimidienkern is van belang vir die antagonisme van adenosienreseptore aangesien hierdie motief voorkom in verbindings met potente adenosien A_{2A} -en/of adenosien A_1 -affiniteit. Geselekteerde 2-aminopirimidinderivate, wat ontwerp en gesintetiseer is tydens 'n vorige studie, het potente adenosien A_{2A} -affiniteite, en ook *in vivo* aktiwiteit, in die haloperidol-geïnduseerde katalepsietoets in rotte getoon. Die eerste doelwit van hierdie studie was om die adenosien A_1 -affiniteite asook die potensiële sitotoksiteit van hierdie verbindings te bepaal. Nadat die 2-amino-4,6-difenielpirimidienkern as 'n geskikte motief vir die ontwerp van dualistiese adenosien A_1 - en A_{2A} -antagoniste geïdentifiseer is, was die hoofdoelwit van hierdie studie dan om die struktuuraktiwiteitsverwantskappe van hierdie aminopirimidienkern, wat betrekking het op hulle potensiaal as adenosien A_1 - en A_{2A} -antagoniste, verder te ondersoek.

Die adenosien A_1 -reseptoraffiniteite van die 2-aminopirimidienkern wat in die voorafgaande studie gesintetiseer is, is bepaal deur gebruik te maak van radioligandbindingstudies. Om binding aan A_1 -reseptore te bepaal, is 1,3- ^3H -dipropiel-8-siklopentielxantien (^3H]DPCPX) gebruik as radioligand. Breinweefsel, verkry vanaf manlike Sprague-Dawley rotte (NWU-0035-10-A5), is gebruik as bron van

reseptore. Hierdie 2-aminopirimidienreë het matig tot goeie affiniteite vir die A_1 -reseptor, met K_i waardes tussen 9.54 nM – 650.1 nM, getoon. Hierdie verbinding is dus belowende dualistiese adenosien A_1 en A_{2A} antagonist, aangesien potente A_{2A} reseptoraffiniteite in die voorafgaande studie verkry is. Hulle was ook relatief non-toksies, aangesien sellewensvatbaarheid in die algemeen groter as 70% was by 'n konsentrasie van 10 μ M, wat ongeveer 1000 keer hoër is as die waargenome K_i waardes.

Drie nuwe amied-, karbamaat- en etergesubstitueerde 2-amino-4,6-difenielpirimidienreë is verder gesintetiseer. Die sintese van die eter en karbamaatderivate het eerstens die reaksie van asetofenoon en 3-hidroksiebensaldehyd, onder basiese kondisies, om (2*E*)-3-(3-hidroksifeniel)-1-fenielprop-2-en-1-oon as voorloper te lewer, behels. Soortgelyk hieraan, is 3-[(1*E*)-3-okso-3-fenielprop-1-en-1-iel]bensoësuur, die voorloper van die amiedreë, gesintetiseer deur die reaksie van 3-formielbensoësuur met asetofenoon, ook onder basiese kondisies. Verskillende karbamoielchloriede, alkylchloriede en amiene is aan die onderskeie voorlopers gekoppel om die chalkoonintermediêre vir die karbaat, eter en amiedreë, respektiewelik, te lewer. Siklisering van hierdie intermediêre met guanidienhidrochloried en natriumhidried in DMF het gelei tot die verkryging van die gewenste 2-aminopirimidienreë. Strukture is bevestig met kernmagnetieseresonansspektroskopie en massaspektrometrie.

Die adenosien A_1 - en A_{2A} -affiniteite van die nuutgesintetiseerde 2-amino-4,6-difenielpirimidienreë is bepaal deur gebruik te maak van radioligandbindingstudies. Die non-selektiewe radioligand, [3 H]5'-N-etielkarboksamied-adenosien ([3 H]NECA), in die teenwoordigheid van N^6 -siklopentieladenosien (CPA), is gebruik om binding aan die adenosien A_{2A} reseptor te meet. Striata, wat verkry is deur disseksie van manlike Sprague-Dawley rotte (NWU-0035-10-A5), het as bron van reseptore gedien. Evaluering van A_1 affiniteite is uitgevoer soos hierbo beskryf. Terwyl verbindinge van die amied- en karbamaatreë matig tot potente dualistiese affiniteite vir die adenosien A_1 - en A_{2A} -reseptore getoon het, was die eteranaloe meer selektief vir die A_1 -reseptor. K_i -waardes vir die A_1 -reseptor was tussen 5.42 - 25.2 nM, 0.175 - 10.7 nM en 5.66 – 48.8 nM vir die amied-, karbamaat- en eterreë, respektiewelik. Matige $A_{2A}K_i$ -waardes van 47.0 – 351 nM is waargeneem vir die eters, terwyl die amiede en karbamate beter affiniteite van tussen 3.37 - 106.5 nM en 1.58 - 451 nM, respektiewelik, getoon het.

Molekulêre passingstudies (C-Docker, Discovery studio 3.1), is verder uitgevoer in 'n poging om 'n rasonale verduideliking vir die resultate van die radioligandbindingstudies te verkry. Die kristalstruktuur van die adenosien A_{2A} -reseptor (PDB 3EML) is hiervoor gebruik. Ongelukkig is die kristalstruktuur van die adenosien A_1 -reseptor nog nie beskikbaar nie. Belangrike ankeringsinteraksies, soos die van die eksosikliese amiengroep en Glu169, sowel as hidrofobiese interaksies tussen die trisikliese ringsisteem en Phe168 is waargeneem vir die meeste verbindinge. Die amied- en karbamaatderivate het verder ook addisionele interaksies tussen die karbonielgroep in die syketting en

Glu169 of Tyr271, wat voorkom in die bindingsetel, getoon, wat aandui dat hierdie interaksie van besondere belang vir A_{2A} -affiniteit is. Daar word gepostuleer dat die afwesigheid van hierdie karbonielgroep in die syketting van die eters verantwoordelik is vir die verlies aan affiniteit vir hierdie reeks, aangesien hierdie interaksie nie meer moontlik is nie.

Die verbindings met die mees belowende dualistiese affiniteit is geselekteer vir *in vivo* toetsing in die haloperidol-geïnduseerde katalepsietoets. Hierdie toets word gereeld gebruik as 'n aanduiding van A_{2A} -reseptorantagonisme, aangesien die toediening van bekende A_{2A} -antagoniste haloperidol-geïnduseerde katalepsie omkeer. Terselfdertyd gee hierdie toets ook 'n aanduiding van biobeskikbaarheid en akute toksiese effekte. Die verbindings [3-(2-amino-6-fenielpirimidien-4-iel)feniel-4-metielpiperasien-1-karboksilaat, 3-(2-amino-6-fenielpirimidien-4-iel)fenielmorfolien-4-karboksilaat en 3-(2-amino-6-fenielpirimidien-4-iel)-*N*-[3-(morfolien-4-iel)propiel]bensamied] is gekies en het *in vivo* aktiwiteit getoon aangesien 'n statisties waarneembare afname in katalepsie waargeneem is, vergeleke met die kontrolegroepe. Een karbamaatverbinding, 3-(2-amino-6-fenielpirimidien-4-iel)feniel 4-metielpiperasien-1-karboksilaat het geen *in vivo* aktiwiteit getoon nie. Beide Log D en wateroplosbaarheid is vir hierdie verbinding bepaal en hierdie waardes het aangetoon dat hierdie derivaat hoogs lipofiel is (Log D = 4.03), met lae wateroplosbaarheid. Daar word gepostuleer dat hierdie ongunstige fisiese-chemiese eienskappe verantwoordelik is vir die afwesigheid van *in vivo* aktiwiteit.

Alle doelwitte, soos gestel, is suksesvol bereik, aangesien 26 nuwe 2-amino-4,6-difenielpirimidien gesintetiseer is en as dualistiese adenosien A_1 - en A_{2A} -antagoniste geëvalueer is. Belowende dualistiese A_1 - en A_{2A} affiniteit en goeie *in vivo* resultate is verkry vir die nuut gesintetiseerde derivate, wat duidelik die potensiaal van die 2-aminopirimidien vir die behandeling van PS aantoon.

Sleutelwoorde

Parkinson se siekte, dualistiese adenosienantagoniste, 2-aminopirimidien, A_{2A} -antagoniste, A_1 -antagoniste

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ABBREVIATIONS

Asn	-	Asparagine
ATP	-	Adenosine triphosphate
cAMP	-	Cyclic adenosine monophosphate
CPM	-	Counts per minute
CNS	-	Central nervous system
COMT	-	Catechol- <i>O</i> -methyltransferase
CPA	-	<i>N</i> ⁶ -cyclopentyladenosine
CSC	-	8-(3-chlorostyryl)caffeine
DAG	-	Diacylglycerol
DAs	-	Dopamine agonists
DMSO	-	Dimethyl sulfoxide
EL	-	Extracellular loop
[³ H]DPCPX	-	1,3-[³ H]-dipropyl-8-cyclopentylxanthine
GABA	-	Gamma-aminobutyric acid
G-protein	-	Guanine nucleotide-binding protein
GPCR	-	G-protein coupled receptor
IL	-	Intracellular loop
IP ₃	-	Inositol triphosphate
L-AAD	-	L-amino acid decarboxylase
LBs	-	Lewy bodies
MAO-B	-	Monoamine oxidase B
MPTP	-	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTT	-	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
[³ H]NECA	-	[³ H]5'- <i>N</i> -ethylcarboxamide-adenosine
PD	-	Parkinson's disease
Phe	-	Phenylalanine
ROS	-	Reactive oxygen species
SNpc	-	Substantia nigra pars compacta
TM	-	Transmembrane

CHAPTER 1

Introduction

1.1 Background

1.1.1 Parkinson's disease

Parkinson's disease (PD) is a chronic neurodegenerative disease characterised by typical motor symptoms such as bradykinesia, muscle rigidity, impairment of postural balance and resting tremor. These motor symptoms are believed to be the result of the deterioration of dopaminergic neurons in the striatum, which leads to a substantial decrease in the dopamine concentration in the brain. Individuals with PD not only suffer from these movement disabilities but generally develop several non-motor symptoms, like depression and loss of cognitive function, especially in the progressive stages of the disease (Dauer & Przedborski, 2003).

Current PD therapies, which are mostly dopaminergic in nature, only provide symptomatic relief (by improving the dopamine deficiency in the brain), and do not have the ability to halt the progression of the disease (Olanow *et al.*, 2008; Schapira *et al.*, 2014). A further limitation of these therapies is that, while providing relief for the motor symptoms, the non-motor symptoms that usually accompany PD are left untreated (Todorova *et al.*, 2014; Jenner *et al.*, 2013, Shook & Jackson, 2011). Additionally, detrimental side effects are often experienced and the therapeutic effect of drugs such as levodopa, decrease over time (Chaudhuri *et al.*, 2006; Adler, 2005). These limitations demonstrate the immense need for the development of alternative drug therapies for PD.

It is believed that over 10 million people worldwide suffer from PD and this number is expected to rise substantially, as the incidence of the disease escalates with the increase in human lifespan (Dorsey *et al.*, 2007). Finding a cure for PD is therefore of utmost importance, since the economic and social burden of the disease will increase substantially in the future.

1.1.2 Adenosine receptors as drug targets in PD

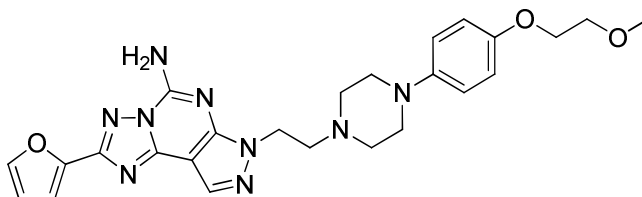
The basal ganglia and its sub-structures, particularly the striatum, plays an imperative role in the control of movement. The appeal of adenosine A₁ and A_{2A} receptors as targets in the treatment of movement disorders are twofold: Firstly, they are localised in the parts of the brain that control movement and secondly, they have the potential to modulate neurotransmission of other neurotransmitters involved in movement control, due to their integrative roles with other receptors such as the dopamine D₂ receptors

(Graybiel *et al.*, 1994). The involvement of both the adenosine A₁ and A_{2A} receptors in motor function are well established and several studies have shown the potential of adenosine antagonists in the treatment of PD (Shook & Jackson, 2011; Müller & Ferré, 2007; Schwarzschild *et al.*, 2006; Fredholm *et al.*, 2005; Shook *et al.*, 2012).

Since antagonism of both adenosine A₁ and A_{2A} receptors individually leads to an improvement in motor function (Pollack & Fink, 1995; Trevitt *et al.*, 2009; Antoniou *et al.*, 2005), it has been suggested that A₁ and A_{2A} receptor antagonists can act synergistically to relieve the motor symptoms associated with Parkinson's disease (Shook *et al.*, 2012). Antagonists of these receptors also have the potential to improve several non-motor symptoms. For example, the anti-depressant effects of the known adenosine A_{2A} antagonist KW6002 was recently reported, providing evidence of a possible role for adenosine A_{2A} receptor antagonists in the treatment of depression (Yamada *et al.*, 2013). Antagonism of the A₁ receptor on the other hand, has been linked to enhancement of cognition (Pereira *et al.*, 2002; Maemoto *et al.*, 2004; Bortolotto *et al.*, 2015). Dual antagonism of A₁ and A_{2A} receptors will therefore offer an added advantage as most PD patients suffer from depression and impaired cognitive abilities, especially in the later stages of the disease. Furthermore, adenosine A_{2A} receptor antagonism may have the ability to halt the progression of PD as neuroprotective properties have been reported in several preclinical studies (Jenner *et al.*, 2009; Popoli *et al.*, 2000; Ascherio *et al.*, 2001). This makes dual adenosine A₁ and A_{2A} antagonism a particularly exciting alternative to current PD therapies, since it may not only improve the motor and non-motor symptoms, but has the potential to halt the progression of the disease.

1.1.3 Design of 2-aminopyrimidines as adenosine A₁ and A_{2A} antagonists

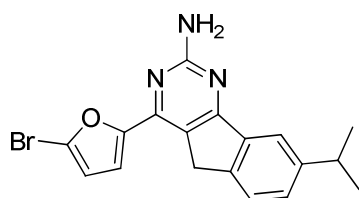
Although none of the drugs currently on the market for the treatment of PD are adenosine antagonists, several have reached clinical trials. The first adenosine antagonists designed for the treatment of PD were xanthines, derived from the known non-selective adenosine antagonist caffeine, and this class of compounds have been extensively researched (Mantri *et al.*, 2008). However, our interest in the design of adenosine antagonists started with the realisation that many high potency adenosine antagonists, and adenosine A_{2A} antagonists in particular, contained a 2-aminopyrimidine moiety in their structures. Preladenant (**1.1**), for example, reached phase 3 clinical trials as a possible antiparkinsonian drug.



1.1 Preladenant

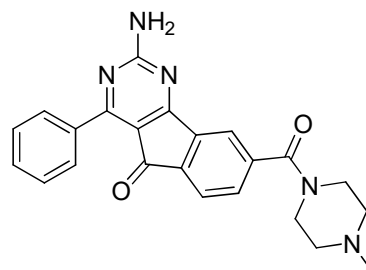
We thus set out to synthesise a preliminary series of 2-aminopyrimidines in order to assess the potential of these compounds as adenosine A_{2A} antagonists (Robinson, 2013). The design of these compounds

was based on similar indenopyrimidines (**1.2**) and indenopyrimidones (**1.3**) for which potent adenosine A_{2A} and in some cases, potent adenosine A₁ affinities have been reported (de Lera Ruiz *et al.*, 2014; Gillespie *et al.*, 2009a; Gillespie *et al.*, 2009b; Matasi *et al.*, 2005; Shook *et al.*, 2010a, Shook *et al.*, 2010b; Shook *et al.*, 2010c; Atack *et al.*, 2014; Shook & Jackson, 2011; Müller & Ferré, 2007; Van Veldhoven *et al.*, 2008; Lim *et al.*, 2011).



1.2

A_{2A} K_i = 0.8 nM

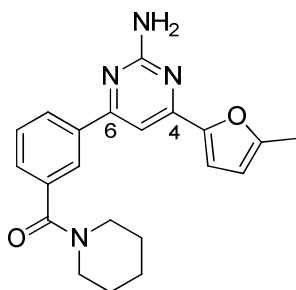


1.3

A₁ K_i = 58.4 nM

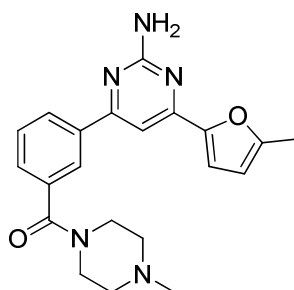
A_{2A} K_i = 8.2 nM

A series of amide derivatives was thus synthesised and compounds with potent adenosine A_{2A} affinity and *in vivo* activity were identified during this preceding study (e.g. **1.4**, **1.5**, **1.6**) establishing the feasibility of this scaffold in the design of adenosine A_{2A} antagonists for future studies.



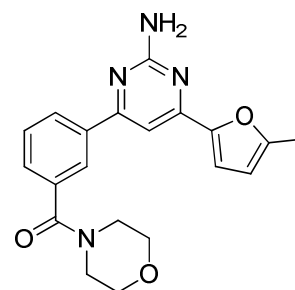
1.4

A_{2A}K_i = 6.34 nM



1.5

A_{2A}K_i = 16.28 nM



1.6

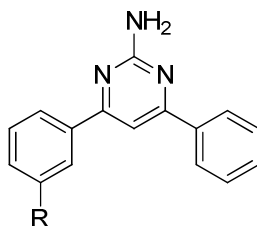
A_{2A}K_i = 29.32 nM

1.2 Aim, rationale and hypothesis

The focus of the preceding study was to identify a suitable scaffold for the design of adenosine antagonists which led to the discovery of the novel 2-amino-6-phenylpyrimidine nucleus. The main aim of this study is to further investigate the structure-activity relationships of this 2-amino-6-phenylpyrimidine scaffold with regards to its potential to antagonise both adenosine A₁ and A_{2A} receptors.

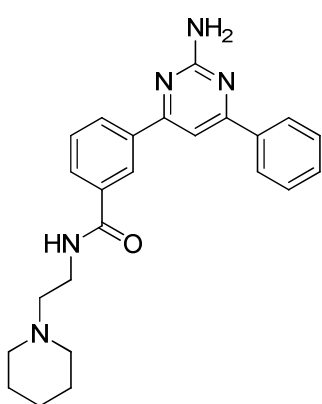
As a validated adenosine A₁ receptor assay was not available at the time of the preceding study, the potential of these 2-aminopyrimidines (e.g. **1.4**, **1.5**, **1.6**) as dual A₁ and A_{2A} antagonists could not be determined. The first objective of the current study is therefore to expand the biological data of these previously synthesised compounds by evaluating their potential as adenosine A₁ antagonists. The adenosine A₁ affinities will be combined with the previously obtained A_{2A} data so that the dual antagonistic potential of these compounds can be assessed and published at the same time. Furthermore, a preliminary cytotoxicity study will be carried out to assess the toxicity profile of these compounds.

To further explore the structure-activity relationships of the 2-aminopyrimidines, the following strategies will be employed: Firstly, it was decided to replace the methyl furan substituent on position 4 with a phenyl ring, as this simplified the synthesis (**1.7**) and results from a related study indicated that this change does not alter affinity to a significant degree (Kleynhans, 2014).

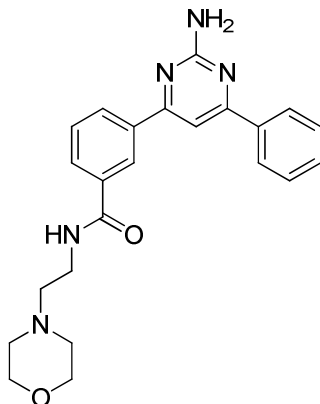


1.7

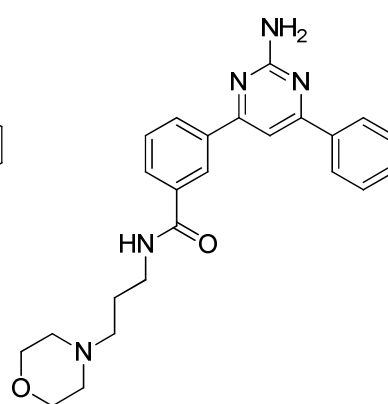
It was subsequently decided that three series of 2-amino-4,6-diphenylpyrimidines will be synthesised and evaluated as dual adenosine antagonists. In the first series, the effect of lengthening the amide side chain on both adenosine A₁ and A_{2A} affinity will be evaluated. (e.g. **1.8**, **1.9**, **1.10**).



1.8



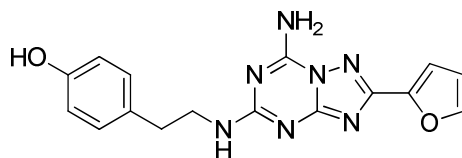
1.9



1.10

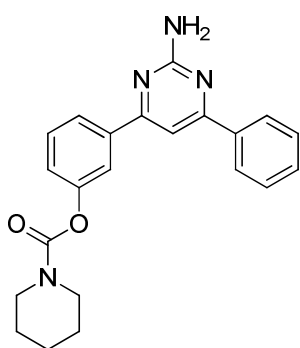
If the structures of other adenosine A_{2A} antagonists, for example preladenant (**1.1**) and ZM241385 (**1.11**), is considered it appears that long chains can often be accommodated in the receptor binding site, and it is postulated that lengthening the side chain may lead to additional interactions with the binding site, which may lead to an improvement in affinity. Since molecular modelling studies done in the

preceding study indicated that the amide carbonyl group is important for binding, this group is retained for this series.

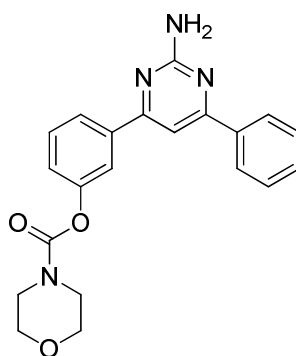


1.11 ZM241385

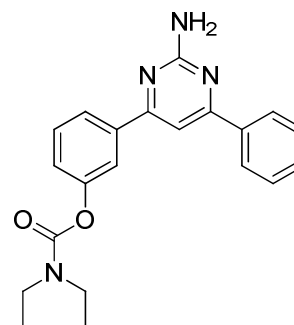
For the second series, different carbamate substituted 2-amino-4,6-diphenylpyrimidines (**1.12**, **1.13**, **1.14**) will be synthesised.



1.12

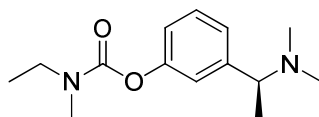


1.13



1.14

The carbamate group is often found in therapeutic agents such as the acetylcholinesterase inhibitor rivastigmine (**1.15**), used in the therapy of Alzheimer's disease.

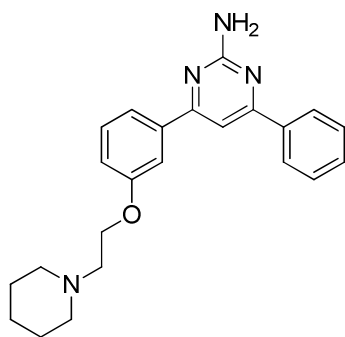


1.15 Rivastigmine

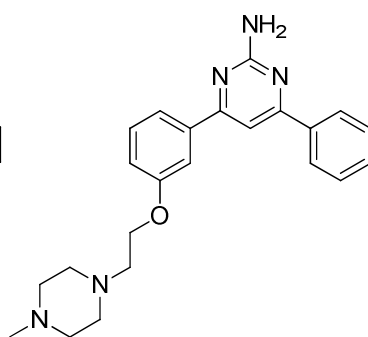
These amide-ester hybrids generally exhibit very good chemical and proteolytic stability as well as increased permeability across cellular membranes (Gosh & Brindisi, 2015). The additional oxygen in the carbamate side chain will alter the position of both the carbonyl oxygen as well as the side chain nitrogen and it is hypothesised that this could lead to additional or different binding interactions which could result in higher affinities.

To assess the importance of the carbonyl in the side chain, the amide group will be replaced with a less rigid ether group (**1.16**, **1.17**, **1.18**) in the third series. The hypothesis is that the carbonyl group is important for high affinity and it is postulated that these derivatives may show decreased affinities. On the other hand, it is possible that the more flexible side chain could bend and rotate to a greater extent

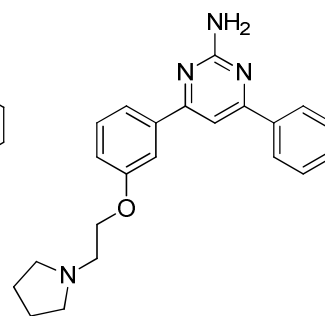
than the more rigid amide and carbamate side chains, allowing an improved fit of these compounds in the binding sites of the adenosine receptors, resulting in improved affinities.



1.16



1.17

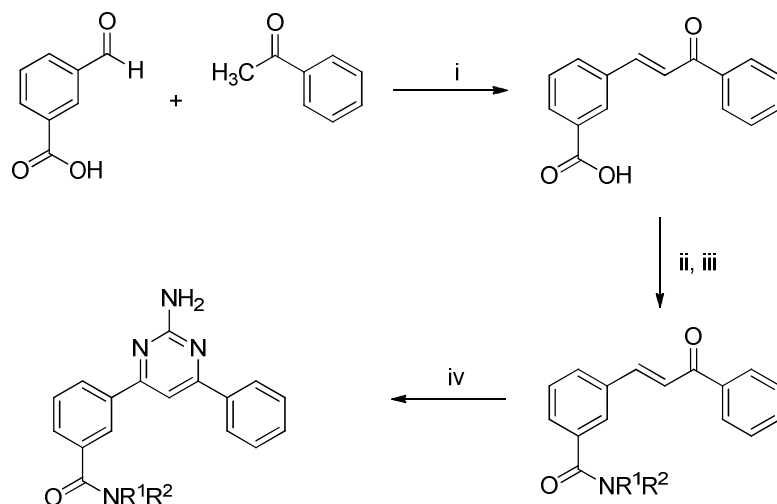


1.18

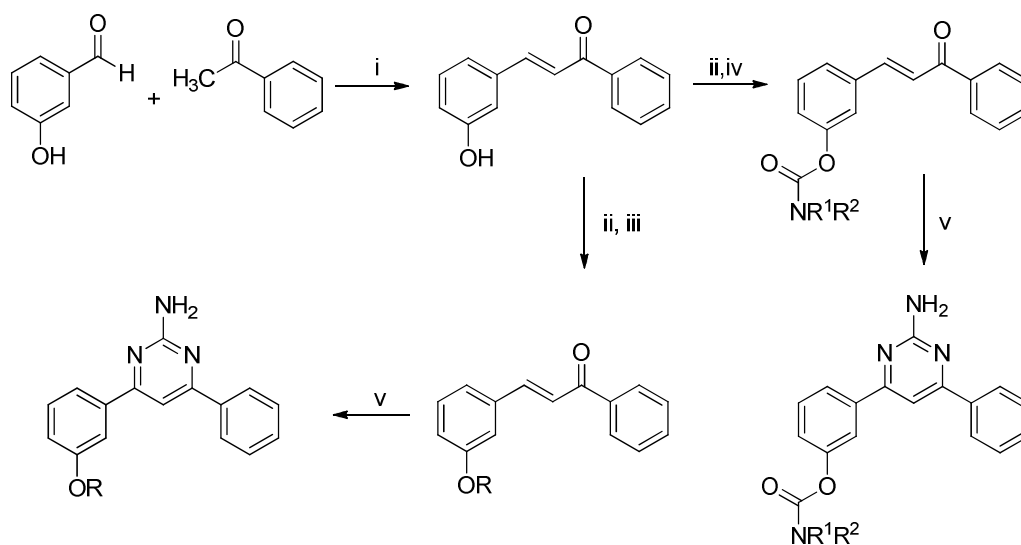
1.3 Objectives

The objectives of this study are summarised below:

- The adenosine A_1 affinities of the compounds synthesised in the preceding study (appendix) will be determined with a radioligand binding assay and their potential as dual antagonists will be assessed.
- Since the safety profile of these previously synthesised derivatives is also unknown, toxicity assays will be performed using the MTT cell viability assay in HELA cells.
- Three series of novel amide, carbamate and ether substituted 2-amino-4,6-diphenylpyrimidines will be synthesised. The selection of derivatives will mostly depend on the availability of starting materials. The synthesis will be performed by employing the general synthetic routes below (Scheme 1.1 and Scheme 1.2):



Scheme 1.1. Synthesis of amide derivatives. Reagents and conditions: (i) NaOH, 1M (2 eq.), MeOH, rt, overnight; (ii) CDI (1.2 eq.), CH₂Cl₂, rt, 4 h; (iii) Amine (1.2 eq.), CH₂Cl₂, rt, overnight; (iv) Guanidine hydrochloride (1.5 eq.), NaH (1.5 eq.), DMF, 110 °C, overnight.



Scheme 1.2. Synthesis of carbamate and ether derivatives. Reagents and conditions: (i) NaOH, 1M (2 eq.), MeOH, 90 °C, 5 days; (ii) K₂CO₃ (2 eq.), CH₃CN, rt, 30 min; (iii) RCl (1.2 eq.), reflux at 90 °C, overnight; (iv) R¹R²NCOCl (1.2 eq.), reflux at 90 °C, overnight; (v) Guanidine hydrochloride (1.5 eq.), NaH (1.5 eq.), DMF, 110 °C, overnight.

- d) Synthesised compounds will be screened *in vitro* using radioligand binding studies to determine their affinities for both adenosine A₁ and A_{2A} receptors.
- e) Compounds with the most promising dual adenosine A₁ and A_{2A} affinity will be evaluated *in vivo* using the haloperidol induced catalepsy assay in rats to assess their bioavailability and to

verify whether they are antagonists. Drug-like properties such as Log D and solubility will be determined for selected compounds if required.

- f) Molecular modelling will further be used to assess probable binding orientations as well as interactions between compounds and the binding site in an attempt to rationalise observed affinities of all compounds.

CHAPTER 2

Literature overview

2.1 Parkinson's disease

2.1.1 General background

Parkinson's disease (PD) is a chronic, neurodegenerative disorder described for the first time in 1817 by James Parkinson. While James Parkinson could only describe the clinical features of the disease, extensive research over the years has established that its primary pathological feature is the deterioration of dopaminergic neurons in an area of the brain known as the substantia nigra. Today PD is the second most common neurodegenerative disorder after Alzheimer's disease and affects approximately 1.5% of the global population over the age of 65. With the current increase in global population age, this number is expected to rise substantially in the coming decades, increasing the social and economic burden associated with the disease (Dauer & Przedborski, 2003; Hindle, 2010).

The most perceptible symptoms of PD include motor deficits such as bradykinesia (slowness), muscle rigidity, tremor during rest and an impairment of postural balance (Schwarzschild *et al.*, 2006). For the diagnosis of PD to be confirmed, at least two of these cardinal features must be present. Most of the motor symptoms can be managed and the prognosis of PD may be altered with effective pharmacological treatments such as levodopa and dopamine agonists. Levodopa, which is a precursor of dopamine, works by restoring the dopamine deficiency in the striatum and is, to date, the most effective option for treating the motor symptoms of PD. Unfortunately, long-term treatment with dopaminergic drugs (like levodopa and dopamine agonists) are associated with the development of motor complications such as dyskinesia as well as several other dopamine related autonomic and neuropsychological side effects. This highlights the importance of research aimed at the discovery of alternative and more effective therapies for PD (Olanow *et al.*, 2013; Fahn, 2008). In addition to the limitation of movement, PD patients also suffer from non-motor symptoms, which is especially troublesome in the more advanced stages of the disease. These symptoms include: neuropsychiatric disturbances such as depression, anxiety, apathy, problems with cognition, thought, behavior, speech and swallowing, as well as several autonomic and sensory disorders (Chaudhuri *et al.*, 2006; Adler, 2005).

2.1.2 Neuropathology

As mentioned earlier, one of the primary hallmarks of PD is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which leads to a substantial reduction in the dopamine

concentration in the striatum. The SNpc forms part of the striatum which is the primary input center (via the nigrostriatal pathway) of the basal ganglia. The main function of the striatum is to facilitate voluntary movements, and therefore a dopamine deficiency in this region of the brain leads to the involuntary movements seen in PD (Parent & Hazrati, 1995). Brain autopsies in patients diagnosed with only a mild degree of PD, have revealed that over 60% of striatal dopaminergic neurons have degenerated. Furthermore, if the possible dysfunction of a percentage of the remaining neurons is taken into account, up to 80% of the striatal dopamine concentration may be lost in these patients (Zigmond & Burke, 2005). It is further estimated that at least 50% of neurons in the SNpc are destroyed before PD symptoms emerge (Mackenzie, 2001).

Examination of the parkinsonian brain further reveals depigmentation of the SNpc as well as the presence of intraneuronal inclusions called “Lewy bodies” (LB’s), (Figure 2.1) (Dauer & Przedborski, 2003).

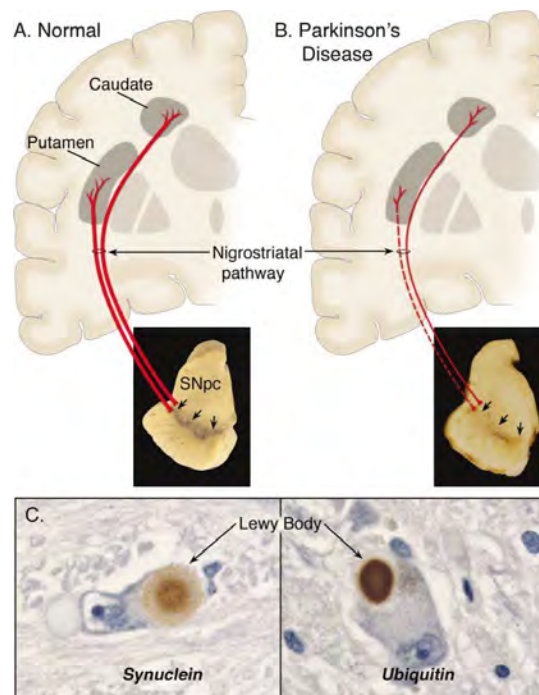


Figure 2.1. Pathophysiology of PD. (A) The human brain with the normal nigrostriatal pathway shown in red and the pigmented SNpc. (B) Represents the brain of a PD patient with the degradation of the nigrostriatal pathway as well as depigmentation of the SNpc. (C) The immunohistochemical labelling of intraneuronal inclusions named Lewy bodies (Dauer & Przedborski, 2003).

LBs are intracytoplasmic eosinophilic inclusions that are also considered to be a pathological hallmark of PD. In fact, some neuropathologists are reluctant to make the diagnosis in their absence (Mackenzie, 2001). It is suggested that LBs are a result of a failed attempt to remove excess proteins produced during PD. These proteins are encapsulated to be removed but become permanent after mechanisms

responsible for their elimination fail (Olanow *et al.*, 2004). These spherical cytoplasmic aggregates measure between 8 μm to 30 μm in diameter and are composed of a variety of proteins such as α -synuclein, ubiquitin, parkin, and neurofilaments. Although LBs can be found in the affected regions of the brain in the majority of PD patients, it has been reported that LBs are not exclusive to PD, and it remains unclear whether they are involved in the cause or formed as a result of the disease (Halliday *et al.*, 2011; Tugwell, 2008).

Although it is commonly thought that the pathology of PD is defined solely by dopaminergic neuron loss (believed to be the cause of typical motor symptoms) and the presence of LB's, there is also progressive degeneration reported in the cholinergic, noradrenergic and serotonergic systems. Post-mortem studies revealed a decline in choline acetyltransferase activity in the hippocampus and cortical areas, believed to be responsible for symptoms such as cognitive dysfunction and hallucinations present in some PD patients (Bosboom *et al.*, 2004). Furthermore, there are reports of impaired noradrenergic intervention in the locus coeruleus and neocortex which is believed to contribute to the cognitive impairment, as well as a serotonergic deficiency in the striatum and mediofrontal cortex that has been associated with depression (Espay *et al.*, 2014; Politis & Loane, 2011). The effects of PD on the noradrenergic and serotonergic systems are however not as clearly defined as that of the dopaminergic systems and are generally thought to occur only in more severe cases or late stages of the disease (Dauer & Przedborski, 2003). There are several other neuropathological features which are also worth mentioning. These include a consistent impairment of glutathione metabolism in the striatum, abnormalities in mitochondrial function, as well as high levels of superoxide dismutase activity together with increased iron concentrations that leads to abnormal hydrogen peroxide processing. The presence of hydrogen peroxide contributes to the formation of damaging cytotoxic free radicals which are believed to be involved in the progression of PD (Jenner *et al.*, 2013; Nikolova, 2012).

There is no standard diagnostic test for PD and the precise neuropathological hallmarks are still hotly debated. Diagnosis of PD is therefore mostly made on clinical grounds. However, a definite diagnosis still entails a post-mortem neuropathological examination of brain tissue from PD patients to identify required features such as dopaminergic neuron loss, the presence of LB's (although not exclusive to PD) and also to exclude histopathological trademarks of other disorders (Michotte, 2003; Gelb *et al.*, 1999).

2.1.3 Aetiology and pathogenesis

Despite decades of intensive research, the exact cause of PD remains unknown (Cieślak *et al.*, 2008) and the question remains whether the degeneration of dopaminergic neurons as observed in PD is primarily caused by ageing, or genetic or various environmental factors (Healy *et al.*, 2008; Talpade *et al.*, 2000). Consequently, several competing hypotheses have emerged, all containing viable evidence to support the separate theories.

2.1.3.1 Age hypothesis

PD can be classified as an age related disorder as the risk of developing the disease increases dramatically after the age of 60. In fact, it is believed that more than 1% of all elderly people have some form of the disease. However, the precise mechanistic correlation between PD and age is still unclear (Abdullah *et al.*, 2015). It has been previously reported that although neural cell death is associated with normal aging, the rate is much higher in patients with PD (Nurmi *et al.*, 2000). This provides evidence that age cannot be the sole cause of PD and that there is still uncertainty on which particular factors contribute to neural cell death.

2.1.3.2 Environmental toxin hypothesis

The environmental toxin hypothesis received intensive attention during the 1980's after it was discovered that prolonged exposure to certain external toxins lead to an elevated risk of developing PD (Dauer & Przedborski, 2003). For example, exposure of young drug addicts to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a contaminant found in a designer drug, resulted in a syndrome almost identical to PD, giving credibility to this hypothesis. After injection, MPTP is metabolised to the toxic metabolite MPP⁺, which accumulates in the brain causing the death of dopaminergic neurons, which in turn precipitates symptoms similar to PD. Numerous other toxins like paraquat (a herbicide structurally related to MPP⁺), rotenone (a garden insecticide) as well as metals, solvents and other pollutants have also been proven to precipitate PD symptoms in animal models (Goldman, 2014). However, there is no conclusive evidence that links any environmental toxin to idiopathic PD and most people exposed to these toxins do not necessarily develop the disease, suggesting that environmental toxins alone, are not enough to cause PD (McCormack & Di Monte, 2003; Tanner, 1992).

2.1.3.3 Genetic hypothesis

The possibility of a genetic aetiology for PD emerged in the last 15 years, after the discovery of a possible genetic basis for several forms of PD and other PD related disorders. Several genes, required for the expression of different proteins, have been identified and linked to PD namely, PARK1 (α -synuclein), PARK2 (parkin), PARK5 (ubiquitin carboxy terminal hydrolase-L1), PARK7 (DJ-1) and PARK 8 (leucine rich repeat kinase 2). From the genes mentioned above, mutations in the PARK-1 gene, encoding for α -synuclein, has probably received the most attention (Steece-Collier *et al.*, 2002). Mutations of α -synuclein are common in all cases of PD, however the extent to which it is involved in the onset of the disease is still unclear (Stefanis, 2012). Evidence suggests that the formation of aggregates may be accelerated due to mutation of α -synuclein, and lead to early-onset PD. As α -synuclein is a component of the LB structure, LB production may also increase as a result of these α -synuclein aggregates (Stefanis, 2012).

Although there is increasing evidence which is indicative of a genetic cause of PD, the pathogenic role of most of these gene mutations appears to be subtle. The majority of PD cases are not directly inherited

and do not have a genetic origin (Tugwell, 2008). In fact, only 5% of all PD cases appear to have a genetic basis. It has been shown that having a parent with PD only increases the risk of developing PD from 2% to 6%. In a study where 14000 pairs of twins 50 years and older were examined, only two twin pairs both had PD (Wirdefeldt *et al.*, 2004). Nonetheless, the identification of risk genes and mutations have provided new insights into PD aetiology and advances in genetics will continue to improve our understanding of the progression, response to treatment and underlying molecular mechanisms of PD (Trinh & Farrer, 2013).

2.1.3.4 Several other hypotheses

Several other hypotheses exist, including one that ascribes the development of PD to the aggregation and misfolding of proteins. Protein aggregation takes place when certain proteins undergo misfolding due to either oxidative stress, thermal stress or several other factors. These aggregated proteins are deposited when the necessary degradation systems responsible for removing them malfunction (Dobson, 2003). Protein aggregates in the brain are present in several age-related diseases and although the composition and location differ from disease to disease, the common presence of these protein deposits suggest that it might play a role in cell degeneration (Dauer & Przedborski, 2003).

Another hypothesis postulates that PD may be caused by endogenous toxins that produce harmful oxidative species. Dopamine, for example, is metabolised by monoamine oxidase to form reactive oxygen species (ROS), which cause damage to dopaminergic neurons (Youdim *et al.*, 2006). Another example is that of the excitatory neurotransmitter, glutamate, which causes excitotoxic cell death in excessive amounts (Foran & Trotti, 2009).

Neuro-inflammation has recently also been implicated in the development of PD. The tightly regulated immune responses within the brain are partly dependant on the blood-brain barrier as well as a number of resident cells in the brain such as microglia, astrocytes and oligodendrocytes (Taylor *et al.*, 2013). Under regular circumstances, when an immune response is initiated (due to toxic protein accumulation, pathogen invasion or injury) tissue repair is instigated to clear out debris as well as apoptotic cells. Once the initial stress has been removed, the innate response should resolve, however, persistence or a failure in this inflammatory process will lead to an overproduction of neurotoxic factors such as prostaglandins, cytokines and chemokines. These inflammatory responses generate ROS which are believed to contribute to neural cell death (Taylor *et al.*, 2013).

Furthermore, there are theories regarding the role of energy metabolism, viral causes, immune regulation and several other hypotheses, all of which have some credible role in the aetiology of PD (Standaert & Young, 2006; Hirsch & Hunot, 2009; Armentero *et al.*, 2011).

Not one of these hypotheses can however, on its own, adequately explain the cause of PD, which suggests that a cascade of events that includes the interaction between some or even all the above could potentially be responsible for the onset of the disease.

2.1.4 Treatment

There is no cure for PD and none of the currently approved drugs are registered as neuroprotective agents. However, evidence suggests that mono-amine oxidase inhibitors may have neuroprotective properties (described in section 2.1.4.3). These effects however have only been observed in animal models and cell cultures, and actual evidence of neuroprotection in humans is still to be reported (Youdim *et al.*, 2006; Baker *et al.*, 2012; Youdim *et al.*, 2005).

Current therapies are therefore only symptomatic, aimed at relieving the motor and in some cases the non-motor symptoms of the disease. Drugs presently on the market are mostly dopaminergic in nature, and are aimed at improving dopaminergic signalling in the striatum. This is done by either increasing the supply of dopamine (with drugs such as levodopa), by stimulating the dopamine receptors directly (dopamine agonists) or by inhibiting the metabolism of dopamine (monoamine oxidase inhibitors) (Lees, 2005). Several symptomatic drug therapies thus exist; however, the extent of the disease ultimately determines which therapy best suits the unique needs of each patient so that quality of life can be improved. Several common drugs clinically used today are discussed below.

2.1.4.1 Levodopa

Levodopa is still, more than 40 years after its first introduction to the market, the gold standard in PD therapy. It is the metabolic precursor of dopamine and is metabolised to dopamine by L-amino acid decarboxylase (L-AAD) after crossing the blood-brain barrier, which dopamine is unable to do (Figure 2.2) (Chen & Swope, 2007).

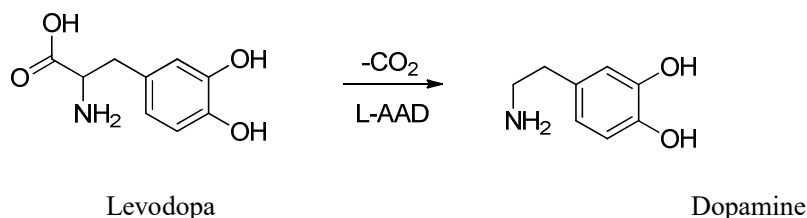
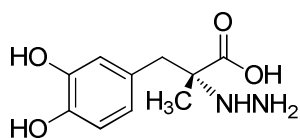


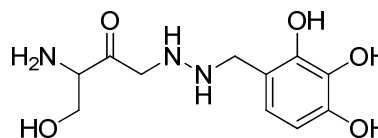
Figure 2.2: The metabolism of Levodopa to dopamine by L-AAD

The supply of dopamine in the brain therefore increases directly with the administration of levodopa, reducing symptoms like muscle rigidity and bradykinesia. However, after long-term treatment, patients usually develop side effects such as fluctuations in motor function, dyskinesia, neurobehavioural problems as well as nausea, which are ascribed to the peripheral conversion of levodopa to dopamine. The latter can be reduced by combining levodopa with drugs like carbidopa (2.1) and benserazide (2.2) which inhibit L-AAD peripherally, resulting in higher levels of levodopa in the brain (Standaert &

Young, 2006; Guttman *et al.*, 2003). The motor complications associated with levodopa treatment, especially dyskinesia, are common, difficult to treat and are often troublesome (Fabbrini *et al.*, 2007). The motor fluctuations are believed to be the result of a combination of the chronic intermittent delivery of the short half-life levodopa (+/- 90 minutes) and the progressive nature of PD (Smith *et al.*, 2003). In the early stages of PD, the remaining natural dopamine release is sufficient enough to compensate for the fluctuations of plasma levels caused by the short pharmacological half-life of levodopa. However, in advanced PD, the physiological dopamine-release continues to decrease and after a while reaches a point where it can no longer buffer the unstable plasma levels of levodopa. This manifests as involuntary movements or the so called “wearing-off” phenomenon (decrease of therapeutic effect with each dose of levodopa) as well as levodopa-induced dyskinesia. Strategies to increase the half-life of levodopa are therefore needed if a more stable clinical response is to be seen in advanced PD patients (Huot *et al.*, 2013; Brotchie, 2005).



2.1 Carbidopa

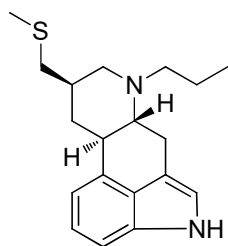


2.2 Benserazide

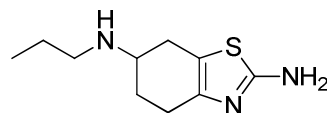
2.1.4.2 Dopamine D₂ agonists

Dopamine agonists (DAs) are divided into two classes, the ergot derived DAs such as bromocriptine and pergolide (2.3), and the newer non-ergot DAs like ropinirole and pramipexole (2.4). Although the use of ergot DAs is effective in the management of PD, reports of adverse fibrotic side effects in patients have restricted their use as PD therapy. Like most DAs, the non-ergot derivatives more commonly used to treat PD have a high affinity for the dopamine D₂ receptor, and are used to improve both motor symptoms and the “wearing-off phenomenon” experienced during levodopa therapy (Zhou *et al.*, 2014; Vijverman & Fox, 2014). Dopamine D₂ agonists mimic the function of dopamine by stimulating dopaminergic D₂ receptors directly. The therapeutic effect of DAs is generally less dramatic when compared to the clinical effects seen with levodopa, but does result in less dyskinesia. This may be attributed to the fact that DAs have preferential selectivity for the D₂ receptor, resulting in less motor side-effects and a weaker symptomatic effect compared to dopamine produced from levodopa, which interacts equally with all five dopamine receptor subtypes as well as with other neurotransmitting systems (Misu *et al.*, 2002). DAs are frequently used, mainly in combination with levodopa, although they are effective as monotherapy in mild-to-moderate cases of PD. With combination therapy, the levodopa dosage may additionally be lowered, especially when initialising treatment. When DAs are prescribed as first-line treatment it is mostly for patients under the age of 55, since monotherapy provokes less dyskinesia. However, levodopa remains the therapy of choice in older patients, as they

are more susceptible to the cognitive side effects associated with dopamine agonists (Lees, 2005; Standaert & Young, 2006).



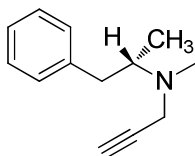
2.3 Pergolide



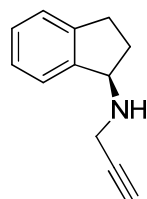
2.4 Pramipexole

2.1.4.3 Monoamine oxidase B (MAO-B) inhibitors

MAO-B inhibitors decrease the oxidative metabolism of dopamine in the striatum and consequently prolong the activity of the available endogenous dopamine. Since the metabolism of amines leads to the production of ROS (believed to contribute to neurodegeneration), MAO-B inhibitors may also, in theory, reduce neurodegeneration. The two drugs of this class currently used are selegiline (**2.5**) and rasagiline (**2.6**). These drugs can be given as monotherapy in the early stages of PD, delaying the need for levodopa, or they can decrease the motor fluctuations associated with long-term use of levodopa, if co-administered (Youdim *et al.*, 2006; Miyasaki *et al.*, 2002; Suchowersky *et al.*, 2006). Unfortunately, several undesirable psychotoxic and cardiovascular side effects are associated with the use of MAO-B inhibitors. Furthermore, since these drugs bind irreversibly to the MAO-B enzyme, a slow recovery rate of enzyme activity is expected after treatment is terminated (Tipton *et al.*, 2004).



2.5 Selegiline

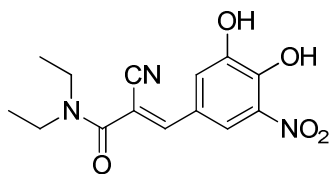


2.6 Rasagiline

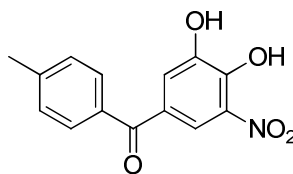
2.1.4.4 Catechol-O-methyltransferase (COMT) inhibitors

COMT inhibitors such as entacapone (**2.7**) and tolcapone (**2.8**) inhibit the peripheral metabolism of catecholamines such as dopamine and levodopa. This class of drugs can be given in combination with levodopa, since the peripheral metabolism of levodopa will be decreased, resulting in higher levels of levodopa that reaches the brain. COMT inhibitors however have no effect on PD as monotherapy and side effects are mostly dopaminergic in nature (nausea, vomiting, dyskinesias, hallucination), since it is usually used in combination with levodopa treatment. These side effects are usually moderate,

however, the use of tolcapone are under strict regulations with regards to liver enzyme monitoring, as cases of fatal hepatotoxicity have been reported (Haasio, 2010).



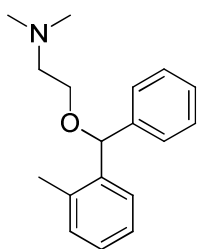
2.7 Entacapone



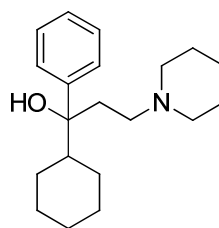
2.8 Tolcapone

2.1.4.5 Anticholinergics

Antagonists of muscarinic acetylcholine receptors such as orphenadrine (2.9) and trihexyphenidyl (2.10) are generally reserved as last resort for patients suffering from tremors that are resistant to dopaminergic therapy (Rezак, 2007; Rao *et al.*, 2006). Tremor is a symptom of increased cholinergic activity caused by the dopamine deficiency in PD. Inhibition of cholinergic activity will therefore be therapeutic, however, this class of compounds is also associated with numerous side effects. The most severe adverse effects include sedation, mental confusion, vision disturbances, constipation and urinary retention which usually limit the use of these compounds, especially in elderly patients (Chen & Swope, 2007).



2.9 Orphenadrine



2.10 Trihexyphenidyl

2.1.4.6 Novel therapies

Several new therapeutic strategies are currently being investigated as alternative drug treatment options for PD, including new formulations of existing drugs like levodopa and dopamine agonists (Vijverman & Fox, 2014). Most PD drugs currently used have numerous pharmacokinetic and pharmacodynamic challenges. New formulations of existing drugs are therefore investigated in an attempt to bypass these clinical shortcomings and to optimise the benefits. IPX066 (approved in January 2015 by the FDA) is an extended-release formulation of levodopa combined with carbidopa that contains both immediate and extended release components. This prolongs the duration of sustained release of levodopa and decreases the wearing-off phenomenon. Another example of an old drug with new possibilities is APL-130277, which is a novel formulation of apomorphine for sublingual administration, currently in phase 2 clinical trials. Apomorphine is an agonist of the D₁ and D₂ receptors (with a preference toward the

latter) used as rescue therapy, much like insulin for diabetics, to temporarily treat the refractory motor fluctuations that develop in between levodopa dosages. Although Apomorphine is effective, it remains largely underused, mostly due to its short dose dependent duration (mean 40 min), side effects like nausea (usually administered with an anti-emetic) and unpleasant administration (subcutaneous injection or infusion). The main goal of this newly developed sublingual preparation of apomorphine is to retain the quick onset of action, to improve duration of therapeutic effect as well as simplifying administration and improving the delivery of apomorphine (Katzenschlager, 2009; Vijverman & Fox, 2014).

There are also novel therapeutic strategies that focus on alternative non-dopaminergic targets for the treatment of PD which include adenosine A_{2A} antagonists, glutamate inhibitors, adrenergic receptor antagonists, iron chelators, calcium channel blockers and several others (Stayte & Vissel, 2014; Vijverman & Fox, 2014). In recent years, adenosine A_{2A} antagonism in particular has received attention (Pinna, 2014). So far, KW6002 (istradefylline) is the only adenosine A_{2A} antagonist approved (in Japan) as adjunctive PD treatment, however tozadenant, another A_{2A} antagonist currently in phase 3 clinical trials, is expected to be approved in the near future if all goes well. Although KW6002 was only permitted in combination treatment of PD (Stayte & Vissel, 2014), several preclinical studies have reported that adenosine A_{2A} antagonism independently can improve motor symptoms (Shiozaki *et al.*, 1999; Kanda *et al.*, 1998a; Kanda *et al.*, 1998b; Schwarzschild *et al.*, 2006; Wardas *et al.*, 2001; Bara-Jimenez, *et al.*, 2003), non-motor symptoms (Yamada *et al.*, 2013) and furthermore has the possibility to exert neuroprotective effects in PD (Ascherio *et al.*, 2001; Jenner *et al.*, 2009; Müller & Ferré, 2007), making antagonism of adenosine A_{2A} receptors an especially appealing strategy.

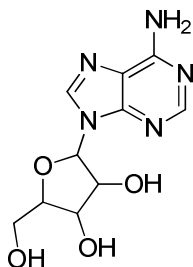
2.1.4.7 Conclusion

Although all of the above-mentioned drugs alleviate the symptoms of PD and improve the quality of life for a short while, none of them can stop the progressive neurodegeneration in PD. Furthermore, all of these drugs come with their own array of side effects which adds to the patient's suffering. This clearly emphasises the need for more effective drug therapies that can not only treat the symptoms of the disease, but also have the potential to be neuroprotective. Adenosine receptors have recently emerged as a promising non-dopaminergic alternative target for PD, with the potential to relieve motor as well as non-motor symptoms and in addition, have the potential to provide neuroprotection. The focus of this study is thus to further investigate antagonism of adenosine receptors as an alternative therapy option for PD.

2.2 Adenosine and its receptors

2.2.1 Background

Adenosine is an essential endogenous nucleoside composed of the purine base adenine attached to a ribose sugar (2.11). It is present throughout the human body where it has numerous functions, in both the central nervous system (CNS) and a large variety of peripheral tissues.



2.11 Adenosine

In the CNS, adenosine acts as a neuromodulator by regulating the release of several neurotransmitters presynaptically. It is thus implicated in the regulation of sleep, basal ganglia function, locomotion, cerebral blood flow, nociception, arousal as well as providing neuroprotection during hypoxic, ischemic and oxidative stress events (Cunha, 2005; Ferreira & Paes-de-Carvalho, 2001; Xu *et al.*, 2005; Jaakola *et al.*, 2008). Peripherally, adenosine plays a role in cell growth, pain perception, inflammation, apoptosis and affects the cardiovascular as well as gastro-intestinal systems (Schulte & Fredholm, 2003). These biological effects of adenosine are initiated via four receptor subtypes which have been denoted A₁, A_{2A}, A_{2B}, and A₃ receptors. All four adenosine receptor subtypes are members of the heterotrimeric guanine nucleotide-binding protein (G protein) coupled receptor (GPCR) family, each having their own distinct subcellular localization, transduction pathways, different G-protein binding preferences, as well as unique ligand binding- and acting profiles (briefly summed up in Table 2.1) (Hernan *et al.*, 2002; Fredholm *et al.*, 2001; Fredholm *et al.*, 2005).

Table 2.1: The distribution, function and different G-protein binding preferences of each adenosine receptor subtype (Fredholm *et al.*, 2001; Xu *et al.*, 2005; Poulsen & Quinn, 1998; Cacciari *et al.*, 2005)

<i>Receptor subtype</i>	<i>G-protein</i>	<i>Signalling pathway</i>	<i>Role and/or Function</i>	<i>Brain distribution</i>	<i>Peripheral distribution</i>
A ₁	G _i	cAMP* ↓	Inhibition of neurotransmitter release, ischemic preconditioning, symptomatic and parasymptomatic activation, bradycardia, sleep, tubuloglomerular feedback, analgesia, inhibition of lipolysis and glucagon release	Densely expressed throughout the CNS (particularly in neocortex hippocampus and cerebellum).	Skeletal muscles, lungs, liver, pancreas kidneys and adipose tissue
A _{2A}	G _s	cAMP ↑	Neurodegeneration, locomotion, inhibition of platelet aggregation, wakefulness, immunosuppression, vasodilation, defense against ischemic damage, activation of sensory nerve activity and angiogenesis	Densely expressed in striatum. Also present in olfactory tubercle, nucleus accumbens, caudate putamen and brain endothelial cells	Blood platelets, leukocytes, thymus, spleen, heart, lungs, blood vessels neutrophils, microglia, astrocytes, oligodendrocytes, platelets and vascular smooth muscle
A _{2B}	G _s	cAMP ↑	Vascular integrity, relaxation of smooth muscle in intestine and vasculature, pain, inhibition of monocytes and macrophages and activation of mast cell release.	Widespread (low concentration in all areas)	Caecum, lungs, colon, blood vessels, bladder, eye, mast cells, adipose tissue, kidney and adrenal gland

A ₃	G _i	cAMP ↓	Boost mediator release from mast cells, inflammatory pain, white cell chemotaxis and airway contraction	Widespread (Higher concentrations in hippocampus and cerebellum)	Testis, thyroid, mast cells, spleen, kidney heart and liver.
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*cAMP = cyclic adenosine monophosphate

The adenosine A_{2B} and A₃ receptors possess relatively low affinity for adenosine when compared to the superior affinities of the A₁ and A_{2A} receptors (Fredholm *et al.*, 2011). This suggests that both the adenosine A₁ and A_{2A} receptors play the dominant role in the central nervous system under normal physiological conditions. Furthermore, the distinct localisation of the adenosine A_{2A} receptor in the striatum and its specific role in neurodegeneration, (discussed in section 2.3.5) as well as locomotion (Table 2.1) is indicative of its potential as a target in the treatment of PD. Recently, the role of adenosine A₁ receptors in PD has also emerged (discussed in section 2.4.3). There is evidence that suggests that antagonism of both adenosine A₁ and A_{2A} receptors during dual therapy could promote the stimulation of movement (discussed in section 2.5). In addition, epidemiological studies reported that regular consumers of caffeine, which is a dual adenosine A₁ and A_{2A} antagonist, have a lower risk of developing PD, which is indicative of a neuroprotective role in PD (Hernan *et al.*, 2002; Ascherio *et al.*, 2004).

A detailed description of the properties, processes, functions as well as the particular role that the adenosine A₁ and A_{2A} receptors play in PD, are discussed below.

2.2.2 Overview of the basal ganglia structures, pathways and their role in PD

Adenosine A_{2A} receptors are concentrated in the brain regions where dopamine is most abundant, such as the striatum and other parts of the basal ganglia (Table 2.1). They are co-localised with dopamine D₂ receptors and are involved in the modulation of dopaminergic neurotransmission. The richly interconnected neural network of the basal ganglia allows these receptors to play a part in adaptive control of behaviour through interactions with sensorimotor, cognitive and motivational brain areas (Graybiel *et al.*, 1994). The major input centre of the basal ganglia is the striatum, which plays a critical role in the control of movement. The dorsal part of the striatum is responsible for the selection and initiation of motor functions as well as for the development of skills and habits (Graybiel *et al.*, 1994; Graybiel, 1995; White, 1997). On the other hand, the ventral striatum plays a central role in motivation and reward (Schultz *et al.*, 2003).

Two important pathways, namely the direct striatonigral and the indirect striatopallidal pathway connect the output structures of the basal ganglia and the dorsal striatum. The direct pathway expresses the stimulatory dopamine D₁ as well as the inhibitory adenosine A₁ receptor subtypes. In contrast, the indirect pathway expresses the inhibitory D₂ and opposing stimulatory adenosine A_{2A} receptors. Under normal conditions, binding of dopamine to D₁ receptors thus result in activation of the direct pathway,

while binding to D₂ receptors results in deactivation of the indirect pathway. Adenosine on the other hand deactivates the direct pathway by binding to A₁ receptors and activates the indirect pathway by binding to A_{2A} receptors. Activation of the direct pathway is believed to stimulate normal movement while activation of the indirect pathway inhibits movement. Signalling occurs in both these pathways during movement and should therefore be in balance for movement to be coordinated (Figure 2.3 A) (Müller & Ferré, 2007; Xu *et al.*, 2005). For example, when a person is walking, activation of the direct pathway mobilises the right sets of muscles so that normal forward movement can take place, while simultaneous activation of the indirect pathway leads to inhibition of other opposing muscles preventing disruption of the intended movement (Wang *et al.*, 2015). (It should be noted that this is a very simplified representation of the mechanisms involved in coordinated movement, which is a highly complicated process).

The substantial loss of dopaminergic input in the striatum experienced during Parkinson's disease causes an imbalance between the two pathways as adenosine signalling is now basically unopposed. As a result, the activity of the direct pathway decreases and the activity of the indirect pathway increases, resulting in disruption of movement (Figure 2.3 B). Current dopaminergic treatment therapies used in PD, such as levodopa, improve motor function by re-activating the direct pathway (D₁ receptors) and inhibiting the indirect pathway (D₂ receptors) and as a result restore the imbalance between the two pathways (Figure 2.3 C). Dopamine D₂ agonists on the other hand, improve motor symptoms of PD by binding to inhibitory D₂ receptors, deactivating the overactive indirect pathway, thus restoring some of the imbalance between the two pathways (Figure 2.3 D) (Müller & Ferré, 2007).

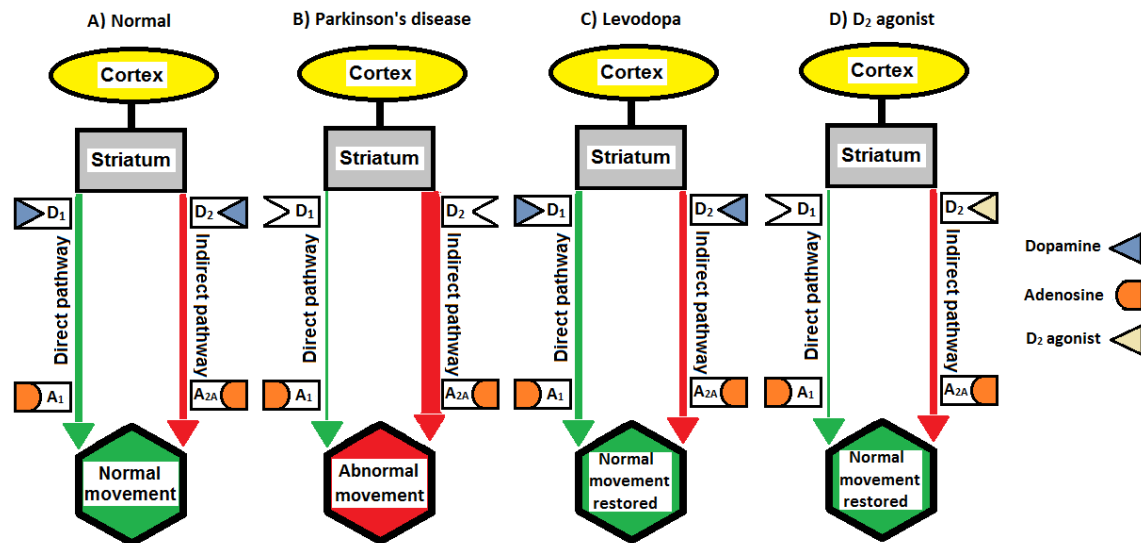


Figure 2.3: Schematic diagram illustrating the direct and indirect pathways under (A) normal conditions, (B) during Parkinson's disease (C) with the administration of levodopa agent, restoring the imbalance and (D) administration of a D₂ agonist restoring only some of the imbalance between the direct and indirect pathways. Blue arrows indicate stimulation of movement and red arrows inhibition of movement.

Adenosine A_{2A} antagonists act in a similar manner, deactivating the indirect pathway by blocking the stimulatory adenosine A_{2A} receptors, thus also restoring some of the imbalance between the pathways (Figure 2.4 C) (Xu *et al.*, 2005; Mori & Shindou, 2003). In theory, adenosine A_1 antagonism would also be able to restore the motor symptoms present in PD since antagonism of A_1 receptors would increase the activity of the direct pathway by binding to the inhibitory adenosine A_1 receptors, also improving some of the balance between the pathways (Figure 2.4 D).

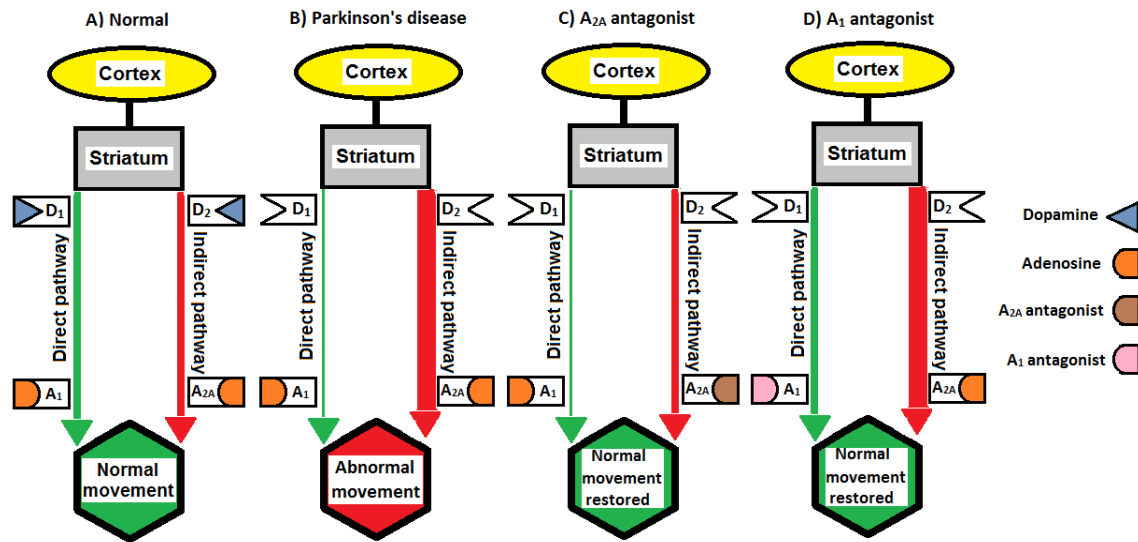


Figure 2.4: Schematic diagram illustrating the direct and indirect pathways under (A) normal conditions, (B) during Parkinson's disease (C) with the administration of an A_{2A} antagonist, restoring the imbalance and (D) administration of an A_1 antagonist restoring only some of the imbalance between the direct and indirect pathways. Blue arrows indicate stimulation of movement and red arrows inhibition of movement.

The study of these pathways and the understanding of their functions thus provides the basis for the design of adenosine antagonists as a treatment option for PD patients.

2.3 Adenosine A_{2A} receptor

2.3.1 Structure

The adenosine A_{2A} receptor gene has been cloned from numerous species including human, rat, mouse, guinea pig and dog, and a high degree of structural homology exists between A_{2A} receptors from rat, mouse and human (Fredholm *et al.*, 2000). All the adenosine receptor subtypes exhibit characteristics similar to class A G-protein coupled receptors. Similarity of the overall amino acid sequence identity between the adenosine A_{2A} receptor and the A_1 , A_{2B} and A_3 receptor subtypes are 49%, 58% and 41%, respectively (Katrictch *et al.*, 2010). Even though all subtypes of adenosine receptors have a high sequence similarity, A_{2A} receptors have a much greater weight (45 kDa) compared to the rest. This superior weight is attributed to a longer cytosolic carboxy terminus (C-terminus) present in the A_{2A}

receptor structure. The structure of the A_{2A} receptor is further composed of a central core consisting of seven transmembrane (TM1-7) α -helices (each with 20 to 27 amino acids), three intercellular (IL1-3), and three extracellular loops (EL1-3), an additional short membrane-associated helix (helix 8) that runs parallel to the cytoplasmic membrane surface, as well as an extracellular amino-terminus (*N*-terminus) (Pirainen *et al.*, 2011). The extracellular loops link the TM domains (which are mainly α -helices) where TM3 and EL2 contain cysteine residues forming a disulfide bond (Pirainen *et al.*, 2011).

Mutagenesis data on agonist and antagonist binding as well as high resolution crystal structures aided in the identification of the specific receptor residues in the ligand-binding cavity that are important for binding to different ligands (Keränen *et al.*, 2014). One such example is the availability of the crystal structure of the human adenosine A_{2A} receptor crystallised with the known A_{2A} antagonist ZM241385 (Jaakola *et al.*, 2008) (Figure 2.5). Several important interactions were observed between ZM241385 and the binding site. These include interactions such as aromatic stacking interactions between Phe168 and the triazolotriazine core and hydrogen bonds between the exocyclic amino group of ZM241385 and Glu169 as well as Asn253. These interactions are believed to anchor the ligand in the active site and are also important binding interactions observed for other high affinity antagonists (Zhang *et al.*, 2014). Jaakola and co-workers (2010) further reported that interactions with residues in the lower region of the binding site of the A_{2A} receptor determine the affinity, while selectivity is mostly controlled by the less conserved upper part (Figure 2.5).

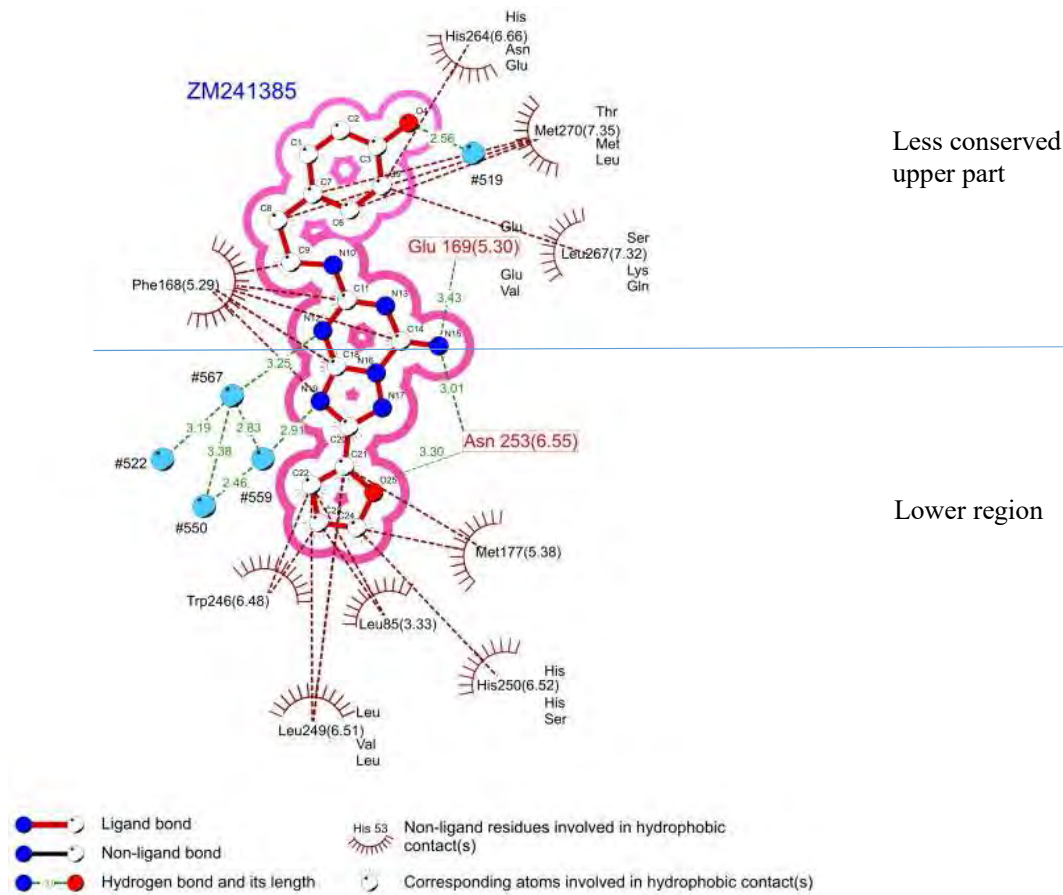


Figure 2.5: ZM241385 docked in the active site of the human A_{2A} receptor. Green lines: hydrogen bonding interactions; Blue lines: hydrophobic interactions; Orange lines: π stacking (Pirainen *et al.*, 2011).

2.3.2 Brain Distribution of the adenosine A_{2A} receptor

In the central nervous system, A_{2A} receptors are abundantly expressed in the striatum as well as other parts of the basal ganglia such as the nucleus accumbens and olfactory bulb, where they are mostly co-localised with dopamine D₂ receptors (Rosin *et al.*, 2003). In fact, the density of A_{2A} receptors in the basal ganglia is about 20 times greater than any other part of the brain (Lopes *et al.*, 2004). Distribution in these specific parts of the brain that mostly control movement, implicate these receptors in neurological diseases such as PD. High expression of adenosine A_{2A} receptors has also been reported in neutrophils, microglia, astrocytes, oligodendrocytes, platelets and vascular smooth muscle which all play key roles during inflammation. A_{2A} receptors are also abundantly present in brain endothelial cells where they are ideally localised to alter the lipid and/or cholesterol metabolism in the blood-brain barrier, further implicating them in the regulation of inflammatory responses (Schaddelee *et al.*, 2003; Varani *et al.*, 2010; Lappas *et al.*, 2005; Varani *et al.*, 2009; Stolp & Dziegielewska, 2009; Weiss *et al.*, 2009; Carvey *et al.*, 2005).

The specific distribution of adenosine A_{2A} receptors in the brain therefore supports its role in neural development, neuroprotection, as well as its significant role in movement and is therefore ideal as a target for PD.

2.3.3 Role of adenosine A_{2A} antagonism in PD

Adenosine A_{2A} receptor antagonism is relevant to the treatment of PD in several ways. Firstly, there is a strong antagonistic interaction between dopamine D₂ receptors and adenosine A_{2A} receptors in the indirect striatopallidal pathway, effecting the regulation of normal movement (discussed in section 2.2.2). Results from biochemical studies further illustrated that stimulation of A_{2A} receptors decreases the affinity of dopamine for D₂ receptors as well as its signal transduction to the G protein. Antagonism of adenosine A_{2A} receptors would thus lead to an increase in dopamine's affinity for the D₂ receptor (Ferrè *et al.*, 1991; Salim *et al.*, 2000).

Secondly, preclinical studies have shown that A_{2A} antagonism has the ability to improve several of the primary motor symptoms of PD while also reducing motor fluctuations (“wearing-off” phenomenon) and dyskinesias which develop with levodopa therapy (Hickey & Stacy, 2012). Parkinsonian rest tremor, which is normally resistant to dopamine replacement therapies, was stabilised in rodent models of parkinsonian tremor after administration of A_{2A} antagonists (Correa *et al.*, 2004; Salamone *et al.*, 2008). This was validated in a clinical setting, as KW6002 in combination with subthreshold doses of levodopa, counteracted resting tremor effectively (Bara-Jimenez, *et al.*, 2003). In addition, muscle rigidity induced in rodents by the dopamine depleting drug reserpine can be reduced with the administration of an A_{2A} antagonist or completely eliminated in combination with levodopa (Wardas *et al.*, 2001).

Thirdly, a recent study has shown that A_{2A} antagonism results in a reduction of non-motor symptoms such as depression that often accompany PD. An anti-depressant effect in rodents was illustrated for KW6002, at doses comparable to equivalent doses of known tricyclic anti-depressants such as desipramine and imipramine. KW6002 may further enhance the effects of other antidepressants since a significant improvement in rodents was seen during combination therapy with drugs like paroxetine, fluoxetine and deprenyl at doses that do not normally show anti-depressant effects (Yamada *et al.*, 2013).

Fourthly, and probably the most exciting role of A_{2A} antagonism, is its ability to exert a neuroprotective effect. A detailed discussion of the neuroprotective properties and underlying mechanisms are provided in section 2.3.5.

2.3.4 A_{2A} antagonism and its interactions with other neurotransmitter receptors in PD

A receptor heteromer is defined as a macromolecular complex, composed of no less than two functional receptors, where stimulation results in a physiological outcome that is noticeably different from those of its individual effects (Ferrè *et al.*, 2009). Adenosine A_{2A} receptors form several heteromers with other

neurotransmitter receptors which include D₁, D₂, A₁, cholinergic, glutamatergic, opioid, vasoactive intestinal peptide and calcitonin gene-related peptide receptors. Some of these receptor interactions play distinct roles in PD and are discussed in this section.

The interaction between adenosine A_{2A} and dopamine D₂ receptors has already been described in sections 2.2.2 and 2.3.2. These receptors can however also interact on the level of the second messenger systems. For example, the activation of adenosine A_{2A} receptors results in the formation of cAMP, while activation of dopamine D₂ receptor inhibits cAMP formation. The response to D₂ receptor activation can thus be mimicked by antagonising adenosine A_{2A} receptors, since this will also result in inhibition of cAMP production (Lee *et al.*, 2002). Furthermore, as mentioned in section 2.3.3, the antagonism of A_{2A} receptors increases the binding affinity of dopamine for the D₂ receptor and also increases the G-protein coupling of the D₂ receptor. This interaction between the A_{2A} and D₂ receptors implies functional interdependence, however data suggest that striatal adenosine A_{2A} receptors are not entirely dependent and can exhibit antiparkinsonian activities with either D₂-independent or D₂-dependent mechanisms (Aoyama *et al.*, 2002; Chen *et al.*, 2001a; Xu *et al.*, 2005). This is one of the most studied receptor heteromers in the striatum and their malfunction plays a key role in neurodegenerative disorders like PD (Ferrè *et al.*, 2008; Fuxe *et al.*, 2007).

Another interesting interaction that plays a role in PD is the one between the adenosine A_{2A} receptor and the cholinergic system. Anticholinergic drugs are given to patients to decrease the overactive cholinergic system present in PD. Adenosine A_{2A} receptors can also influence the cholinergic system as the levels of acetylcholine in the striatum decrease during antagonism, restoring motor deficits associated with an overactive cholinergic output (Kirkpatrick & Richardson, 1993; Xu *et al.*, 2005).

Antagonism of the adenosine A_{2A} receptor further decreases extracellular gamma-aminobutyric acid (GABA) levels. In PD, the central nervous system is inhibited by excessive amounts of GABA which result in CNS suppression. Adenosine A_{2A} antagonism can therefore restore these levels and in this way also improve the motor effects caused by the GABA mediated CNS suppression (Díaz-Cabiale *et al.*, 2002; Xu *et al.*, 2005).

A strong synergistic relationship exists between the metabotropic glutamate receptor and the adenosine A_{2A} receptor. Glutamate is one of the major excitatory neurotransmitters in the brain, involved in the repair of the central nervous system, memory and learning, as well as synaptic plasticity (Armentero *et al.*, 2011). Whereas stimulation of the adenosine A_{2A} receptor results in glutamate release, which in the long run causes neurotoxicity, adenosine A_{2A} antagonism will decrease the excitatory output of glutamate and reduce glutamate mediated neurotoxicity.

There are also other possible neuroprotective benefits of adenosine A_{2A} antagonism, these will be discussed in the next section.

2.3.5 A_{2A} antagonism and neuroprotection

The neuroprotective properties of adenosine A_{2A} receptor antagonism was first illustrated in a model of cerebral ischemic injury (Gao & Phillis, 1994) and since then, neuroprotective properties have been illustrated in several other animal models.

For example, the deleterious impact of chronic noxious insults to brain tissue under various conditions such as neuro-inflammation (Yu *et al.*, 2008), disrupted blood-brain barrier (Chen *et al.*, 2008; Chen *et al.*, 2010; Stolp & Dziegielewska, 2009; Weiss *et al.*, 2009; Carvey *et al.*, 2005), combined free radical/excitotoxic injury (Behan & Stone, 2002), glutamate excitotoxicity (Domenici *et al.*, 2007; Popoli *et al.*, 2003; Stone & Behan, 2007) 6-hydroxydopamine toxicity and MPTP toxicity (Chen *et al.*, 2001b; Ikeda *et al.*, 2002; Xu *et al.*, 2002) showed promising results after treatment with an A_{2A} antagonist. Considering the above, the role that adenosine A_{2A} antagonism may play in neuroprotection can be summarised as follows:

Firstly, adenosine A_{2A} antagonism may prevent neuro-inflammation, which is believed to be a contributing factor in the development of PD. Neuro-inflammation leads to the disruption of normal mechanisms of control, and toxic metabolites are produced that can increase the underlying pathology (Armentero *et al.*, 2011). In addition, dopaminergic cell death is also stimulated by inflammation (Hirsch & Hunot, 2009). Adenosine A_{2A} receptors are ideally situated to influence inflammation in PD since they are found in cells such as astrocytes (Brambilla *et al.*, 2003; Fiebich *et al.*, 1996) oligodendrocytes (Stevens *et al.*, 2002) and microglia (Fiebich *et al.*, 1996), which are associated with neuro-inflammation. The positive effects of adenosine A_{2A} antagonism on neuro-inflammation was, for example, illustrated in a study where the administration of the known adenosine A_{2A} receptor antagonist KW6002, resulted in a reduction of large activated microglial cells as well as a decrease in neuro-inflammation (Yu *et al.*, 2008).

Adenosine A_{2A} antagonism can, in addition, assist in the conservation and protection of the blood-brain barrier. Several reports have illustrated that the blood-brain barrier is disrupted during PD, which further contributes to the progression of the disease (Stolp & Dziegielewska, 2009; Weiss *et al.*, 2009; Carvey *et al.*, 2005). For example, it was illustrated in a mouse model of PD, that chronic ingestion of caffeine protected against blood-brain barrier dysfunction (Chen *et al.*, 2008). Although the precise mechanism by which caffeine protects against blood-brain barrier dysfunction remains unclear, it is reported that this protective effect is most likely due to the blockade of adenosine A_{2A} receptors (Chen *et al.*, 2010). Antagonism of adenosine A_{2A} receptors may therefore aid in maintaining this important defence mechanism, as well as shield it from any destructive factors that may be triggered during PD.

Adenosine A_{2A} antagonism may also potentially prevent brain damage caused by free radical oxygen species. In a study where rats received quinolinic acid (an endogenous excitotoxin present in low levels in the CNS), as well as a mixture of xanthine and xanthine oxidase (which is known to generate free

radicals), over 80% neural loss resulted. Under normal physiological conditions, the concentration of quinolinic acid in the CNS is too low to be neurotoxic. However, the neurotoxic properties of quinolinic acid at even low concentrations can be enhanced significantly when combined with free radicals which are produced, for instance, during neuro-inflammation. In this study, the administration of an A_{2A} antagonist protected against this combined damage, suggesting that antagonism of the A_{2A} receptor has potential value in the prevention of brain damage caused by combined excitotoxic/free radical injury (Behan & Stone, 2002).

As previously discussed, another mechanism by which A_{2A} antagonism may exert neuroprotection is through the inhibition of glutamate release (see section 2.3.4). The increased release of excitatory transmitters, like glutamate, is an important cause of excitotoxic neuronal cell death (Popoli *et al.*, 2003; Chen *et al.*, 2001b). Adenosine A_{2A} antagonism may therefore provide neuroprotection by decreasing the excessive amounts of glutamate and reducing the deleterious effect it has on neurons.

Additional mechanisms may also be involved since it is not clear why A_{2A} antagonism prevents the effects of MPTP and 6-hydroxydopamine induced toxicity in rodents, however, several intriguing possibilities have emerged (Chen *et al.*, 2001b; Ikeda *et al.*, 2002). For example, it has been suggested that A_{2A} modulation of dopaminergic neurotoxicity is indirect. A_{2A} receptor stimulation enhances GABA release in the striatum and may therefore facilitate the indirect pathway's disinhibition which in turn may contribute to the excitotoxic injury of dopaminergic neurons in the substantia nigra (Piallat *et al.*, 1996). Antagonism of the A_{2A} receptors would therefore decrease the proposed dopaminergic toxicity produced through this circuit (Sebastiao & Ribeiro, 1996; Chen *et al.*, 2001b).

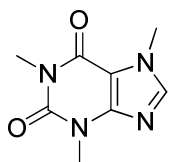
Although the neuroprotective properties of A_{2A} antagonism have been illustrated in various animal models, the most compelling evidence of the neuroprotective effect can be found in the results of epidemiological studies. Numerous studies have been performed over the years and a definite relationship between the consumption of the non-selective adenosine A₁ and A_{2A} antagonist caffeine, and a decreased risk of developing PD has been established (Hernan *et al.*, 2002; Ross *et al.*, 2000; Palacios *et al.*, 2010; Ascherio *et al.*, 2001). For example, in a 30 year follow up study where data from 8004 Japanese-American men were analyzed, it was confirmed that coffee drinkers had a lower risk of developing PD than non-coffee drinkers (Ross *et al.*, 2000). Results showed that the age-adjusted incidence of PD decreased consistently with an increase in coffee intake. These results are also supported by numerous other large, prospective, more ethnically diverse cohorts (Ascherio *et al.*, 2001; Hernan *et al.*, 2002). For example, the results from another study which investigated the effects of coffee consumption and the risk of developing PD in both men (197 with a mean age of 71) as well as women (120 with a mean age of 69) (Palacios *et al.*, 2010), indicated that coffee consumption was associated with a significantly reduced risk of developing PD in men, and a marginally reduced risk in

women. These findings occurred with a mean caffeine intake of approximately 3 cups of coffee a day and were conducted over 8 years (Palacios *et al.*, 2010).

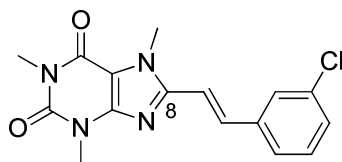
The precise mechanism by which caffeine exerts neuroprotection is still unknown, and remains the object of scientific debate. However, the neuroprotective effect of caffeine is linked to its ability to antagonise the adenosine A_{2A} receptor, rather than its ability to block the A₁ receptor, which most likely is responsible for its motor effects. (Jenner *et al.*, 2009).

2.3.6 Adenosine A_{2A} antagonists in PD

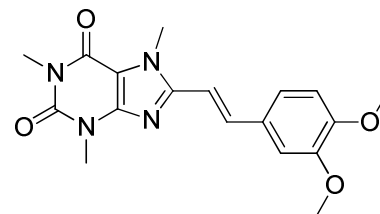
The focus of this section will mainly be on adenosine A_{2A} antagonists that have progressed to clinical trials. The first class of compounds that was designed with PD therapy in mind were the xanthine derivatives, such as caffeine (**2.12**). This class of compounds mostly represents compounds containing a caffeine core, commonly substituted with a styryl moiety at position 8 of the purine. An example of such a styryl xanthine is 8-(3-chlorostyryl)caffeine (CSC) (**2.13**) which also served as a lead compound for the development of compounds such as KW6002 (**2.14**), which is the only compound in this class that has reached clinical trials and the only A_{2A} antagonist currently registered as an antiparkinsonian drug (Stayte & Vissel, 2014).



2.12 Caffeine

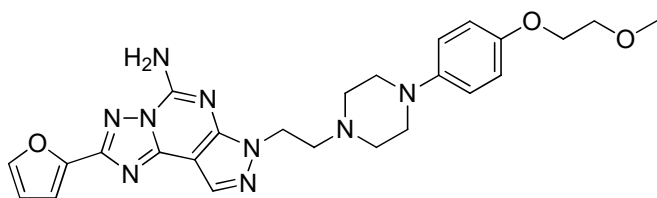


2.13 CSC

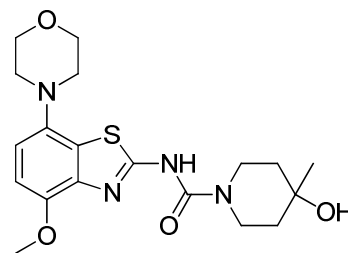


2.14 KW6002

These xanthine derivatives often suffer from inadequate water solubility, as well as photoinstability caused by photoisomerization. A wide range of nonpurine heterocycles that are remarkably diverse in structure were also developed (Preti *et al.*, 2015; Shook & Jackson, 2011). Of particular importance to this study is the number of these heterocyclic adenosine A_{2A} antagonists with potent A_{2A} and/or A₁ affinities that contain a 2-aminopyrimidine motif (de Lera Ruiz *et al.*, 2014; Gillespie *et al.*, 2009a; Gillespie *et al.*, 2009b; Matasi *et al.*, 2005; Shook *et al.*, 2010a; Shook *et al.*, 2010b; Shook *et al.*, 2010c; Atack *et al.*, 2014; Shook & Jackson, 2011; Müller & Ferré, 2007; Van Veldhoven *et al.*, 2008; Lim *et al.*, 2011). These include several promising candidates such as preladenant (**2.15**) that reached phase 3 clinical trials before termination of further studies due to insufficient effectivity (Merck, 2013). Another example is tozadenant which was selected for phase 3 clinical trials, starting in July 2015 (**2.16**) (Biote therapies, 2015). It is thus evident that the 2-aminopyrimidine scaffold is a privileged structure when aiming for adenosine receptor affinity.



2.15 Preladenant



2.16 Tozadenant

In the previous paragraphs the possibilities of antagonism of adenosine A_{2A} receptors in the treatment of PD has been discussed. In the next section, the role of the adenosine A_1 receptor will be discussed.

2.4 Adenosine A_1 receptor

2.4.1 Overview

Adenosine A_1 receptors are the most conserved adenosine receptor subtype and can be found throughout the body, with the highest levels expressed in the central nervous system, including the cortex, hippocampus and striatum (Fredholm *et al.*, 2001). Adenosine A_1 receptors are G_i coupled receptor subtypes which means that their activation results in inhibition of adenylyl cyclase activity, activation of potassium channels, an increase in inositol-1,4,5-trisphosphate (IP_3) through the activation of phospholipase C and blockade of transient Ca^{2+} channels, resulting in an increase in intracellular calcium (Fredholm *et al.*, 1996). Adenosine A_1 receptors play a particularly important role in the brain as stimulation of these receptors inhibits the influx of calcium Ca^{2+} ions which leads to a reduction in the release of excitatory transmitters (presynaptically) as well as counteracting their excitatory effects (postsynaptically) (Fredholm *et al.*, 1996). The A_1 receptor also plays a role in many other organs including the heart and liver and is implicated in conditions such as renal dysfunction in patients with acute heart failure (Chen *et al.*, 2013).

2.4.2 Structure of the adenosine A_1 receptor

As mentioned in section 2.3.1, the adenosine A_1 receptor shares a 49% amino acid sequence similarity with the A_{2A} receptor. Reports on the size of the adenosine A_1 receptor vary, with reported values between 34-38 kDa, which is rather low in comparison to the mass obtained for the A_{2A} receptor (45-47 kDa) (Barrington *et al.*, 1989; Nanoff *et al.*, 1991). Similar to the adenosine A_{2A} receptor, the A_1 receptor also displays the typical pattern of seven transmembrane (TM1-7) α -helices each with 20 to 27 hydrophobic residues, three intracellular loops and three extracellular loops. The distinctive N -terminus, carboxy tail, as well as the additional cytoplasmic loop are also present in the A_1 receptor, however these particular substructures are all very short in comparison with those of the A_{2A} receptor, which further supports the low mass obtained for the A_1 receptor (Pirainen *et al.*, 2011; Raymond *et al.*, 1990).

Since no crystal structure currently exists for the adenosine A_1 receptor, it is quite difficult to define the receptor binding site or to design ligands that can antagonise this important drug target.

2.4.3 Role of adenosine A₁ antagonism in PD

Adenosine A₁ receptor antagonism may relieve several symptoms of PD in three separate ways. Firstly, it may improve the impaired motor function in PD patients. Several studies reported that motor activation in animals was stimulated with the administration of a selective adenosine A₁ antagonist (Trevitt *et al.*, 2009; Antoniou *et al.*, 2005). Adenosine A₁ receptor antagonism can thus be of particular importance since it can potentially reduce the primary motor symptoms of PD. Secondly, A₁ receptor antagonism facilitates dopamine release in the striatum and potentiates the response to dopamine (Nikodijević *et al.*, 1991; Ferré *et al.*, 2001; Shook *et al.*, 2012). This means that the low concentration of dopamine observed in PD patients may be restored and that the desensitising of dopamine receptors (observed during long-term dopaminergic treatment) would be counteracted as dopamine mediated-responses also improve with the administration of an adenosine A₁ antagonist. Thirdly, adenosine A₁ receptor antagonism has the potential to improve non-motor symptoms associated with PD. Recent studies illustrated that the antagonism of central adenosine A₁ receptors can lead to enhanced cognition (Bortolotto *et al.*, 2015). In fact, a short open-labelled study in Parkinson's disease patients illustrated the improvement of both mental and motor impairment scores with the administration of the non-selective (A₁/A_{2A}) adenosine antagonist theophylline (Mally & Stone, 1994). Since administration of the selective adenosine A_{2A} antagonist KW6002 resulted in no cognitive improvement in animal models, the improved cognition observed in PD patients was most likely a result of the A₁ antagonism (Shook *et al.*, 2012). Several other studies performed in animals also illustrated improved cognition with the administration of an adenosine A₁ antagonist (Pereira *et al.*, 2002; Maemoto *et al.*, 2004; Bortolotto *et al.*, 2015). Adenosine A₁ receptor antagonism can therefore provide an added benefit as the cognitive dysfunction present in PD patients, especially in the later stages of the disease, would be improved.

2.5 Dual adenosine A₁ and A_{2A} antagonism

2.5.1 Examples and benefits of dual antagonism

When looking at PD as a whole, the disease seems incredibly complicated with symptoms ranging from motor disturbances such as tremor and dyskinesia, to debilitating non-motor symptoms including, depression and cognitive impairment. This is complicated further by the fact that the cause of PD remains unclear, there is involvement of several neurotransmitter systems and the disease is progressive in nature. A multi-target approach for the therapy of PD may thus be more beneficial than the usual single target approaches to address the multifactorial nature of the disease.

Dual antagonism of adenosine A₁ and A_{2A} receptors presents an exciting alternative non-dopaminergic therapy for Parkinson's disease. It has been proposed that antagonism of the A₁ and A_{2A} receptors will be synergistic as each of these receptors has the ability on their own to improve motor function (Shook

et al., 2012). The main characteristic motor symptoms of PD can therefore be reduced with dual adenosine A₁ and A_{2A} antagonism.

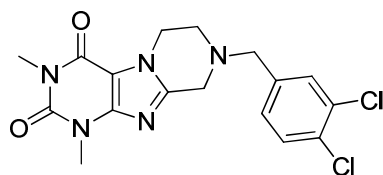
Dual antagonism will most likely not only lead to an improvement in motor function, but several non-motor symptoms may potentially be improved as well. It has already been mentioned that antagonism of A₁ receptors results in improved cognition (discussed in section 2.4.3) and since a decrease in cognition is often found in PD with a progressive form of the disease, this would be an added benefit.

Furthermore, it has been suggested that A_{2A} antagonism may have anti-depressant effects. A recent study reported the anti-depressant activity of the known adenosine A_{2A} antagonist KW6002 (Yamada *et al.*, 2013) providing additional evidence that A_{2A} antagonism may also have the ability to alter non-motor symptoms of PD.

Then there is also the possibility that A_{2A} antagonism may provide neuroprotection (discussed in section 2.3.5), which means that dual antagonism may not only be beneficial for the motor and non-motor symptoms of PD, but may potentially inhibit the progression of PD.

Caffeine is an example of a dual adenosine antagonist and provides proof of concept as numerous studies have illustrated its value in PD. For example, reports indicate that caffeine improved motor activity in PD patients (Postuma *et al.*, 2012) as well as in toxin-induced models of PD (Yu *et al.*, 2006), drug induced parkinsonism models (Moo-Puc, 2003) and models of dopamine-deficient mice (Kim & Palmiter, 2003). There are also studies that showed improvement of cognition (Abreu *et al.*, 2011; Hogervorst *et al.*, 2008) and decreased risk of developing depression (Lucas *et al.*, 2011) with the consumption of caffeine, illustrating the potential of dual adenosine antagonism for treatment of non-motor symptoms of PD. Caffeine further may have a role in delaying the neurodegeneration as it has been shown in rat models of PD, that the administration of caffeine after the start of disease progression prevented the loss of dopaminergic neurons (Li *et al.*, 2008; Morelli *et al.*, 2012; Sonsalla *et al.*, 2012). In addition, it has been shown that caffeine is a down-regulator of neuro-inflammatory responses and nitric oxide production which are believed to contribute to many underlying pathological processes in PD, including cell death (Salvemini *et al.*, 2013; Yadav *et al.*, 2012; Tsutsui *et al.*, 2004). Caffeine may furthermore increase the bioavailability as well as prolong the clinical effect of levodopa (Deleu *et al.*, 2006) illustrating its value in combination therapy. The ample benefits of the dual adenosine A₁ and A_{2A} antagonist caffeine in PD are clear and illustrate the potential of dual adenosine antagonism as non-dopaminergic alternative in PD therapy.

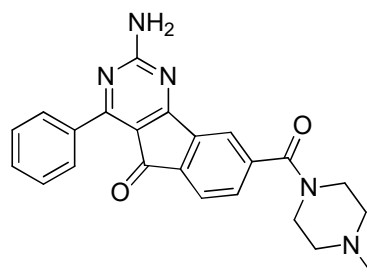
Since the potential of caffeine in PD was discovered, several dual adenosine antagonists have been developed, such as compound **2.17**, a xanthine derived from caffeine, and particularly important to this study, heterocyclic compounds containing a 2-aminopyrimidine motif such as compound **2.18** (Prete *et al.*, 2015; Shook *et al.*, 2010a).



2.17

$A_1 K_i = 351 \text{ nM}$

$A_{2A} K_i = 322 \text{ nM}$



2.18

$A_1 K_i = 58.4 \text{ nM}$

$A_{2A} K_i = 8.2 \text{ nM}$

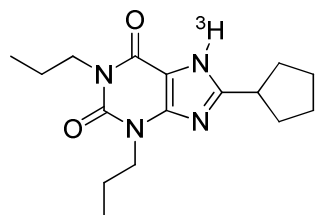
2.5.2 Possible side effects of dual adenosine A_1 and A_{2A} antagonism

Adenosine receptors are not only expressed in the central nervous system, but also occur in various organs and major peripheral tissue such as the heart, lungs, liver as well as the immune system (Ledent *et al.*, 1997; Lee *et al.*, 2003). Adenosine A_{2A} and A_1 receptors play multiple roles in these peripheral parts of the body, which suggests that antagonising them may have an influence on these areas. The best example of the safety of a dual antagonist is caffeine, which is the most consumed psychoactive substance in the world. Caffeine is relatively safe with moderate intake, however, some caution is advised as feelings of anxiety, restlessness, agitation, excitement and insomnia were reported with excessive caffeine consumption (Winston *et al.*, 2005; Yacoubi *et al.*, 2000). Caffeine is however a weak antagonist, and further investigation of the effects of potent dual antagonists are required.

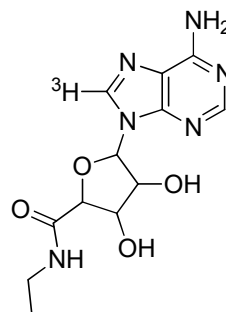
2.6 Evaluation of synthesised compounds as dual adenosine A_1 and A_{2A} antagonists

2.6.1 *In vitro* assays

A Radioligand binding study is a cell-free method which can be used to determine whether ligands have affinities for specific target receptors and is suitable for any G-protein coupled receptor (Zhang & Xie, 2012). These assays are used to determine the affinities of either agonists or antagonists, but do not distinguish between agonistic, antagonistic or inverse agonistic interactions. The availability of radiolabelled ligands can be a limitation in the use of ligand binding assays. However, there are several radioligands available for both adenosine A_1 and A_{2A} receptor subtypes, such as 1,3- ^3H -dipropyl-8-cyclopentylxanthine (^3H]DPCPX) (**2.19**) (A_1 specific) and ^3H]5'-*N*-ethylcarboxamide-adenosine (^3H]NECA) (**2.20**) (non-specific).



2.19 [³H]DPCPX



2.20 [³H]NECA

These radioligands bind to the specific receptor targets and the measured radiation in the absence of a test compound then represents total (100%) binding. The affinity of a test compound is determined by the amount of radioligand that it is able to displace from the target receptors. If a compound has high affinity for the target receptor it would displace almost all of the radioligand, resulting in a low radiation reading and *vice versa* (Bruns *et al.*, 1986; Bruns *et al.*, 1987; Van der Walt *et al.*, 2013). Historically, radioligand binding assays have been the gold standard in determining affinities of test compounds for their target receptors, and are the most established assay for this particular purpose (Zhang & Xie, 2012).

2.6.2. Determination of agonistic and antagonistic effects with functional assays

The results of radioligand binding assays only provide information regarding the affinity of a compound for the target receptor, and do not distinguish between agonistic and antagonistic activity. It also does not give an indication of the test compound's bioavailability or its potential to reverse PD symptoms *in vivo* (Baraldi *et al.*, 1995). Additional assays are thus performed in an attempt to determine these characteristics of the test compounds. G-protein dependent functional assays determine the biological responses after ligand binding (to its target receptor) revealing important information about the characteristics of the ligand-receptor interaction (for example, agonistic or antagonistic interaction). These characteristics are determined by either measuring the G-protein activation or G-protein-mediated events including second messenger generation (Zhang & Xie, 2012). The cAMP assay is one example of a G-protein dependant functional assay that measures the cellular levels of cAMP. These levels are dependent on the adenylyl cyclase activity mediated by G-protein coupled receptors (G_s, G_i, G_o). This assay is often used and it is generally straightforward to screen G_s-coupled receptors. However screening G_{i/o}-coupled receptors with cAMP assays can be difficult as the inhibition signal needs to be enhanced with a direct activator of adenylyl cyclase, such as forskolin, which needs to be titrated during optimization of the assay (Thomsen *et al.*, 2005). In addition, a highly sensitive instrument is also needed to detect the low levels of cAMP that accumulate from adenosine receptor stimulation or inhibition, making it a relatively expensive and complex assay (Williams, 2004).

Numerous *in vivo* rodent models for PD also exist. These range from acute pharmacological models including the reserpine akinesia-(causes akinesia and hind limb rigidity) or haloperidol induced catalepsy (induces muscle rigidity and catalepsy) models, where one or more PD symptoms are induced. Models that mimic the destruction of the dopaminergic nigro-striatal pathway present in PD also exist, such as the 6-OHDA rat and the MPTP mouse models (Duty & Jenner, 2011). Although the latter mentioned models are the most popular experimental models of PD when it comes to preclinical testing of new symptomatic therapies, they also have shortcomings. For example, 6-OHDA cannot be administered systemically since it poorly crosses the blood-brain barrier and causes a chemical sympathectomy by damaging the peripheral nervous system. The acquired route of administration used to circumvent this problem is thus by directly injecting 6-OHDA into the brain by either free hand or by stereotaxic means, which is difficult (Bové *et al.*, 2005). The MPTP model on the other hand has no effect on rats, as the dopaminergic neurons are for some unknown reason relatively resistant in these rodents. The effects of MPTP are therefore mostly tested in nonhuman primates and small vertebrate animals such as mice. The sensitivity to the MPTP toxin varies considerably between the distinct mouse strains and studies have reported the spontaneous recovery of mice after MPTP administration. The use of nonhuman primates is the understandable alternative and although they are highly effective, they are limited due to obvious financial and ethical implications (Antony *et al.*, 2011). Care also has to be taken when using MPTP as it is a highly toxic substance.

The haloperidol-catalepsy assay employed for this study, is a popular model of choice for assessing the potential symptomatic efficacy of novel non-dopaminergic agents such as A_{2A} antagonists (Duty & Jenner, 2011). For this assay, the D₂ antagonist haloperidol is administered to rats to induce catalepsy and inhibit locomotion. The effects of the haloperidol should effectively be reversed with the administration of an adenosine A_{2A} antagonist, as indicated by several literature results (Correa *et al.*, 2004; Ishiwari *et al.*, 2007; Salamone *et al.*, 2008). The mechanism by which A_{2A} antagonism reverses catalepsy can be seen in Figure 2.6.

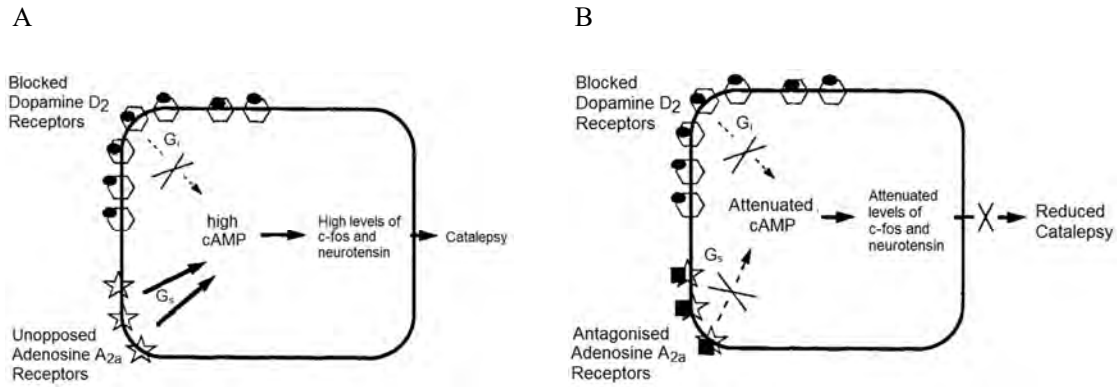


Figure 2.6: Schematic diagram illustrating A) the mechanism by which dopamine D₂ antagonism exerts catalepsy as well as B) the reversal effect that A_{2A} antagonism has on induced catalepsy (Ward & Dorsa, 1999).

Haloperidol antagonises the D₂ receptor and eliminates the inhibitory G_i-coupled effect of normal endogenous dopamine. The unopposed signalling of adenosine G_s-coupled A_{2A} receptors lead to increased cAMP levels in the striatopallidal neurons and result in catalepsy. Antagonism of the A_{2A} receptor will therefore block the G_s-coupled stimulation and reverse catalepsy by normalising the cAMP input in the striatopallidal neuron (Ward & Dorsa, 1999).

Choice of the type of model to use depends on the specific pathophysiological state or symptom of PD that needs to be assessed, as well as several other ethical, financial and time dependant elements. However, there is still much progress to be made as no single model fully represents the complexity of PD (Blesa *et al.*, 2012).

2.7 Summary

In this chapter, the need for the development of alternative drug therapies for PD has clearly been illustrated. A particular promising alternative to current dopaminergic therapies is dual adenosine A₁ and A_{2A} antagonism, as it has the potential to confer neuroprotection and presents the possibility of addressing non-motor symptoms concomitant to improvements in motor symptoms.

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CHAPTER 3

Article 1

2-Aminopyrimidines as dual adenosine A₁/A_{2A} antagonists

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Abstract

In this study thirteen 2-aminopyrimidine derivatives were synthesised and screened as potential antagonists of adenosine A₁ and A_{2A} receptors in order to further investigate the structure activity relationships of this class of compounds. 4-(5-Methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**) was identified as a compound with high affinities for both receptors, with an A_{2A}K_i value of 6.34 nM and an A₁K_i value of 9.54 nM. The effect of selected compounds on the viability of cultured cells was assessed and preliminary results indicate low cytotoxicity. *In vivo* efficacy at A_{2A} receptors was illustrated for compounds **8k** and **8m** since these compounds attenuated haloperidol induced catalepsy in rats. A molecular docking study revealed that the interactions between the synthesised compounds and the adenosine A_{2A} binding site most likely involve Phe168 and Asn253, interactions which are similar for structurally related adenosine A_{2A} receptor antagonists.

Keywords: Adenosine A_{2A} antagonist, Adenosine A₁ antagonist, 2-aminopyrimidine, Parkinson's disease

1. Introduction

Parkinson's disease is a complex, chronic neurodegenerative disorder, mainly characterised by a decline in motor function, but also associated with non-motor manifestations such as cognitive deficits (e.g. dementia) and neuropsychiatric symptoms, such as depression [1]. Management of the non-motor symptoms in particular is challenging and represents an important unmet medical need [2,3,4]. The neuropathology of the disease is hallmarked by the selective degeneration of dopaminergic neurons of the nigrostriatal pathway and the resulting deficiency of dopamine in the basal ganglia [5]. However, the importance of other neurotransmitter systems and organs in Parkinson's disease pathogenesis are increasingly recognised [6,7,8].

Nevertheless, current treatments are still focused on enhancing dopaminergic neurotransmission and include the use of levodopa, (which is still the gold standard nearly 50 years since its introduction), dopamine agonists, catechol-*O*-methyltransferase and monoamine oxidase inhibitors. Since the chronic nature of the disease requires long-term use of medication, the occurrence of side-effects is unavoidable, and levodopa especially is associated with the development of debilitating dyskinesias [9, 10]. Limited progress has been made in altering the progress of neurodegeneration, and to date, no agent has been established as neuroprotective or disease modifying [11,12]. Non-dopaminergic targets for the disease are thus particularly appealing; especially if these would also improve non-motor symptoms and provide neuroprotection [13]. In recent years, antagonists of the adenosine A_{2A} receptor in particular has received attention as a promising non-dopaminergic alternative (for examples of reviews see [3, 4, 14-17] and several of these agents such as istradefylline (KW-6002), preladenant and tozadenant have been investigated clinically with promising results. Istradefylline for example has been approved for use as adjunctive treatment for Parkinson's disease in Japan [18-22].

Adenosine receptors are G-protein coupled receptors and consist of four subtypes, namely A₁, A_{2A}, A₃ and A_{2B} [23,24]. Of particular importance to Parkinson's disease are adenosine A_{2A} receptors which are concentrated in the indirect striatopallidal GABAergic pathway. This pathway also expresses the D₂ dopamine receptor and enkephalin [25-27]. Antagonism of A_{2A} receptors potentiate dopamine mediated responses and partly restores the imbalance between the hypoactive direct striatonigral and hyperactive indirect striatopallidal pathways that develops in Parkinson's disease, thus relieving motor symptoms [14, 16, 28, 29]. Both epidemiological and experimental data have shown that adenosine A_{2A} antagonists exert a neuroprotective effect [16]. Furthermore, it has been reported that the adenosine A_{2A} antagonist KW-6002, exhibit antidepressant properties in animal models of depression [30,31] and that adenosine A_{2A} antagonists have potential in the management of dyskinesias [29,32]. This illustrates the promise of these agents as multifactorial non-dopaminergic treatment of Parkinson's disease.

Adenosine A₁ receptors on the other hand, are expressed throughout the brain, including the cortex, hippocampus and striatum [23]. Since A₁ receptor antagonism also results in motor activation in animals

[33,34] it has been suggested that dual antagonism of A₁ and A_{2A} receptors would act synergistically in improving motor deficits in Parkinson's disease [4]. Furthermore, since the A₁ receptor also occurs in systems that are important for cognitive function, adenosine A₁ antagonism may improve cognitive deficits experienced in Parkinson's disease, as illustrated in animals [35-37]. Several dual adenosine A₁/A_{2A} antagonists have in fact been investigated in animals and has not only shown effectiveness in improving motor disabilities [4, 38-43], but has also illustrated effectiveness in enhancing cognition [38]. In summary, dual adenosine A₁ and A_{2A} antagonists would thus not only treat the motor symptoms of Parkinson's disease and potentially be neuroprotective, but may also improve non-motor symptoms.

The 2-aminopyrimidine motif frequently occurs in compounds (e.g. **1-6**) that exhibit potent adenosine A_{2A} and/or adenosine A₁ affinity (Figure 1) and indicates that this scaffold is privileged for antagonism of these receptors [4, 15, 17, 40-48]. Of particular relevance to this paper are the findings of Van Veldhoven, Matasi and Shook and co-workers [40-42, 46, 47], who synthesised a number of 2-aminopyrimidines (e.g. **3**), indenopyrimidones (e.g. **4, 5**) and indenopyrimidines (e.g. **6**), respectively.

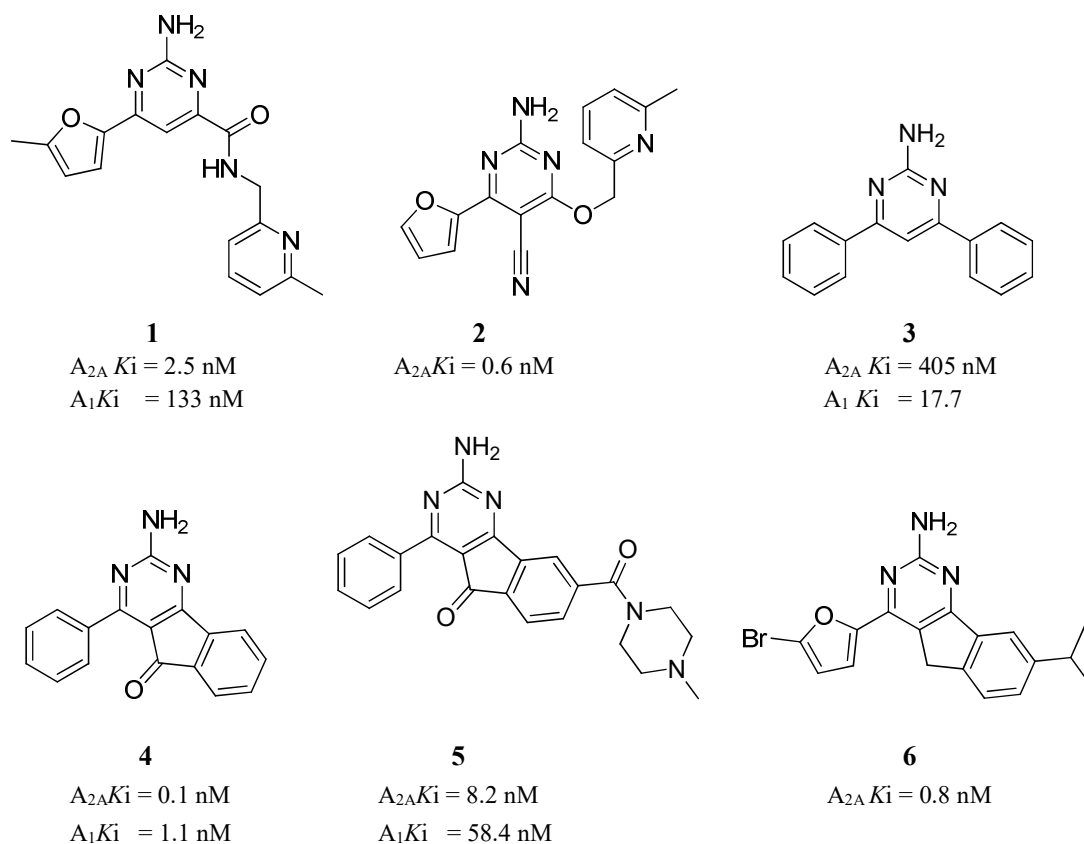


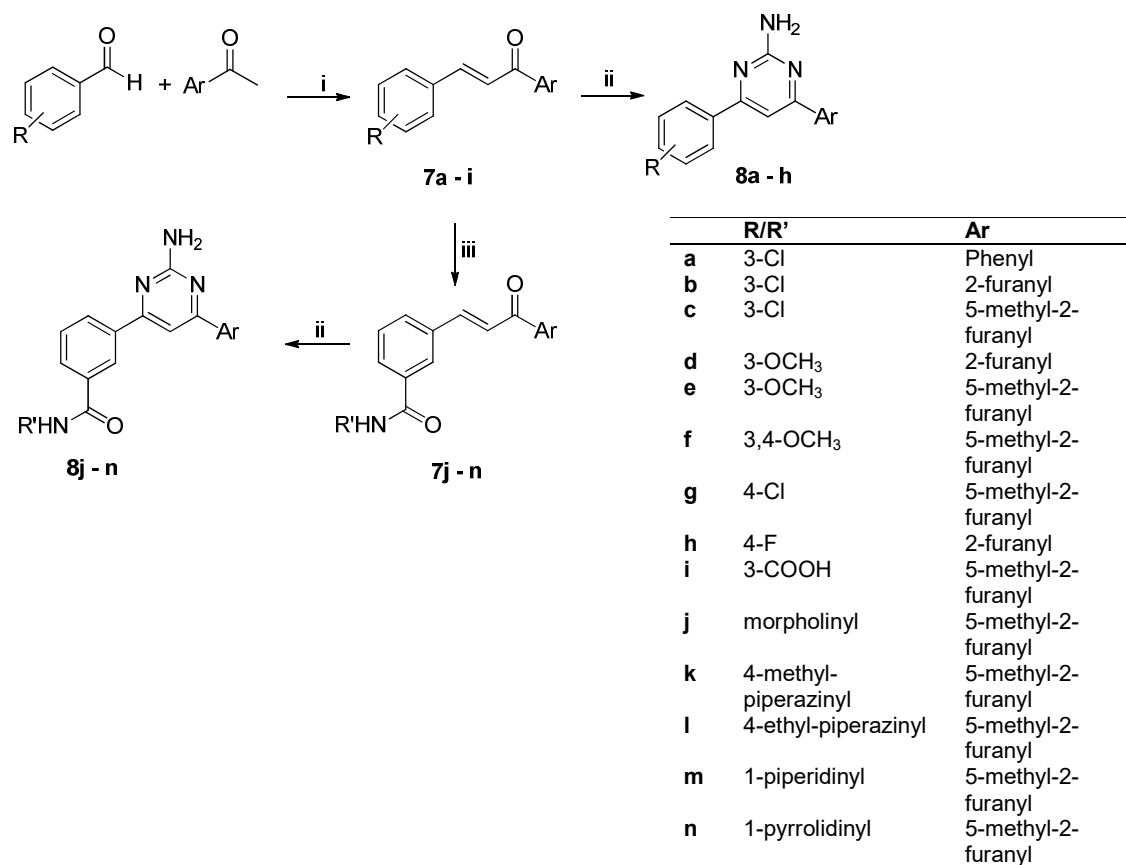
Figure 1: Adenosine A_{2A} antagonists containing the 2-aminopyrimidine moiety

Our research group has been interested in the design, synthesis and evaluation of heterocycles as antagonists of adenosine receptors. Based on the aforementioned findings, the aim of the present study was to explore the necessity of the methylene bridge as present in the indenopyrimidine or

indenopyrimidone scaffolds previously synthesised [40-42, 46] and to further investigate the structure-activity relationships of the 2-aminopyrimidine scaffold for dual antagonism of adenosine A₁ and A_{2A} receptors. We thus set out to synthesise firstly, a set of 2-aminopyrimidines substituted with simple electron withdrawing and donating groups (**8a - h**), and secondly an amide substituted series (**8j - n**), related to the indenopyrimidones (e.g. **5**) as synthesised by Shook and co-workers [41]. We report herein their affinities for adenosine A_{2A} and A₁ receptors, the results of docking selected compounds into the adenosine A_{2A} receptor's binding site, as well as the *in vivo* activities of selected compounds.

2. Chemistry

As shown in scheme 1, the 2-aminopyrimidines were readily synthesised in two or three steps, albeit in low yields. Firstly, a Claisen-Schmidt condensation, using commercially available ketones and aldehydes under basic conditions [49] yielded the desired intermediate chalcones (**7a - i**). For the amide derivatives (**8j - n**), the condensation reaction was followed by an amide coupling reaction mediated by 1,1'-carbonyldiimidazole (CDI) resulting in chalcones **7j - n**. All chalcones were cyclised with guanidine hydrochloride in the presence of sodium hydride [50] to yield the desired 2-aminopyrimidines (**8a - n**), since the use of sodium hydroxide in ethanol resulted in complicated mixtures of products.



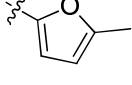

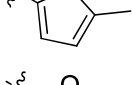
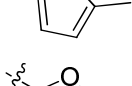
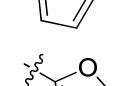

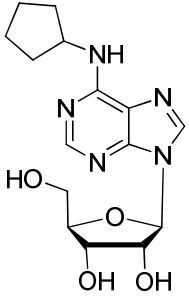


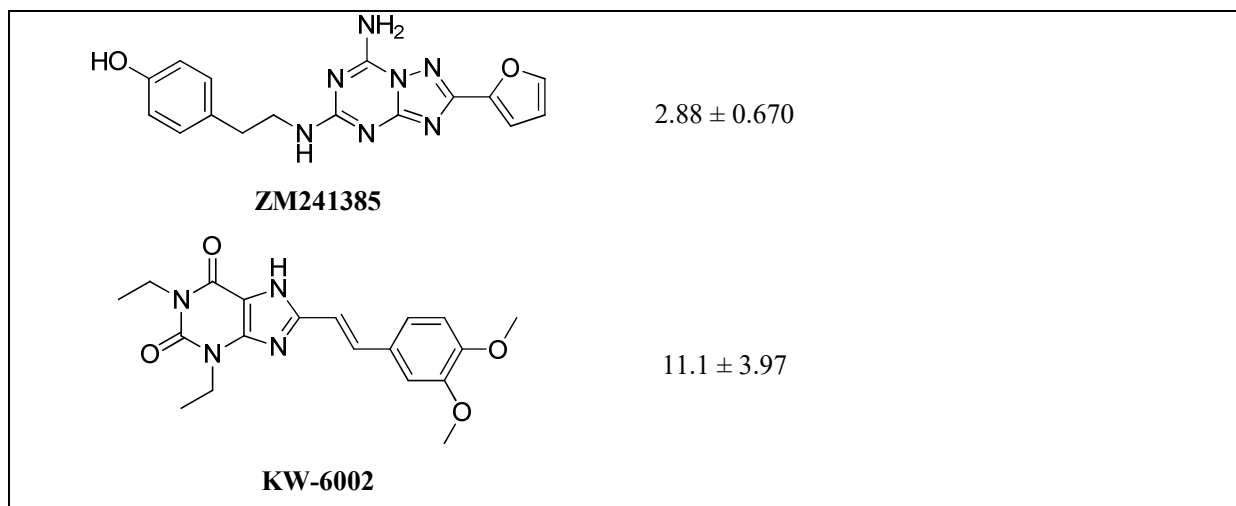
Scheme 1. Synthesis. Reagents and conditions of 2-aminopyrimidine derivatives **8**. Reagents and Conditions: (i) NaOH, EtOH/MeOH, rt, 3 h; (ii) Guanidine hydrochloride, NaH, DMF, 110 °C, 24 h. (iii) CDI, CH₂Cl₂, NHR, rt, 5 h.

3. Results and discussion

The affinities of the 2-aminopyrimidines for the adenosine A_{2A} and A₁ receptors were determined by radioligand binding and are expressed as the receptor-ligand dissociation constants (*K_i*, nM) (Tables 1 and 2). Adenosine A_{2A} receptor affinity was determined using the non-selective adenosine antagonist, [³H]5'-N-ethylcarboxamide-adenosine ([³H]NECA) in the presence of N⁶-cyclopentyladenosine (CPA), and A₁ receptor affinity was determined using 1,3-[³H]-dipropyl-8-cyclopentylxanthine ([³H]DPCPX) [51-53].

Table 1: Adenosine receptor affinities (K_i) of compounds **8a - h**.

Comp.	R ¹	R ²	R ³	CPA		
				A _{2A} K _i (nM) ^a	A ₁ K _i (nM) ^a	SI ^b A _{2A} K _i /A ₁ K _i
8a	-H	-Cl		948 ± 141	22.8 ± 5.51	42
8b	-H	-Cl		245 ± 21.1	39.1 ± 3.64	6.3
8c	-H	-Cl		399 ± 151	129 ± 12.1	3.1
8d	-H	-OCH ₃		249 ± 79.6	61.4 ± 0.435	4.1
8e	-H	-OCH ₃		409 ± 217	145 ± 19.6	2.8
8f	-OCH ₃	-OCH ₃		2778 ± 375	650 ± 63.0	4.3
8g	-Cl	-H		3320 ± 484	434 ± 13.0	7.6
8h	-F	-H		257 ± 36.4	158 ± 12.1	1.6
					10.4 ± 1.57	
			CPA			



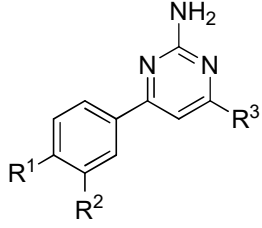
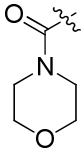
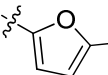
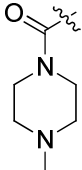
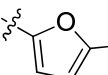
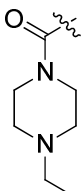
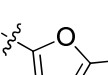
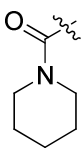
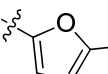
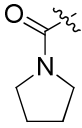
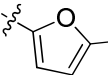
^aValues are given as mean ± SEM of duplicate determinations

^bSelectivity index

The 2-aminopyrimidines of series 1 (**8a - h**) exhibited moderate to weak affinities for the adenosine A_{2A} receptor and moderate to good affinities for A_1 receptors with $A_{2A}K_i$ values ranging from approximately 3 μ M (**8f**, **8g**) to approximately 250 nM (**8b**, **8d**, **8h**) and A_1K_i values ranging from 23 nM (**8a**) to 650 nM (**8f**). These compounds all have higher affinities for the adenosine A_1 receptor than for the adenosine A_{2A} receptor, with compound **8a** being the most selective (selectivity index of 42). For adenosine A_{2A} receptor affinity, the replacement of the phenyl substituent (R^3) with either a furan or methyl furan group, resulted in improved affinity (compound **8a** vs. compounds **8b** and **8c**), whereas the opposite was true for A_1 affinity, where methyl furan substitution in particular proved to be detrimental. When the affinities of compounds **8b** and **8c**, and compounds **8d** and **8e** are compared, it is clear that methyl substitution of the furan ring results in decreased affinity, especially for the adenosine A_1 receptor. Based on these results, it thus appears that for R^3 , furan substitution is preferable for A_{2A} affinity, while phenyl substitution is optimal for A_1 receptor affinity. These results are in agreement with literature since the preference of the adenosine A_{2A} receptor for furan substitution is well documented [e.g. 46]. The affinity is apparently not significantly affected by the electronic effects of the substituent on the phenyl ring (R^2) as compounds **8b** and **8d** ($A_{2A}K_i$ values approx. 250 nM, A_1K_i values 40 - 60 nM) as well as compounds **8c** and **8e** ($A_{2A}K_i$ values approx. 400 nM, A_1K_i values 130- 140 nM) had similar affinities for A_{2A} and A_1 receptors, respectively.

On the other hand, the position of the substituent on the phenyl ring seems to have a significant effect on both A_1 and A_{2A} affinity, as substitution on the *meta* position (**8c**, $R^1 = H$, $R^2 = Cl$) is superior to substitution on the *para* position (**8g**, $R^1 = Cl$, $R^2 = H$). A similar observation is made when the affinities of compounds **8e** and **8f** are compared, where addition of a second methoxy group, in the *para* position, results in much weaker A_1 and A_{2A} affinity. However, since only a limited number of derivatives have been synthesised, these structure-activity relationships should be seen as preliminary.

Table 2: Adenosine receptor affinities (K_i) of compounds **8j - n**.

						
Comp.	R ¹	R ²	R ³	A _{2A} K _i (nM) ^a	A ₁ K _i (nM) ^a	SI ^b A _{2A} K _i /A ₁ K _i
8j	-H			29.3 ± 0.978	52.1 ± 16.9 ^c	0.6
8k	-H			16.3 ± 2.18	136 ± 1.88	0.1
8l	-H			30.9 ± 3.10	37.5 ± 0.857	0.8
8m	-H			6.34 ± 0.532	9.54 ± 1.34	0.7
8n	-H			58.2 ± 19.9	36.8 ± 5.42	1.6
		CPA			10.4 ± 1.57	
		ZM241385		2.88 ± 0.670		
		KW-6002		11.1 ± 3.97		

^aValues are given as mean ± SEM of duplicate determinations

^bSelectivity index

^cn= 4

For compounds **8j** - **n**, the 5-methyl-2-furanyl group was selected as the R³ substituent, since it was synthetically easier to work with than furan and is also less likely to present with metabolic liabilities [40]. Gratifyingly, this amide series showed improved adenosine A_{2A} affinity, while still retaining good A₁ affinity. The most promising candidate was compound **8m**, with dual affinity for both A_{2A} and A₁ receptors with K_i values of 6.34 nM and 9.54 nM, respectively. For adenosine A_{2A} affinity, the amine groups yielded the following order of affinity: piperidine > methyl piperazine > morpholine = ethyl piperazine > pyrrolidine. Piperidine substitution was also most advantageous for A₁ affinity, while methyl piperazine substitution was least favourable. The affinities of this series of compounds are quite similar to those reported for related arylindenopyrimidones [41], and indicate that the presence of the five-membered indenyl ring (e.g as present in **5** and **6**) is not an absolute requirement for dual affinity.

To gain an indication of potential cytotoxicity of the amide derivatives, the effect of these compounds on the viability of cultured HeLa cells were measured. For this purpose, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay was used [54]. Cell viability was generally above 60% when exposed for 24 h to either 1 μM or 10 μM of test compound (Table 3). These concentrations are almost 1000-fold higher than the reported K_i values of these compounds. Cytotoxicity at the doses required to obtain adenosine A₁ and A_{2A} affinity should thus not be problematic.

Table 3: The percentage viable cells remaining after treatment with amide derivatives (**8j** - **8n**), as compared to untreated cells (100%).

Comp.	% Viable cells	
	1 μM ^a	10 μM ^a
8j	98.6 ± 4.45	81.7 ± 5.97
8k	106.6 ± 9.76	76.8 ± 18.1
8l	65.7 ± 4.95	60.1 ± 0.00
8m	64.3 ± 4.74	61.6 ± 4.46
8n	101 ± 6.22	67.9 ± 7.09

^aValues are given as mean ± SD of triplicate determinations

Selected representatives **8m** (the compound with best dual affinity, A_{2A}K_i = 6.34 nM, A₁K_i = 9.54 nM) and **8k** (A_{2A}K_i = 16.3 nM), were subjected to *in vivo* studies to determine the effectiveness of these compounds as dual adenosine A₁/A_{2A} antagonists. For these studies the effect of the test compounds on haloperidol induced catalepsy in rats was investigated [33].

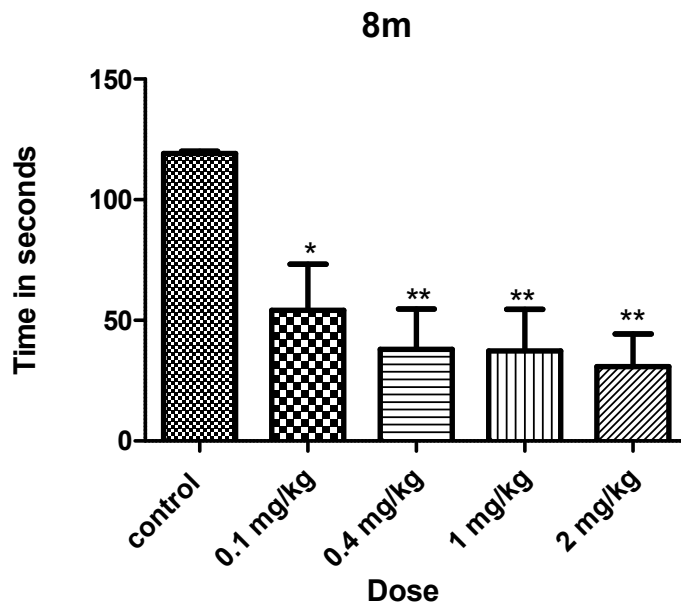


Figure 2a. Reduction in haloperidol induced-catalepsy in male Sprague-Dawley rats by compound **8m**. Time to descend from the bar was measured in haloperidol (5 mg/kg, ip) treated rats after ip administration of compound **8m** (0.1, 0.4, 1, 2 mg/kg). Each bar represents average time (\pm SEM) of (n = 6) rats in the cataleptic position (*,** indicate significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [F (4, 25) = 5.893; P < 0.005] followed by Dunnet's post test with *P = 0.01 - 0.05 and **P = 0.001 - 0.01).

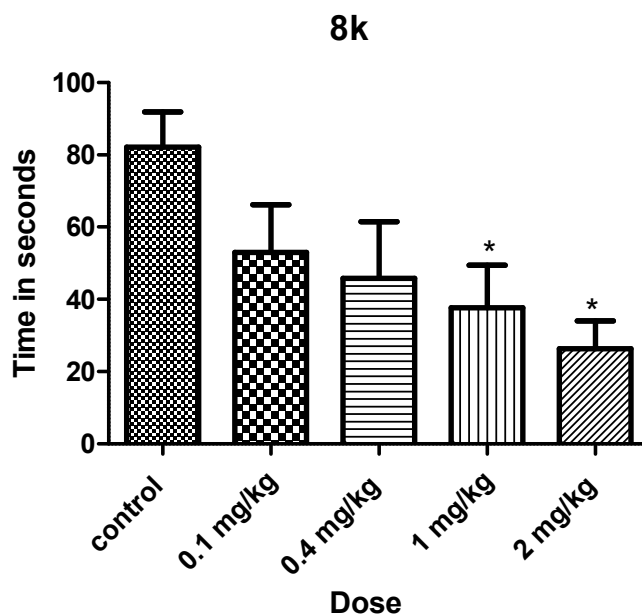


Figure 2b. Reduction in haloperidol induced-catalepsy in male Sprague-Dawley rats by compound **8k**. Time to descend from the bar was measured in haloperidol (5 mg/kg, ip) treated rats after ip administration of compound **8k** (0.1, 0.4, 1, 2 mg/kg). Each bar represents average time (\pm SEM) of (n = 6) rats in the cataleptic

position (* indicates significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [F (4, 25) = 3.103; P < 0.05] followed by Dunnet's post test with P = 0.01 – 0.05).

As depicted in figure 2, a significant reduction in catalepsy time was observed for **8m** at all intraperitoneal (ip) administered doses while a significant reduction in catalepsy time was only observed for the highest two doses (1 and 2 mg/kg) for compound **8k** (Figures 2a and b).

The results obtained with compounds **8m** and **8k** are thus similar to those obtained with other adenosine A_{2A} antagonists where catalepsy is reversed in the presence of A_{2A} antagonists or dual adenosine A_{2A}/A₁ antagonists [33,34,43], and provides evidence of *in vivo* efficacy of these compounds as antagonists.

In order to rationalise the results obtained in the radioligand binding studies, a docking study was performed using CDOCKER (Discovery Studio 3.1). The synthesised compounds were docked into a model of the binding site of the adenosine A_{2A} receptor (PDB code: 3PWH). Visual inspection of the docked poses with most favourable CDOCKER interaction energy firstly revealed that the three-membered ring system of all derivatives (**8a - n**), undergo π - π interactions with Phe168. Hydrogen bonding interactions also occur between the exocyclic amino group and Asn253 for most derivatives. These interactions most likely anchor the aminopyrimidine in the binding site and are also important binding interactions predicted for other 2-aminopyrimidine antagonists [55] (Figure 3 and 4). Additionally, hydrogen bonding interactions were also observed with His250, Ser67, Glu169, Phe168 and Ala63 for some compounds. Interestingly, for compounds **8a - h** there were two different orientations, one where the C-6 substituent was orientated towards His250 and another where the C-4 substituent was orientated towards His250 (Figure 3). The CDOCKER interaction energies of these two poses were in all cases very similar. The docking results gave no clear explanation for the observed higher affinity of the amide derivatives (**8j - n**) compared to the series 1 compounds (**8a - h**). However, when ranked according to CDOCKER interaction energy, most of the series 2 compounds ranked above the series 1 compounds, except for compound **8f**, which ranked above compound **8n**. It would thus appear that the interaction between the amide derivatives (**8j - n**) and the receptor is generally more favourable than for the “smaller” derivatives and provides some explanation for the superior affinity of compounds **8j - n**.

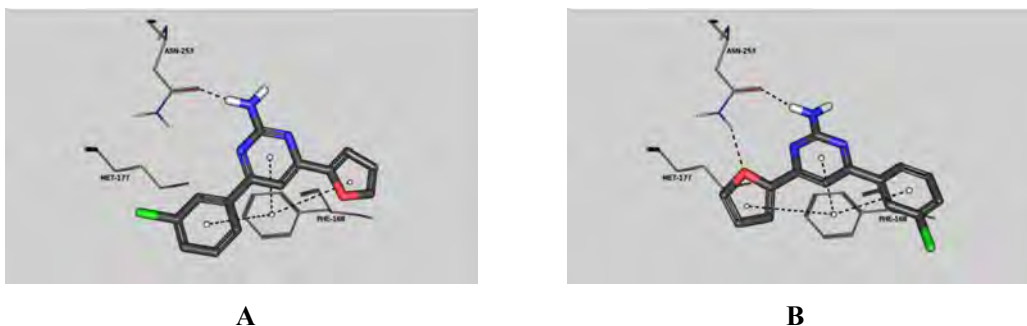


Figure 3: Illustration of the different orientations of compound **8b** in the binding site of the A_{2A} receptor. Intermolecular hydrogen bond interactions are observed between the exocyclic amino group and Asn253, while $\pi=\pi$ stacking occurs between the three ring systems and Phe168 (**A**). In orientation **B**, an additional hydrogen bonding interaction is observed between the furan oxygen and Asn253 (Figure generated using Pymol).

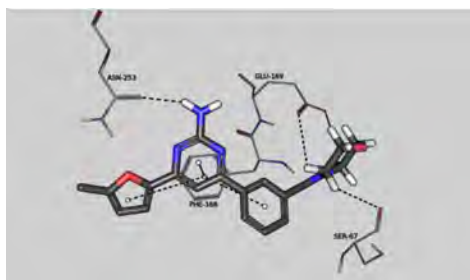


Figure 4: Docking orientation of compound **8j** in the active site of the adenosine A_{2A} receptor. An intermolecular hydrogen bond interaction is observed between the exocyclic amino group and Asn253, while $\pi=\pi$ stacking occurs between the three ring systems and Phe168. Additional hydrogen bond interactions occur between the morpholine in the side chain and Glu169 and Ser67 (Figure generated using Pymol).

4. Conclusion

This series of 2-aminopyrimidines, particularly the amide derivatives (**8j** - **n**), which are related to previously synthesised arylindenopyrimidines, retain affinity for both adenosine A_1 and A_{2A} receptors. The 2-aminopyrimidine scaffold can thus be optimised and the presence of a five-membered “linker ring” is not an absolute requirement for affinity. *In vivo* activity is indicative of adenosine antagonism rather than agonism and has been illustrated for compounds **8k** and **8m** with $A_{2A}K_i$ values of 16.3 nM and 6.34 nM and A_1K_i values of 136 nM and 9.54 nM, respectively. Docking results indicate that these compounds are predicted to bind in a fashion similar to that illustrated for other compounds of the 2-aminopyrimidine class.

5. Experimental section

5.1 Chemistry

Chemical reagents were purchased from Sigma-Aldrich, and used without further purification. Reactions were routinely monitored on TLC using precoated Kieselgel 60 F254 sheets with ethyl

acetate: petroleum ether (1:4) as mobile phase for series 1 (**8a - h**) and dichloromethane: methanol (9:1) as mobile phase for series 2 compounds (**8j - n**). Melting points were determined using a Buchi B-545 apparatus, and are uncorrected. Mass spectra were obtained on a dual focusing DFS magnetic sector mass spectrometer in EI+ mode. The mass spectrum for compound **7n** was obtained with a Bruker micrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively. Samples were dissolved in either deuteriochloroform (CDCl_3) or deuterated dimethylsulfoxide ($\text{DMSO-}d_6$). ^1H NMR data are reported in parts per million (ppm) and the following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), dd (doublet of doublets), t (triplet), br t (broad triplet), q (quartet), p (pentet/quintet) or m (multiplet). Chemical shifts are referenced to the residual solvent signal (CDCl_3 7.26 and 77.0 ppm for ^1H and ^{13}C respectively; $\text{DMSO-}d_6$: 2.5 and 39.5 ppm for ^1H and ^{13}C , respectively). Assignments were based on data obtained from 1D (^1H , ^{13}C , DEPT) and 2D (HSQC, HMBC, COSY) NMR experiments. HPLC analyses were conducted with an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector. A Venusil XBP C18 column (4.60 \times 150 mm, 5 μm) was used with a solvent gradient program (30% acetonitrile and 70% MilliQ water initially) at a flow rate of 1 ml/min. The concentration of acetonitrile in the mobile phase was linearly increased up to 85% over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. The test compound was injected (20 μl , 1 mM) into the HPLC system and the eluent was monitored at a wavelength of 254 nm.

5.1.1 Procedure for the synthesis of 3-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (**7i**)

A solution of 4% (w/v) sodium hydroxide (34 mmol) was added to a suspension of 3-formylbenzoic acid (17 mmol) and 1-(5-methyl-2-furyl)ethanone (17 mmol) in methanol (100 ml). The mixture was stirred at room temperature for 24 h and acidified with concentrated hydrochloric acid to a pH of 1-2. The precipitate that formed was filtered, rinsed with water and recrystallised from methanol to afford **7i**.

5.1.1.1 3-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (**7i**)

Yield 68%; Pale yellow crystals; mp 191.7-194.1 $^\circ\text{C}$ (methanol); ^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 13.20 (br s, 1H, OH), 8.33 (br s, 1H, H-2'), 8.07 (br d, $J = 7.8$ Hz, 1H, H-6'), 7.98 (dt, $J = 1.4, 7.7$ Hz, 1H, H-4'), 7.82 (d, $J = 3.5$ Hz, 1H, H-3''), 7.75 (d, $J = 16.0$ Hz, 1H, H-7 or H-8), 7.71 (d, $J = 16.0$ Hz, 1H, H-7 or H-8), 7.57 (t, $J = 7.7$ Hz, 1H, H-5'), 6.42 (dd, $J = 1.1, 3.5$ Hz, 1H, H-4''), 2.40 (s, 3H, CH₃). ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 175.6 (C-1), 167.0 (acid C=O), 158.8 (C-5''), 151.8 (C-2''),

141.2 (C-3), 135.0 (C-1'), 132.8 (C-6'), 131.6 (C-3'), 131.0 (C-4'), 129.2 (C-2', C-5'), 123.1 (C-2), 121.9 (C-3''), 109.6 (C-4''), 13.8 ($\underline{\text{C}}\text{H}_3$). EI-HRMS m/z: calcd for $\text{C}_{15}\text{H}_{12}\text{O}_4$, 256.07356, found 256.07292; Purity (HPLC): 100%.

5.1.2 General procedure for the synthesis of chalcones (**7j** – **7n**)

1,1'-Carbonyldiimidazole (CDI) (7.0 mmol) was added to a suspension of the acid (**7i**) (5.8 mmol) in dichloromethane (70 ml). The reaction mixture was stirred under nitrogen at room temperature for 2 h and the appropriate amine (7.0 mmol) was added. The mixture was then stirred for a further 3 h. The reaction was quenched by the addition of brine and the aqueous phase extracted with dichloromethane (2 × 20 ml). The combined organic fractions were washed once with saturated sodium hydrogen carbonate and twice with brine. The organic fraction was concentrated (*in vacuo*), purified with column chromatography [dichloromethane: methanol (98:2)] and recrystallised from methanol.

5.1.2.1 (2E)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one (**7j**)

The title compound was prepared from (1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and morpholine in a yield of 54%: mp 149.1-149.9 °C (methanol), pale yellow crystals. ^1H NMR (600 MHz, CDCl_3) δ 7.80 (d, $J = 15.8$ Hz, 1H, H-3), 7.69 (br t, $J = 1.6$ Hz, 1H, H-2'), 7.65 (dt, $J = 1.6, 7.7$ Hz, 1H, H-6'), 7.47 – 7.36 (m, 3H, H-2, H-5', H-4'), 7.25 (br d, $J = 3.4$ Hz, 1H, H-3''), 6.21 (dd, $J = 1.0, 3.4$ Hz, 1H, H-4''), 3.93 – 3.31 (m, 8H, 4 x morpholine $\underline{\text{C}}\text{H}_2$), 2.43 (s, 3H, $\underline{\text{C}}\text{H}_3$). ^{13}C NMR (151 MHz, CDCl_3) δ 176.8 (C-1), 169.6 (amide C=O), 158.4 (C-5''), 152.3 (C-2''), 141.8 (C-3), 136.0 (C-1' or C-3'), 135.4 (C-1' or C-3'), 129.9 (C-6'), 129.1, 128.5 (C-4', C-5')*, 126.6 (C-2'), 122.4 (C-2), 119.8 (C-3''), 109.4 (C-4''), 66.8, 48.2, 42.5 (morpholine $\underline{\text{C}}\text{H}_2$). EI-HRMS m/z: calcd for $\text{C}_{19}\text{H}_{19}\text{NO}_4$, 325.13141, found 325.13031; Purity (HPLC): 100%. * In no particular order.

5.1.2.2 (2E)-1-(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one (**7k**)

The title compound was prepared from (1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and 1-methylpiperazine in a yield of 67%: mp 131.0-132.2 °C (methanol), yellow crystals. ^1H NMR (600 MHz, CDCl_3) δ 7.79 (d, $J = 15.8$ Hz, 1H, H-3), 7.68 (br t, $J = 1.6$ Hz, 1H, H-2'), 7.63 (dt, $J = 1.6, 7.7$ Hz, 1H, H-6'), 7.45 – 7.35 (m, 3H, H-4', H-5', H-2), 7.25 (d, $J = 3.5$ Hz, 1H, H-3''), 6.20 (d, $J = 3.5$ Hz, 1H, H-4''), 3.81 (br s, 2H, CONCH_2), 3.44 (br s, 2H, CONCH_2), 2.50-2.27 (m, 10H, 2 x $\underline{\text{C}}\text{H}_2\text{NCH}_3$, 2 x $\underline{\text{C}}\text{H}_3$). ^{13}C NMR (151 MHz, CDCl_3) δ 176.8 (C-1), 169.5 (amide C=O), 158.3 (C-5''), 152.3 (C-2''), 141.9 (C-3), 136.5 (C-1' or C-3'), 135.3 (C-1' or C-3'), 129.7 (C-6'), 129.0 (C-4' or C-5'), 128.5 (C-4' or C-5'), 126.6 (C-2'), 122.3 (C-2), 119.8 (C-3''), 109.4 (C-4''), 55.2 ($\underline{\text{C}}\text{H}_2\text{NCH}_3$), 54.6 ($\underline{\text{C}}\text{H}_2\text{NCH}_3$), 47.6 (CONCH_2), 45.9 (piperazine $\underline{\text{C}}\text{H}_3$), 42.0 (CONCH_2), 14.1 (furan $\underline{\text{C}}\text{H}_3$). EI-HRMS m/z: calcd for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_3$, 338.16304, found 338.16270; Purity (HPLC): 97%.

5.1.2.3 (2E)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7l**)

The title compound was prepared from (1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and 1-ethylpiperazine in a yield of 63%: mp 98.5-98.8 °C (methanol), orange solid. ¹H NMR (600 MHz, CDCl₃) δ 7.80 (d, *J* = 15.9 Hz, 1H, H-3), 7.68 (br s, 1H, H-2'), 7.63 (br d, *J* = 7.6 Hz, 1H, H-6'), 7.45 – 7.36 (m, 3H, H-4', H-5', H-2), 7.25 (d, *J* = 3.5 Hz, 1H, H-3''), 6.21 (d, *J* = 3.5 Hz, 1H, H-4''), 3.81 (br s, 2H, CONCH₂), 3.44 (br s, 2H, CONCH₂), 2.55 – 2.33 (m, 9H, 2 x CH₂NCH₃, NCH₂CH₃, furan CH₃), 1.08 (t, *J* = 7.2 Hz, 3H, NCH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 176.8 (C-1), 169.4 (amide C=O), 158.3 (C-5''), 152.3 (C-2''), 141.9 (C-3), 136.5 (C-1' or C-3'), 135.3 (C-1' or C-3'), 129.8 (C-6'), 129.0 (C-4' or C-5'), 128.5 (C-4' or C-5'), 126.6 (C-2'), 122.3 (C-2), 119.8 (C-3''), 109.4 (C-4''), 53.1 (CH₂NCH₂), 52.3 (CH₂NCH₂), 52.2 (NCH₂CH₃), 47.7 (CONCH₂), 42.1 (CONCH₂), 14.1 (furan CH₃), 11.8 (piperazine CH₃). EI-HRMS *m/z*: calcd for C₂₁H₂₄N₂O₃, 352.17869, found 352.17790; Purity (HPLC): 98%.

5.1.2.4 (2E)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one (**7m**)

The title compound was prepared from (1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and piperidine in a yield of 78%: mp 144.7 – 145.1 °C (methanol), pale yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 7.81 (d, *J* = 15.8 Hz, 1H, H-3), 7.68 (br s, 1H, H-2'), 7.63 (br d, *J* = 7.8 Hz, 1H, H-6'), 7.45 – 7.35 (m, 3H, H-4', H-5', H-2), 7.25 (d, 1H, *J* = 3.4 Hz, H-3''), 6.21 (d, *J* = 3.4 Hz, 1H, H-4''), 3.71 (s, 2H, CONCH₂), 3.33 (s, 2H, CONCH₂), 2.43 (s, 3H, CH₃), 1.71 – 1.42 (m, 6H, 3 x piperidine CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 176.9 (C-9), 169.5 (amide C=O), 158.3 (C-5''), 152.3 (C-2''), 142.1 (C-3), 137.2 (C-3'), 135.2 (C-1'), 129.5 (C-6'), 128.9 (C-4' or C-5'), 128.3 (C-4' or C-5'), 126.4 (C-2'), 122.2 (C-2), 119.8 (C-3''), 109.4 (C-4''), 48.7 (CONCH₂), 43.1 (CONCH₂), 26.5, 25.5, 24.5 (3 x piperidine CH₂), 14.2 (CH₃). EI-HRMS *m/z*: calcd for C₂₀H₂₁NO₃, 323.15214, found 323.15116; Purity (HPLC): 96%.

5.1.2.5 (2E)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one (**7n**)

The title compound was prepared from (1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and pyrrolidine in a yield of 74%: mp 120.8-122.6 °C (methanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.82 – 7.76 (m, 2H, H-2', H-3), 7.62 (br d, *J* = 7.7 Hz, 1H, H-6'), 7.49 (br d, *J* = 7.7 Hz, 1H, H-4'), 7.44 – 7.37 (m, 2H, H-5', H-2), 7.24 (d, *J* = 3.5 Hz, 1H, H-3''), 6.2 (d, *J* = 3.5 Hz, 1H, H-4''), 3.63 (t, *J* = 7.1 Hz, 2H, CONCH₂), 3.41 (t, *J* = 6.6 Hz, 2H, CONCH₂), 2.41 (s, 3H, CH₃), 1.95 (p, *J* = 6.9 Hz, 2H, CH₂CH₂CH₂), 1.86 (p, *J* = 6.7 Hz, 2H, CH₂CH₂CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 176.9 (C-1), 168.9 (amide C=O), 158.3 (C-5''), 152.3 (C-2''), 142.1 (C-3), 137.8 (C-3'), 135.0 (C-1'), 129.9 (C-6'), 128.8 (C-4' or C-5'), 128.6 (C-4' or C-5'), 126.6 (C-2'), 122.1 (C-2), 119.8 (C-3''),

109.4 (C-4''), 49.5 (CONCH₂), 46.2 (CONCH₂), 26.3 (CH₂CH₂CH₂), 24.3 (CH₂CH₂CH₂), 14.1 (CH₃). APCI-HRMS m/z: calcd for C₁₉H₁₉NO₃ (M + H)⁺, 310.1438, found 310.1448; Purity (HPLC): 93%.

5.1.3 General procedure for the synthesis of 2-aminopyrimidines

Guanidine hydrochloride (4.6 mmol) was dissolved in a small amount of DMF (15 ml), and the appropriate chalcone (3.1 mmol) and sodium hydride (9.2 mmol) were added while stirring. The reaction mixture was heated (110 °C) for 24 h under nitrogen, allowed to cool to room temperature and then diluted with equal volumes of ethyl acetate and water. The aqueous phase was extracted with ethyl acetate (twice) and the organic layers were combined. All traces of DMF were removed by washing the combined organic layers with water (4 times). The organic layer was concentrated *in vacuo* and the crude product was purified with column chromatography [petroleum ether: ethyl acetate (4:1)] and recrystallised from ethanol.

5.1.3.1 4-(3-chlorophenyl)-6-phenylpyrimidin-2-amine (8a)

The title compound was prepared from (2E)-3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (7a) in a yield of 15%: mp 128.6-131.4 °C (ethanol), (lit. 132 – 133 °C) [56] white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.05 (m, 3H, Ar-H), 7.92 (dt, *J* = 1.5, 7.6 Hz, 1H, H-4'/6'), 7.53 – 7.39 (m, 6H, Ar-H, H-5), 5.42 (s, 2H, NH₂). ¹³C NMR (151 MHz, CDCl₃) δ 166.5, 164.64, 163.6 (C-2, C-4, C-6)*, 139.5, 137.4, 134.8 (C-1', C-1'', C-3'')*, 130.6, 130.3, 130.0, 128.8 (2C), 127.2, 127.1 (2C), 125.1 (Ar-C), 104.1 (C-5). EI-HRMS m/z: calcd for C₁₆H₁₂ClN₃, 281.07198, found 281.07167; Purity (HPLC): 99%.* In no particular order.

5.1.3.2 4-(3-chlorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (8b)

The title compound was prepared from (2E)-3-(3-chlorophenyl)-1-(furan-2-yl)prop-2-en-1-one (7b) in a yield of 22%: mp 144.1-144.4 °C (ethanol), light yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.05 (br t, *J* = 1.9 Hz, 1H, H-2'), 7.91 (dt, *J* = 1.45, 7.6 Hz, 1H, H-6'), 7.59 (dd, *J* = 0.8, 1.8 Hz, 1H, H-5''), 7.42 (ddd, *J* = 8.0, 2.1, 1.2 Hz, 1H, H-4'), 7.38 (br t, *J* = 7.8 Hz, 1H, H-5'), 7.34 (s, 1H, H-5), 7.20 (dd, *J* = 0.8, 3.5 Hz, 1H, H-3''), 6.56 (dd, *J* = 1.8, 3.5 Hz, 1H, H-4''), 5.41 (s, 2H, NH₂). ¹³C NMR (151 MHz, CDCl₃) δ 164.6 (C-2 or C-4), 163.4 (C-2 or C-4), 157.24 (C-6), 152.0 (C-2''), 144.6 (C-5''), 139.3 (C-1'), 134.8 (C-3'), 130.4 (C-4' or C-5'), 129.9 (C-4' or C-5'), 127.2 (C-2'), 125.1 (C-6'), 112.3 (C-3'' or C-4''), 111.80 (C-3'' or C-4''), 101.94 (C-5). EI-HRMS m/z: calcd for C₁₄H₁₀ClN₃O, 271.05124, found 271.05049; Purity (HPLC): 98%.

5.1.3.3 4-(3-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8c**)

The title compound was prepared from (2*E*)-3-(3-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7c**) in a yield of 45%: mp 170.5-172.1 °C (ethanol), yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.05 (br s, 1H, H-2'), 7.92 (d, *J* = 7.6 Hz, 1H, H-6'), 7.46 – 7.37 (m, 2H, H-4', H-5'), 7.31 (s, 1H, H-5), 7.11 (br s, 1H, H-3''), 6.17 (br s, 1H, H-4''), 5.26 (s, 2H, NH₂), 2.43 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 164.3 (C-2 or C-4), 163.3 (C-2 or C-4), 157.3 (C-6), 155.4 (C-5''), 150.5 (C-2''), 139.4 (C-1'), 134.8 (C-3'), 130.3 (C-4' or C-5'), 129.9 (C-4' or C-5'), 127.2 (C-2'), 125.1 (C-6'), 113.3 (C-3''), 108.8 (C-4''), 101.6 (C-5), 14.0 (CH₃). EI-HRMS *m/z*: calcd for C₁₅H₁₂ON₃Cl, 285.06690, found 285.06556; Purity (HPLC): 100%.

5.1.3.4 4-(3-methoxyphenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8d**)

The title compound was prepared from (2*E*)-1-(furan-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (**7d**) in a yield of 19%: mp 113.8-116.8 °C (ethanol), dark brown crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.64 – 7.57 (m, 3H, H-2', H-4', H-5''), 7.40 – 7.37 (m, 2H, H-5, H-5'), 7.19 (dd, *J* = 0.82, 3.4 Hz, 1H, H-3''), 7.03 (ddd, *J* = 0.96, 2.59, 8.1 Hz, 1H, H-6'), 6.55 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4''), 5.41 (s, 2H, NH₂), 3.89 (s, 3H, OCH₃). ¹³C NMR (151 MHz, CDCl₃) δ 165.92 (C-4), 163.35 (C-2), 159.96 (C-3'), 156.43 (C-6), 152.15 (C-2''), 144.51 (C-5''), 138.89 (C-1'), 129.68 (C-5'), 119.47 (C-4'), 116.54 (C-6'), 112.21 (C-2'/C-3''/C-4''), 112.21 (C-2'/C-3''/C-4''), 112.07 (C-2'/C-3''/C-4''), 102.18 (C-5), 55.37 (OCH₃). EI-HRMS *m/z*: calcd for C₁₅H₁₃N₃O₂, 267.10078, found 267.09949; Purity (HPLC): 92%.

5.1.3.5 4-(3-methoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8e**)

The title compound was prepared from (2*E*)-3-(3-methoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7e**) in a yield of 43%: mp 149.0 -150.6 °C (ethanol), light orange crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.64 – 7.58 (m, 2H, H-2', H-4'), 7.38 (br t, *J* = 7.9 Hz, 1H, H-5'), 7.33 (s, 1H, H-5), 7.09 (d, *J* = 3.3 Hz, 1H, H-3''), 7.02 (dd, *J* = 2.6, 8.2 Hz, 1H, H-6'), 6.15 (dd, *J* = 0.9, 3.3 Hz, 1H, H-4''), 5.44 (s, 2H, NH₂), 3.88 (s, 3H, OCH₃), 2.44 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 165.6 (C-4), 163.4 (C-2), 159.9 (C-3'), 157.0 (C-6), 155.1 (C-5''), 150.6 (C-2''), 139.1 (C-1'), 129.6 (C-5'), 119.4 (C-4'), 116.3 (C-6'), 113.0 (C-3''), 112.1 (C-2'), 108.7 (C-4''), 101.7 (C-5), 55.4 (OCH₃), 14.0 (CH₃). EI-HRMS *m/z*: calcd for C₁₆H₁₅N₃O₂, 281.11643, found 281.11556 ; Purity (HPLC): 100%.

5.1.3.6 4-(3,4-dimethoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8f**)

The title compound was prepared from (2*E*)-3-(3,4-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7f**) in a yield of 27%: mp 167.4-168.1 °C (ethanol), dark yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.68 (d, *J* = 2.0 Hz, 1H, H-2'), 7.61 (dd, *J* = 2.1, 8.4 Hz, 1H, H-6'), 7.29 (s, 1H, H-5), 7.08 (d, *J* = 3.3 Hz, 1H, H-3''), 6.93 (d, *J* = 8.4 Hz, 1H, H-5'), 6.15 (dd, *J* = 0.8, 3.3 Hz, 1H, H-4''), 5.35 (s, 2H, NH₂), 3.98 (s, 3H, OCH₃), 3.93 (s, 3H, OCH₃), 2.42 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 165.2 (C-4), 163.3 (C-2), 156.7 (C-6), 155.0 (C-5''), 151.1, 150.6 (C-4' or C-3' and C-2'')*, 149.0 (C-3' or C-

4'), 130.2 (C-1'), 120.1 (C-6'), 112.8 (C-3''), 110.7 (C-5'), 109.8 (C-2'), 108.7 (C-4''), 100.8 (C-5), 56.0 (OCH₃), 55.9 (OCH₃), 14.00 (CH₃). EI-HRMS m/z: calcd for C₁₇H₁₇N₃O₃, 311.12699, found 311.12618; Purity (HPLC): 100%* In no particular order.

5.1.3.7 4-(4-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (8g)

The title compound was prepared from (2E)-3-(4-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7g) in a yield of 30%: mp 204.6 – 205.8 °C (ethanol), dark yellow powder. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.14 (d, *J* = 8.6 Hz, 2H, H-2', H-6'), 7.56 (d, *J* = 8.6 Hz, 2H, H-3', H-5'), 7.39 (s, 1H, H-5), 7.21 (d, *J* = 3.3 Hz, 1H, H-3''), 6.76 (s, 2H, NH₂), 6.32 (dd, *J* = 1.0, 3.3 Hz, 1H, H-4''), 2.37 (s, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 163.9 (C-2 or C-4), 163.1 (C-2 or C-4), 156.7 (C-6), 154.7 (C-5), 150.4 (C-2''), 136.0 (C-1'), 135.2 (C-4'), 128.8 (C-2', C-6' or C-3', C-5'), 128.6 (C-2', C-6' or C-3', C-5'), 113.3 (C-3''), 108.9 (C-4''), 99.4 (C-5), 13.7 (CH₃). EI-HRMS m/z: calcd for C₁₅H₁₂ClN₃O, 285.06689, found 285.06633; Purity (HPLC): 99%.

5.1.3.8 4-(4-fluorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (8h)

The title compound was prepared from (2E)-1-(4-fluorophenyl)-3-(furan-2-yl)prop-2-en-1-one (7h) in a yield of 41%: mp 162.3-162.5 °C (lit. 170 °C) [57] (ethanol), faded yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.09 – 8.03 (m, 2H, H-2', H-6'), 7.59 (dd, *J* = 0.8, 1.8 Hz, 1H, H-5''), 7.37 (s, 1H, H-5), 7.19 – 7.13 (m, 3H, H-3'', H-3', H-5'), 6.57 (dd, *J* = 1.8, 3.4 Hz, 1H, H-4''), 5.28 (s, 2H, NH₂). ¹³C NMR (151 MHz, CDCl₃) δ 165.0 (C-2 or C-4), 164.4 (d, *J*_{C-F} = 250.5 Hz, C-4'), 163.3 (C-2 or C-4), 157.1 (C-6), 152.2 (C-2''), 144.6 (C-5''), 133.5 (d, *J*_{C-F} = 3.5 Hz, C-1'), 129.1 (d, *J*_{C-F} = 8.5 Hz C-2', C-6'), 115.7 (d, *J*_{C-F} = 21.3 Hz, C-3', C-5'), 112.3 (C-3''), 111.6 (C-4''), 101.7 (C-5). EI-HRMS m/z: calcd for C₁₄H₁₀FN₃O, 255.08079, found 255.07982; Purity (HPLC): 100%.

5.1.3.9 4-(5-methylfuran-2-yl)-6-[3-(morpholine-4-carbonyl)phenyl]pyrimidin-2-amine (8j)

The title compound was prepared from (2E)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one (7j) in a yield of 20%: mp 205.9 – 206.4 °C (methanol), yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.11 (m, 2H, H-2', H-6'), 7.55 – 7.46 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, *J* = 3.4 Hz, 1H, H-3''), 6.16 (br d, *J* = 3.4 Hz, 1H, H-4''), 5.32 (s, 2H, NH₂), 3.87 – 3.43 (m, 8H, morpholine CH₂), 2.42 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 170.0 (C=O), 164.6 (C-2 or C-6), 163.4 (C-2 or C-6), 157.3 (C-4), 155.3 (C-5''), 150.4 (C-2''), 138.1 (C-1'), 135.7 (C-3'), 128.8, 128.7, 128.4, 125.9 (C-2', C-4', C-5', C-6')*, 113.2 (C-3''), 108.7 (C-4''), 101.5 (C-5), 66.8, 48.2, 42.6 (morpholine CH₂) 14.0 (CH₃). EI-HRMS m/z: calcd for C₂₀H₂₀N₄O₃, 364.15354, found 364.15292; Purity (HPLC): 92%. * In no particular order.

5.1.3.10 4-(5-methylfuran-2-yl)-6-[3-(4-methylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8k**)

The title compound was prepared from (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one (**7k**) in a yield of 57%: 194.3-194.4 °C (methanol), cream solid. ¹H NMR (600 MHz, CDCl₃) δ 8.13 – 8.07 (m, 2H, H-2', H-6'), 7.53 – 7.45 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, *J* = 3.4 Hz, 1H, H-3"), 6.15 (br d, *J* = 3.4 Hz, 1H, H-4"), 5.30 (s, 2H, NH₂), 3.83 (br s, 2H, CONCH₂), 3.46 (br s, 2H, CONCH₂), 2.60 – 2.19 (m, 10H, 2 x CH₂NCH₃, 2 x CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 169.8 (C=O), 164.7 (C-2 or C-6), 163.4 (C-2 or C-6), 157.3 (C-4), 155.3 (C-5"), 150.5 (C-2"), 138.1 (C-1'), 136.2 (C-3'), 128.8, 128.7, 128.2, 125.9 (C-2', C-4', C-5', C-6')*, 113.2 (C-3"), 108.8 (C-4"), 101.6 (C-5), 55.2 (CH₂NCH₃), 54.6 (CH₂NCH₃), 47.7 (CONCH₂), 46.0 (piperazine CH₃), 42.1 (CONCH₂), 14.0 (furan CH₃). EI-HRMS *m/z*: calcd for C₂₁H₂₃N₅O₂, 377.18518, found 377.18422; Purity (HPLC): 85%. *In no particular order.

5.1.3.11 4-(5-methylfuran-2-yl)-6-[3-(4-ethylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8l**)

The title compound was prepared from (2*E*)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7l**) in a yield of 16%: 178.1-178.4 °C (methanol), light orange solid. ¹H NMR (600 MHz, CDCl₃) δ 8.12 – 8.07 (m, 2H, H-2', H-6'), 7.52 – 7.44 (m, 2H, H-4', H-5'), 7.34 (s, 1H, H-5), 7.08 (d, *J* = 3.4 Hz, 1H, H-3"), 6.14 (dd, *J* = 1.0, 3.4 Hz, 1H, H-4"), 5.38 (s, 2H, NH₂), 3.83 (br s, 2H, CONCH₂), 3.46 (s, 2H, CONCH₂), 2.62 – 2.21 (m, 9H, 2 x CH₂NCH₃, NCH₂CH₃, furan CH₃), 1.07 (t, *J* = 7.4 Hz, 3H, NCH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 169.7 (C=O), 164.7 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-5"), 150.4 (C-2"), 138.0 (C-1'), 136.2 (C-3'), 128.7, 128.7, 128.2, 125.9 (C-2', C-4', C-5', C-6')*, 113.1 (C-3"), 108.7 (C-4"), 101.5 (C-5), 53.0 (CH₂NCH₂), 52.3 (CH₂NCH₂), 52.2 (NCH₂CH₃), 47.7 (CONCH₂), 42.1 (CONCH₂), 14.0 (furan CH₃), 11.8 (NCH₂CH₃). EI-HRMS *m/z*: calcd for C₂₂H₂₅N₅O₂, 391.20083, found 391.20052; Purity (HPLC): 100%. *In no particular order.

5.1.3.12 4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**)

The title compound was prepared from (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one (**7m**) in a yield of 56%: 179.2-180.5 °C (methanol), orange crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.12 – 8.05 (m, 2H, H-2', H-6'), 7.52 – 7.44 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, *J* = 3.4 Hz, 1H, H-3"), 6.15 (dd, *J* = 1.1, 3.4 Hz, 1H, H-4"), 5.35 (s, 2H, NH₂), 3.73 (br s, 2H, CONCH₂), 3.36 (br s, 2H, CONCH₂), 2.42 (s, 3H, CH₃), 1.77 – 1.39 (m, 6H, 3 x piperidine CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 169.8 (C=O), 164.9 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-5"), 150.4 (C-2"), 137.9 (C-1'), 136.8 (C-3'), 128.7, 128.5, 127.9 125.7 (C-2', C-4', C-5', C-6'), 113.1 (C-3"), 108.7 (C-4"), 101.6 (C-5), 48.8 (CONCH₂), 43.1 (CONCH₂), 26.5, 25.5, 24.5 (3 x piperidine CH₂), 14.00 (CH₃). EI-HRMS *m/z*: calcd for C₂₁H₂₂N₄O₂, 362.17428, found 362.17292; Purity (HPLC): 97%.

5.1.3.13 4-(5-methylfuran-2-yl)-6-[3-(pyrrolidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8n**)

The title compound was prepared from (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one (**7n**) in a yield of 27%: 212.7 – 213.4 °C (methanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.20 (t, *J* = 1.8 Hz, 1H, H-2'), 8.10 (dt, *J* = 1.5, 7.8 Hz, 1H, H-6'), 7.59 (dt, *J* = 1.4, 7.7 Hz, 1H, H-4'), 7.49 (t, *J* = 7.7 Hz, 1H, H-5'), 7.36 (s, 1H, H-5), 7.08 (d, *J* = 3.4 Hz, 1H, H-3''), 6.14 (dd, *J* = 1.2, 3.3 Hz, 1H, H-4''), 5.37 (s, 2H, NH₂), 3.66 (t, *J* = 7.0 Hz, 2H, CONCH₂), 3.44 (t, *J* = 6.7 Hz, 2H, CONCH₂), 2.41 (s, 3H, CH₃), 1.95 (p, *J* = 7.0 Hz, 2H, CH₂CH₂CH₂), 1.86 (p, *J* = 6.7 Hz, 2H, CH₂CH₂CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 169.2 (C=O), 164.8 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-4''), 150.5 (C-1''), 137.7 (C-1' or C-3'), 137.6 (C-1' or C-3'), 128.8, 128.5, 128.3, (C-4', C-5', C-6')*, 125.9 (C-2'), 113.1 (C-3''), 108.7 (C-4''), 101.5 (C-5), 49.6 (CONCH₂), 46.2 (CONCH₂), 26.3 (CH₂CH₂CH₂), 24.4 (CH₂CH₂CH₂), 14.0 (CH₃). EI-HRMS *m/z*: calcd for C₂₀H₂₀N₄O₂, 348.15863, found 348.15730; Purity (HPLC): 95%. *In no particular order.

5.2 Biological methods

5.2.1. Materials

Adenosine deaminase (type X from calf spleen), N⁶-cyclopentyladenosine (CPA), anhydrous magnesium chloride, Trizma® Base, Trizma® Hydrochloride, silicone solution (Sigma-cote), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), phosphate-buffered saline and isopropanol were obtained from Sigma Aldrich. Dimethyl sulfoxide (DMSO), Whatman® GF/B 25 mm diameter filters and formic acid were obtained from Merck. Filtercount scintillation fluid, radioligands, [³H]5'-N-ethylcarboxamide-adenosine {[³H]NECA, 25 Ci/mmol (250 μCi)} and 1,3-[³H]-dipropyl-8-cyclopentylxanthine ([³H]DPCPX, 120 Ci/mmol) were obtained from Separation Scientific SA. Cell culture media (Dulbecco's Modified Eagle Medium), fungizone, trypsin/EDTA, streptomycin, fetal bovine serum and penicillin were obtained from Gibco and Merck. Well-plates (24 and 96) and culture flasks were obtained from Corning. Sterile syringe filters (0.22 μM) were obtained from Pall Corporation Life Sciences.

5.2.2 Radioligand binding studies

Tissue preparation for binding studies

Radioligand binding studies were carried out as reported in literature [51-53]. The Animal Research Ethics Committee of the North-West University (NWU-0035-10-A5) approved the collection of animal tissue required for these assays. Adult male Sprague-Dawley rats were obtained from the Vivarium of the North-West University, Potchefstroom campus. Striata (for the adenosine A_{2A} assays) and whole brains (for the adenosine A₁ assay) were dissected on ice and immediately snap frozen with liquid nitrogen and then stored at -70 °C until required. The frozen striata as well as the whole brain tissue were suspended in ice-cold 50 mM Tris buffer (pH 7.7 at 25 °C) and homogenised using a Polytron PT-

10 homogeniser (Brinkman) to yield final suspensions of 1 g/5 ml, which were aliquoted and stored at -70 °C until required. Test compounds were dissolved (10 mM) and further diluted in DMSO, with the final concentration of DMSO in the incubations being 1%. All pipette tips as well as the 4 ml polypropylene tubes used for the incubations were coated with Sigmacote®. The incubations were prepared using 50 mM Tris buffer (pH 7.7 at 25 °C). For the adenosine A_{2A} assay, the final volume of the incubations was 1 ml and each incubation contained test compound (0-100 µM), membrane suspension yielding ~10 mg of original tissue weight of rat striata, 10 mM MgCl₂, 0.2 units of adenosine deaminase, 50 nM CPA and 4 nM [³H]NECA. The MgCl₂ (A_{2A} assay) and adenosine deaminase (A_{2A} and A₁ assays) were firstly added to the membrane suspension, and this mixture was subsequently added to the incubations. The order of addition was test compound, membrane suspension, CPA and [³H]NECA. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 µM replacing the test compound). For the adenosine A₁ assay, the final volume of the incubations was also 1 ml and each incubation contained test compound (0-100 µM), membrane suspension yielding 5 mg of original tissue weight of rat whole brain, 0.1 units of adenosine deaminase and 0.1 nM [³H]DPCPX. The order of addition was test compound, membrane suspension and [³H]DPCPX. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 µM replacing the test compound). After incubation for 1 h (with vortexing after 30 minutes), the incubations were rapidly filtered through Whatman® GF/B 25 filters (25 mm diameter) fitted on a Hoffeler vacuum system. The damp filters were placed into scintillation vials and scintillation fluid (4 ml) was added. The vials were shaken thoroughly and left for 2 h. A Packard Tri-CARB 2100 TR scintillation counter was used to count the radioactivity retained on the filters. Specific binding was defined as total binding minus non-specific binding, and was expressed as counts per minute (CPM).

Data analysis

Using the one site competition model of the Prism 5 software package (GraphPad) the CPM values were plotted against the logarithm of the ligand concentration to give a sigmoidal dose response curve, from which the IC₅₀ values were determined. For adenosine A₁ binding, K_i values were calculated from the IC₅₀ values by using the Cheng-Prusoff equation [58], as applicable to radioligand binding assays [51]. K_d, the equilibrium dissociation constant for the radioligand [³H]DPCPX, was taken as 0.36 nM [52]. Since adenosine A_{2A} binding was performed in the presence of CPA, an adapted version of the Cheng-Prusoff equation was used. The K_d of the radioligand, [³H]NECA, was taken as 15.3 nM and a K_c value of 685 nM was used for CPA [51]. The binding affinities of the known adenosine A₁ agonist, CPA, and the A_{2A} antagonists, KW-6002 and ZM241385 were also determined as controls. The results of the radioligand binding studies are reported as the mean ± standard error of the mean (SEM) of duplicate determinations.

5.2.3 MTT cell viability assay

Cell culture

HeLa cells were maintained in 250 cm² flasks in DMEM media (30 ml) containing 10% fetal bovine serum, 1% penicillin (10 000 units/ml)/streptomycin (10 mg/ml), and fungizone (250 µg/ml). The cells were incubated at 37 °C in an atmosphere of 10% CO₂. The media was replaced once a week and cells were allowed to reach confluency before use in assays.

Preparation of compounds

Stock solutions of test compounds were prepared in DMSO (10 mM) and further diluted in DMSO to concentrations of 1 µM and 10 µM. These solutions were filtered via a syringe filter before addition to the cell cultures.

MTT Assay

Once confluent, cells were detached with 3 ml trypsin/EDTA (0.25%/0.02%) and seeded in 24-well plates at 500 000 cells/ well. Plates were then incubated for 24 h and the wells subsequently rinsed with 0.5 ml DMEM free from fetal bovine serum. A volume of 0.99 ml DMEM (free from fetal bovine serum) was subsequently added to each well followed by 10 µl of the test compound. In each 24-well plate, wells were reserved as either positive control (100% cell death via lyses with 0.03% formic acid) or negative controls (100% cell viability as a result of no drug treatment). The plates were then incubated at 37 °C for a further 24 h where after media was aspirated from each well.

The wells were then washed twice with 0.5 ml/well PBS and 200 µl of 0.5% MTT (prepared in PBS) was added to each well. The well-plates were incubated at 37 °C for 2 h in the dark, where after the residual MTT was aspirated and 250 µl isopropanol was added to dissolve the formed formazan crystals. The well-plates were then incubated at room temperature for 5 min to allow dissolution of the blue formazan crystals, where after 100 µl of the isopropanol solution of each well plate was transferred to a corresponding well in a 96-well plate. The absorbance was measured spectrophotometrically at 560 nm (using a Labsystems Multiscan RC UV/V spectrophotometer), with the absorbance of the negative control signifying 100% viability and the absorbance of the positive control signifying 0% viability. The effects of the test compounds were evaluated in triplicate and the residual cell viabilities reported as the mean ± SD of the percentage viable cells compared to the negative control (100%).

5.2.4 *In vivo* assays

Animals

Sprague-Dawley rats were given free access to standard laboratory food and water until the required weight was obtained (240 g - 300 g). All efforts were made to minimise animal suffering as experiments

were carried out in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the North-West University ethical committee (NWU-00035-10-A5).

Compounds

To induce catalepsy a dose of 5 mg/kg haloperidol (Serenace Injection; 5 mg/ml) was administered intraperitoneally (ip) [33]. A vehicle solution was prepared by mixing a 1:1:4 solution of DMSO, Tween 80 and saline. Compounds (**8k** and **8m**) were dissolved in a required amount of vehicle solution to yield concentrations of 0.1, 0.4, 1 and 2 mg/ml. A suitable volume of these solutions was injected depending on the weight of the rat, resulting in final dose concentrations of 0.1, 0.4, 1 and 2 mg/kg. All injections were administered ip.

Catalepsy test

The experiments were carried out between 8:00 and 15:30 in a lit room with a controlled temperature. All the rats were drug naïve and were only used once. Haloperidol induced catalepsy was measured with the standard bar test, in a Perspex chamber (length, 23 cm; width, 10.5 cm; height, 9 cm) with a horizontal plastic bar (diameter, 1 cm; length, 10.5 cm) fixed at 9 cm above the floor, and at 7 cm from the back of the box.

Animals were divided into 5 groups, each group containing 6 rats. The 5 groups were treated with 0, 0.1, 0.4, 1 and 2 mg/kg of the test compound, respectively. All rats received ip injections of haloperidol (5.0 mg/kg) to induce catalepsy. 30 Minutes later, the rats in each group received ip injections of the compound. The vehicle solution was administered to rats in the control group. Catalepsy was measured 60 min. after the haloperidol injections by placing the rats in the Perspex box with their front paws on the horizontal bar. Catalepsy was measured as the time the animal maintained its position on the bar. Time was recorded until one or both of the rat's front paws were removed from the bar, or up to 120 seconds.

The results of the animal experiments are reported as the mean \pm standard error of the mean (SEM). Data were analysed by means of one-way analysis of variance (ANOVA) across all groups, and were subsequently subjected to Dunnett's post-test to determine if statistical differences exist between mean values. A p value < 0.05 is judged as being statistically significant. These analyses were carried out with the Prism software package.

5.3 Molecular docking

Molecular docking studies were carried out with the Windows based Accelrys[®] Discovery Studio 3.1 software. The crystal structure of the adenosine A_{2A} receptor [Protein Data Bank (PDB) code 3PWH]

co-crystalised with the known A_{2A} antagonist ZM241385 was used. This receptor was prepared with the 'Clean protein' function to correct problems such as incomplete amino acid side chains and typed with the CHARMM forcefield. A fixed atom constraint was applied to the backbone and a minimisation was then carried out using the Generalised Born approximation with Molecular Volume (GBMV) as the solvent model to obtain a receptor at energetic minimum. A binding sphere with a radius of 5 Å was defined using the existing ligand (ZM241385) before it was removed from the receptor. Selected inhibitors were cleaned and prepared for docking with the 'Prepare ligand' protocol to correct valences and remove duplicates whereafter ligands were visually inspected and remaining errors corrected. The CDOCKER protocol was used for the docking of ligands. The orientations, CDOCKER and CDOCKER interaction energies of the ten different conformers of each ligand were considered and the best conformation for each ligand selected. An *in situ* ligand minimisation was then performed on the selected conformers and minimised conformers visually inspected and compared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.ejmech.2015.09.035>. These data include the NMR data of the intermediates (**7a** – **7h**) and examples of sigmoidal dose response curves obtained during the radioligand binding assays.

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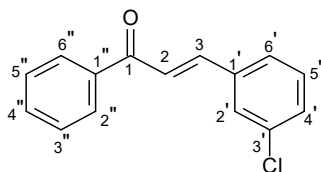
Supplementary material

Synthetic procedures

General procedure for the synthesis of chalcones (**7a** – **7h**)

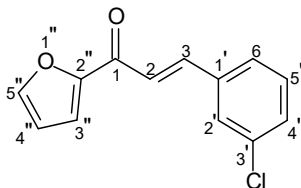
Ketone (8.57 mmol, 1 equiv) and benzaldehyde (8.57 mmol, 1 equiv) were dissolved in ethanol, and stirred at room temperature. To this mixture, a solution of 40% (w/v) sodium hydroxide (0.5 equiv) was added drop wise. After the reaction mixture was stirred at room temperature for 3 hours, the residue that formed was filtered and washed with cold ethanol. The resulting solid was recrystallised from ethanol.

(*E*)-3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (**7a**)



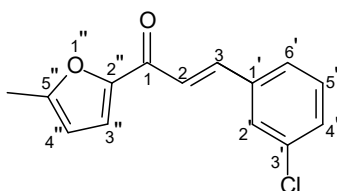
The title compound was prepared from acetophenone and 3-chlorobenzaldehyde in a yield of 34%: mp 74.7-75.1 °C (ethanol), pale yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.05 – 8.00 (m, 2H, H-2'', H-6''), 7.73 (d, *J* = 15.7 Hz, 1H, H-3), 7.65 – 7.57 (m, 2H, H-2', H-4''), 7.56 – 7.47 (m, 4H, H-2, H-3'', H-5'', H-6'), 7.40 – 7.32 (m, 2H, H-4', H-5'), ¹³C NMR (151 MHz, CDCl₃) δ 190.0 (C-1), 143.0 (C-3), 137.8 (C-1''), 136.7 (C-1'), 134.9 (C-3'), 133.0 (C-4''), 130.3, 130.2 (C-4', C-5'), 128.7, 128.5 (C-2'', C-6'' and C-3'', C-5''), 127.9 (C-2'), 126.8 (C-6'), 123.2 (C-2). EI-HRMS *m/z*: calcd for C₁₅H₁₁ClO, 242.04984, found 242.04878; Purity (HPLC): 93%.

(*E*)-3-(3-chlorophenyl)-1-(furan-2-yl)prop-2-en-1-one (**7b**)



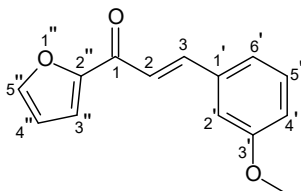
The title compound was prepared from 2-acetylfuran and 3-chlorobenzaldehyde in a yield of 17%: mp 76.2-77.1 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.78 (d, *J* = 15.8 Hz, 1H, H-3), 7.67 – 7.65 (m, 1H, H-5''), 7.62 (br t, *J* = 1.9 Hz, 1H, H-2') 7.49 (br dt, *J* = 1.6, 7.3 Hz, 1H, H-6'), 7.43 (d, *J* = 15.8 Hz, 1H, H-2), 7.39 – 7.31 (m, 3H, H-4', H-5', H-3''), 6.60 (dd, *J* = 1.7, 3.6 Hz, 1H, H-4''); ¹³C NMR (151 MHz, CDCl₃) δ 177.5 (C-1), 153.5 (C-2''), 146.7 (C-5''), 142.2 (C-3), 136.5 (C-1'), 134.9 (C-3'), 130.4, 130.1 (C-4', C-5'), 127.9 (C-2'), 126.9 (C-6'), 122.3 (C-2), 117.8 (C-3''), 112.6 (C-4''). EI-HRMS *m/z*: calcd for C₁₃H₉ClO₂, 232.02911, found 232.02796; Purity (HPLC): 92%.

(2E)-3-(3-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7c**)



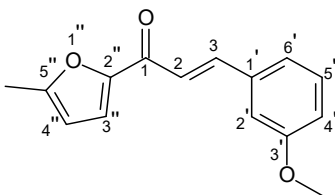
The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 3-chlorobenzaldehyde in a yield of 12%: mp 91.4-92.3 °C (ethanol), orange crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.77 (d, *J* = 15.7 Hz, 1H, H-3), 7.65 – 7.61 (m, 1H, H-2'), 7.52 – 7.47 (m, 1H, H-6'), 7.41 – 7.32 (m, 3H, H-4', H-5', H-2), 7.31 – 7.27 (m, 1H, H-3''), 6.24 (m, 1H, H-4''), 2.46 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 176.7 (C-1), 158.4 (C-2'' or C-5''), 152.3 (C-2'' or C-5''), 141.4 (C-3), 136.6 (C-1'), 134.8 (C-3'), 130.2, 130.1 (C-4' and C-5'), 127.7 (C-2'), 126.8 (C-6'), 122.5 (C-2), 119.9 (C-3''), 109.4 (C-4''), 14.2 (CH₃). EI-HRMS *m/z*: calcd for C₁₄H₁₁ClO₂, 246.04476, found 246.04435; Purity (HPLC): 100%.

(2E)-1-(furan-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (**7d**)



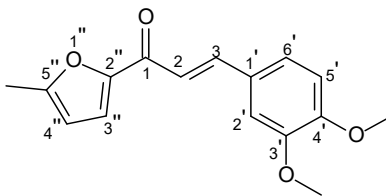
The title compound was prepared from 2-acetylfuran and 3-methoxybenzaldehyde in a yield of 22%: mp 65.4-66.4 °C (ethanol), amber crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.83 (d, *J* = 15.8 Hz, 1H, H-3), 7.65 (dd, *J* = 0.8, 1.7 Hz, 1H, H-5''), 7.42 (d, *J* = 15.8 Hz, 1H, H-2), 7.35 – 7.29 (m, 2H, H-5', H-3''), 7.25 – 7.22 (br d, 7.5 Hz, 1H, H-6'), 7.15 (br t, *J* = 2.0 Hz, 1H, H-2'), 6.95 (ddd, *J* = 0.9, 2.6, 8.2 Hz, 1H, H-4'), 6.59 (dd, *J* = 1.7, 3.6 Hz, 1H, H-4''), 3.84 (s, 3H, OCH₃). ¹³C NMR (151 MHz, CDCl₃) δ 177.9 (C-1), 159.8 (C-3'), 153.6 (C-2''), 146.5 (C-5''), 143.9 (C-3), 136.0 (C-1'), 129.9 (C-5'), 121.3 (C-6' or C-2), 121.1 (C-6' or C-2), 117.6 (C-3''), 116.3 (C-4'), 113.4 (C-2'), 112.5 (C-4''), 55.3 (OCH₃). EI-HRMS *m/z*: calcd for C₁₄H₁₂O₃, 228.07864, found 228.07796; Purity (HPLC): 94%.

(2E)-3-(3-methoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7e**)



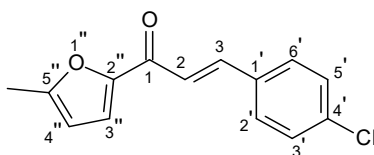
The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 3-methoxybenzaldehyde in a yield of 46%: mp 96.9-97.5 °C (ethanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.79 (d, *J* = 16.1 Hz, 1H, H-3), 7.35 (d, *J* = 16.1 Hz, 1H, H-2), 7.31 (t, *J* = 8.0 Hz, 1H, H-5'), 7.24 (d, 1H, *J* = 3.5 Hz, H-3''), 7.22 (br d, 1H, *J* = 7.7 Hz, H-6'), 7.13 (br s, 1H, H-2'), 6.94 (dd, *J* = 2.6, 8.2 Hz, 1H, H-4'), 6.20 (dd, *J* = 0.8, 3.4 Hz, 1H, H-4''), 3.83 (s, 3H, OCH₃), 2.43 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 177.1 (C-1), 159.8 (C-3'), 158.2 (C-5''), 152.4 (C-2''), 143.1 (C-3), 136.1 (C-1'), 129.8 (C-5'), 121.5 (C-2), 121.0 (C-6'), 119.6 (C-3''), 116.0 (C-4'), 113.4 (C-2'), 109.3 (C-4''), 55.3 (OCH₃), 14.1 (CH₃). EI-HRMS *m/z*: calcd for C₁₅H₁₄O₃, 242.09429, found 242.09396; Purity (HPLC): 100%.

(2E)-3-(3,4-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7f**)



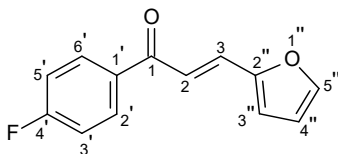
The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 3,4-dimethoxybenzaldehyde in a yield of 35%: mp 116.3-117 °C (ethanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.78 (br d, *J* = 15.7 Hz, 1H, H-3), 7.26 – 7.16 (m, 3H, H-6', H-2, H-3''), 7.11 (br s, 1H, H-2'), 6.85 (br d, *J* = 8.4 Hz, 1H, H-5'), 6.19 (br s, 1H, H-4''), 3.93 (s, 3H, OCH₃), 3.90 (s, 3H, OCH₃), 2.40 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 177.2 (C-1), 157.8 (C-5''), 152.5 (C-2''), 151.2 (C-3' or C-4'), 149.1 (C-3' or C-4'), 143.3 (C-3), 127.7 (C-1'), 123.0 (C-2), 119.1 (C-6' or C-3''), 119.2 (C-6' or C-3''), 111.0 (C-5'), 110.1 (C-2'), 109.2 (C-4''), 55.9 (OCH₃), 55.9 (OCH₃), 14.1 (CH₃). EI-HRMS *m/z*: calcd for C₁₆H₁₆O₄, 272.10486, found 272.10447; Purity (HPLC): 90%.

(2E)-3-(4-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7g**)



The title compound was prepared from 1-(5-methyl-2-furyl)ethanone and 4-chlorobenzaldehyde in a yield of 41%: mp 151.4-151.9 °C (ethanol), pale yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 7.70 (d, *J* = 15.7 Hz, 1H, H-3), 7.49 (m, 2H, H-2', H-6'), 7.29 – 7.24 (m, 3H, H-2, H-3', H-5'), 7.18 (d, *J* = 3.4 Hz, 1H, H-3''), 6.14 (dd, *J* = 0.9, 3.4 Hz, 1H, H-4''), 2.36 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 176.9 (C-1), 158.3 (C-5''), 152.4 (C-2''), 141.7 (C-3), 136.2 (C-4'), 133.3 (C-1'), 129.5 (C-2', C-6' or C-3', C-5'), 129.1 (C-2', C-6' or C-3', C-5'), 121.7 (C-2), 119.7 (C-3''), 109.4 (C-4''), 14.2 (CH₃). EI-HRMS *m/z*: calcd for C₁₄H₁₁ClO₂, 246.04476, found 246.04409; Purity (HPLC): 96%.

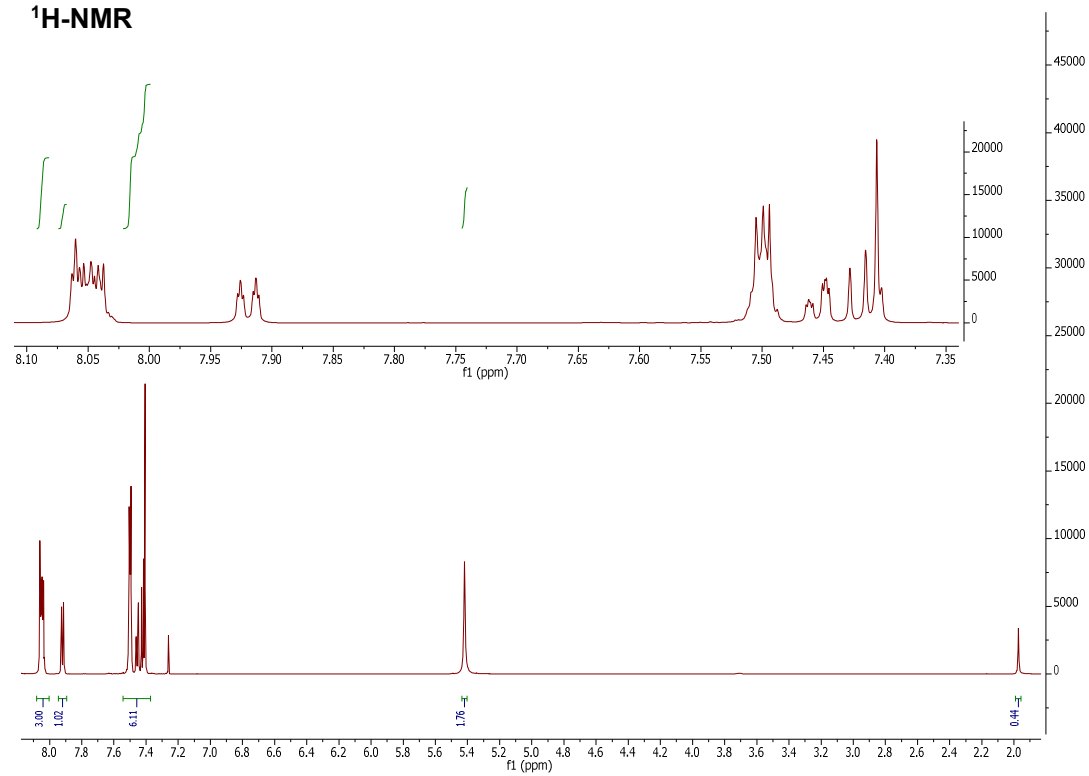
(2E)-1-(4-fluorophenyl)-3-(furan-2-yl)prop-2-en-1-one (**7h**)



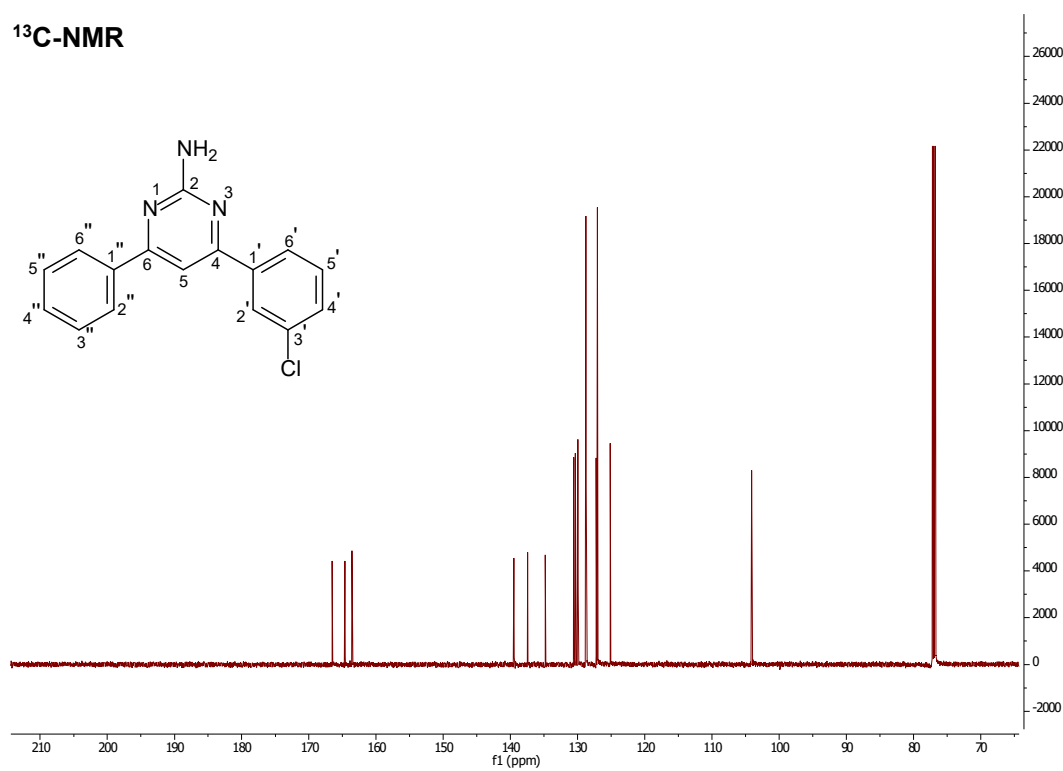
The title compound was prepared from 1-(4-fluorophenyl)ethanone and furan-2-carbaldehyde in a yield of 40%: mp 59.9-60.5 °C (ethanol), dark orange solid. ¹H NMR (600 MHz, CDCl₃) δ 8.09 – 8.02 (m, 2H, H-2', H-6'), 7.59 (d, *J* = 15.3 Hz, 1H, H-3), 7.52 (d, *J* = 1.7 Hz, 1H, H-5''), 7.42 (d, *J* = 15.3 Hz, 1H, H-2), 7.19 – 7.12 (m, 2H, H-3', H-5'), 6.72 (d, *J* = 3.4 Hz, 1H, H-3''), 6.51 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4''). ¹³C NMR (151 MHz, CDCl₃) δ 188.0 (C-1), 165.5 (d, *J*_{C-F} = 253.5 Hz, C-4'), 151.5 (C-2''), 145.0 (C-5''), 134.4 (d, *J*_{C-F} = 3.2 Hz, C-1'), 130.96 (d, *J*_{C-F} = 9.6 Hz, C-2', C-6'), 130.8 (C-3), 118.7 (C-2), 116.4 (C-3''), 115.6 (d, *J*_{C-F} = 21.9 Hz, C-3', C-5'), 112.7 (C-4''). EI-HRMS *m/z*: calcd for C₁₃H₉FO₂, 216.05866, found 216.05805; Purity (HPLC): 91%.

4-(3-chlorophenyl)-6-phenylpyrimidin-2-amine (8a)

¹H-NMR

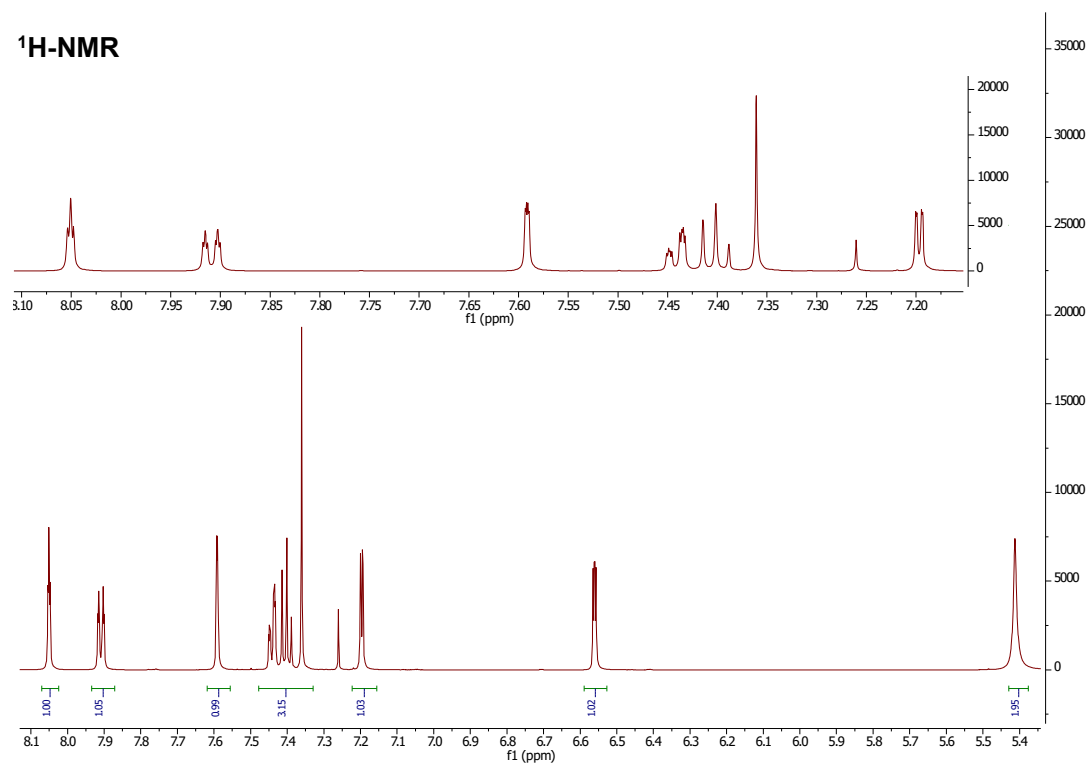


¹³C-NMR

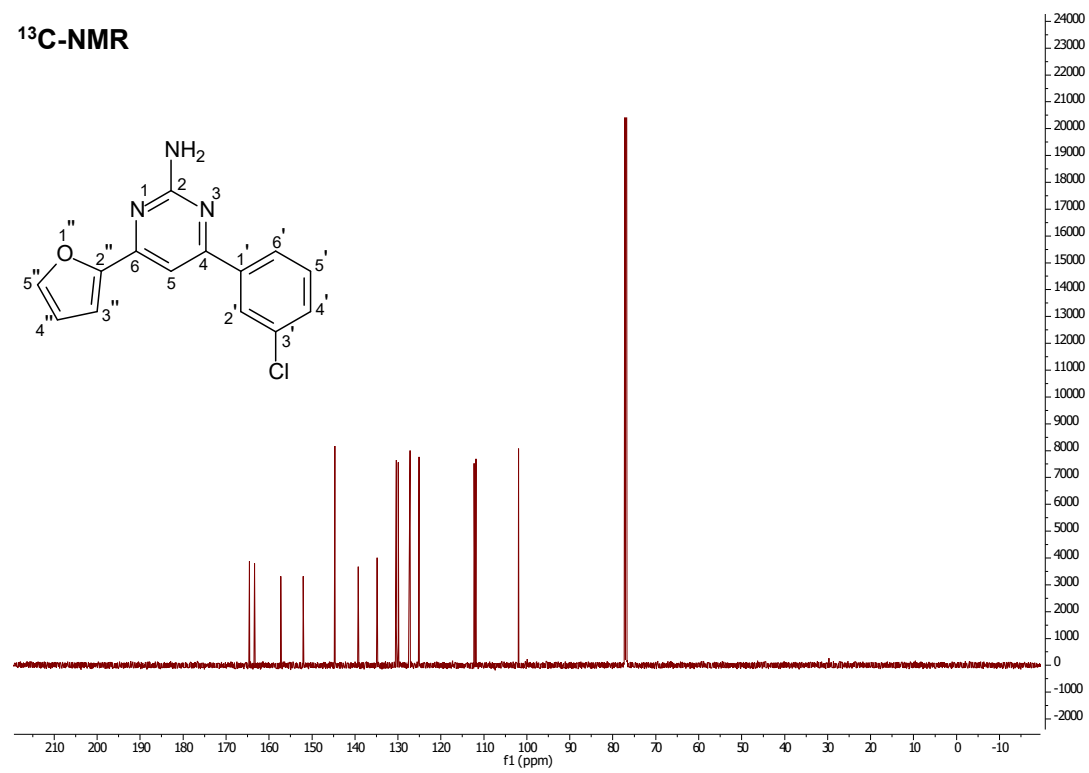


4-(3-chlorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8b**)

¹H-NMR

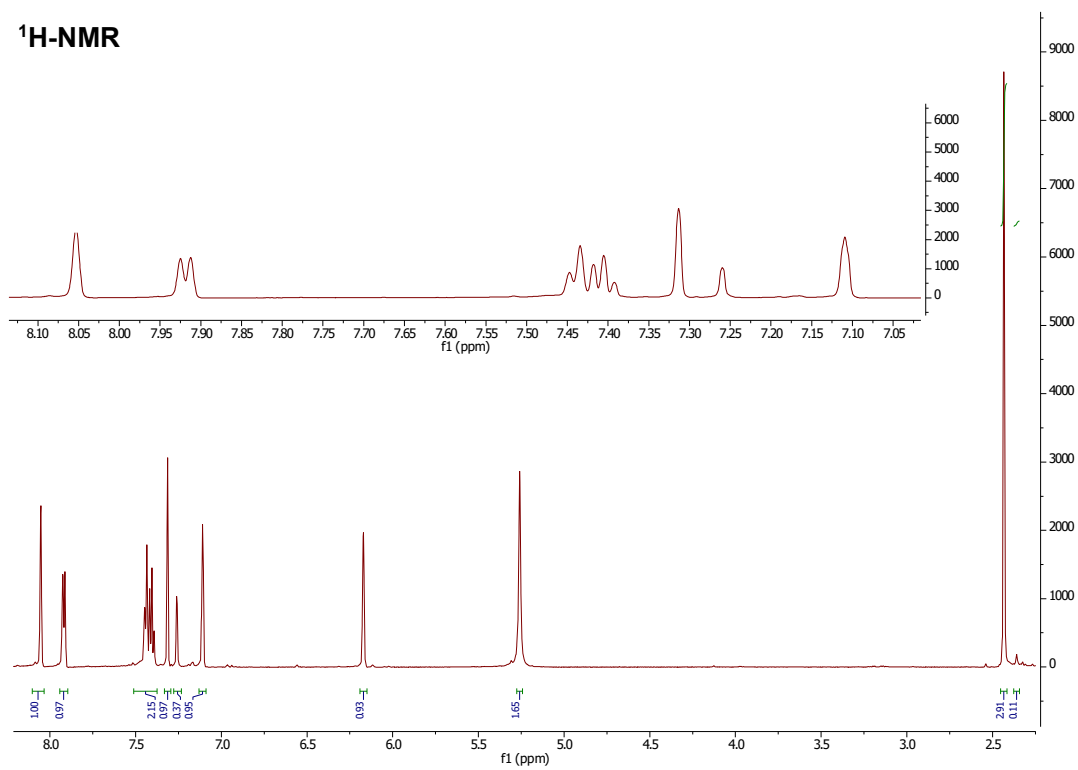


¹³C-NMR

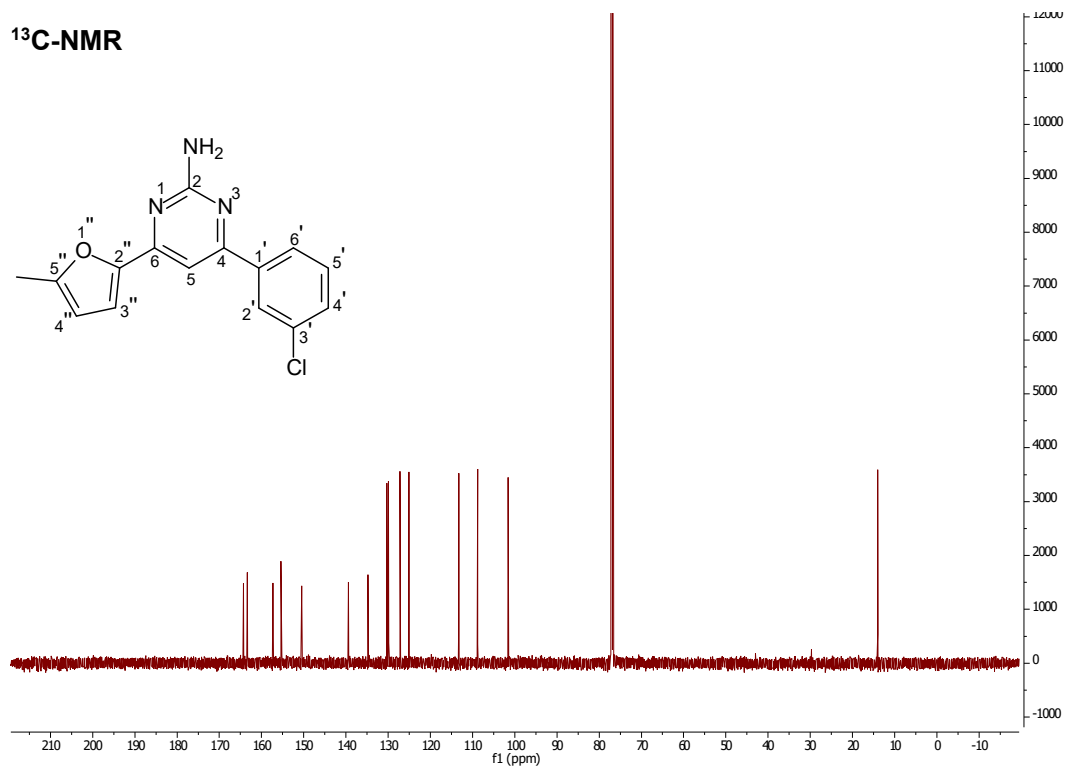


4-(3-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8c**)

¹H-NMR

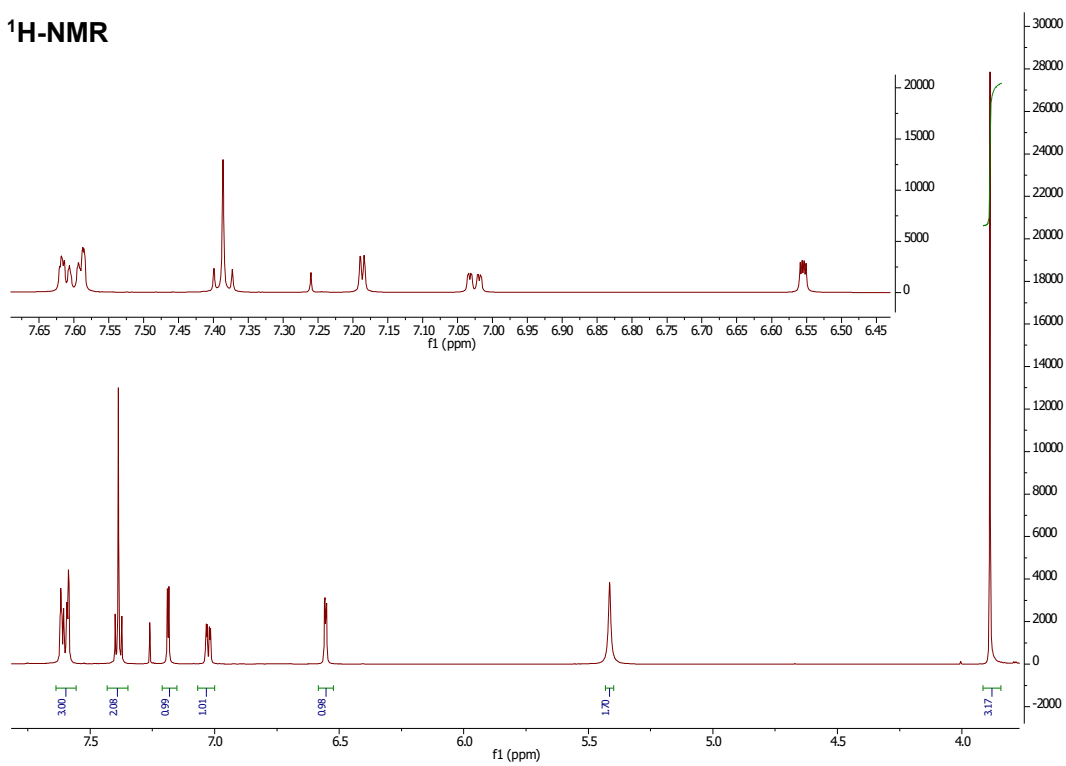


¹³C-NMR

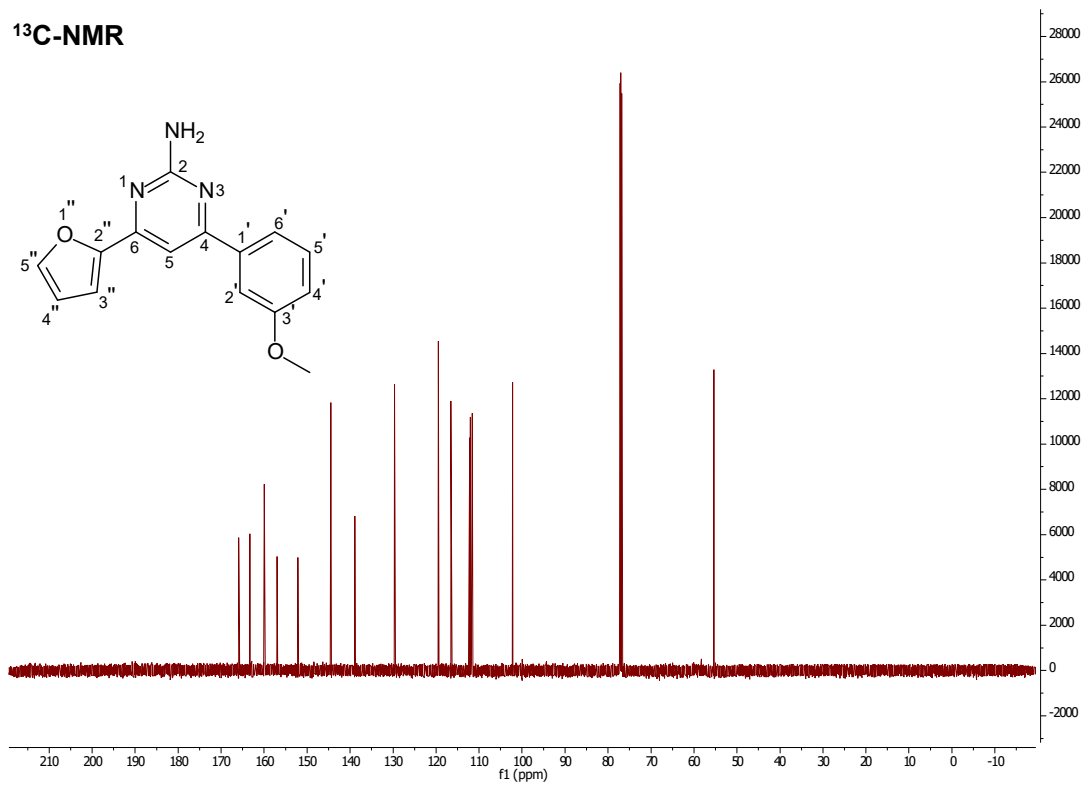


4-(furan-2-yl)-6-(3-methoxyphenyl)pyrimidin-2-amine (**8d**)

¹H-NMR

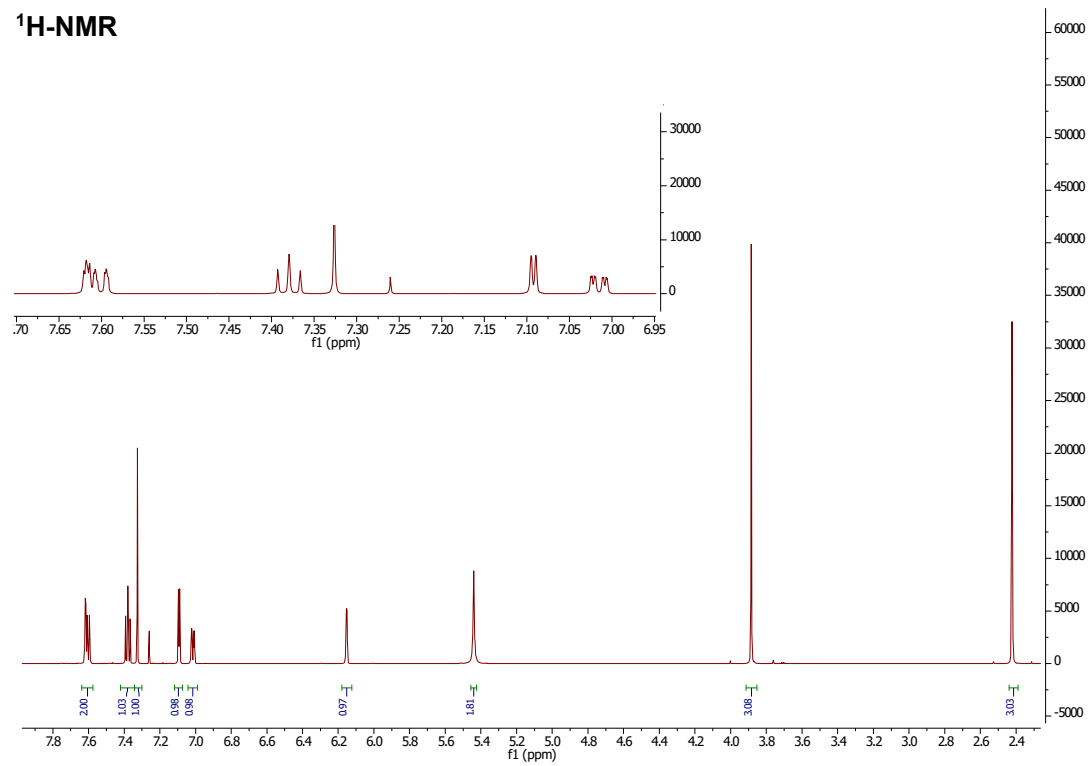


¹³C-NMR

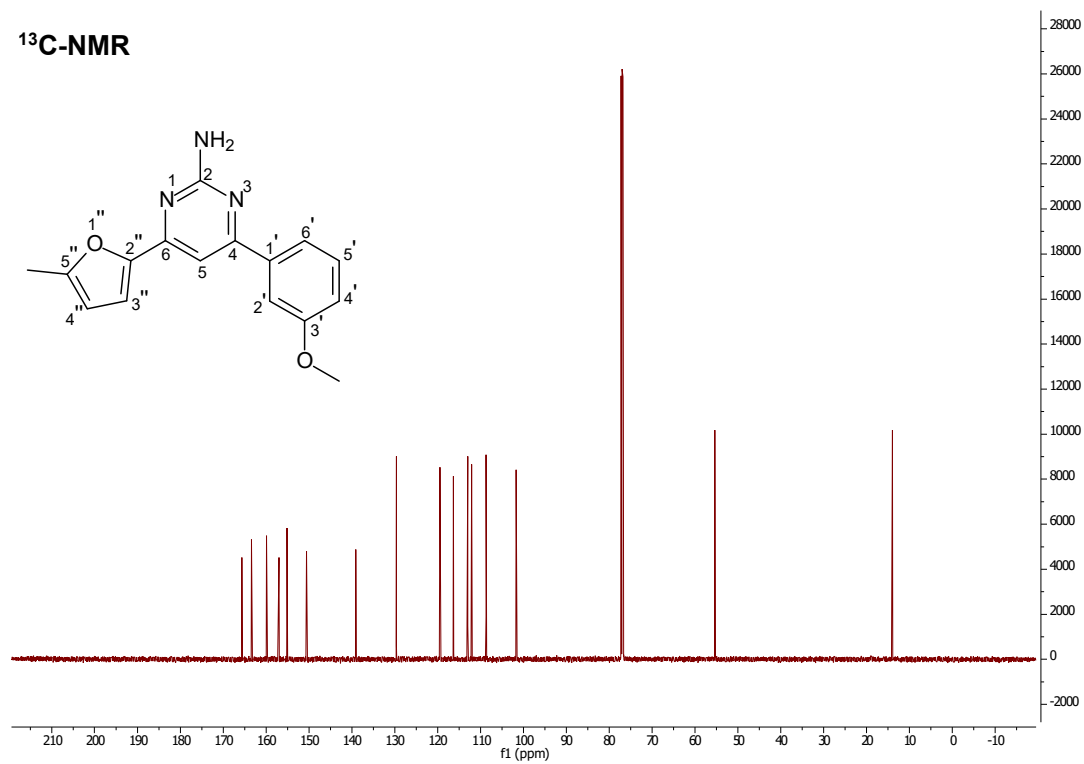


4-(3-methoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8e**)

¹H-NMR

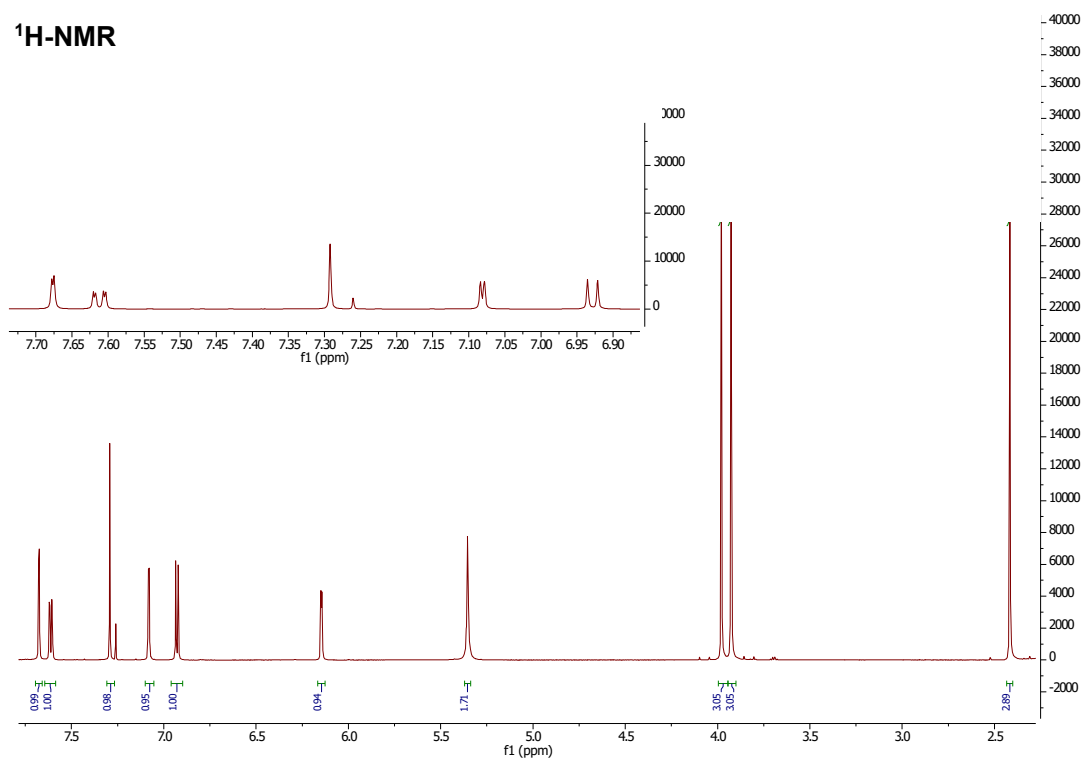


¹³C-NMR

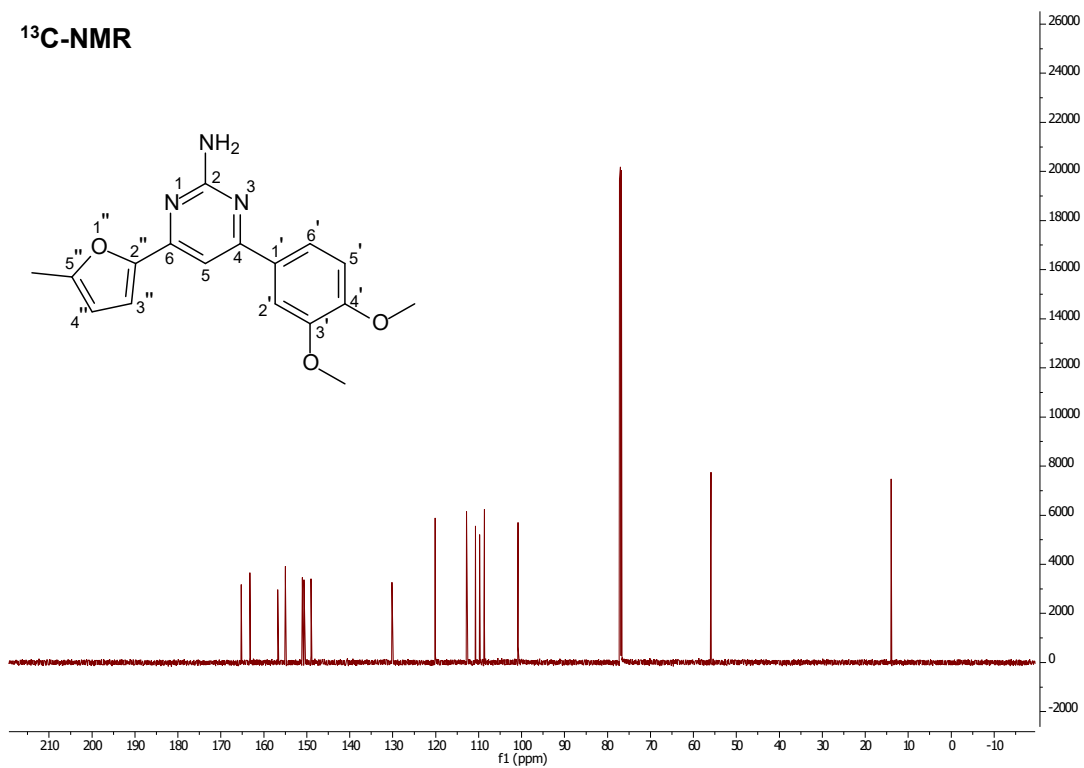


4-(3,4-dimethoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8f**)

¹H-NMR

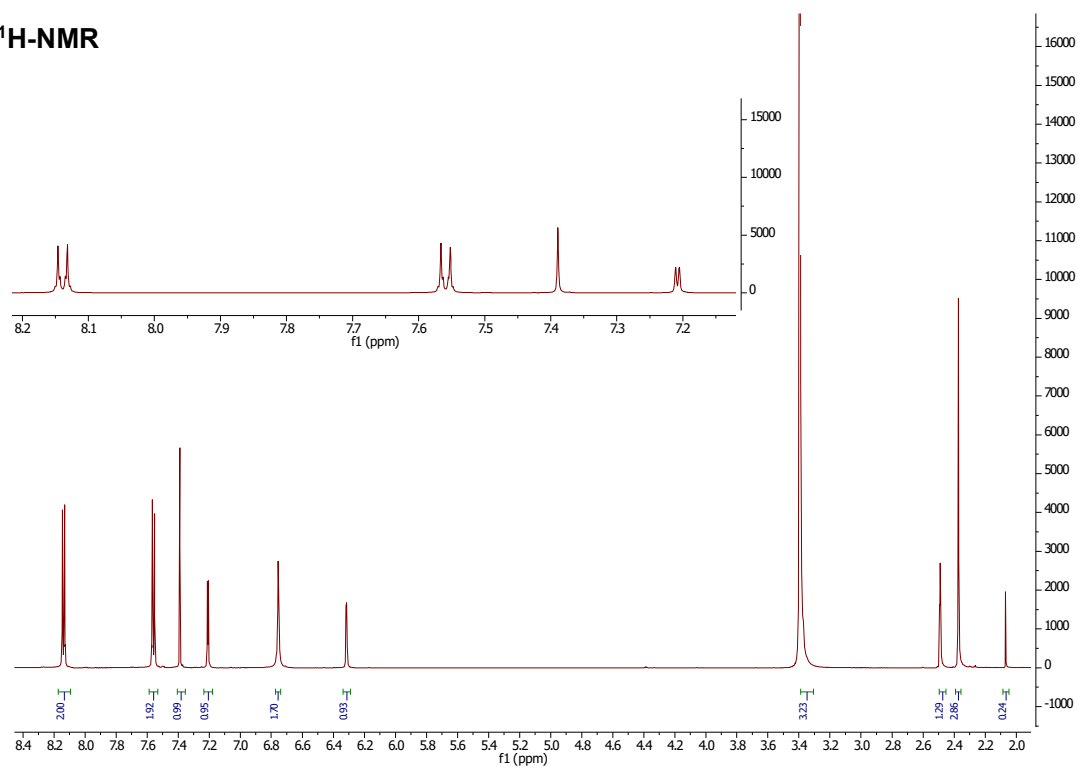


¹³C-NMR

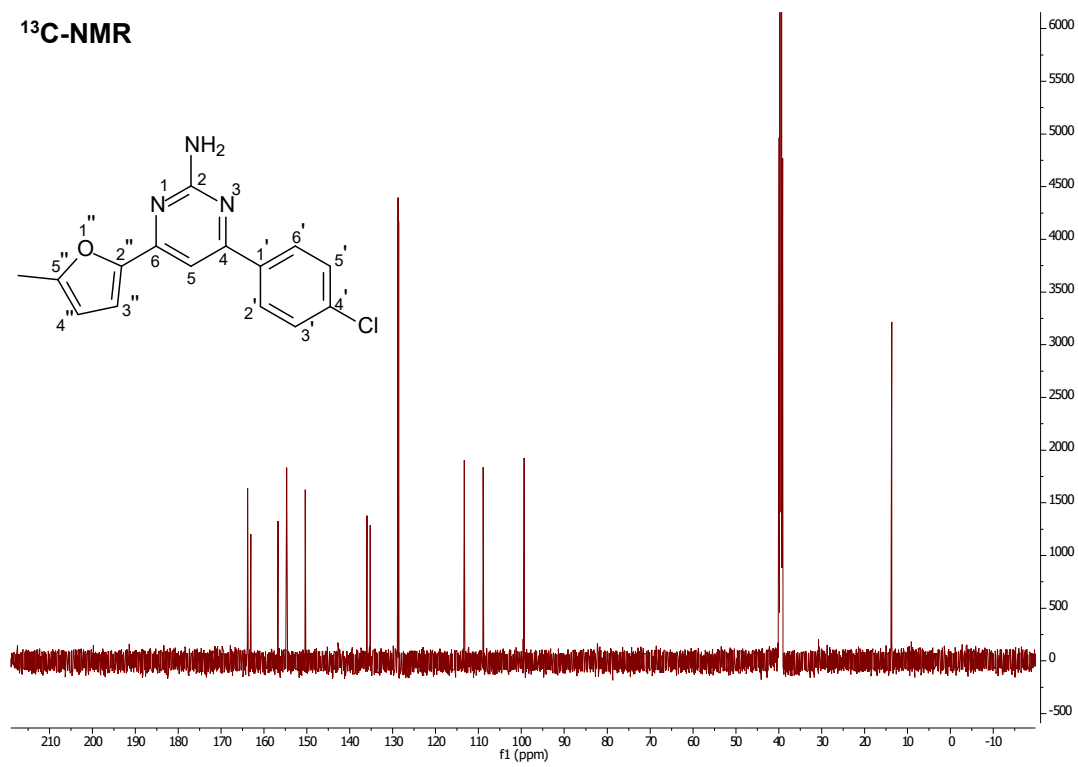


4-(4-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8g**)

¹H-NMR

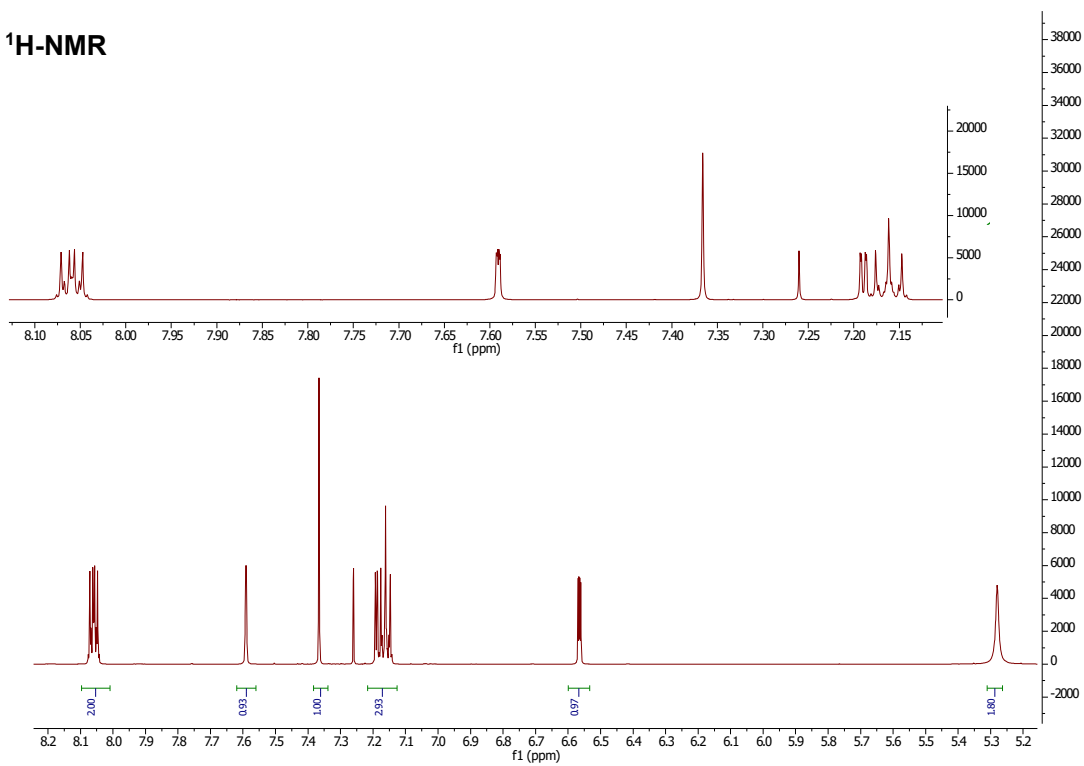


¹³C-NMR

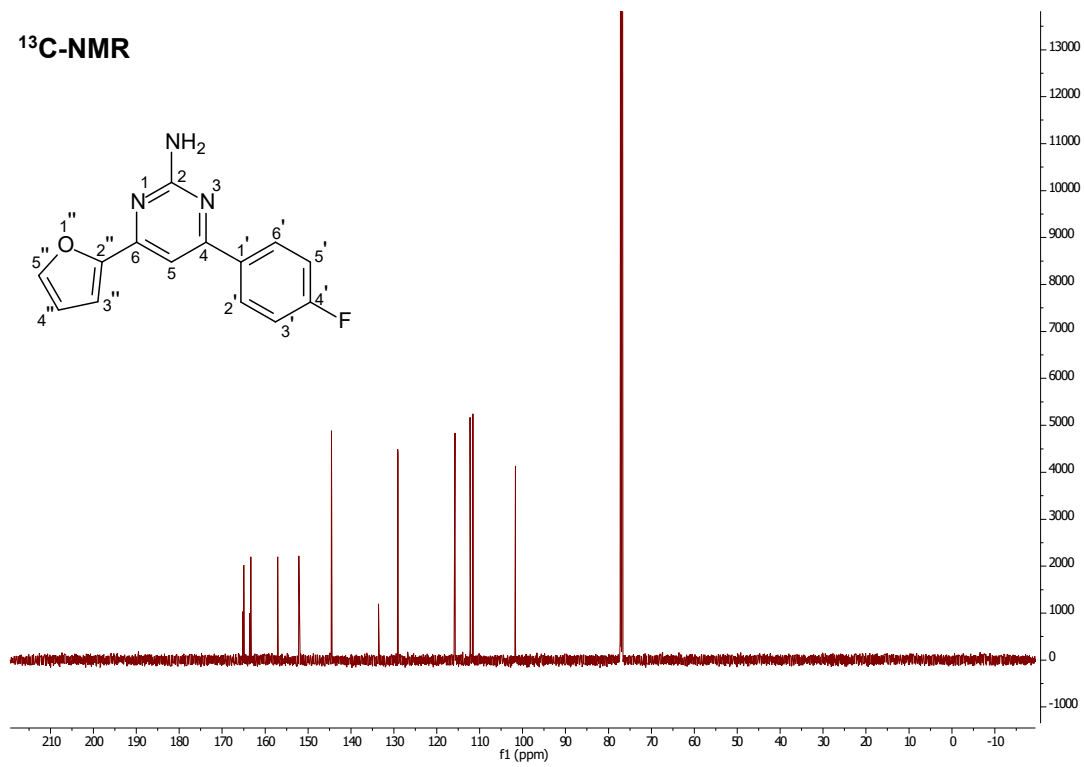


4-(4-fluorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8h**)

¹H-NMR

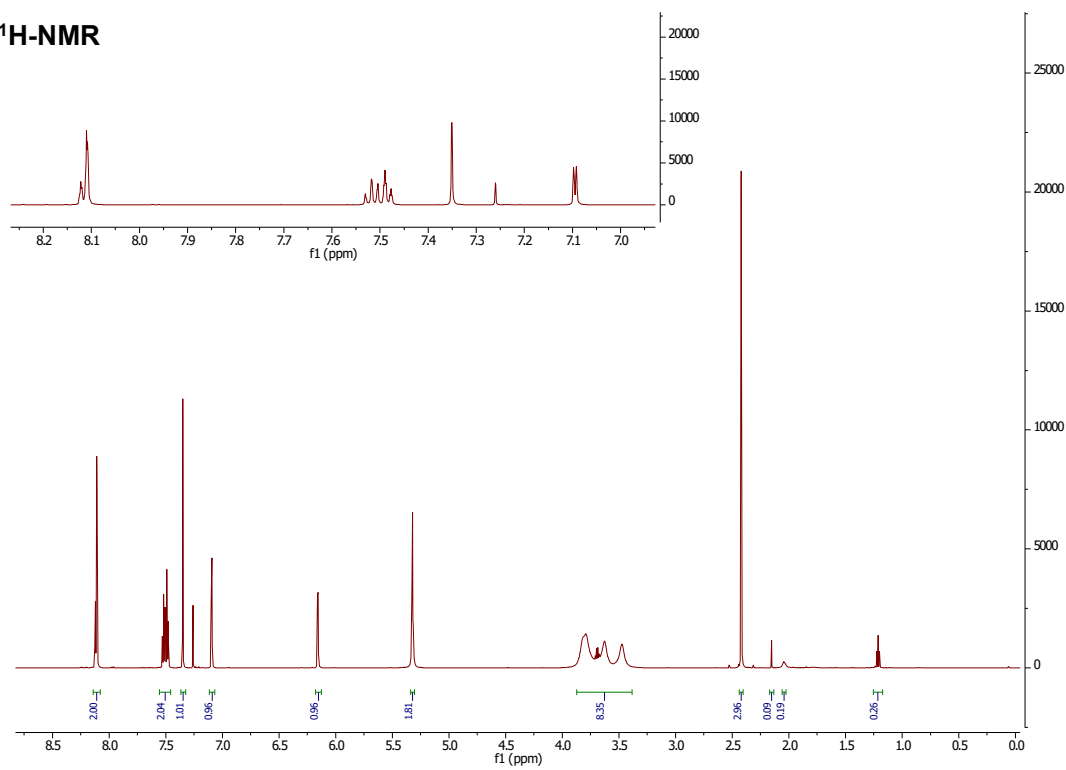


¹³C-NMR

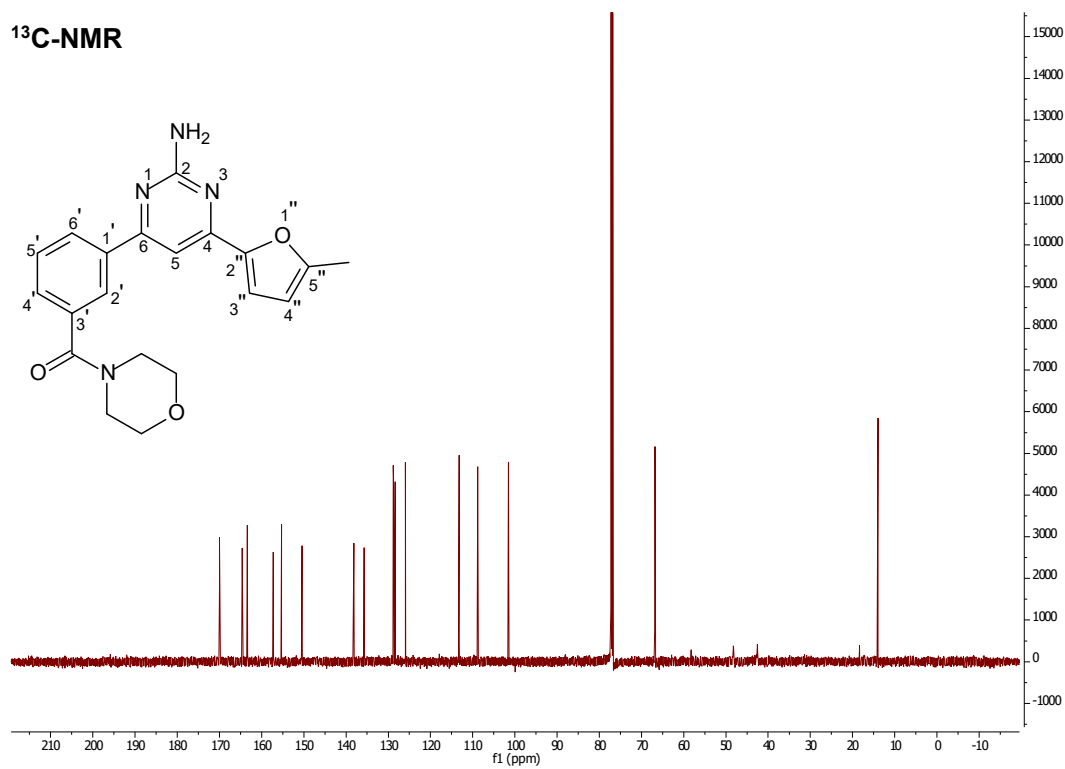


4-(5-methylfuran-2-yl)-6-[3-(morpholine-4-carbonyl)phenyl]pyrimidin-2-amine (**8j**)

¹H-NMR

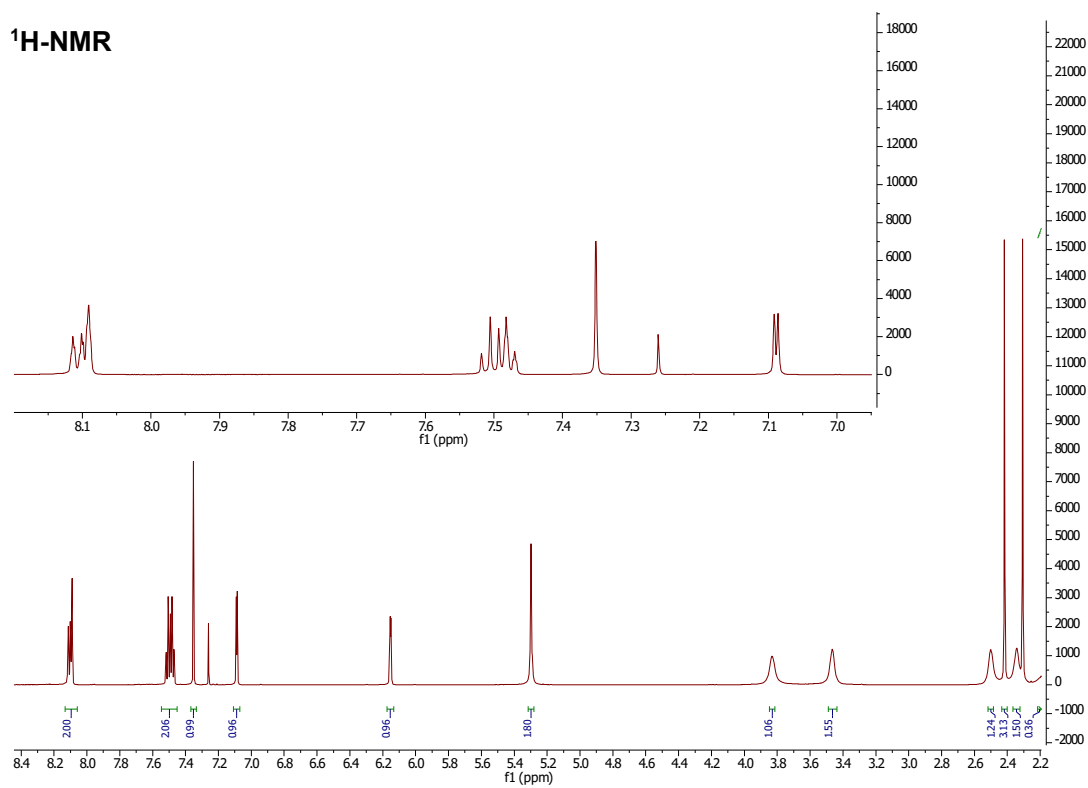


¹³C-NMR

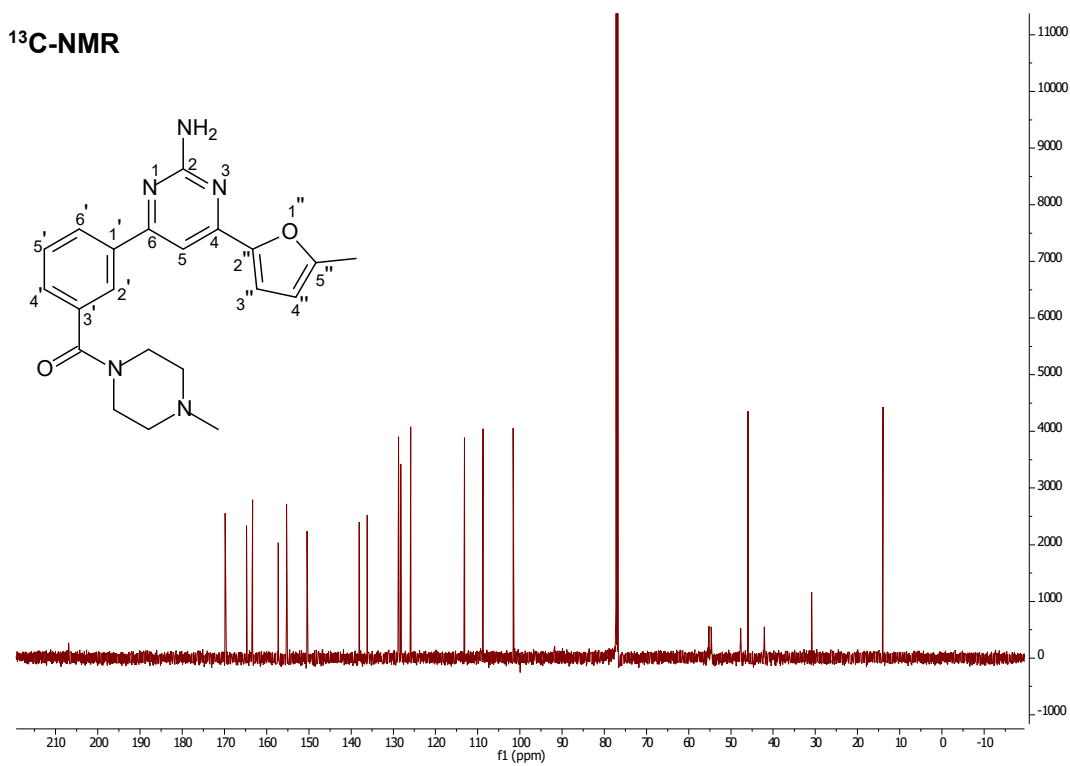


4-(5-methylfuran-2-yl)-6-[3-(4-methylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8k**)

¹H-NMR

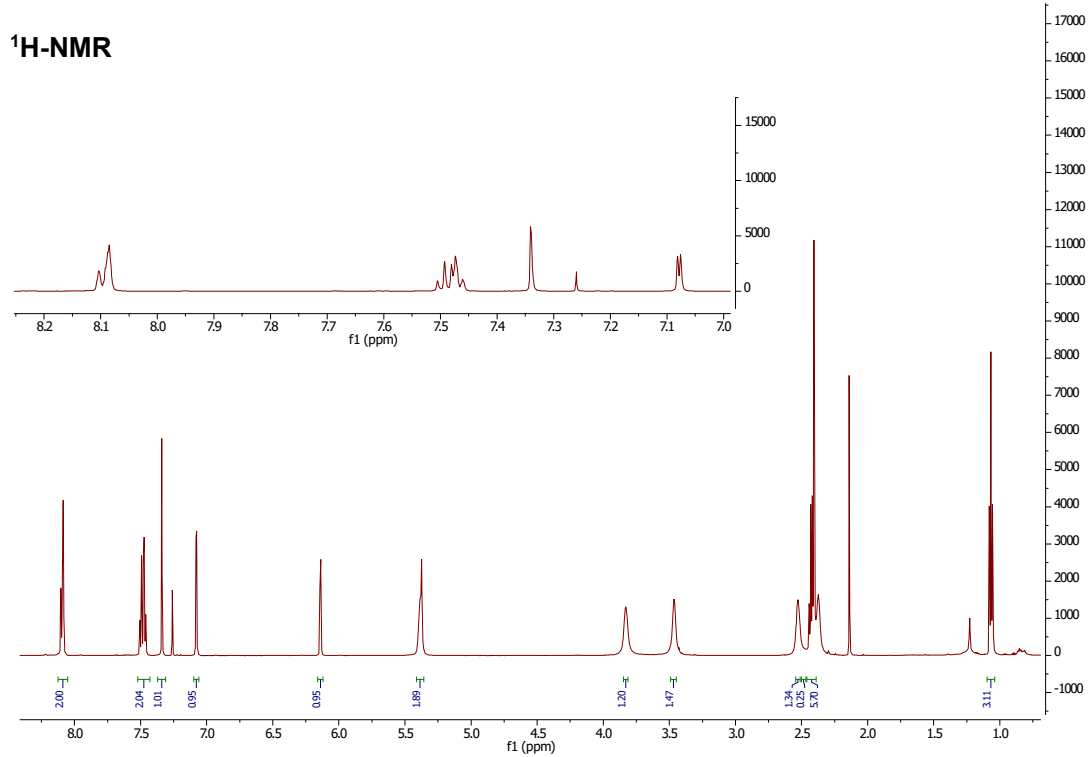


¹³C-NMR

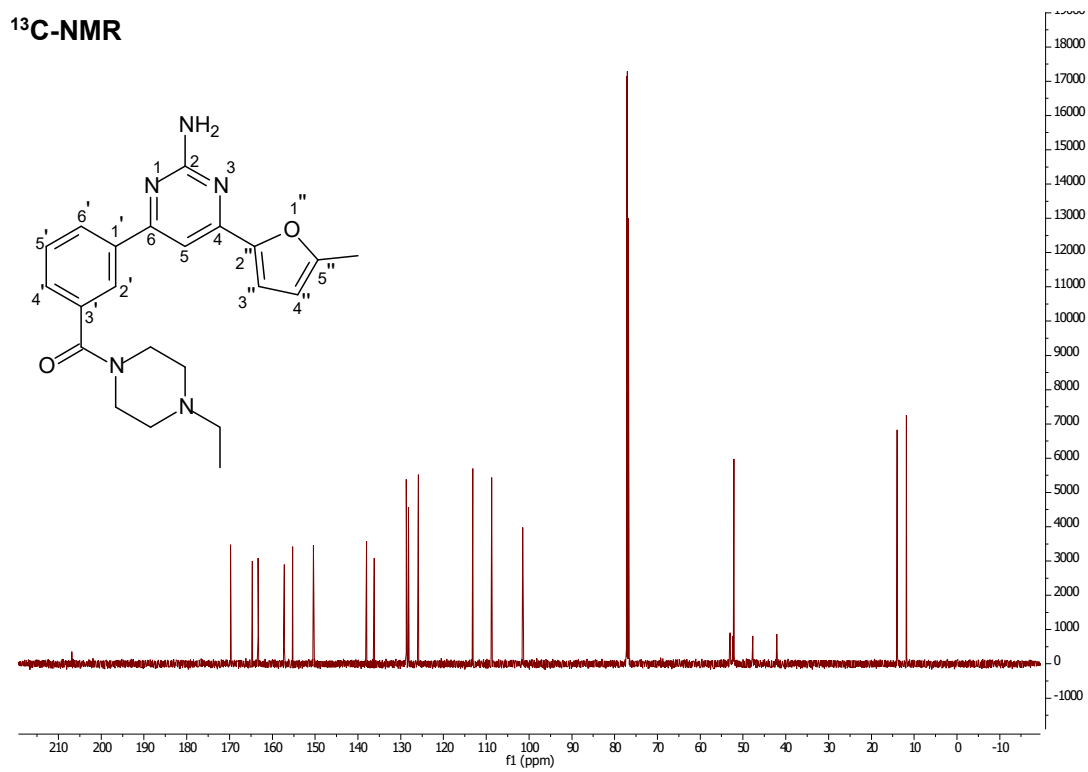


4-(5-methylfuran-2-yl)-6-[3-(4-ethylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (81)

¹H-NMR

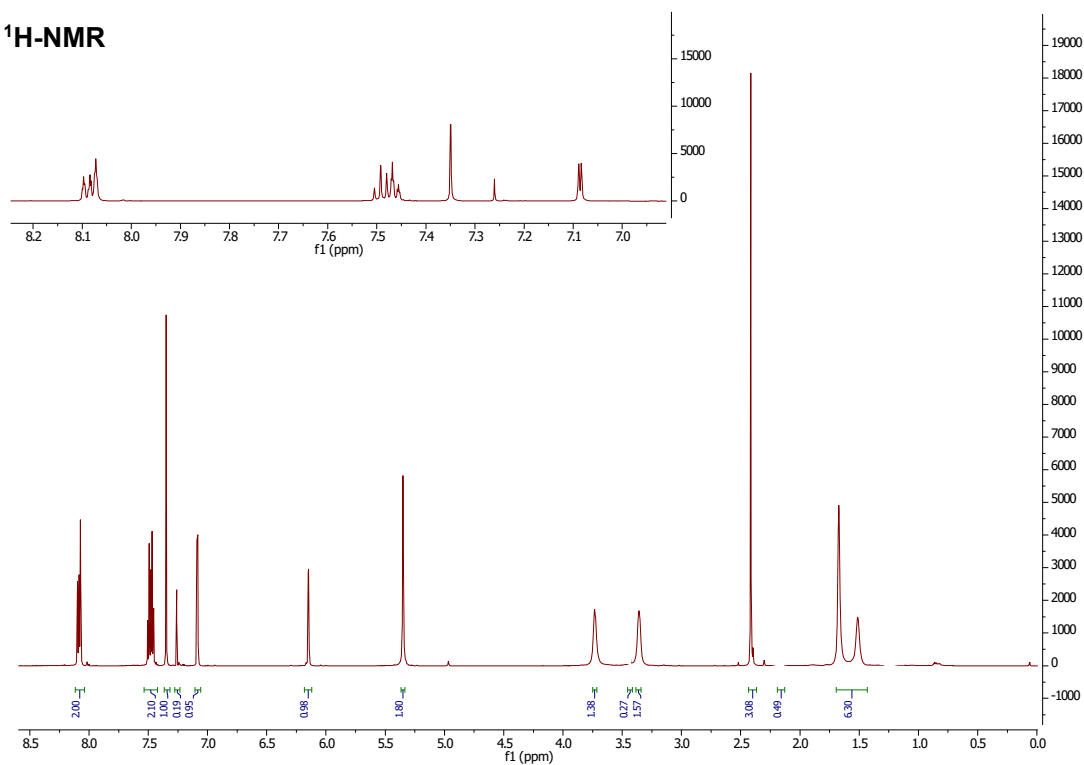


¹³C-NMR

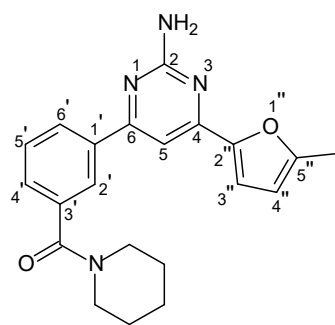
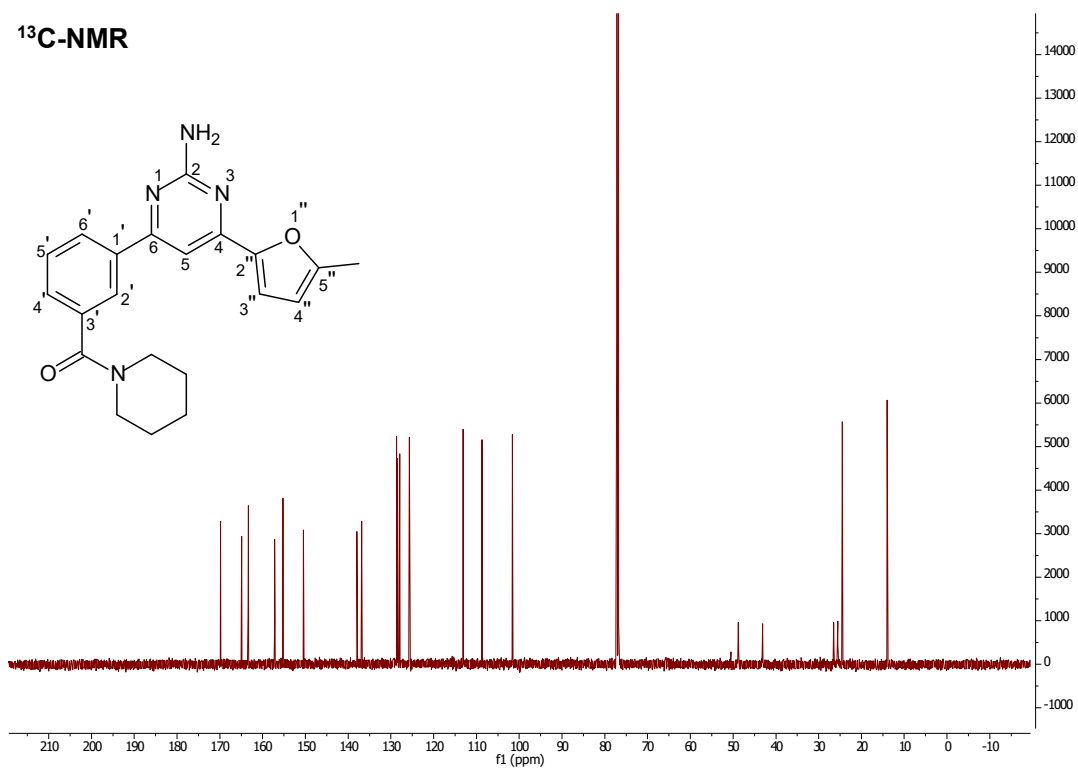


4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**)

¹H-NMR

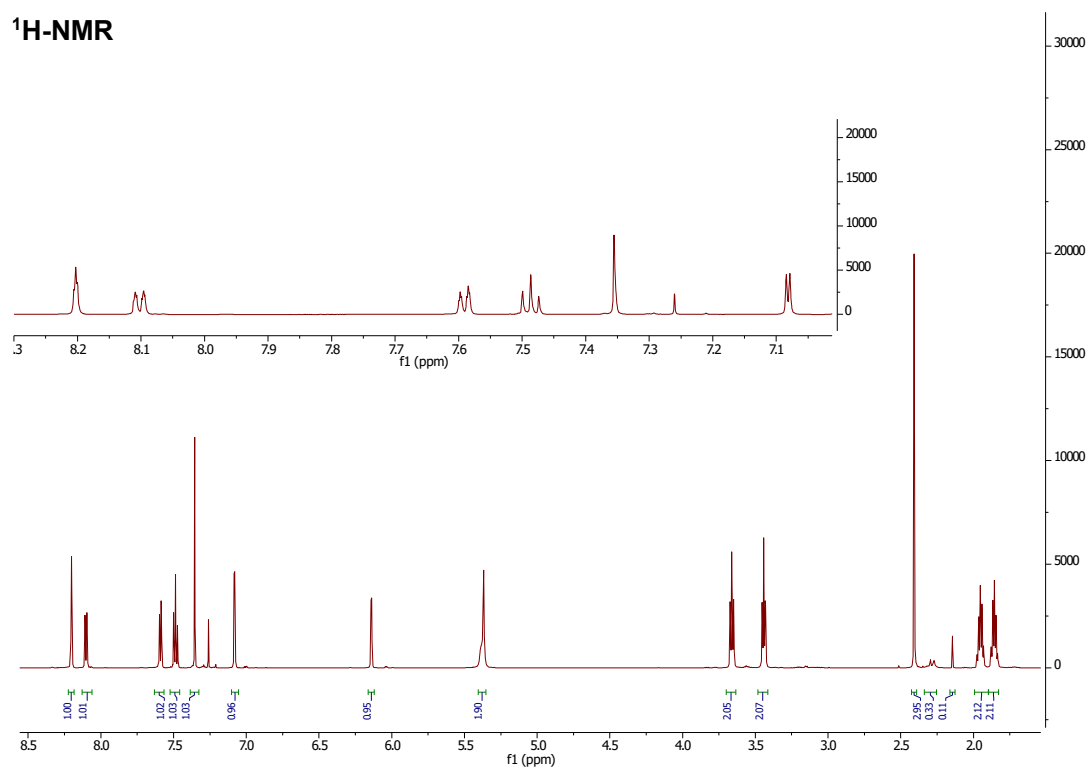


¹³C-NMR

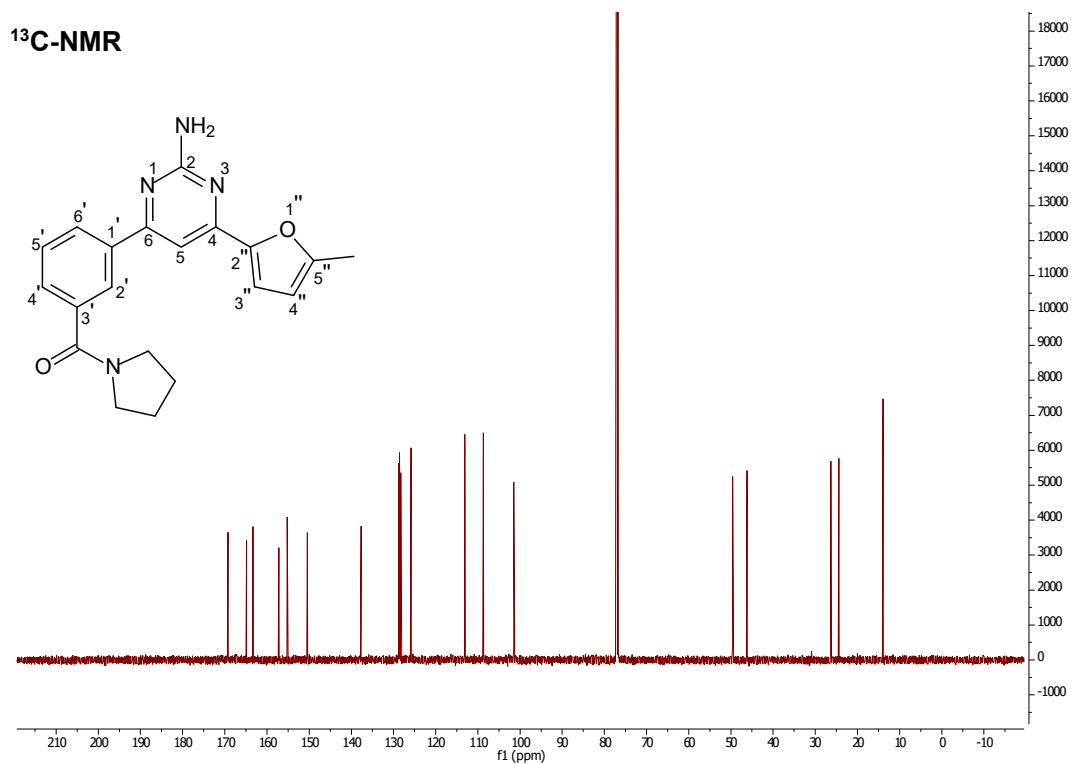


4-(5-methylfuran-2-yl)-6-[3-(pyrrolidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8n**)

¹H-NMR



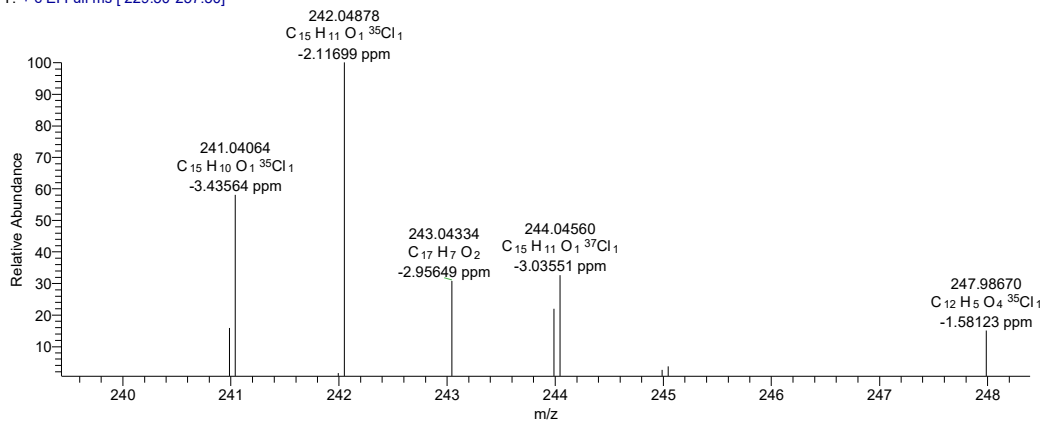
¹³C-NMR



Mass spectra

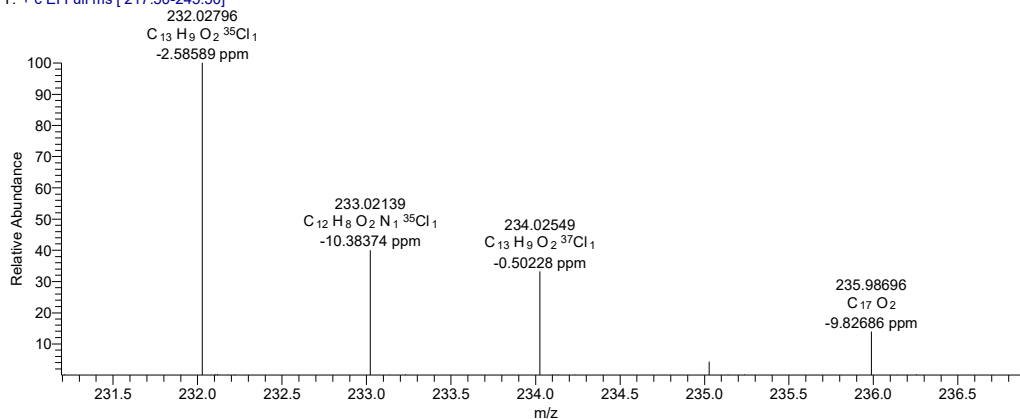
(2E)-3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (7a)

SJ2_HR-c1 #29 RT: 0.77 AV: 1 NL: 1.72E5
T: + c EI Full ms [229.50-257.50]



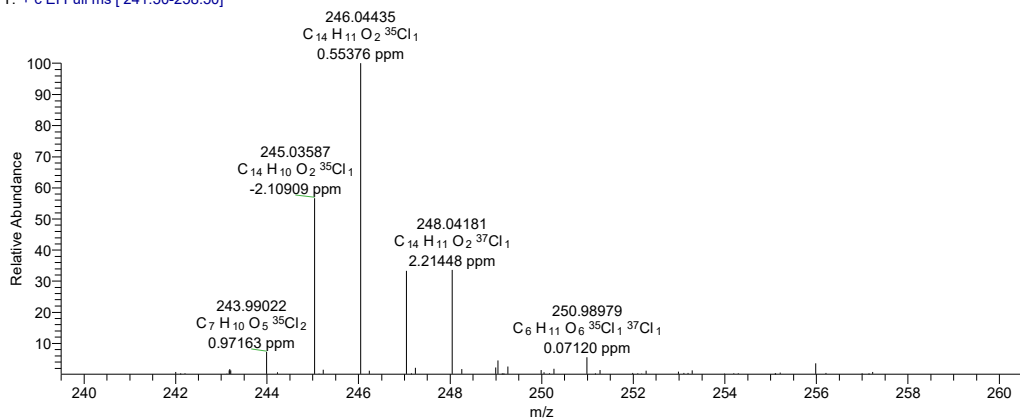
(2E)-3-(3-chlorophenyl)-1-(furan-2-yl)prop-2-en-1-one (7b)

SJ8_HR-c1 #91 RT: 1.65 AV: 1 NL: 2.70E6
T: + c EI Full ms [217.50-245.50]



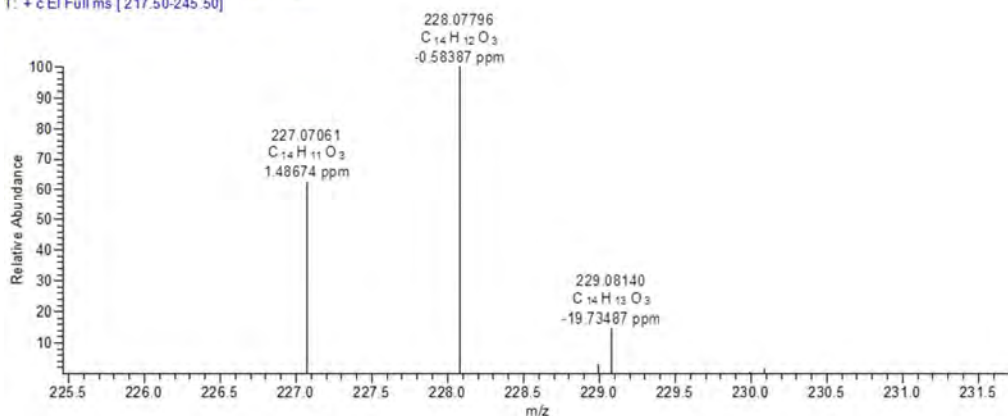
(2E)-3-(3-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7c)

SJ20_HR-c1 #40 RT: 0.69 AV: 1 NL: 8.70E5
T: + c EI Full ms [241.50-258.50]



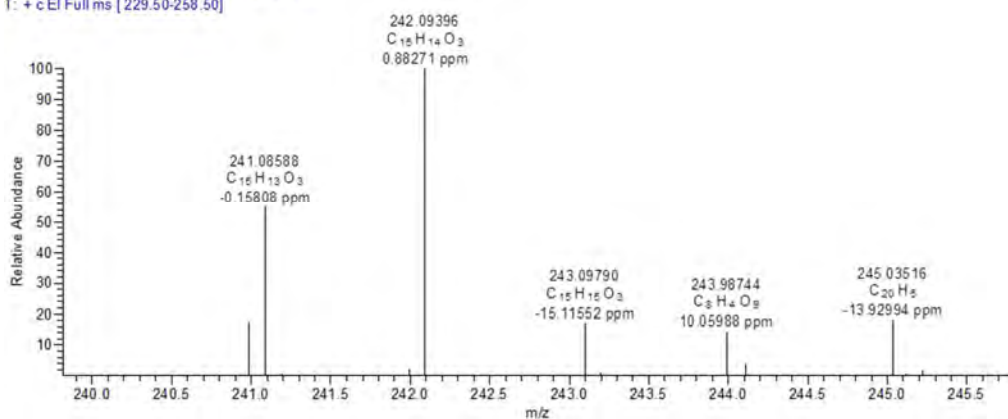
(2E)-1-(furan-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (7d)

SJ10 HR-c1 #20 RT: 0.55 AV: 1 NL: 2.94E6
T: + c EI Full ms [217.50-245.50]



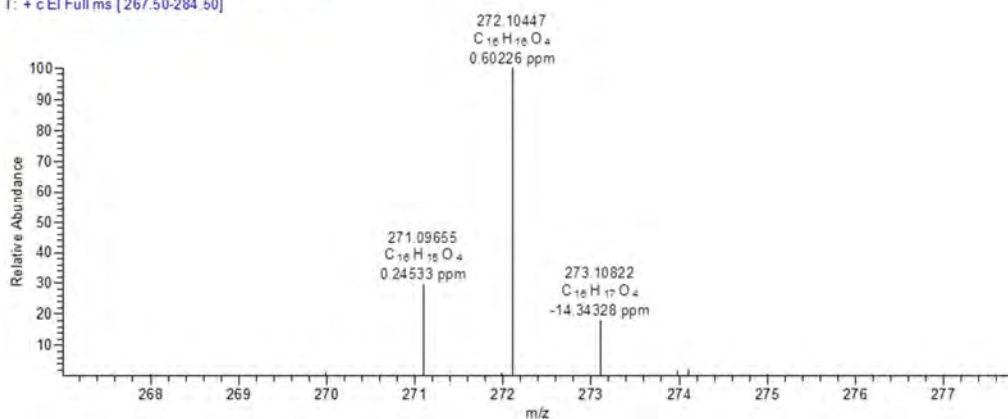
(2E)-3-(3-methoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7e)

SJ22 HR-c1 #30 RT: 0.83 AV: 1 NL: 4.11E5
T: + c EI Full ms [229.50-258.50]



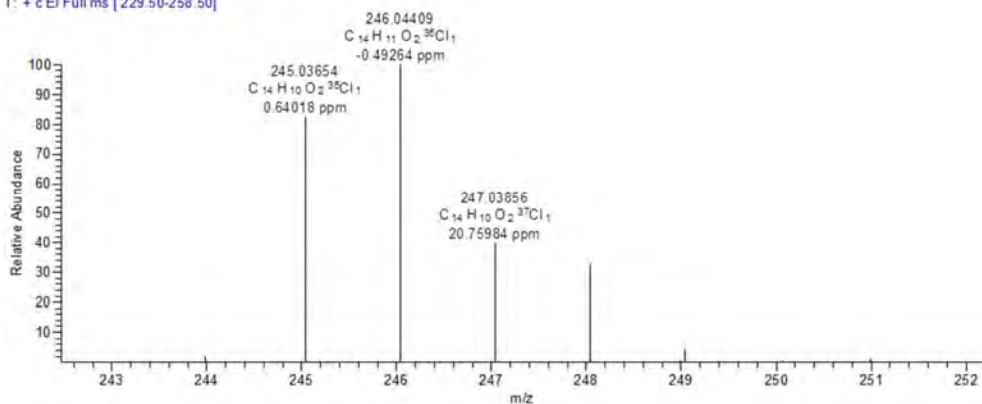
(2E)-3-(3,4-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7f)

SJ40 HR-c1 #46 RT: 0.72 AV: 1 NL: 9.51E5
T: + c EI Full ms [267.50-284.50]



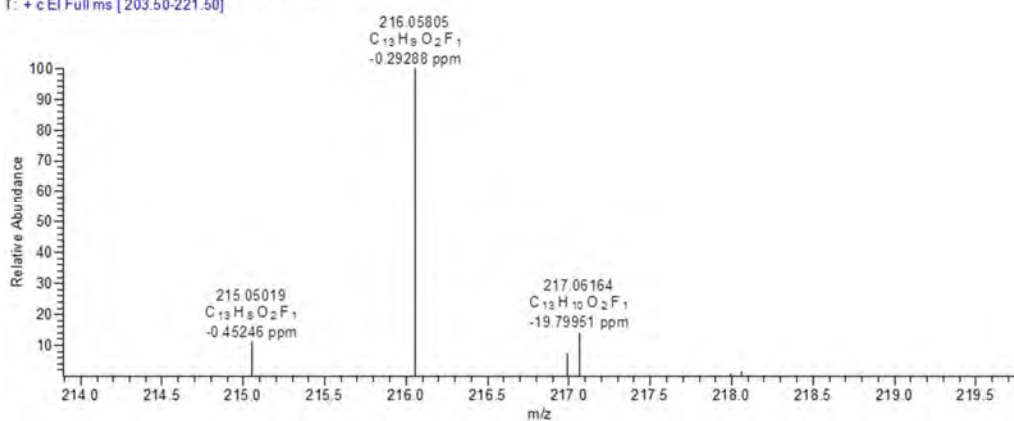
(2E)-3-(4-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7g)

SJ30 HR-c1 #22 RT: 0.60 AV: 1 NL: 2.07E6
T: + c EI Full ms [229.50-258.50]



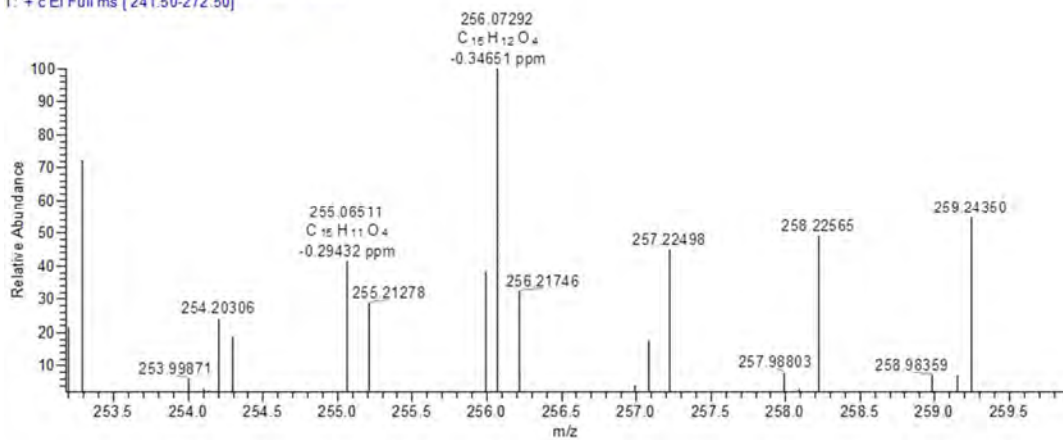
(2E)-1-(4-fluorophenyl)-3-(furan-2-yl)prop-2-en-1-one (7h)

SJ24 HR-c1 #18 RT: 0.37 AV: 1 NL: 2.68E6
T: + c EI Full ms [203.50-221.50]



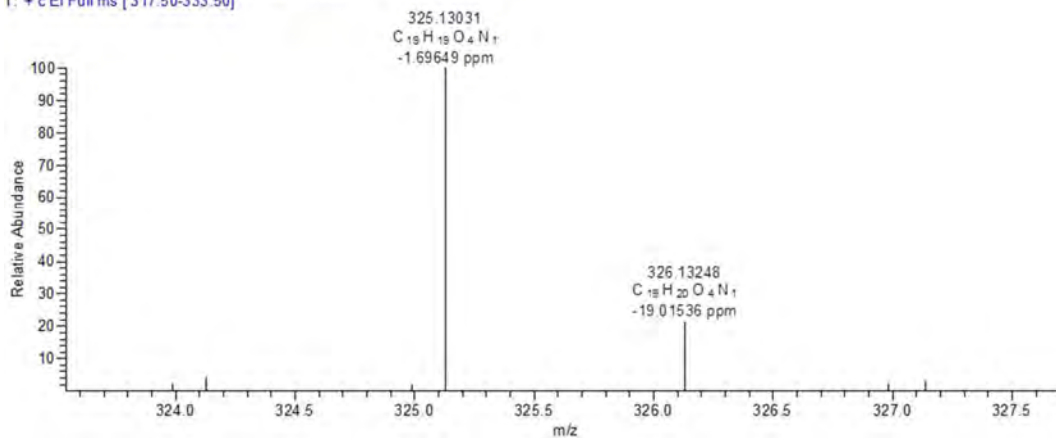
3-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (7i)

SJ32 HR1-c1 #34 RT: 0.95 AV: 1 NL: 1.31E5
T: + c EI Full ms [241.50-272.50]



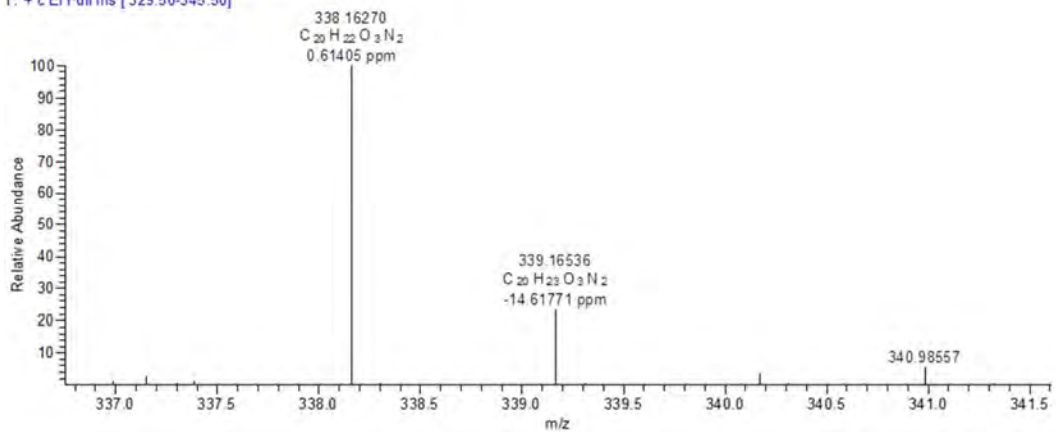
(2E)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one (7j)

SJ36 HR1-c1 #84 RT: 1.13 AV: 1 NL: 1.80E6
T: + c EI Full ms [317.50-333.50]



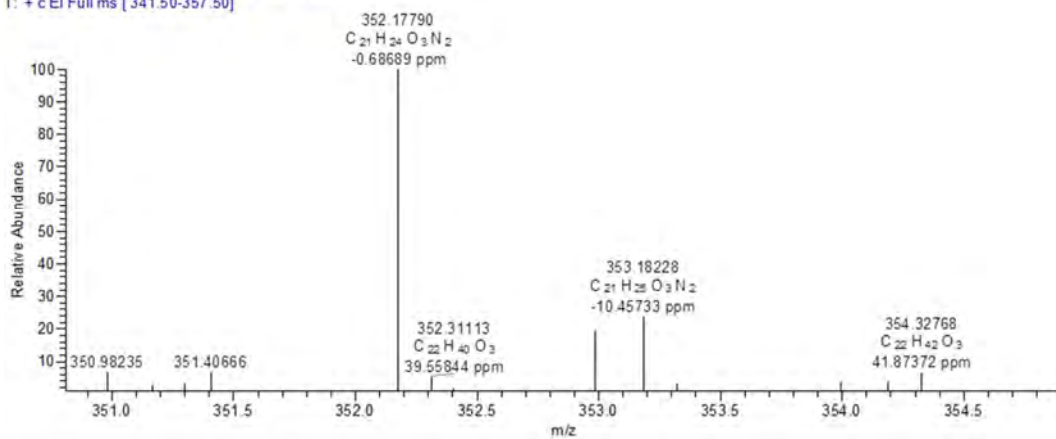
(2E)-1-(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one (7k)

SJ46 HR1-c1 #58 RT: 0.75 AV: 1 NL: 1.80E6
T: + c EI Full ms [329.50-345.50]



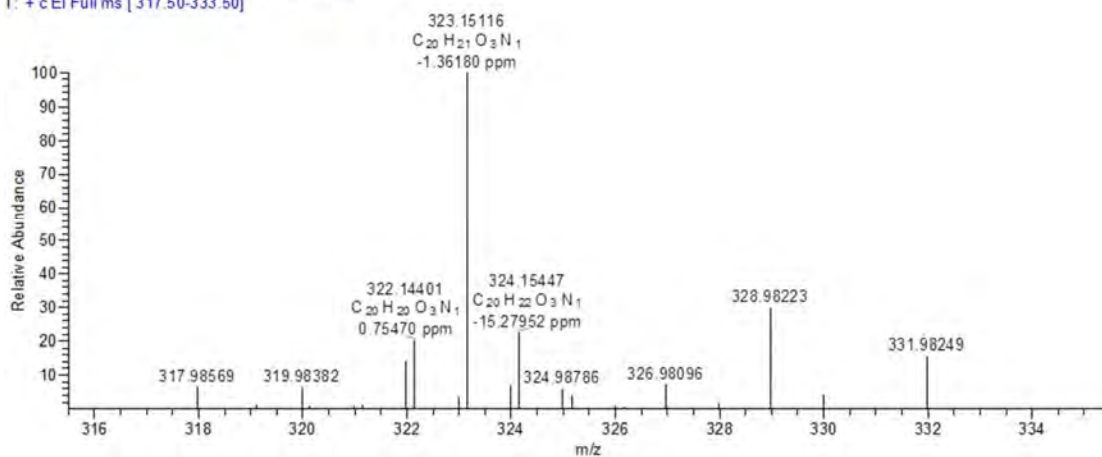
(2E)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one (7l)

SJ48 HR1-c1 #49 RT: 0.61 AV: 1 NL: 3.20E5
T: + c EI Full ms [341.50-357.50]

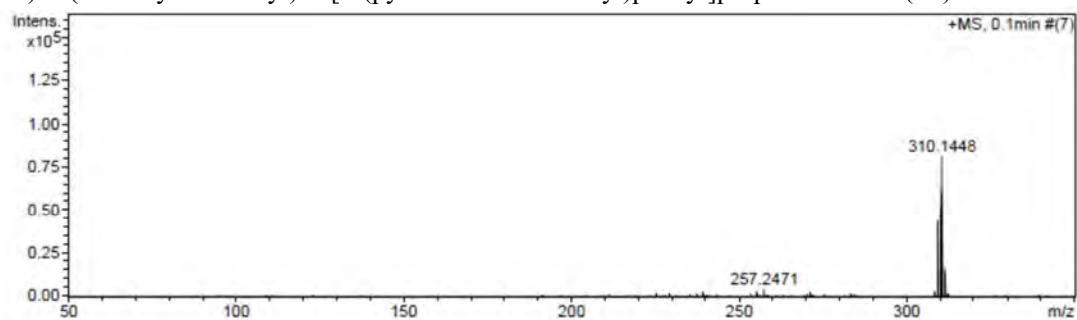


(2E)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one (**7m**)

SJ58 HR-c1 #132 RT: 1.77 AV: 1 NL: 1.38E6
T: + c EI Full ms [317.50-333.50]



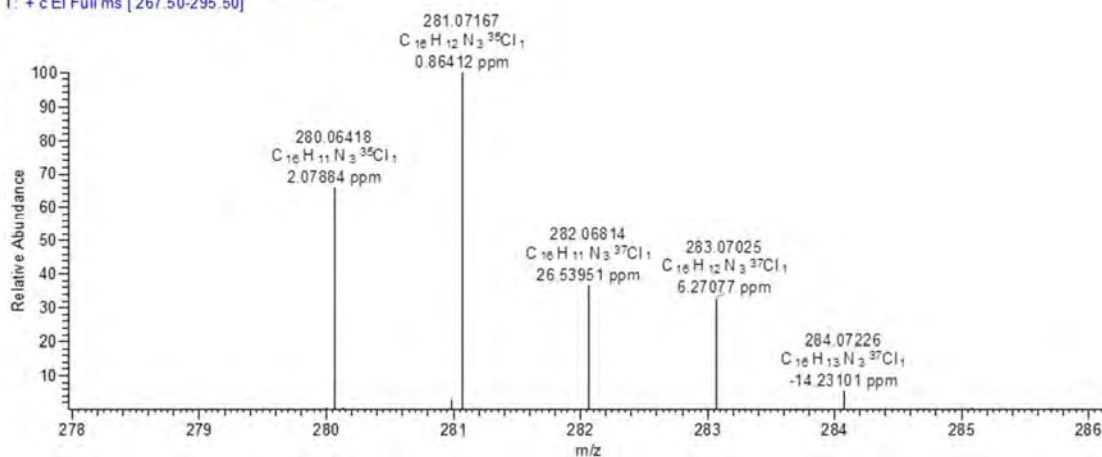
(2E)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one (**7n**)



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻ Conf	N-Rule
310.1448	1	C ₁₉ H ₂₀ N ₁ O ₃	100.00	310.1438	-1.0	-3.4	4.9	10.5	even	ok
	2	C ₁₅ H ₁₆ N ₁ O	9.62	310.1411	-3.7	-12.0	8.8	11.5	even	ok

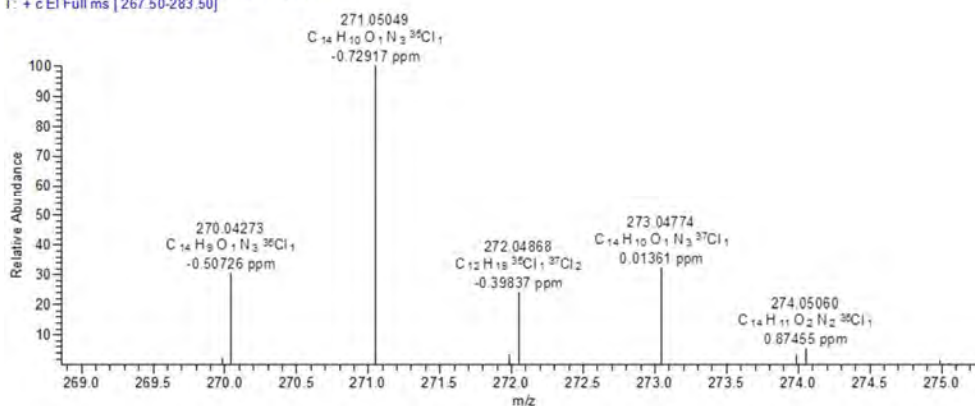
4-(3-chlorophenyl)-6-phenylpyrimidin-2-amine (**8a**)

SJ6 HR-c2 #34 RT: 0.80 AV: 1 NL: 1.92E6
T: + c EI Full ms [267.50-295.50]



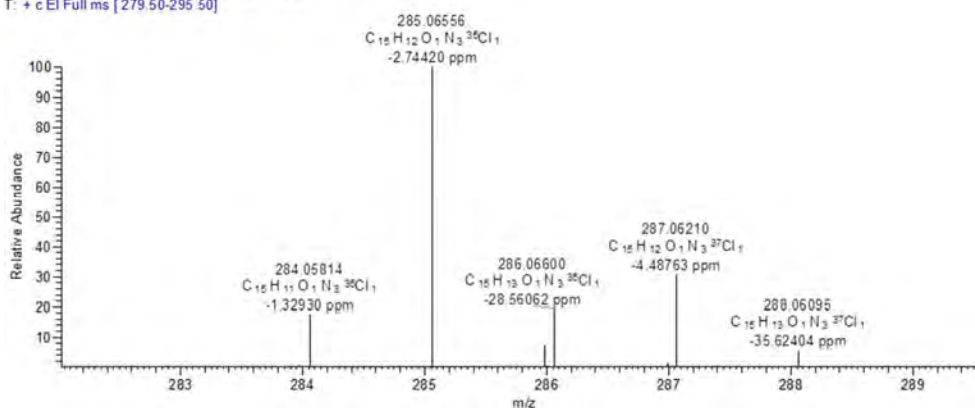
4-(3-chlorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8b**)

SJ16 HR-c1 #54 RT: 0.81 AV: 1 NL: 1.90E6
T: + c EI Full ms [267.50-283.50]



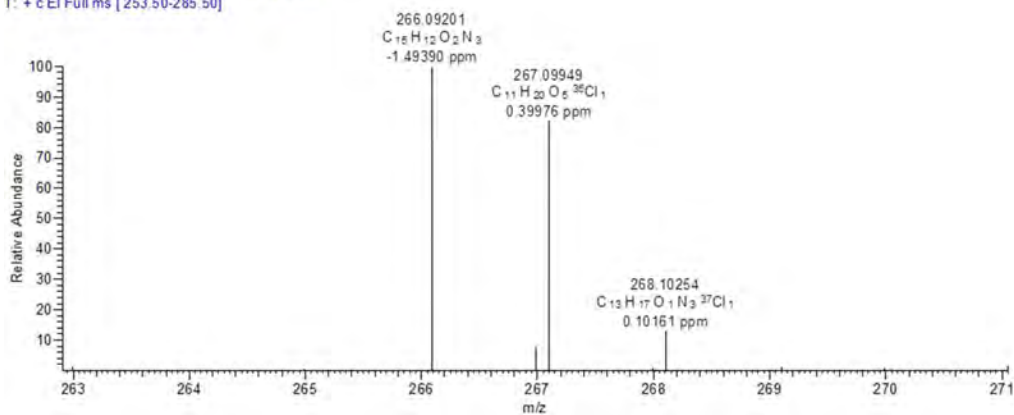
4-(3-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8c**)

SJ44 HR-c1 #71 RT: 1.03 AV: 1 NL: 3.50E5
T: + c EI Full ms [279.50-295.50]



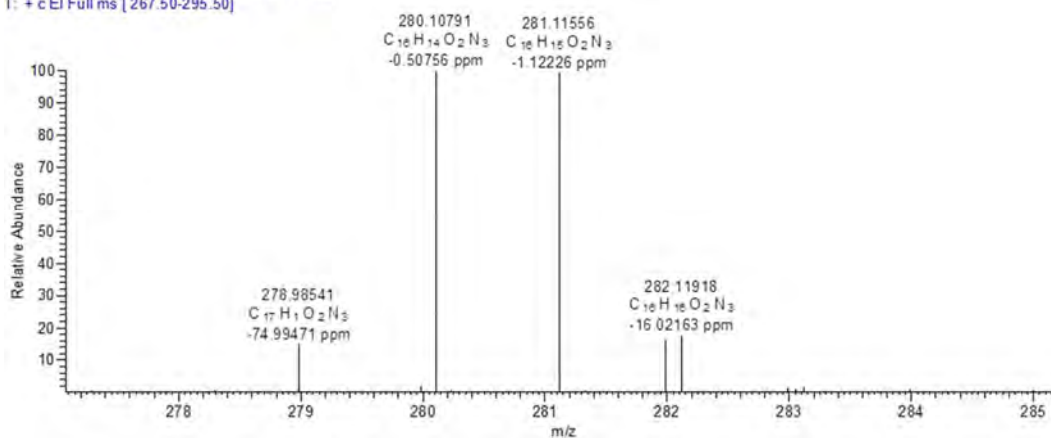
4-(3-methoxyphenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8d**)

SJ18 HR-c1 #47 RT: 0.96 AV: 1 NL: 4.70E6
T: + c EI Full ms [253.50-285.50]



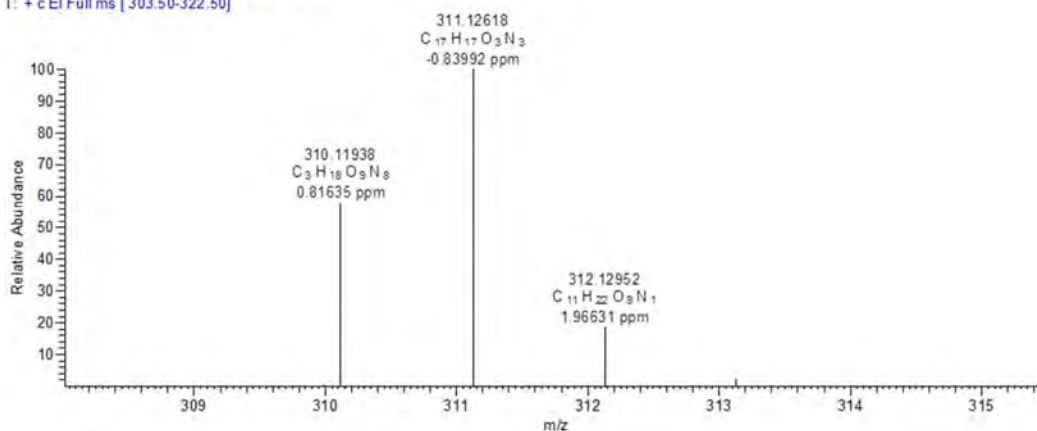
4-(3-methoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8e**)

SJ26 HR-c1 #55 RT: 1.31 AV: 1 NL: 4.70E5
T: + c EI Full ms [267.50-295.50]



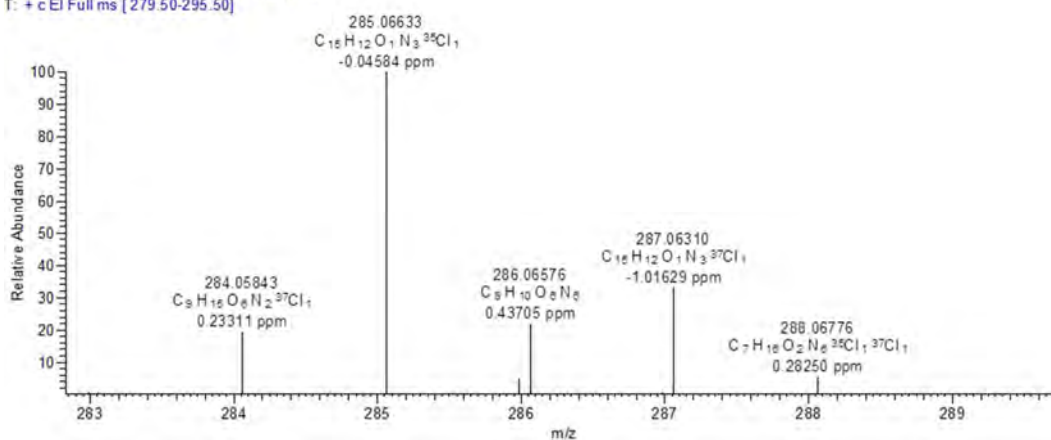
4-(3,4-dimethoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8f**)

SJ42 HR-c1 #62 RT: 0.97 AV: 1 NL: 3.09E5
T: + c EI Full ms [303.50-322.50]



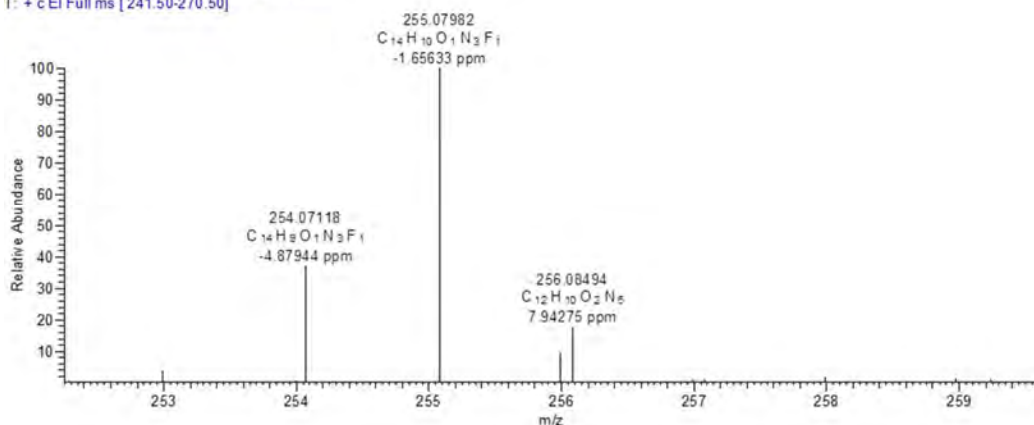
4-(4-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (**8g**)

SJ34 HR-c1 #53 RT: 0.77 AV: 1 NL: 6.16E5
T: + c EI Full ms [279.50-295.50]



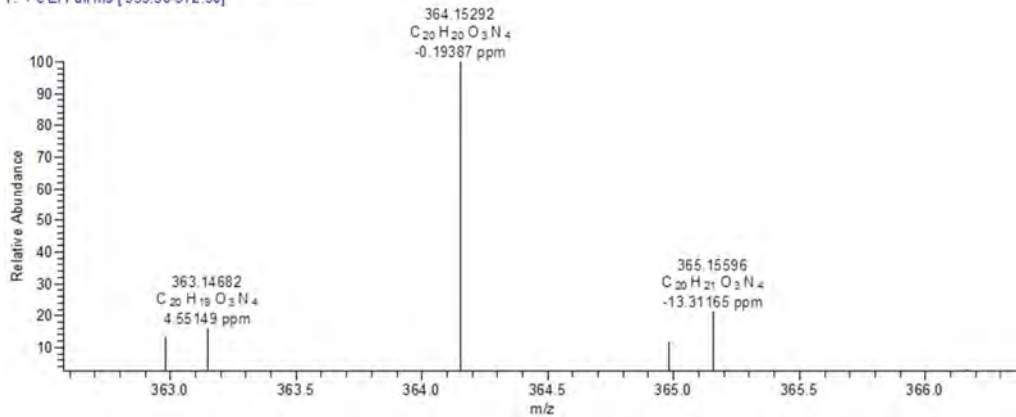
4-(4-fluorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (**8h**)

SJ28 HR-c1 #42 RT: 1.14 AV: 1 NL: 2.51E5
T: + c EI Full ms [241.50-270.50]



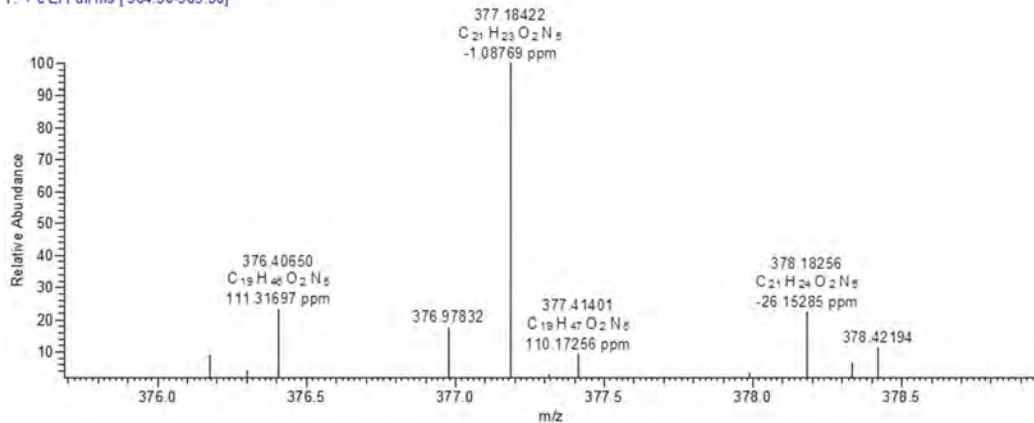
4-(5-methylfuran-2-yl)-6-[3-(morpholine-4-carbonyl)phenyl]pyrimidin-2-amine (**8j**)

SJ38 HR-c1 #72 RT: 1.01 AV: 1 NL: 7.98E4
T: + c EI Full ms [353.50-372.50]



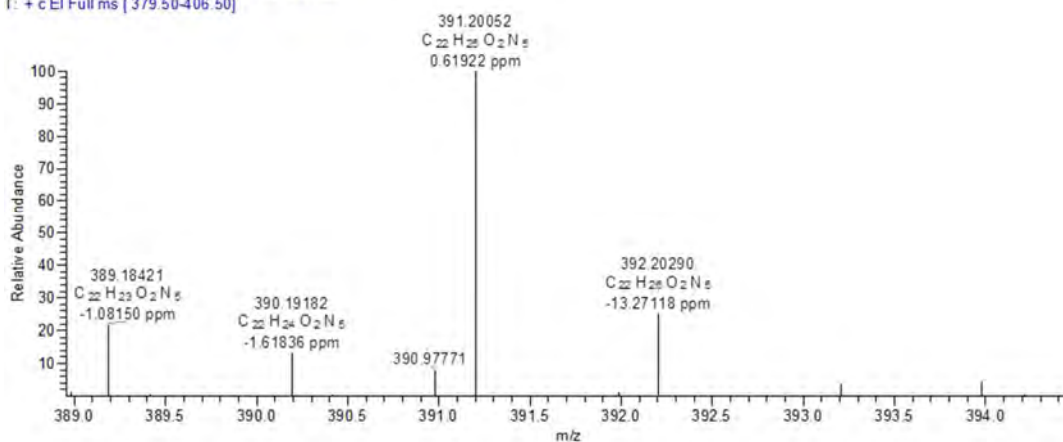
4-(5-methylfuran-2-yl)-6-[3-(4-methylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8k**)

SJ50 HR1-c1 #92 RT: 1.37 AV: 1 NL: 1.33E5
T: + c EI Full ms [364.50-385.50]



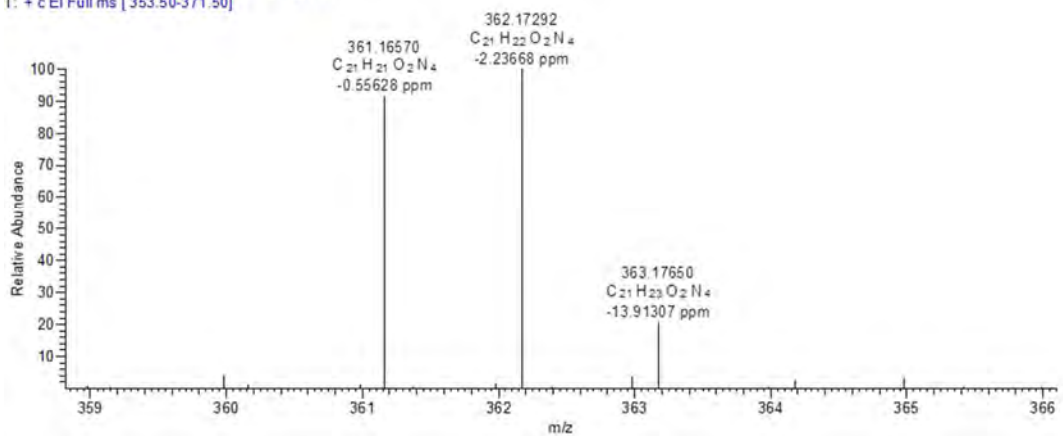
4-(5-methylfuran-2-yl)-6-[3-(4-ethylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (**8l**)

SJ54 HR-c1 #91 RT: 1.60 AV: 1 NL: 3.59E6
T: + c EI Full ms [379.50-406.50]



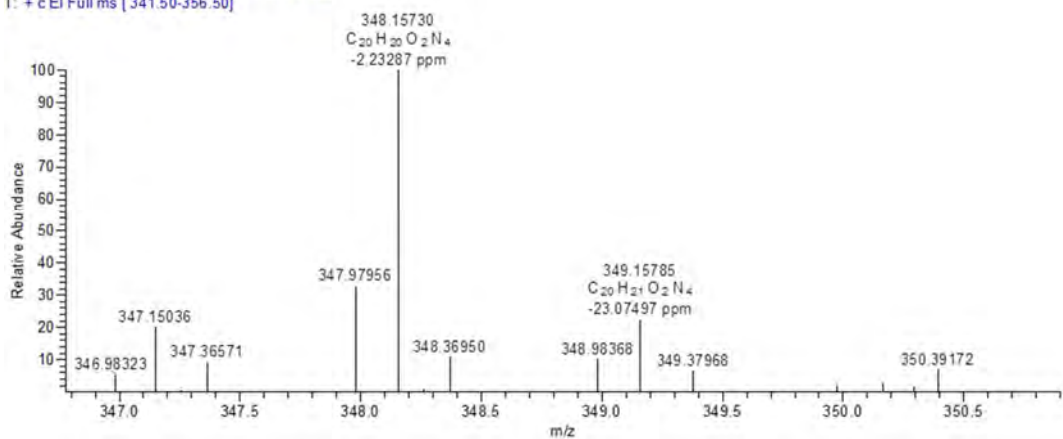
4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**)

SJ62 HR-c1 #61 RT: 0.82 AV: 1 NL: 4.26E6
T: + c EI Full ms [353.50-371.50]

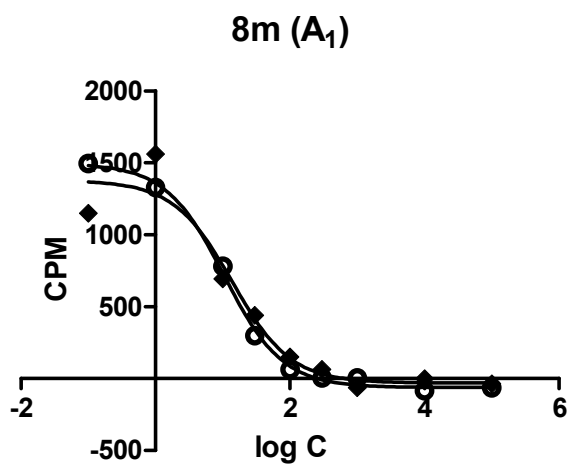


4-(5-methylfuran-2-yl)-6-[3-(pyrrolidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8n**)

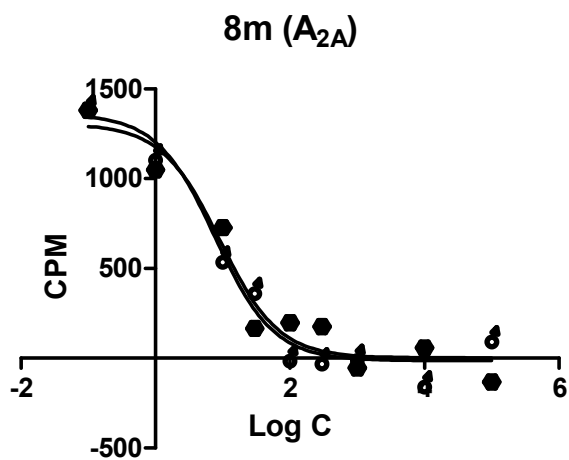
SJ56 HR-c1 #94 RT: 1.14 AV: 1 NL: 5.93E5
T: + c EI Full ms [341.50-356.50]



Examples of sigmoidal dose response curves



Sigmoidal dose response curve obtained during the determination of A_1 affinity of compound **8m** in the radioligand binding assay .



Sigmoidal dose response curve obtained during the determination of A_{2A} affinity of compound **8m** in the radioligand binding assay.

CHAPTER 4

Article 2

Amide substituted 2-amino-4,6-diphenylpyrimidines as adenosine receptor antagonists

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Abstract

Recently, dual antagonism of adenosine A₁ and A_{2A} receptors has gained credibility as an alternative to dopaminergic therapies for the treatment of Parkinson's disease. This study illustrates the synthesis of a novel series of amide substituted 2-amino-4,6-diphenylpyrimidines and their evaluation as potential dual adenosine A₁ and A_{2A} antagonists. Promising dual affinities were obtained for nearly all amide derivatives with 3-(2-amino-6-phenylpyrimidin-4-yl)-N-[3-(morpholin-4-yl)propyl]benzamide (**6b**) identified as the most potent compound, exhibiting an A₁ K_i of 7.53 nM and an A_{2A} K_i value of 3.37 nM. Encouraging *in vivo* results obtained for compound **6b** illustrated the potential of these compounds as alternative drug candidates for Parkinson's disease.

Keywords: 2-aminopyrimidine; amide, adenosine A₁ and A_{2A} receptor; dual; antagonist.

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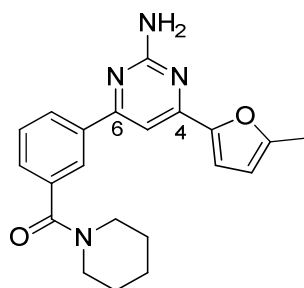
Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, affecting the lives of over ten million people worldwide.¹ The primary motor manifestations of PD which include bradykinesia, muscle rigidity, postural instability and resting tremor, are attributable to the deterioration of dopaminergic neurons in the substantia nigra, resulting in low levels of dopamine in the striatum.² Although these motor symptoms are predominant, patients also suffer from non-motor complications such as cognitive deficiencies and depression.³ Dopamine replacement therapy such as levodopa and dopamine agonists are currently the mainstay of PD treatment, however, after long-term use, patients present with autonomic and neuropsychological side effects as well as the development of motor complications.⁴ Furthermore, these dopamine replacement therapies only provide symptomatic relief and have no effect on the non-motor symptoms associated with PD.³

Non-dopaminergic targets, such as adenosine A₁ and A_{2A} receptors, are increasingly being investigated as alternative therapies for PD.⁵ The adenosine A_{2A} receptor is particularly interesting due to its specific localisation in the striatum and its ability to modulate dopaminergic neurotransmission.⁶ Antagonism of the A_{2A} receptor in animal models extends the efficacy of levodopa and prevents the onset of motor complications such as dyskinesia.⁷ Furthermore, it has been suggested that adenosine A_{2A} antagonism may be neuroprotective, which suggests that the rate of progression of PD may be reduced.^{8,9} Recently the antidepressant properties of the known A_{2A} antagonist KW6002 have been reported and since depression is one of the co-morbidities associated with PD, adenosine A_{2A} antagonism may provide an additional benefit.¹⁰

Antagonism of the adenosine A₁ receptor, which is widely expressed in the brain, may also be advantageous in the treatment of PD.¹¹ A₁ antagonism improves cognition, which is relevant as most PD patients suffer from cognitive deficits, especially in the later stages of the disease.¹²⁻¹⁵ Since A₁ antagonism also activates motor function in animals, adenosine A₁ receptor antagonists can in addition interact synergistically with adenosine A_{2A} receptor antagonists to improve motor symptoms of PD.^{16,17} The synergistic benefits that dual antagonists of the adenosine A₁ and A_{2A} receptors offer, are indicative of the potential of these agents as alternative PD therapies.

Only a handful of adenosine antagonists have reached clinical trials, and most of these are selective towards the adenosine A_{2A} receptor.¹⁸⁻²⁰ Our research group is interested in the design, synthesis and evaluation of heterocyclic adenosine antagonists, and in particular the activity of 2-aminopyrimidines. Recently, we have identified a potent dual affinity 2-aminopyrimidine derivative (**1**).²¹



$$K_iA_1 = 9.54 \text{ nM}$$

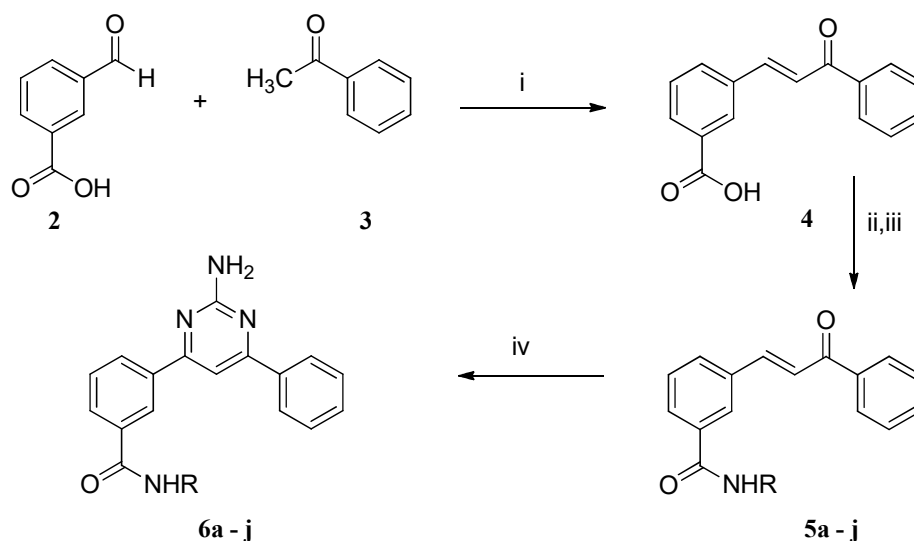
$$K_iA_{2A} = 6.34 \text{ nM}$$

1

In order to further investigate the ability of the adenosine receptor binding site to accommodate these types of compounds, a series of 2-aminopyrimidines with extended amide side chains were synthesised. It was decided to replace the methyl furan substituent at the 4-position of the pyrimidine ring with a phenyl ring, which simplified the synthesis. An initial study suggested that this modification does not alter the affinities for adenosine receptors to a large degree.

Experimental

Synthesis

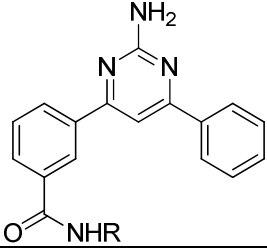
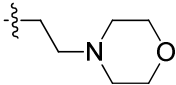
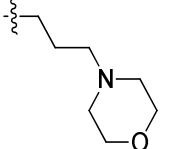
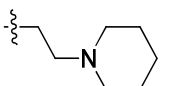
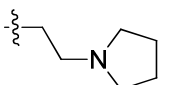
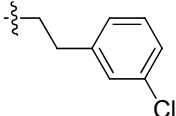
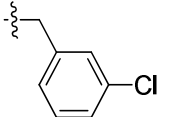
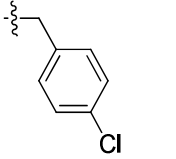
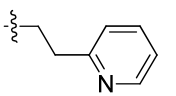


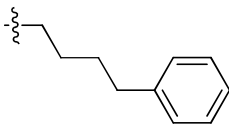
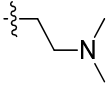
Scheme 1. Synthesis. Reagents and conditions: (i) NaOH, 1 M (2 eq.), MeOH, rt, overnight (67%); (ii) CDI (1.2 eq.), CH₂Cl₂, rt, 4 h; (iii) NH₂R (1.2 eq.), CH₂Cl₂, rt, overnight (60-80%); (iv) Guanidine hydrochloride (1.5 eq.), NaH (1.5 eq.), DMF, 110 °C, overnight (15-30%).

Ten amide substituted 2-amino-4,6-diphenylpyrimidines were successfully synthesised as follows: a Claisen-Schmidt condensation of 3-formylbenzoic acid (**2**) and acetophenone (**3**) yielded chalcone **4**,²² which was coupled to different amines using CDI to obtain the desired amides (**5**).²³ The final

aminopyrimidines (**6**) were obtained by cyclization of the amides (**5**) with guanidine hydrochloride in the presence of sodium hydride in DMF (Scheme 1).²⁴ The structures of the synthesised compounds (Table 1) were confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry.

Table 1: Adenosine A₁ and A_{2A} receptor affinities of the synthesised 2-aminopyrimidines

				
Compound	R	A ₁ ^a K _i (nM)	A _{2A} ^a K _i (nM)	SI ^b (A _{2A} /A ₁)
6a		25.2 ± 1.58	15.0 ± 7.37	0.60
6b		7.53 ± 2.11	3.37 ± 1.63	0.45
6c		6.00 ± 1.11	45.5 ± 3.64	7.6
6d		6.93 ± 0.85	25.2 ± 1.30	3.6
6e		14.5 ± 2.02	21.0 ± 4.37	1.5
6f		11.5 ± 1.00	7.54 ± 2.83	0.66
6g		5.42 ± 0.60	9.09 ± 0.84	1.7
6h		18.5 ± 1.15	26.2 ± 3.27	1.4

6i		20.0 ± 1.77	1.67 ± 0.73	0.084
6j		13.5 ± 2.50	107 ± 16.2	7.9
^cCPA		8.48 ± 0.30		
^dZM241385			1.20 ± 0.39	

^aAll values are expressed as the mean ± SD of triplicate determinations.

^bSelectivity index = $A_{2A}K_i/A_1K_i$

^c*N*⁶-Cyclopentyladenosine, a known adenosine *A*₁ agonist, used as reference for *A*₁ receptor affinity – literature $K_i = 7.9$ nM.²⁵

^dA known adenosine *A*_{2A} receptor antagonist, used as reference for *A*_{2A} affinity – literature $K_i = 2$ nM.²⁶

Radioligand binding assays were performed with the selective *A*₁ radioligand 1,3-³H-dipropyl-8-cyclopentylxanthine (³H]DPCPX) for binding to adenosine *A*₁ receptors and the non-selective radioligand [³H]5'-*N*-ethylcarboxamide-adenosine (³H]NECA) for binding to adenosine *A*_{2A} receptors.^{25,26} Striata from male Sprague-Dawley rats were used as receptor source for *A*_{2A} receptor binding, while whole brains were utilised in *A*₁ receptor binding assays (Ethics number: NWU-0035-10-A5). IC₅₀ values were calculated from sigmoidal dose response curves using the Prism 5 software package (GraphPad) and the Cheng-Prusoff equation was used to calculate K_i values.^{25,27,28} The results are given in Table 1.

From these results it is clear that most of these compounds exhibit potent dual affinity with adenosine *A*₁ affinities ranging from 5.42 nM (**6g**) to 25.2 nM (**6a**) and adenosine *A*_{2A} affinities ranging from 1.67 nM (**6i**) to 107 nM (**6j**). Compounds **6b** ($A_1K_i = 7.53$ nM, $A_{2A}K_i = 3.37$ nM), **6f** ($A_1K_i = 11.5$ nM, $A_{2A}K_i = 7.54$ nM) and **6g** ($A_1K_i = 5.42$ nM, $A_{2A}K_i = 9.09$ nM) are the most promising candidates for dual antagonistic activity with K_i values very similar to those of the reference compounds, CPA ($A_1K_i = 8.48$ nM) and ZM241385 ($A_{2A}K_i = 1.20$ nM). No clear structure-activity relationships emerged for this study. However, when the *A*₁ affinities of the 2-carbon chain derivatives e.g. **6a**, **6c**, **6d**, **6e**, **6h** and **6j** are compared, it seems that piperidine (**6c**, $K_i = 6.00$ nM) or pyrrolidine (**6d**, $K_i = 6.93$ nM) substitution is most favourable and substitution with either morpholine (**6a**, $K_i = 25.2$ nM), *m*-chlorophenyl (**6e**, $K_i = 14.5$ nM), 2-pyridine (**6h**, $K_i = 18.5$ nM) or the *N,N*-dimethyl (**6j**, $K_i = 13.5$ nM) group results in lower affinity. Interestingly, when the affinities of the two morpholine derivatives (**6a** and **6b**) are compared, it appears that extension of the carbon chain by one carbon (**6b**, $K_i = 7.53$ nM) improves affinity to a value similar to that observed for both the 2-carbon piperidine (**6c**, $K_i = 6.00$ nM) and pyrrolidine (**6d**,

$K_i = 6.93$ nM) derivatives. When comparing the affinity of compound **6g**, which has the most potent A_1 affinity ($K_i = 5.42$ nM), to that of compound **6f** ($K_i = 11.5$ nM) it also appears that *para* substitution of the phenyl ring (**6g**) is preferred over *meta* substitution (**6f**).

The range of observed A_1 receptor affinities for these compounds (**6a** – **6j**) is however, reasonably small, [ranging from 5.42 nM (**6g**) to 25.2 nM (**6a**)] and it would thus appear that the adenosine A_1 receptor is capable of accommodating a variety of substituents equally well in this position. On the other hand, larger substituents are preferable for good A_{2A} affinity. For example, compounds **6i** ($K_i = 1.67$ nM) and **6b** ($K_i = 3.37$ nM) with the longest chains, exhibited the most potent adenosine A_{2A} affinities of the series, while compound **6j** ($A_{2A}K_i = 107$ nM) with the “smallest” substituent exhibited the weakest adenosine A_{2A} affinity. These results suggest that the binding site of the adenosine A_{2A} receptor is more sensitive to changes in the nature of the amide substituent than the binding site of the adenosine A_1 receptor.

Molecular docking studies were performed to rationalise the results obtained from the radioligand binding assays. The structure of the human adenosine A_{2A} receptor crystallised with the known A_{2A} antagonist ZM241385 was used (PDB code: 3EML) for these studies. The C-DOCKER function of Discovery Studio 3.1 (Accelrys) was employed to dock all compounds into the binding site in order to investigate the receptor-ligand interactions of the synthesised compounds. The following interactions were observed for all compounds: i) intermolecular hydrogen bonding between the exocyclic amino group of the pyrimidine ring and Glu169, ii) π - π interactions between the three-membered ring system and Phe168, and iii) hydrogen bonding interactions between one of the pyrimidine ring nitrogens and Asn253 (Figure 1A). These interactions were also observed with known adenosine A_{2A} antagonists and are believed to be responsible for anchoring the aminopyrimidine moiety in the binding site.²⁹ Hydrogen bonding was also observed between the amide carbonyl and Tyr271 (Figure 1A) and in most cases, where this carbonyl interaction was absent, an alternative hydrogen bonding interaction occurred between the amide nitrogen and Tyr271, as illustrated for compound **6f** (Figure 1B).

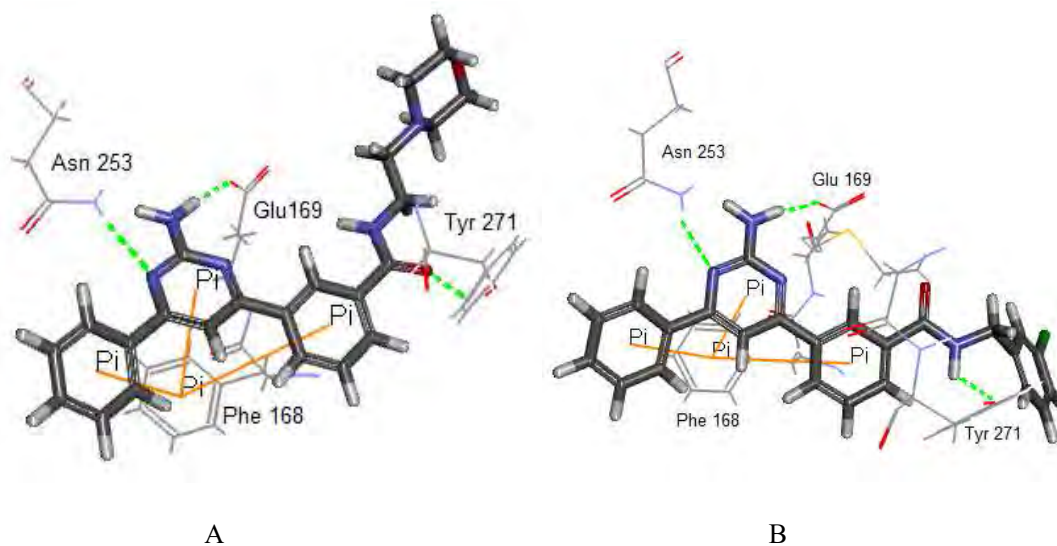


Figure 1: **A.** Compound **6a** docked in the binding site of the human A_{2A} receptor. **B.** Compound **6f** docked in the binding site illustrating the alternative interaction between the amide nitrogen and Tyr271. Orange lines illustrate hydrophobic interactions. Green lines illustrate intermolecular hydrogen bonding.

None of the interactions observed explain the differences in observed affinities, however, it does illustrate the ability of these compounds to bind in the binding site and therefore confirms the good affinities obtained with the radioligand binding assays.

Compound **6b** was subsequently selected to be evaluated *in vivo* since it was identified as the most promising dual affinity candidate *in vitro*. Reversal of haloperidol induced catalepsy was determined with the standard bar test (results shown in Figure 2) to evaluate the potential of compound **6b** to act as an antagonist of the A_{2A} receptor. Three groups ($n = 10$) of drug-naïve Sprague-Dawley rats each received a 5.0 mg/kg intraperitoneal injection of haloperidol to induce catalepsy. Thirty minutes later the animals from two groups received 0.4 and 2 mg/kg test compound intraperitoneally, respectively, and one group (control group) received the vehicle, DMSO (0.3 ml), also intraperitoneally. Catalepsy for each rat was determined 60 minutes after the first injection.¹⁶

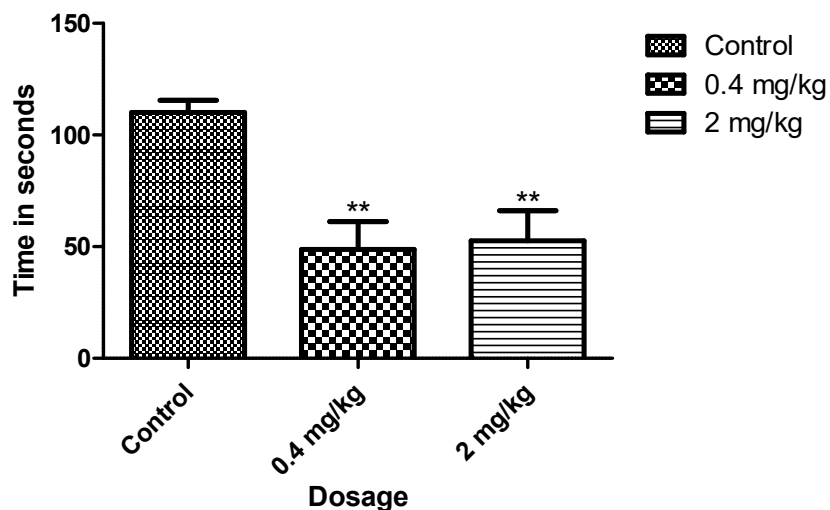


Figure 2: Graph illustrating a significant reduction of catalepsy with both concentrations of compound **6b**. (** indicates significant differences compared to the control group as determined by one-way ANOVA [F (2,27) = 9.634] followed by Dunnet's post-test with $p = 0.001$ and 0.002 for the two dosages respectively).

Compared to the control group, a significant reduction in catalepsy can be observed for both concentrations of compound **6b** administered intraperitoneally. The results provide evidence of bioavailability and indicate that compound **6b** is an antagonist of A_{2A} receptors.

Conclusion

Ten amide substituted 2-amino-4,6-diphenylpyrimidines were successfully synthesised and evaluated as potential dual adenosine A_1 and A_{2A} antagonists. The potent dual affinity previously reported for a related series were improved in *in vitro* assays, again illustrating the potential of the 2-aminopyrimidine scaffold as a source for new antiparkinsonian drugs. Subsequent data from an *in vivo* assay provided evidence that these compounds have the potential to be bioavailable and that these compounds are antagonists of adenosine A_{2A} receptors. Molecular docking results indicate that these compounds bind to the adenosine A_{2A} binding site in a similar fashion as reported for other adenosine A_{2A} antagonists and correlated with the good affinities obtained in *in vitro* assays.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at..... These data include the NMR spectroscopic data of the intermediates and examples of sigmoidal dose response curves obtained during the radioligand binding assays.

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Supplementary material

1. Materials and instrumentation
2. Synthesis
3. Physical data
4. Radioligand binding assays
5. Molecular docking
6. Haloperidol induced catalepsy assay
7. NMR spectra

1. Materials and instrumentation

All chemicals were purchased from Sigma-Aldrich and used without any further purification. Solvents for synthesis and chromatography were obtained from Associated Chemical Enterprises while deuterated solvents for nuclear magnetic resonance (NMR) spectroscopy were purchased from Merck.

Thin layer chromatography (TLC):

Precoated Kieselgel 60 F254 plates (Merck) were used to monitor reactions. Detection was done by UV light at a wavelength of 254 nm.

Melting points:

The melting points of all compounds were determined using a Buchi B-545 apparatus, and are uncorrected.

Mass spectrometry (MS):

The mass spectra for all compounds were obtained with a Bruker micrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode.

Nuclear magnetic resonance (NMR) spectroscopy:

A Bruker Avance III 600 spectrometer was used to record proton (^1H) and carbon (^{13}C) NMR spectra at frequencies of 600 MHz and 151 MHz, respectively. Samples were dissolved in either deuterated dimethylsulfoxide (DMSO-*d*6) or deuterated chloroform (CDCl_3). Reported ^1H NMR spectroscopic data indicate the chemical shift (δ) in ppm, the integration (e.g. 1H), the multiplicity and the coupling constant (J) in Hz. The following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), br t (broad triplet), q (quartet), p (pentet/quintet) or m (multiplet). Chemical shifts are referenced to the residual solvent signal (DMSO-*d*6: 2.5 and 39.5 ppm for ^1H and ^{13}C respectively; CDCl_3 7.26 and 77.0 ppm for ^1H and ^{13}C respectively). An interesting coupling was observed between the amide proton and the

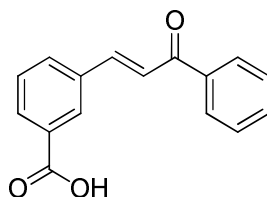
adjacent CH₂ carbons of the side chains, resulting in the observed quartet signals for some compounds at approximately 3-4 Hz.

High performance liquid chromatography (HPLC):

The purity of most of the synthesised aminopyrimidines was assessed by HPLC analysis with an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector. HPLC grade acetonitrile (Merck) and Milli-Q water (Millipore) was used for the chromatography. A Venusil XBP C18 column (4.60 × 150 mm, 5 μm) was used with an initial mobile phase of 30% acetonitrile and 70% MilliQ water at a flow rate of 1 ml/min. At the start of each HPLC run, a solvent gradient program was initiated. The concentration of acetonitrile in the mobile phase was linearly increased up to 85% over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. The test compound was injected (20 μl, 1 mM) into the HPLC system and the eluent was monitored at wavelengths of 210, 254 and 300 nm.

2. Synthesis

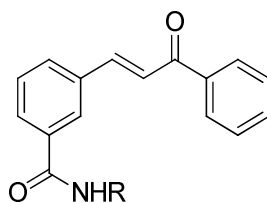
General procedure for the synthesis of 3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid (**4**)



4

A solution of 4% (w/v) sodium hydroxide (33.4 mmol, 2 equiv) was added to a suspension of 3-formylbenzoic acid (**4**) (16.7 mmol 1 equiv) and acetophenone (**5**) (24.5 mmol, 1.5 equiv) in methanol (100 ml). The mixture was stirred at room temperature overnight and then acidified by the dropwise addition of concentrated hydrochloric acid to a pH of 1-2. The resulting precipitate was filtered, rinsed with water and recrystallised from methanol.

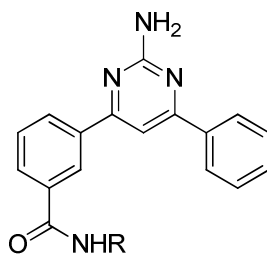
General procedure for the synthesis of chalcones (**5a-j**)



5a-j

A mixture of dichloromethane (70 ml), acid (**4**) (5.83 mmol, 1 equiv) and carbonyldiimidazole (7.00 mmol, 1.2 equiv) was stirred at room temperature for 2 hours. The required amine (7.00 mmol, 1.2 equiv) was subsequently added and the reaction mixture was stirred for a further 3 hours. The reaction was quenched by the addition of water and the aqueous phase was extracted with dichloromethane (20 ml x 3). The organic fractions were combined, washed twice with brine and concentrated on a rotary evaporator. The resulting solid was purified by column chromatography (dichloromethane: methanol, 98:2) and recrystallised from methanol.

General procedure for the synthesis of aminopyrimidines (**6a-j**)



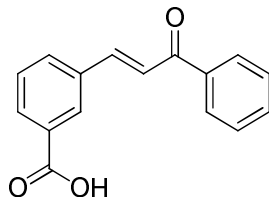
6a-j

The chalcone (**5**) (3.07 mmol, 1 equiv) and sodium hydride (4.61 mmol, 1.5 equiv) were added to a suspension of guanidine hydrochloride (4.61mmol, 1.5 equiv) in DMF (20 ml).The reaction mixture was stirred under nitrogen for 24 hours at 110 °C. Afterwards the reaction was left to cool down, diluted with water and extracted with ethyl acetate (20 ml x 3). The organic layers were combined and all traces of DMF were washed out with water and brine. The organic layer was concentrated *in vacuo* and the crude product was purified by column chromatography (dichloromethane: methanol, 98:2) and recrystallised from ethanol.

3. Physical data

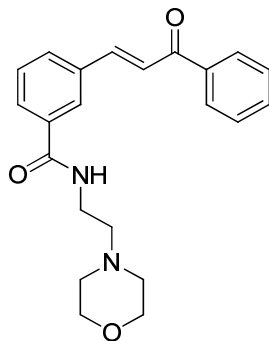
Chalcones:

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid (**4**)



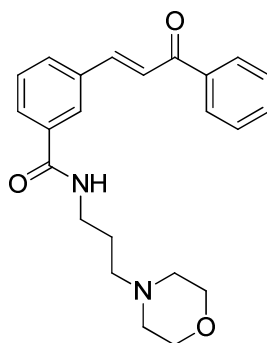
The title compound was prepared from 3-formylbenzoic acid and 1-phenylethan-1-one in a yield of 71%: mp 200.6 – 201.4 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, DMSO-*d*₆) δ 13.16 (br s, 1H), 8.38 (s, 1H), 8.20 – 8.11 (m, 3H), 8.03 – 7.96 (m, 2H), 7.79 (d, *J* = 15.6 Hz, 1H), 7.70 – 7.62 (m, 1H), 7.62 – 7.53 (m, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.2, 167.0, 143.0, 137.4, 135.1, 133.3, 132.9, 131.7, 131.1, 129.6, 129.3, 128.8 (2C), 128.7 (2C), 123.2. APCI-HRMS *m/z*: calcd for C₁₆H₁₃O₃ (M + H)⁺, 253.0859, found 253.0838.

N-[2-(morpholin-4-yl)ethyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5a**)



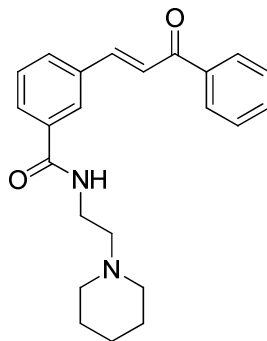
The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and 2-(morpholin-4-yl)ethan-1-amine in a yield of 36%: mp 117.9 – 119.8 °C (ethanol), light yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.09 (s, 1H), 8.03 – 7.98 (m, 2H), 7.82 – 7.69 (m, 3H), 7.62 – 7.55 (m, 2H), 7.52 – 7.44 (m, 3H), 6.96 (t, *J* = 5.0 Hz, 1H), 3.71 (t, *J* = 4.6 Hz, 4H), 3.59 – 3.53 (m, 2H), 2.60 (t, *J* = 6.1 Hz, 2H), 2.52 – 2.47 (m, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 190.1, 166.7, 143.5, 137.8, 135.3, 135.3, 133.0, 131.2, 129.1, 128.6 (2C), 128.4 (2C), 128.3, 126.8, 122.9, 66.9 (2C), 56.8, 53.3 (2C), 36.1. APCI-HRMS *m/z*: calcd for C₂₂H₂₅N₂O₃ (M + H)⁺, 365.1860, found 365.1830.

N-[3-(morpholin-4-yl)propyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5b**)



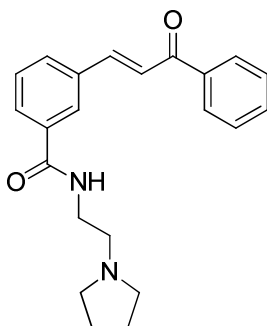
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and 3-(morpholin-4-yl)propan-1-amine in a yield of 24%: mp 110.0 – 111.5 °C (ethanol), light yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.15 – 8.08 (m, 2H), 8.02 – 7.97 (m, 2H), 7.81 – 7.74 (m, 2H), 7.71 (br d, *J* = 7.8 Hz, 1H), 7.61 – 7.54 (m, 2H), 7.51 – 7.42 (m, 3H), 3.65 (t, *J* = 4.7 Hz, 4H), 3.60 – 3.53 (q, *J* = 6.0 Hz, 2H), 2.66 – 2.26 (m, 6H), 1.79 (p, *J* = 6.1 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 190.1, 166.7, 143.5, 137.8, 135.6, 135.3, 132.9, 131.2, 128.9, 128.6 (2C), 128.5 (2C), 128.2, 126.9, 123.0, 66.9 (2C), 58.4, 53.7 (2C), 40.5, 24.2. APCI-HRMS *m/z*: calcd for C₂₃H₂₇N₂O₃ (M + H)⁺, 379.2016, found 379.1998.

3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(piperidin-1-yl)ethyl]benzamide (**5c**)



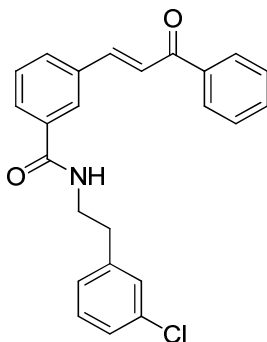
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and 2-(piperidin-1-yl)ethan-1-amine in a yield of 42%: mp 85.2 – 86.9 °C (ethanol), yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.54 (t, *J* = 5.7 Hz, 1H), 8.31 (s, 1H), 8.17 (d, *J* = 6.9 Hz, 2H), 8.06 – 7.96 (m, 2H), 7.90 (br d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 15.6 Hz, 1H), 7.72 – 7.65 (m, 1H), 7.62 – 7.53 (m, 3H), 3.48 – 3.39 (m, 2H), 2.50 – 2.46 (m, 2H), 2.42 (s, 4H), 1.53 – 1.45 (m, 4H), 1.43 – 1.31 (m, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.2, 165.6, 143.4, 137.4, 135.2, 134.7, 133.2, 131.4, 129.3, 129.0, 128.8 (2C), 128.6 (2C), 127.1, 122.9, 57.6, 54.0 (2C), 36.9, 25.4 (2C), 23.9. APCI-HRMS *m/z*: calcd for C₂₃H₂₇N₂O₂ (M + H)⁺, 363.2067, found 363.2085.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(pyrrolidin-1-yl)ethyl]benzamide (**5d**)



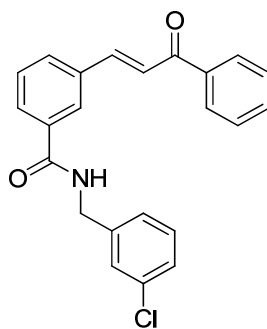
The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and 2-(pyrrolidin-1-yl)ethan-1-amine in a yield of 55%: orange gum. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.62 (t, *J* = 5.7 Hz, 1H), 8.32 (br s, 1H), 8.18 – 8.11 (m, 2H), 8.06 – 7.95 (m, 2H), 7.90 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.77 (d, *J* = 15.6 Hz, 1H), 7.71 – 7.62 (m, 1H), 7.62 – 7.48 (m, 3H), 7.01 (br s, 1H), 3.47 – 3.39 (m, 1H), 2.66 (t, *J* = 6.9 Hz, 2H), 2.56 (d, *J* = 6.1 Hz, 4H), 1.69 (p, *J* = 3.0 Hz, 4H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.2, 165.7, 143.4, 137.5, 135.2, 134.7, 133.3, 131.5, 129.4, 129.0, 128.9 (2C), 128.6 (2C), 127.2, 123.0, 54.8, 53.7 (2C), 38.4, 23.1 (2C). APCI-HRMS *m/z*: calcd for C₂₂H₂₅N₂O₂ (M + H)⁺, 349.1785, found 349.1880.

N-[2-(3-chlorophenyl)ethyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5e**)



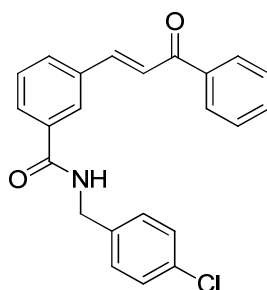
The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and 2-(3-chlorophenyl)ethan-1-amine in a yield of 34%: mp 117.8 – 118.8 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.02 (t, *J* = 1.8 Hz, 1H), 7.99 – 7.94 (m, 2H), 7.74 – 7.68 (m, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.60 – 7.53 (m, 1H), 7.52 (d, *J* = 15.7 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.24 – 7.15 (m, 3H), 7.08 (dt, *J* = 7.1, 1.6 Hz, 1H), 6.81 (t, *J* = 5.8 Hz, 1H), 3.68 (q, *J* = 7.0 Hz, 2H), 2.90 (t, *J* = 7.1 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 166.9, 143.5, 140.8, 137.7, 135.2, 135.1, 134.3, 133.0, 131.5, 129.8, 129.1, 128.8, 128.6 (2C), 128.5, 128.4 (2C), 126.9, 126.7, 126.5, 122.9, 41.0, 35.2. APCI-HRMS *m/z*: calcd for C₂₄H₂₁ClNO₂ (M + H)⁺, 390.1255, found 390.1224.

N-[(3-chlorophenyl)methyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5f**)



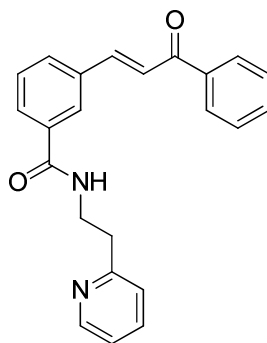
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and (3-chlorophenyl)methanamine in a yield of 55%: mp 126.4 – 127.8 °C (ethanol), yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.13 (br s, 1H), 7.96 – 7.91 (m, 2H), 7.82 (br d, *J* = 7.8 Hz, 1H), 7.74 – 7.63 (m, 2H), 7.59 – 7.50 (m, 2H), 7.48 – 7.36 (m, 4H), 7.27 (br s, 1H), 7.24 – 7.13 (m, 3H), 4.56 (d, *J* = 5.9 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 166.9, 143.5, 140.2, 137.7, 135.2, 134.8, 134.4, 133.0, 131.6, 129.9, 129.2, 128.8, 128.6 (2C), 128.4 (2C), 127.7, 127.6, 126.7, 125.8, 122.9, 43.4. APCI-HRMS *m/z*: calcd for C₂₃H₁₉ClNO₂ (M + H)⁺, 376.1099, found 376.1091.

N-[(4-chlorophenyl)methyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5g**)



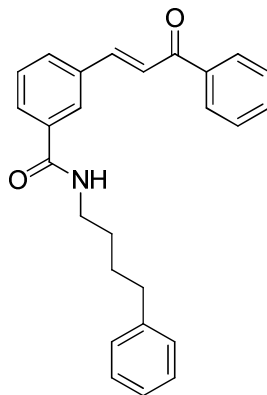
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and (4-chlorophenyl)methanamine in a yield of 53%: mp 191.0 – 192.9 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.16 (t, *J* = 6.0 Hz, 1H), 8.35 (br s, 1H), 8.17 – 8.12 (m, 2H), 8.05 (br d, *J* = 7.8 Hz, 1H), 7.98 (d, *J* = 15.7 Hz, 1H), 7.94 (dt, *J* = 7.8, 1.2 Hz, 1H), 7.78 (d, *J* = 15.7 Hz, 1H), 7.71 – 7.65 (m, 1H), 7.63 – 7.53 (m, 3H), 7.42 – 7.33 (m, 4H), 4.50 (d, *J* = 5.9 Hz, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.3, 165.8, 143.3, 138.6, 137.4, 134.9, 134.8, 133.3, 131.7, 131.4, 129.4, 129.2 (2C), 129.1, 128.9 (2C), 128.6 (2C), 128.3 (2C), 127.2, 123.0, 42.1. APCI-HRMS *m/z*: calcd for C₂₃H₁₉ClNO₂ (M + H)⁺, 376.1099, found 376.1068.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(pyridin-2-yl)ethyl]benzamide (**5h**)



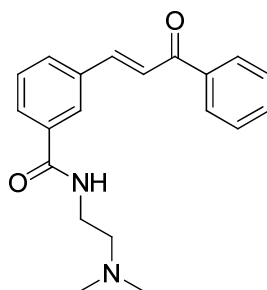
The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and 2-(pyridin-2-yl)ethan-1-amine in a yield of 44%: mp 78.4 – 79.2 °C (ethanol), yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.69 (t, *J* = 5.6 Hz, 1H), 8.52 – 8.48 (m, 1H), 8.27 (br s, 1H), 8.16 – 8.11 (m, 2H), 8.05 – 7.91 (m, 2H), 7.88 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.73 – 7.62 (m, 2H), 7.63 – 7.49 (m, 3H), 7.28 (br d, *J* = 7.8 Hz, 1H), 7.21 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 1H), 3.70 – 3.62 (q, *J* = 7.2 Hz, 2H), 3.03 (t, *J* = 7.4 Hz, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.3, 165.8, 159.1, 149.1, 143.4, 137.5, 136.5, 135.3, 134.7, 133.3, 131.5, 129.3, 129.1, 128.9 (2C), 128.6 (2C), 127.1, 123.2, 122.9, 121.6, 39.4, 37.4. HRMS *m/z*: calcd for C₂₃H₂₁N₂O₂ (M + H)⁺, 357.1598, found 357.1617.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-(4-phenylbutyl)benzamide (**5i**)



The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and 4-phenylbutan-1-amine in a yield of 24%: mp 106.5 – 106.7 °C (ethanol), yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.57 (t, *J* = 5.7 Hz, 1H), 8.30 (br s, 1H), 8.16 (br d, *J* = 7.1 Hz, 2H), 8.06 – 7.96 (m, 2H), 7.91 (br d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 15.6 Hz, 1H), 7.73 – 7.65 (m, 1H), 7.62 – 7.52 (m, 3H), 7.26 (t, *J* = 7.5 Hz, 2H), 7.22 – 7.12 (m, 3H), 3.33 (q, *J* = 6.6 Hz, 2H), 2.61 (t, *J* = 7.5 Hz, 2H), 1.67 – 1.51 (m, 4H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.2, 165.6, 143.4, 142.1, 137.5, 135.4, 134.7, 133.2, 131.3, 129.3, 129.0, 128.8 (2C), 128.5 (2C), 128.3 (2C), 128.2 (2C), 127.2, 125.6, 122.9, 39.1, 34.8, 28.8, 28.5. APCI-HRMS *m/z*: calcd for C₂₆H₂₆NO₂ (M + H)⁺, 384.1945, found 384.1973.

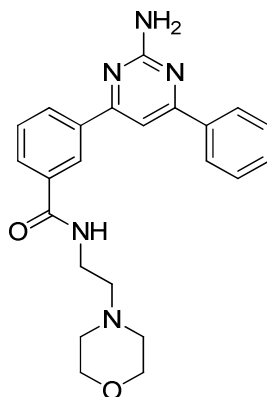
N-[2-(dimethylamino)ethyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5j**)



The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid and (2-aminoethyl)dimethylamine hydrochloride in a yield of 66%: pale yellow gum. ¹H NMR (600 MHz, CDCl₃) δ 8.12 (s, 1H), 8.02 – 7.97 (m, 2H), 7.92 – 7.88 (m, 1H), 7.77 (d, *J* = 15.8 Hz, 1H), 7.72 – 7.67 (m, 1H), 7.62 – 7.52 (m, 2H), 7.50 – 7.40 (m, 3H), 7.19 (s, 1H), 3.52 (q, *J* = 5.5 Hz, 2H), 2.53 (t, *J* = 6.0 Hz, 2H), 2.26 (s, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 190.2, 167.0, 143.6, 137.8, 135.2, 135.1, 132.9, 131.2, 128.7, 128.6 (2C), 128.4 (2C), 127.8, 126.9, 122.9, 57.6, 45.0, 37.2, . APCI-HRMS *m/z*: calcd for C₂₀H₂₃N₂O₂ (M + H)⁺, 323.1754, found 323.1757.

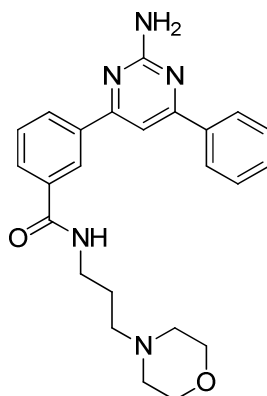
Aminopyrimidines:

3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(morpholin-4-yl)ethyl]benzamide (**6a**)



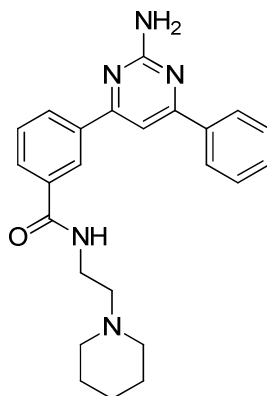
The title compound was prepared from *N*-[2-(morpholin-4-yl)ethyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide in a yield of 14%: mp 192.5 – 193.6 °C (ethanol), white crystals. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.70 (s, 1H), 8.66 (t, *J* = 5.7 Hz, 1H), 8.43 (br d, *J* = 8.0 Hz, 1H), 8.32 – 8.26 (m, 2H), 8.04 (br d, *J* = 7.8 Hz, 1H), 7.83 (s, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.64 – 7.57 (m, 3H), 6.92 (s, 2H), 3.65 (t, *J* = 4.6 Hz, 4H), 3.52 (q, *J* = 6.6 Hz, 2H), 2.62 – 2.42 (m, 6H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.1, 165.1, 164.4, 164.1, 137.5, 137.3, 135.1, 130.5, 129.6, 129.1, 128.7, 128.6 (2C), 127.0 (2C), 125.8, 102.0, 66.2 (2C), 57.4, 53.3 (2C), 36.7. APCI-HRMS *m/z*: calcd for C₂₃H₂₆N₅O₂ (M + H)⁺, 404.2081, found 404.2068; Purity (HPLC): 98%.

3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[3-(morpholin-4-yl)propyl]benzamide (**6b**)



The title compound was prepared from *N*-[3-(morpholin-4-yl)propyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide in a yield of 17%: mp 144.7 – 146.9 °C (ethanol), light yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.58 (t, *J* = 4.6 Hz, 1H), 8.48 (br s, 1H), 8.11 (br d, *J* = 7.7 Hz, 1H), 8.08 (br d, *J* = 7.8 Hz, 1H), 8.06 – 8.02 (m, 2H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.50 – 7.43 (m, 4H), 5.68 (s, 2H), 3.90 (t, *J* = 4.7 Hz, 4H), 3.62 (q, *J* = 5.4 Hz, 2H), 2.74 – 2.30 (m, 6H), 1.79 (p, *J* = 5.9 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 166.4, 166.2, 165.3, 163.9, 138.0, 137.5, 134.9, 130.4, 129.9, 129.9, 128.9, 128.7 (2C), 127.0 (2C), 124.4, 104.0, 66.4 (2C), 59.2, 53.8 (2C), 41.4, 23.6. APCI-HRMS *m/z*: calcd for C₂₄H₂₈N₅O₂ (M + H)⁺, 418.2238, found 418.2229; Purity (HPLC): 99%.

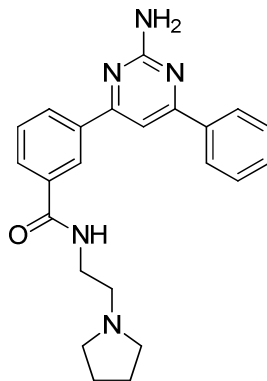
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(piperidin-1-yl)ethyl]benzamide (**6c**)



The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(piperidin-1-yl)ethyl]benzamide in a yield of 12%: mp 109.5 – 110.6 °C (ethanol), orange solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.65 – 8.59 (m, 2H), 8.33 (dt, *J* = 7.8, 1.5 Hz, 1H), 8.23 – 8.18 (m, 2H), 7.95 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.75 (s, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.55 – 7.49 (m, 3H), 6.81 (s, 2H), 2.63 – 2.38 (m, 8H), 1.51 (p, *J* = 5.6 Hz, 4H), 1.38 (q, *J* = 6.0 Hz, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.1, 165.1, 164.4, 164.1, 137.6, 137.3, 135.1, 130.6, 129.6, 129.1, 128.8, 128.7 (2C), 127.0 (2C), 125.8, 102.1,

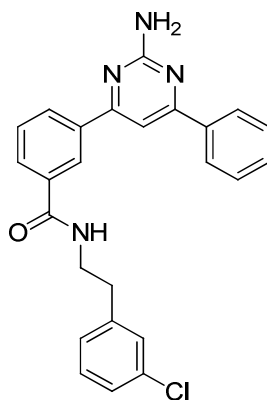
57.5, 53.9 (2C), 36.7, 25.2 (2C), 23.7. APCI-HRMS m/z : calcd for $C_{24}H_{28}N_5O$ ($M + H$)⁺, 402.2302, found 402.2302.

3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(pyrrolidin-1-yl)ethyl]benzamide (**6d**)



The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(pyrrolidin-1-yl)ethyl]benzamide in a yield of 11%: mp 140.6 – 141.7 °C (ethanol), white solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.64 – 8.58 (m, 2H), 8.33 (dt, $J = 7.8, 1.4$ Hz, 1H), 8.24 – 8.17 (m, 2H), 7.95 (dt, $J = 7.7, 1.5$ Hz, 1H), 7.74 (s, 1H), 7.60 (t, $J = 7.7$ Hz, 1H), 7.56 – 7.49 (m, 3H), 6.82 (s, 2H), 3.45 – 3.38 (m, 4H), 2.59 (t, $J = 7.0$ Hz, 2H), 2.51 – 2.45 (m, 2H), 1.66 (p, $J = 3.0$ Hz, 4H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.0, 165.1, 164.5, 164.1, 137.6, 137.3, 135.1, 130.6, 129.6, 129.1, 128.7, 128.7 (2C), 127.0 (2C), 125.8, 102.1, 55.0, 53.7 (2C), 38.8, 23.2 (2C). APCI-HRMS m/z : calcd for $C_{23}H_{26}N_5O$ ($M + H$)⁺, 388.2132, found 388.2153.

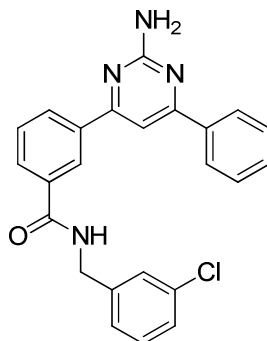
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(3-chlorophenyl)ethyl]benzamide (**6e**)



The title compound was prepared from *N*-[2-(3-chlorophenyl)ethyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide in a yield of 11%: mp 180.7 – 181.3 °C (ethanol), white crystals. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.73 (t, $J = 5.6$ Hz, 1H), 8.60 (s, 1H), 8.35 (br d, $J = 7.9$ Hz, 1H), 8.25 – 8.19 (m, 2H), 7.94 (br d, $J = 7.7$ Hz, 1H), 7.74 (s, 1H), 7.62 (t, $J = 7.7$ Hz, 1H), 7.58 – 7.51 (m, 3H), 7.37 – 7.29 (m,

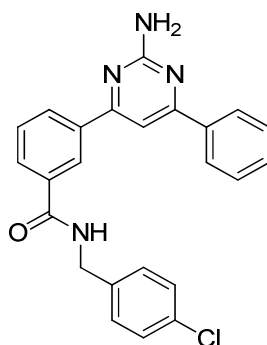
2H), 7.29 – 7.20 (m, 2H), 6.85 (s, 2H), 3.55 (q, $J = 6.9$ Hz, 2H), 2.90 (t, $J = 7.2$ Hz, 2H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 166.1, 165.1, 164.4, 164.1, 142.1, 137.5, 137.3, 135.1, 132.9, 130.5, 130.1, 129.6, 129.0, 128.7, 128.6 (2C), 128.6, 127.5, 127.0 (2C), 126.1, 125.8, 102.0, 40.6, 34.6. APCI-HRMS m/z : calcd for $\text{C}_{25}\text{H}_{22}\text{ClN}_4\text{O}$ ($\text{M} + \text{H}$) $^+$, 429.1477, found 429.1475; Purity (HPLC): 99%.

3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[(3-chlorophenyl)methyl]benzamide (**6f**)



The title compound was prepared from *N*-[(3-chlorophenyl)methyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide in a yield of 22%: mp 182.8 – 184.1 °C (ethanol), white solid. ^1H NMR (600 MHz, DMSO- d_6) δ 9.27 (t, $J = 6.0$ Hz, 1H), 8.70 (s, 1H), 8.39 (br d, $J = 7.8$ Hz, 1H), 8.26 – 8.19 (m, 2H), 8.05 (br d, $J = 7.7$ Hz, 1H), 7.77 (s, 1H), 7.65 (t, $J = 7.7$ Hz, 1H), 7.57 – 7.50 (m, 3H), 7.41 (br s, 1H), 7.40 – 7.34 (m, 1H), 7.37 – 7.29 (m, 2H), 6.86 (s, 2H), 4.55 (d, $J = 5.9$ Hz, 2H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 166.2, 165.1, 164.3, 164.1, 142.2, 137.7, 137.3, 134.6, 133.0, 130.5, 130.2, 129.9, 129.2, 128.8, 128.6 (2C), 127.1, 127.0 (2C), 126.8, 126.0, 125.9, 102.1, 42.3. APCI-HRMS m/z : calcd for $\text{C}_{24}\text{H}_{20}\text{ClN}_4\text{O}$ ($\text{M} + \text{H}$) $^+$, 415.1320, found 415.1304; Purity (HPLC): 99%.

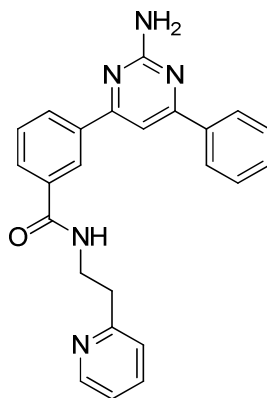
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[(4-chlorophenyl)methyl]benzamide (**6g**)



The title compound was prepared from *N*-[(4-chlorophenyl)methyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide in a yield of 9%: mp 209.5 – 210.9 °C (ethanol), orange solid. ^1H NMR (600 MHz, DMSO- d_6) δ 9.25 (t, $J = 6.0$ Hz, 1H), 8.68 (br s, 1H), 8.38 (br d, $J = 7.8$ Hz, 1H), 8.24 – 8.20 (m, 2H), 8.03 (br d, $J = 7.8$ Hz, 1H), 7.77 (s, 1H), 7.64 (t, $J = 7.8$ Hz, 1H), 7.57 – 7.51 (m, 3H), 7.40 (d, $J = 8.4$ Hz, 2H), 7.38 (d, $J = 8.4$ Hz, 2H), 6.85 (s, 2H), 4.52 (d, $J = 5.9$ Hz, 2H). ^{13}C NMR (151 MHz, DMSO-

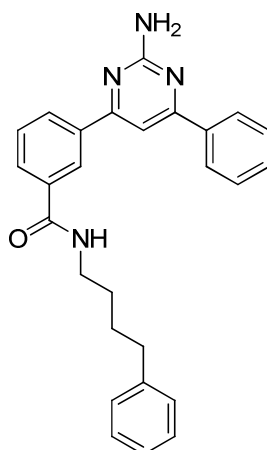
d6) δ 166.1, 165.1, 164.3, 164.0, 138.7, 137.6, 137.3, 134.7, 131.3, 130.5, 129.8, 129.2 (2C), 128.8, 128.6 (2C), 128.3 (2C), 128.2, 127.0 (2C), 125.9, 102.0, 42.1. APCI-HRMS *m/z*: calcd for C₂₄H₂₀ClN₄O (M + H)⁺, 415.1320, found 415.1311; Purity (HPLC): 96%.

3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(pyridin-2-yl)ethyl]benzamide (**6h**)



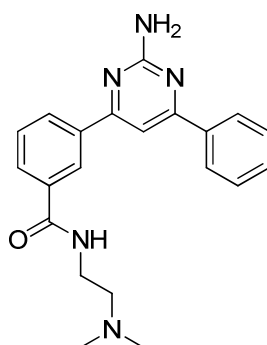
The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(pyridin-2-yl)ethyl]benzamide in a yield of 13%: mp 165.8 – 166.6 °C (ethanol), white solid. ¹H NMR (600 MHz, DMSO-*d6*) δ 8.74 (t, *J* = 5.6 Hz, 1H), 8.59 (t, *J* = 1.8 Hz, 1H), 8.51 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 1H), 8.35 – 8.30 (m, 1H), 8.24 – 8.17 (m, 2H), 7.93 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.74 – 7.66 (m, 2H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.56 – 7.49 (m, 3H), 7.29 (br d, *J* = 7.8 Hz, 1H), 7.21 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 1H), 6.82 (s, 2H), 3.66 (q, *J* = 7.0 Hz, 2H), 3.03 (t, *J* = 7.4 Hz, 2H). ¹³C NMR (151 MHz, DMSO-*d6*) δ 166.1, 165.1, 164.4, 164.1, 159.2, 149.1, 137.6, 137.3, 136.6, 135.2, 130.6, 129.6, 129.1, 128.8 (2C), 128.7, 127.0 (2C), 125.8, 123.2, 121.6, 102.1, 39.4, 37.4. APCI-HRMS *m/z*: calcd for C₂₄H₂₂N₅O (M + H)⁺, 396.1819, found 396.1843; Purity (HPLC): 99%.

3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-(4-phenylbutyl)benzamide (**6i**)



The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-(4-phenylbutyl)benzamide in a yield of 15%: mp 156.9 – 158.2 °C (ethanol), white crystals. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.65 – 8.58 (m, 2H), 8.33 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.24 – 8.18 (m, 2H), 7.95 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.74 (s, 1H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.56 – 7.49 (m, 3H), 7.27 – 7.11 (m, 5H), 6.83 (s, 2H), 3.32 (q, *J* = 6.9 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 1.66 – 1.51 (m, 4H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 166.0, 165.1, 164.5, 164.1, 142.2, 137.5, 137.3, 135.3, 130.5, 129.5, 129.1 (2C), 128.7 (2C), 128.3 (2C), 128.2 (2C), 127.0 (2C), 125.8, 125.7, 102.0, 39.1, 34.9, 28.9, 28.6. APCI-HRMS *m/z*: calcd for C₂₇H₂₇N₄O (M + H)⁺, 423.2179, found 423.2198; Purity (HPLC): 100%.

3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(dimethylamino)ethyl]benzamide (**6j**)



The title compound was prepared from *N*-[2-(dimethylamino)ethyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide in a yield of 14%: mp 133.5 – 134.2 °C (ethanol), orange solid. ¹H NMR (600 MHz, CDCl₃) δ 8.46 (br s, 1H), 8.09 (br d, *J* = 7.7 Hz, 1H), 7.99 – 7.95 (m, 2H), 7.88 (br d, *J* = 7.7 Hz, 1H), 7.59 (t, *J* = 5.2 Hz, 1H), 7.50 – 7.40 (m, 5H), 5.66 (s, 2H), 3.59 (q, *J* = 5.5 Hz, 2H), 2.60 (t, *J* = 5.8 Hz, 2H), 2.30 (s, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 167.3, 166.3, 165.1, 163.6, 137.7, 137.4, 135.0, 130.4, 129.7, 129.0, 128.8, 128.6 (2C), 127.0 (2C), 125.8, 104.0, 58.0, 44.9, 37.1 (2C). APCI-HRMS *m/z*: calcd for C₂₁H₂₄N₅O (M + H)⁺, 362.1975, found 362.1983.

4. Radioligand binding assays

Tissue preparation for binding studies

Radioligand binding studies were performed according to literature procedures.^{1,2} The collection of animal tissue for these assays was approved by The Animal Research Ethics Committee of the North-West University (NWU-0035-10-A5). Adult male Sprague-Dawley rats were obtained from the Vivarium of the North-West University, Potchefstroom campus. Whole brains (for A₁ assay) and striata (for A_{2A} assay) were immediately snap frozen with liquid nitrogen after dissection (on ice), and stored at -70 °C. The frozen whole brains as well as striata were suspended in ice-cold 50 mM Tris buffer (pH 7.7 at 25 °C) and homogenised using a Polytron PT-10 homogeniser (Brinkman) to yield final suspensions of 1 g/5 ml, which were aliquoted and stored at -70 °C until required.

Test incubations contained 1% of DMSO in the final concentration. Sigmacote® was used to coat all pipette tips as well as the 4 ml polypropylene tubes used for the incubations. The incubations were prepared using 50 mM Tris buffer (pH 7.7 at 25 °C). The final incubation volume for the A_{2A} assay was 1 ml; containing test compound (0-100 µM), membrane suspension yielding ~10 mg of original tissue weight of rat striata, 10 mM MgCl₂, 0.2 units of adenosine deaminase, 50 nM CPA and 4 nM [³H]NECA. The MgCl₂ (A_{2A} assay) and adenosine deaminase (A_{2A} and A₁ assays) were first added to the membrane suspension, and this mixture was subsequently added to the incubations. The order of addition was test compound, membrane suspension, CPA and [³H]NECA. All incubations were carried out in triplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 µM replacing the test compound). The final incubation volume for the A₁ assay was also 1 ml, containing test compound (0-100 µM), membrane suspension yielding 5 mg of original tissue weight of rat whole brain, 0.1 units of adenosine deaminase and 0.1 nM [³H]DPCPX. The order of addition was test compound, membrane suspension and [³H]DPCPX. All incubations were carried out in triplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 µM replacing the test compound). Incubations were stopped after 1 hour with rapid filtration through Whatman® GF/B 25 filters (25 mm diameter) fitted on a Hoffeler vacuum system. The damp filters were placed into scintillation vials and 4 ml scintillation fluid was added. Afterwards the vials were shaken thoroughly and left for two hours before placed in a Packard Tri-CARB 2100 TR scintillation counter that counted the radioactivity retained on the filters. Specific binding was defined as total binding minus non-specific binding, and was expressed as counts per minute (CPM).

5. Molecular docking

Molecular docking of compounds in the binding site of the crystal structure of the human A_{2A} receptor was carried out using the Windows based Accelrys® Discovery Studio 3.1 software. The receptor

structure which is co-crystallised with the known A_{2A} antagonist ZM241385 (PDB code: 3EML) was obtained from the Protein data bank. The protein was prepared and errors corrected with the 'Clean protein' function. The receptor was typed with the CHARMM forcefield and the ionisation state of residues was optimised by selecting the 'Calculate Protein Ionization and Residue pK' function. After a fixed atom constraint was applied to the backbone a minimisation was carried out utilising the Generalised Born approximation with Molecular Volume (GBMV) as the solvent model to obtain the energetic minimum of the receptor. The existing ligand (ZM241385) was selected, used to define a binding sphere with a radius of 5 Å and removed from the binding site. All compounds screened *in silico* were drawn in a new molecule window. These ligands were cleaned and prepared for docking with the 'Prepare ligand' protocol. The CDOCKER protocol was used to dock ligands - each with 10 random conformations and using Momany-Rone as Ligand partial charge. C-DOCKER, C-DOCKER_INTERACTION energies as well as orientation of the ten different conformers of each ligand were inspected and the best conformation for each ligand selected for *in situ* ligand minimisation.

6. Haloperidol induced catalepsy assay

The North-West University ethical committee approved the following experimental protocol (NWU-00035-10-A5):

In vivo assay:

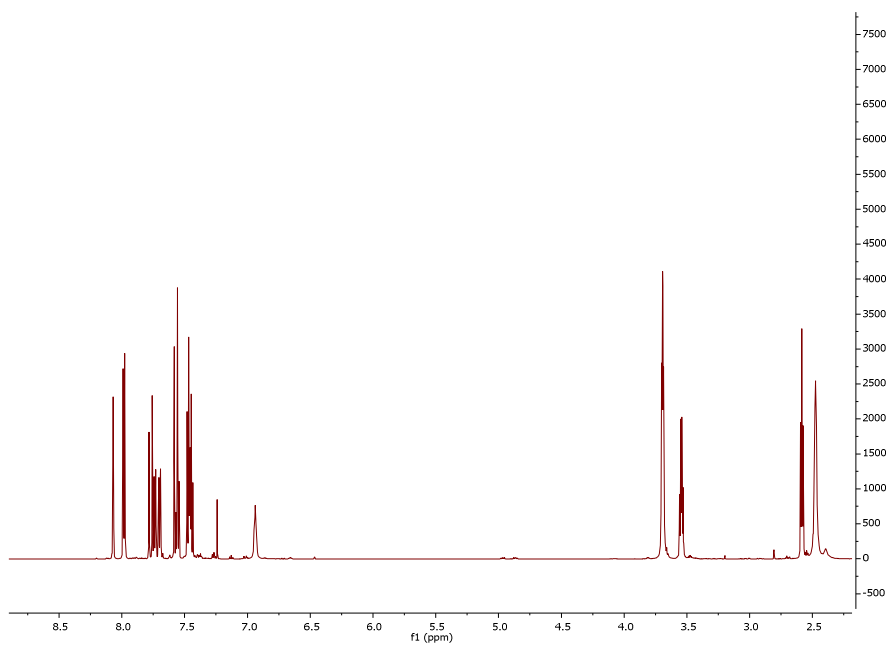
Drug naïve male Sprague-Dawley rats (240 – 300 g) were utilised for the *in vivo* assay. The assay was performed between 8:00 and 15:30 in a lit room with a controlled temperature. The animals were divided into 3 groups (control, 0.4 and 2 mg/kg groups) each containing 10 rats. A dose of 5 mg/kg haloperidol was administered intraperitoneally to all rats to induce catalepsy.³ Thirty minutes later each animal from their respective groups received another i.p injection. The control group receiving DMSO and the other 2 groups the corresponding concentration of test compound. Catalepsy was measured 60 minutes after the first i.p injection (Haloperidol) with the standard bar test, in a Perspex chamber (length, 23 cm; width, 10.5 cm; height, 9 cm) with a horizontal plastic bar (diameter, 1 cm; length, 10.5 cm) fixed at 9 cm above the floor, and at 7 cm from the back of the box. This was performed by placing the animals in the Perspex box with their front paws on the horizontal bar. The time the animal maintained its position on the bar or until both paws were removed from the bar, was recorded up to 120 seconds. To compare between the control group and the compound treated groups a 1 way ANOVA test followed by Dunnett's multiple comparison's test was performed. A probability of $p < 0.05$ was used to declare statistically significant differences.

7. NMR spectra

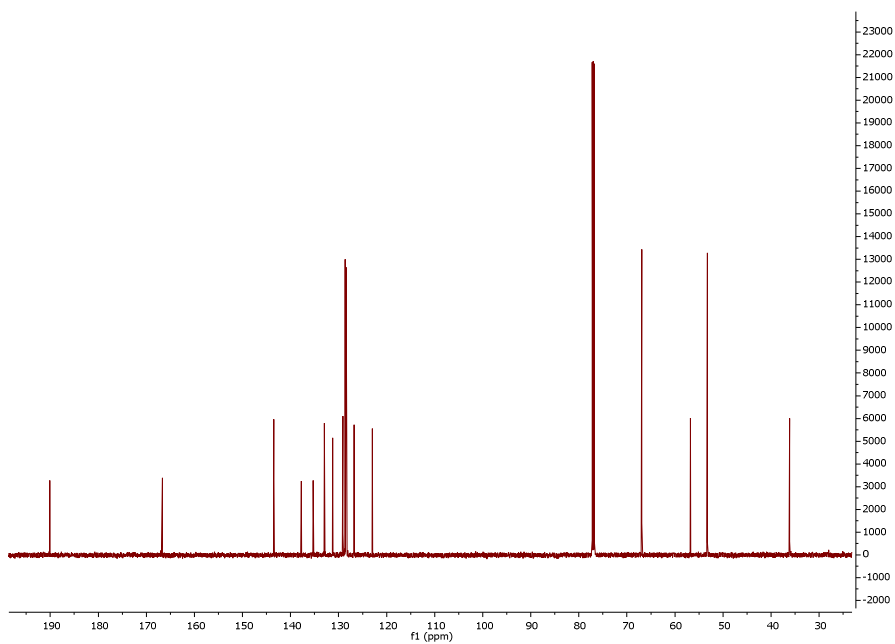
Chalcones

N-[2-(morpholin-4-yl)ethyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5a**)

¹H NMR

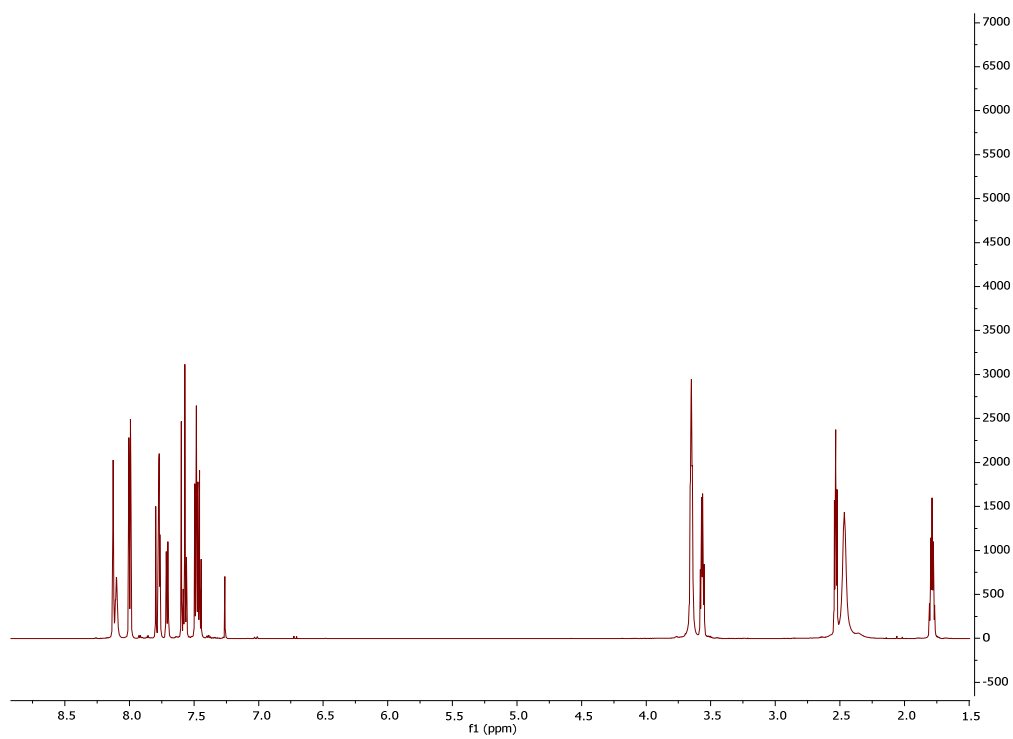


¹³C NMR

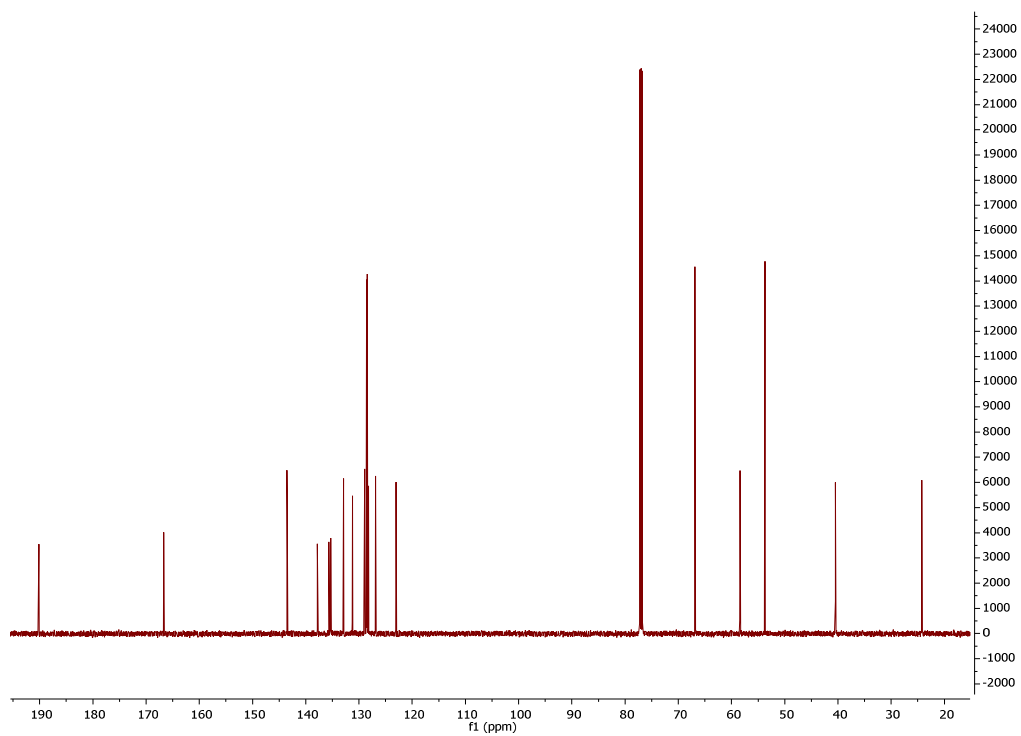


N-[3-(morpholin-4-yl)propyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5b**)

¹H NMR

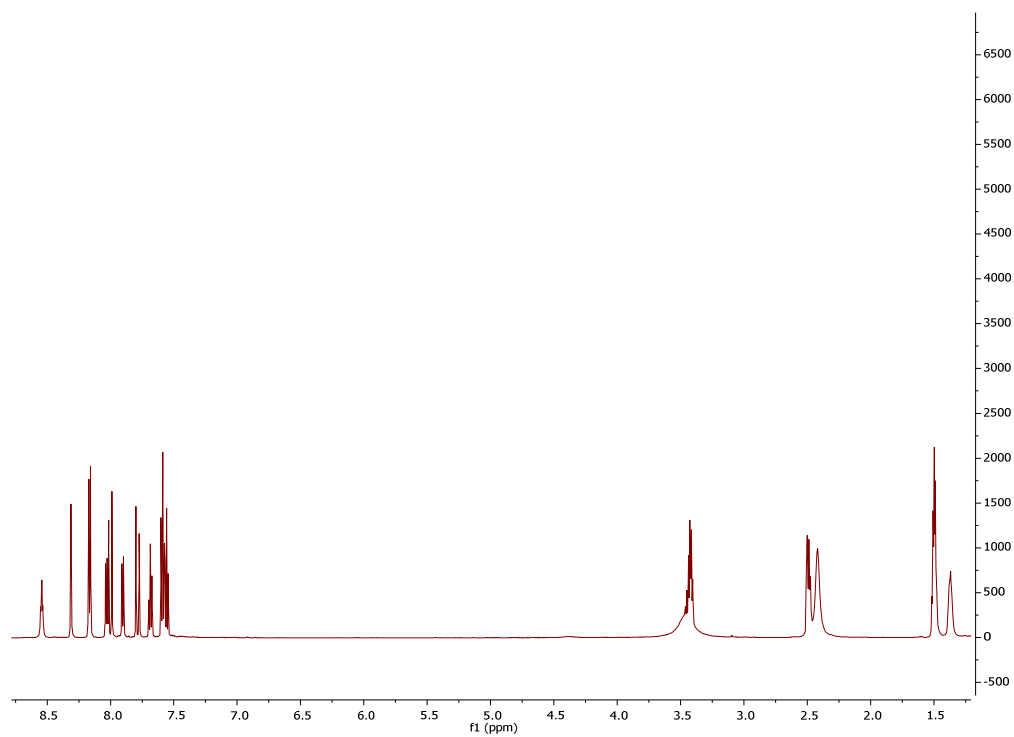


¹³C NMR

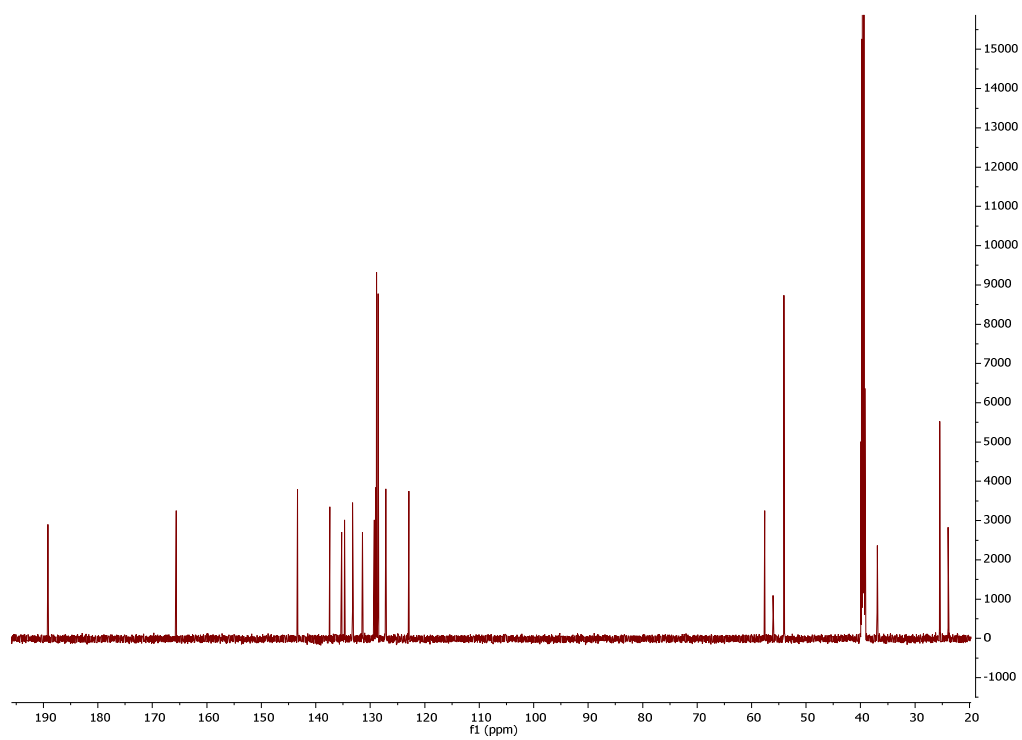


3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]-N-[2-(piperidin-1-yl)ethyl]benzamide (**5c**)

¹H NMR

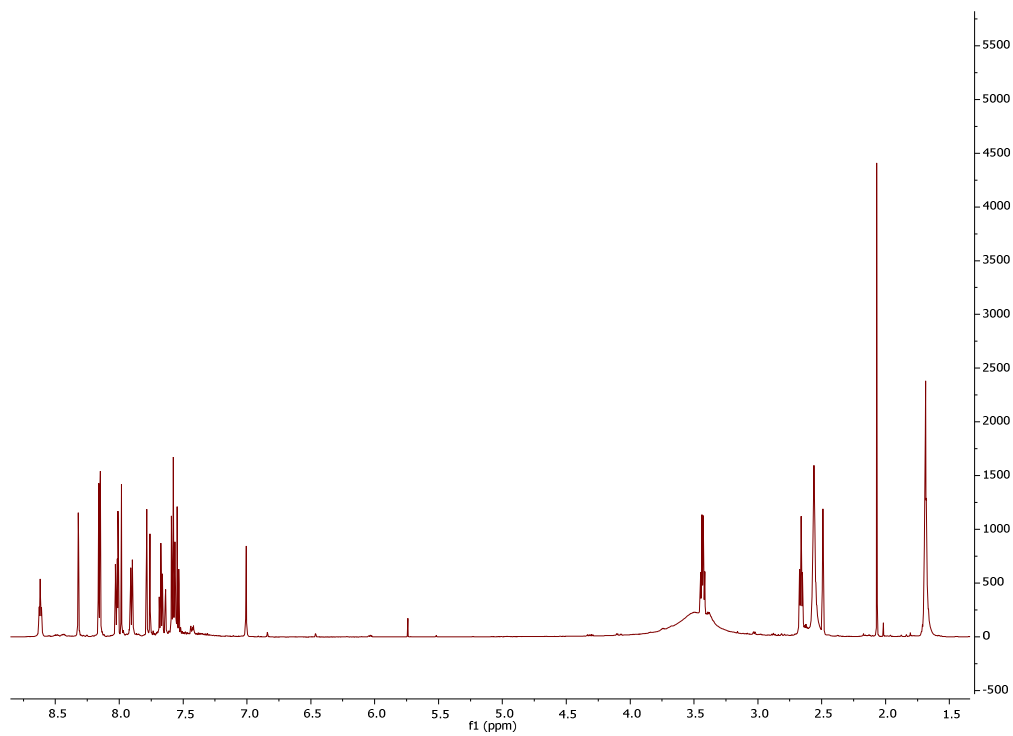


¹³C NMR

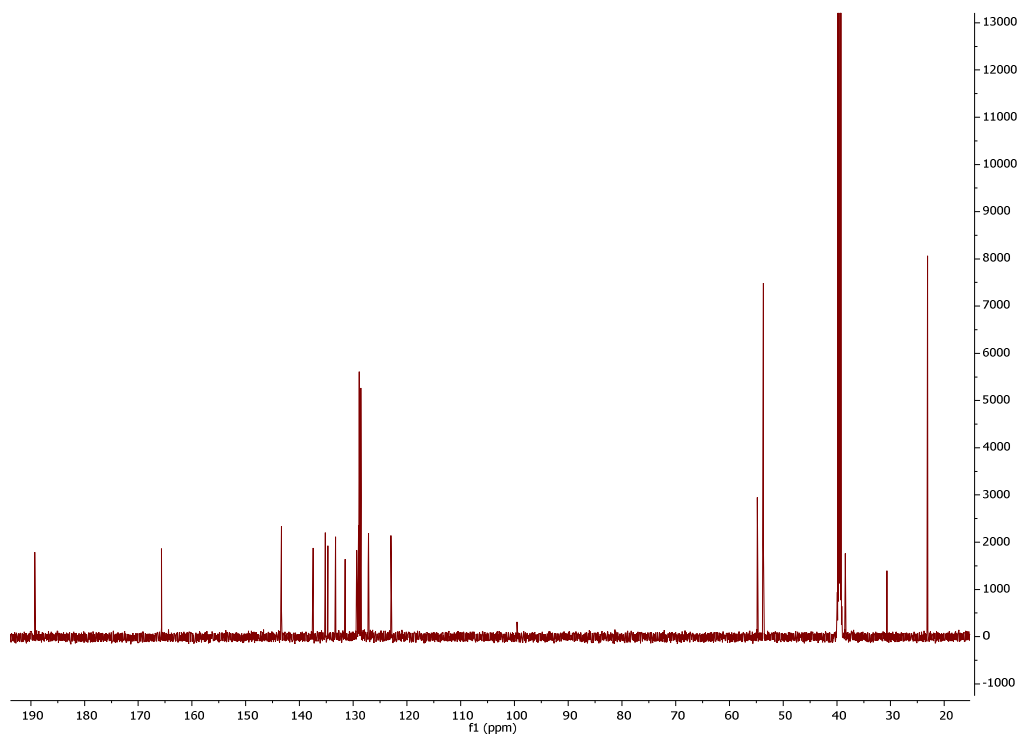


3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(pyrrolidin-1-yl)ethyl]benzamide (**5d**)

¹H NMR

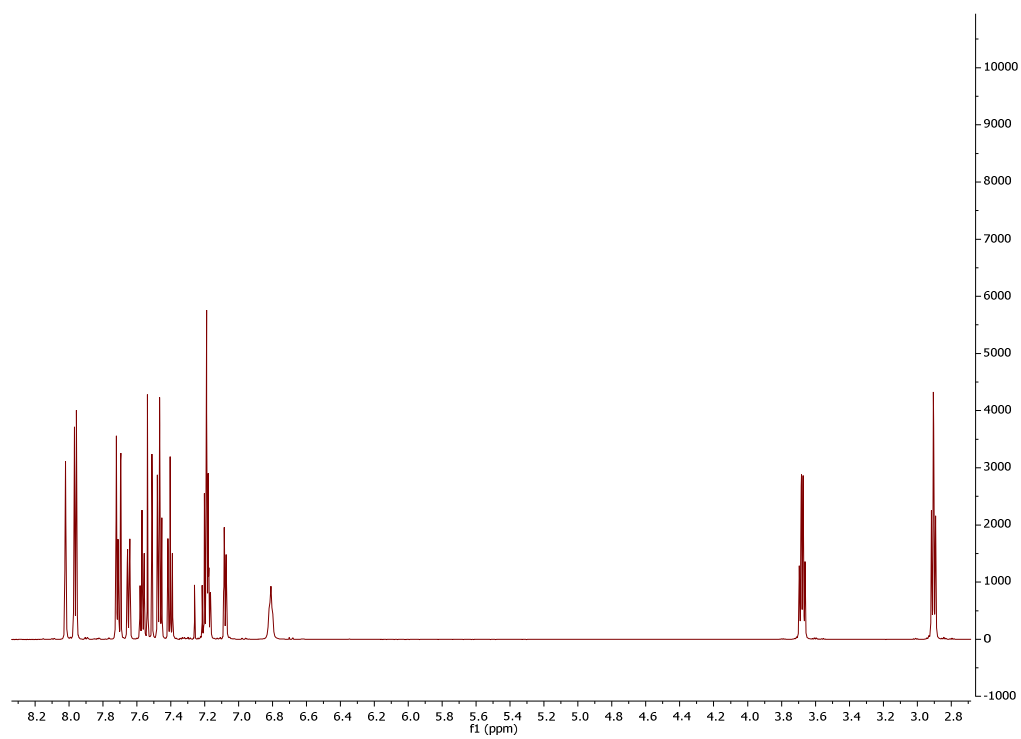


¹³C NMR

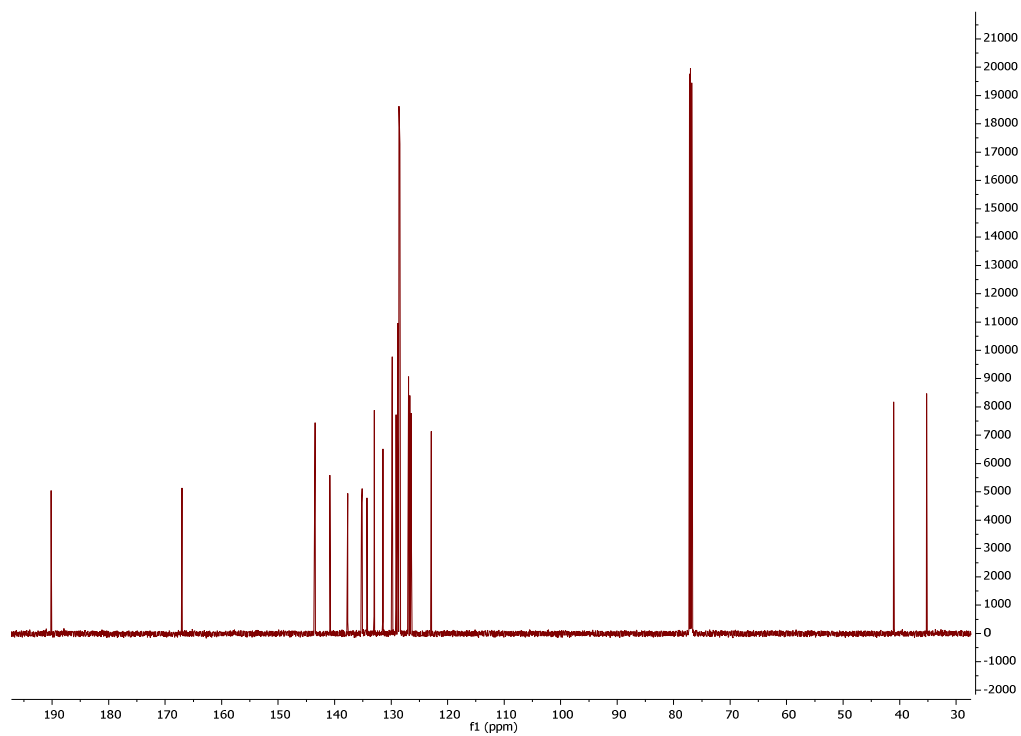


N-[2-(3-chlorophenyl)ethyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5e**)

¹H NMR

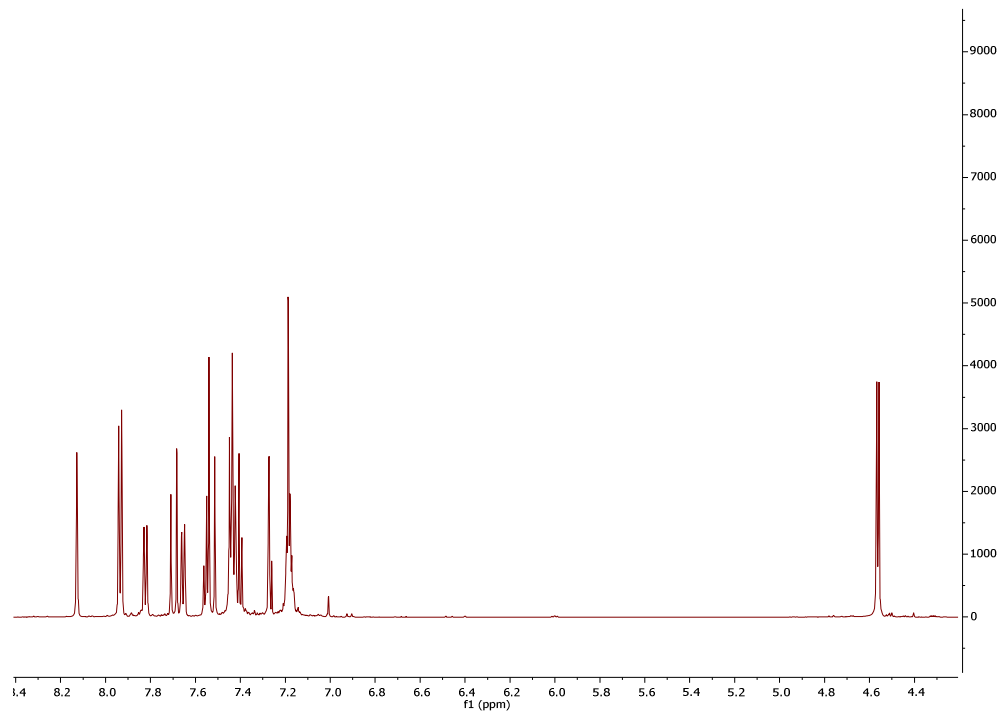


¹³C NMR

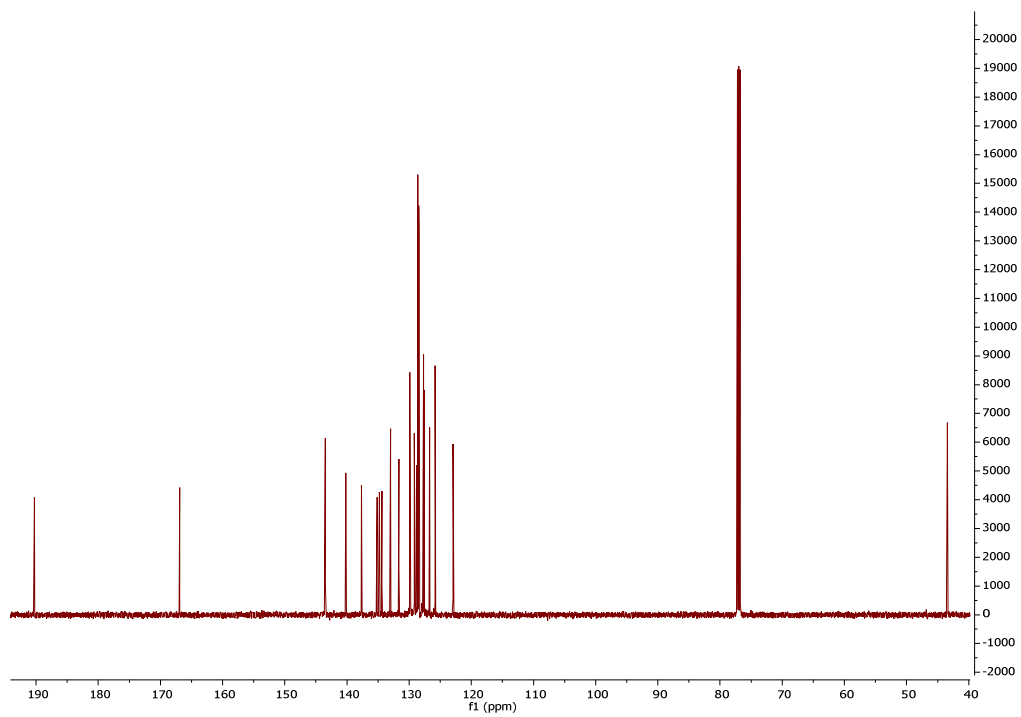


N-[(3-chlorophenyl)methyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5f**)

¹H NMR

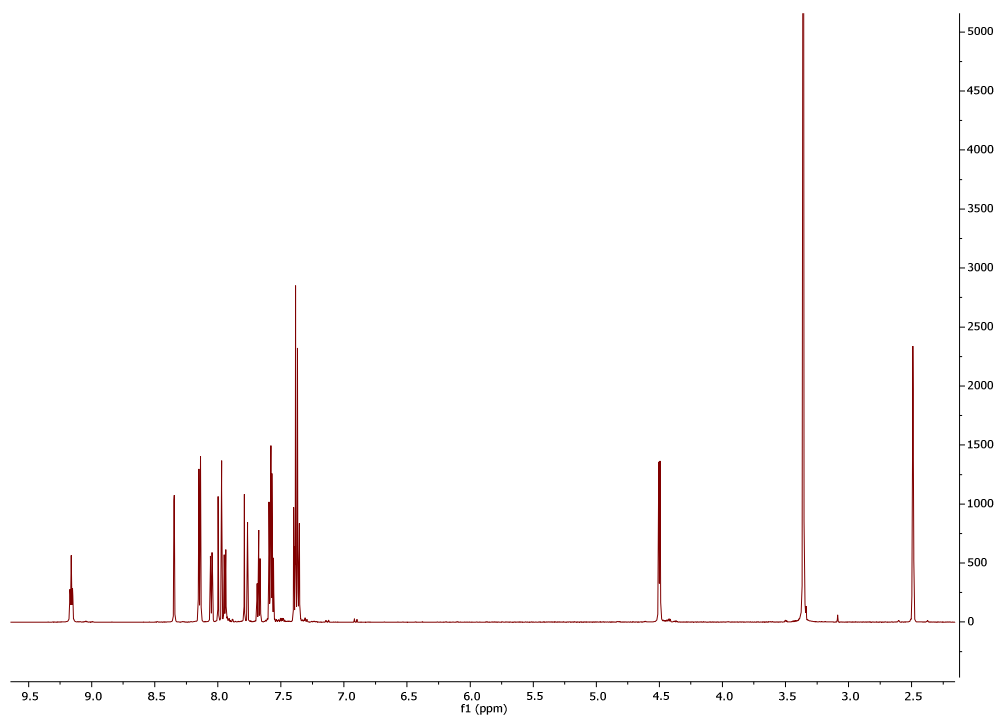


¹³C NMR

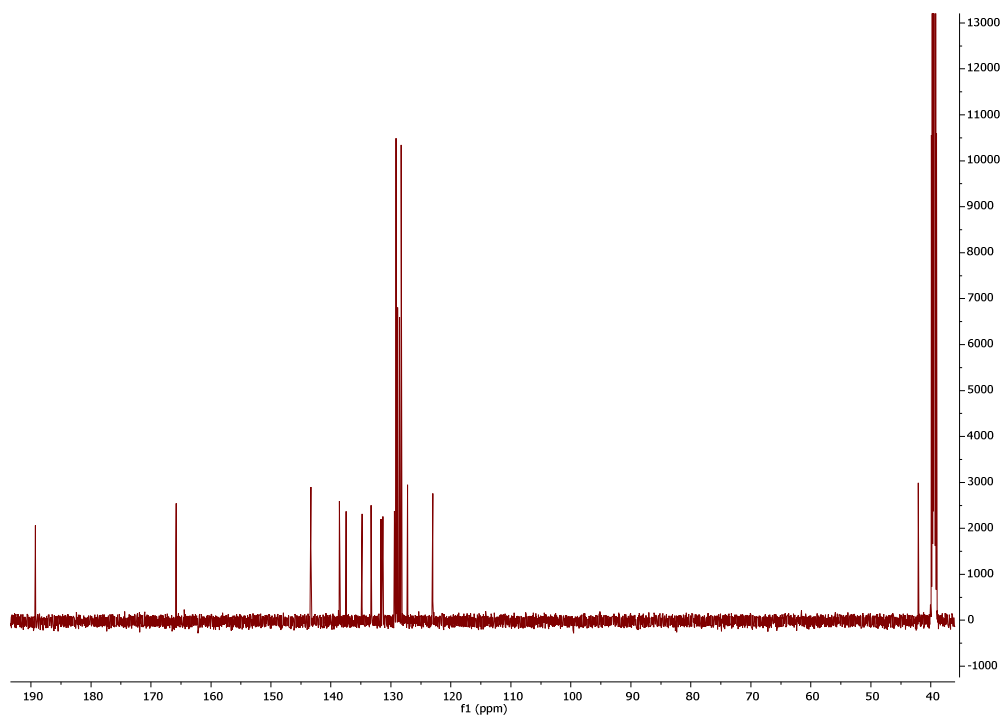


N-[(4-chlorophenyl)methyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5g**)

¹H NMR

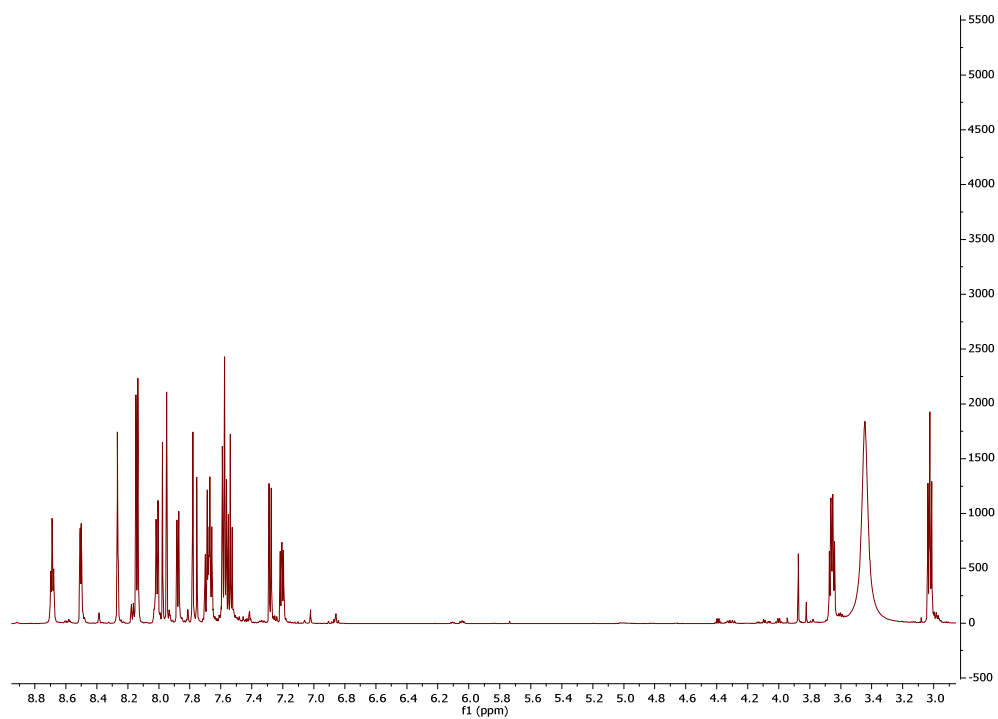


¹³C NMR

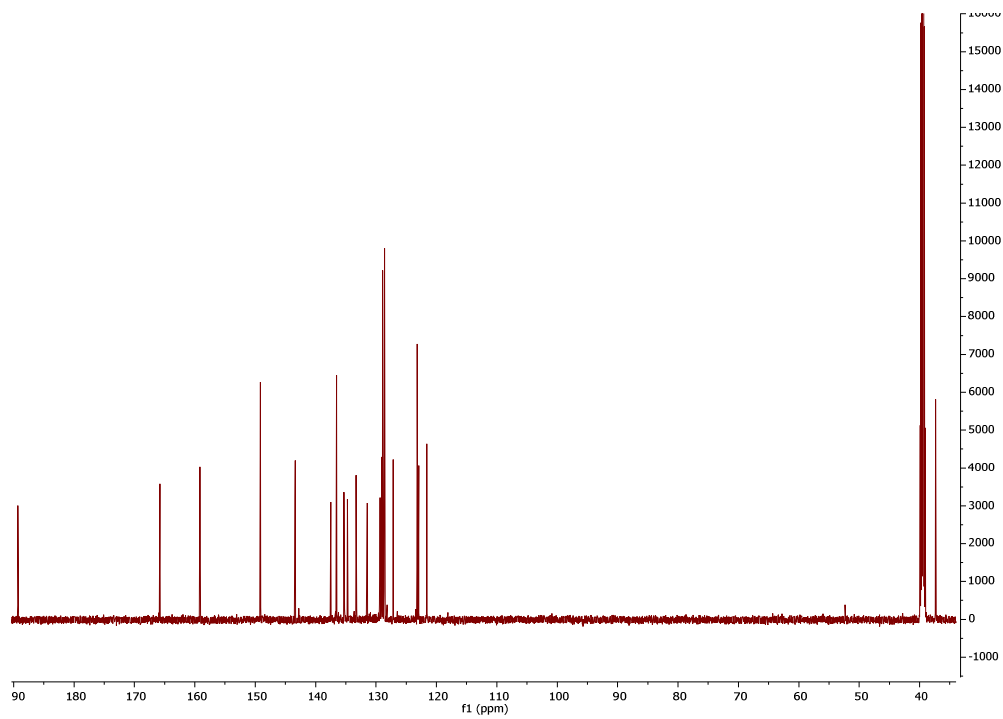


3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(pyridin-2-yl)ethyl]benzamide (**5h**)

¹H NMR

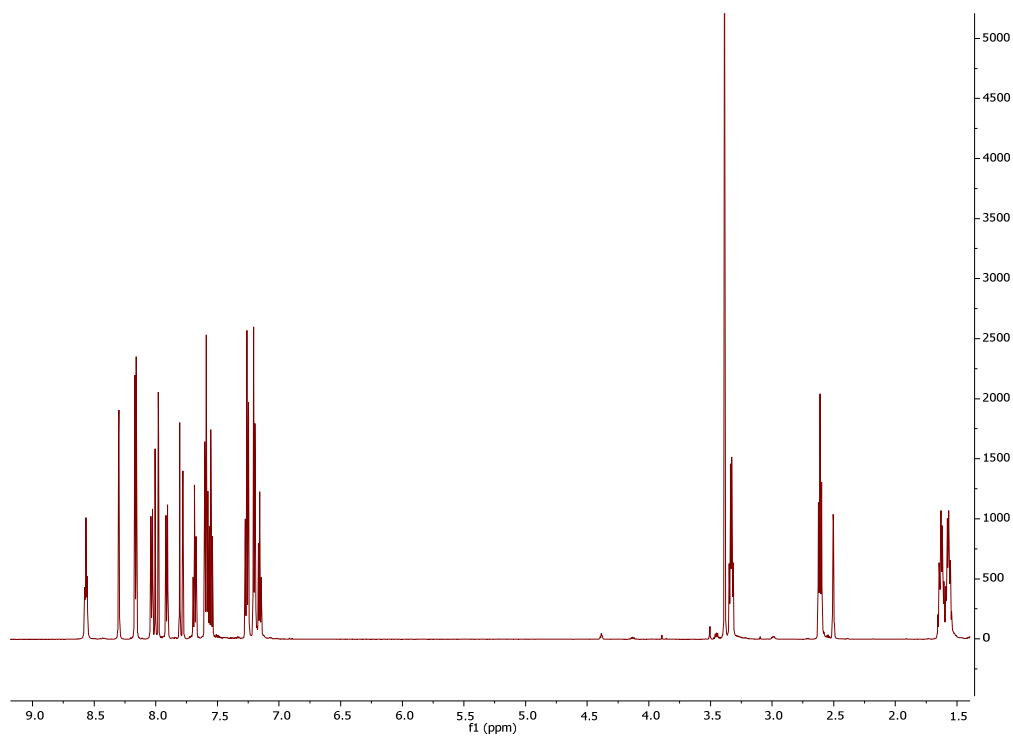


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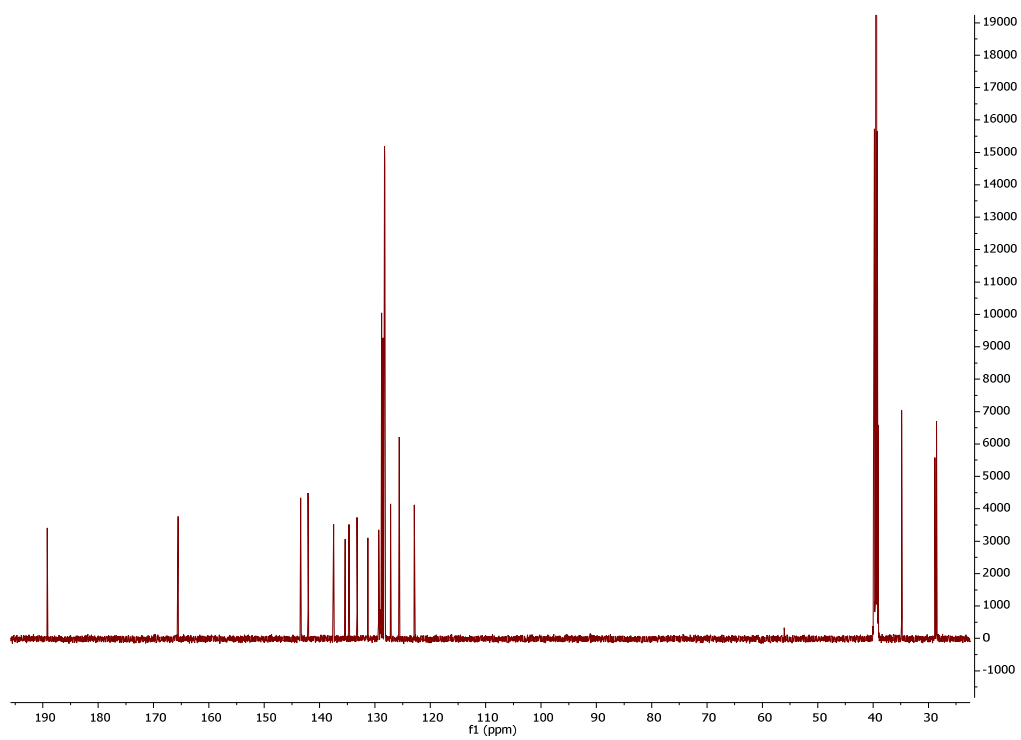


3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-(4-phenylbutyl)benzamide (**5i**)

¹H NMR

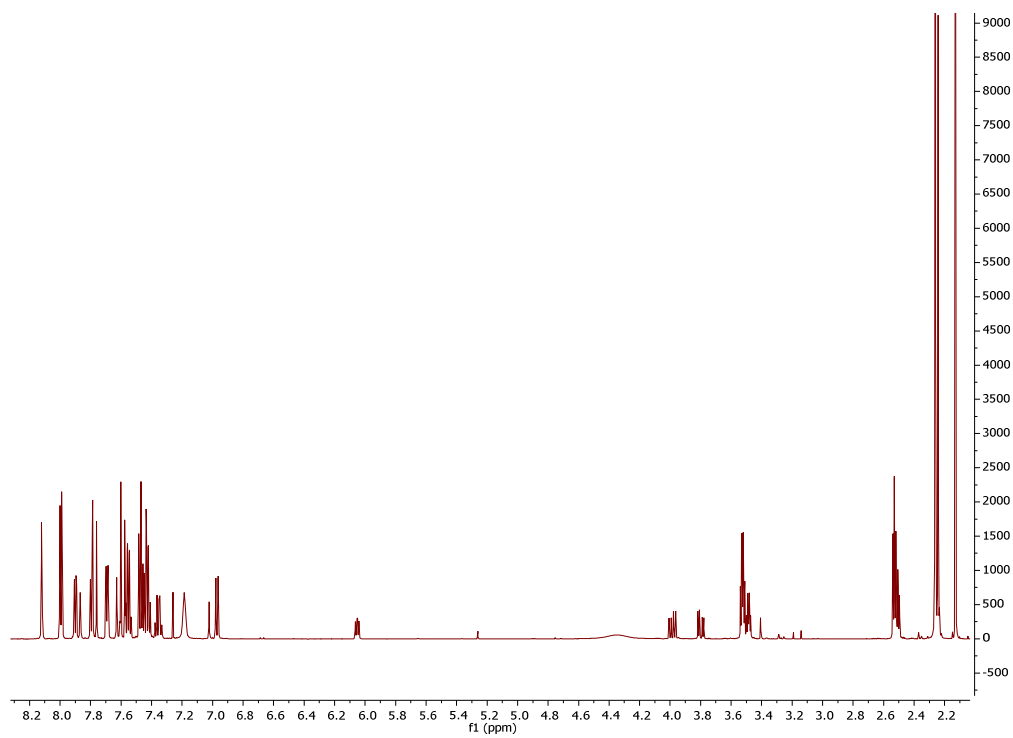


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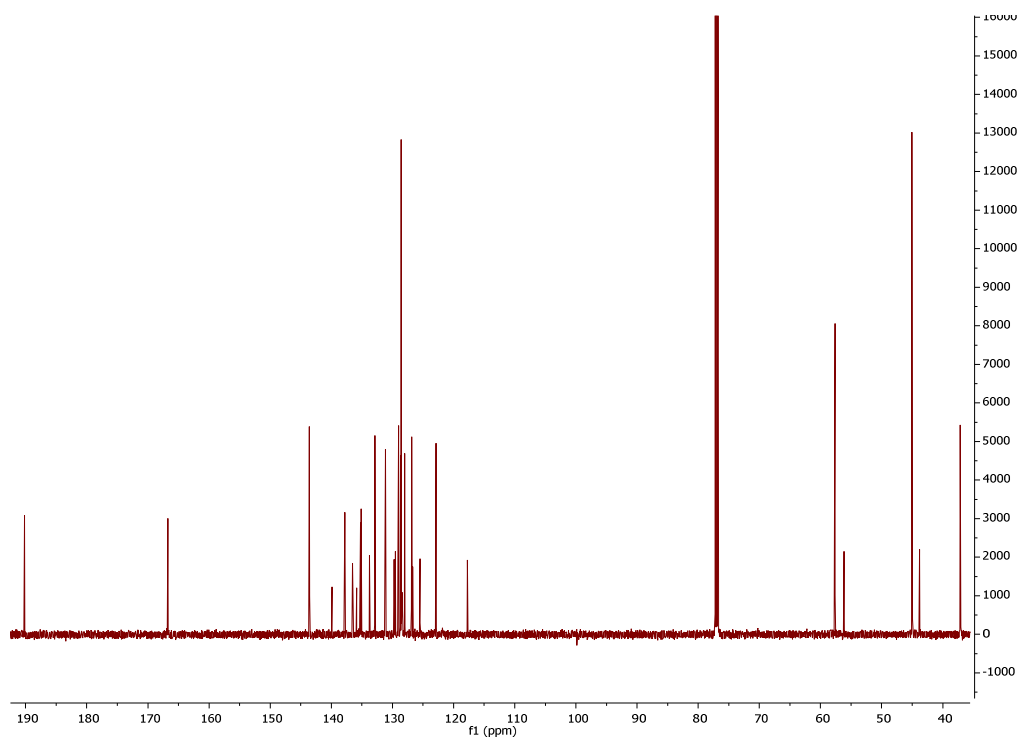


N-[2-(dimethylamino)ethyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5j**)

¹H NMR



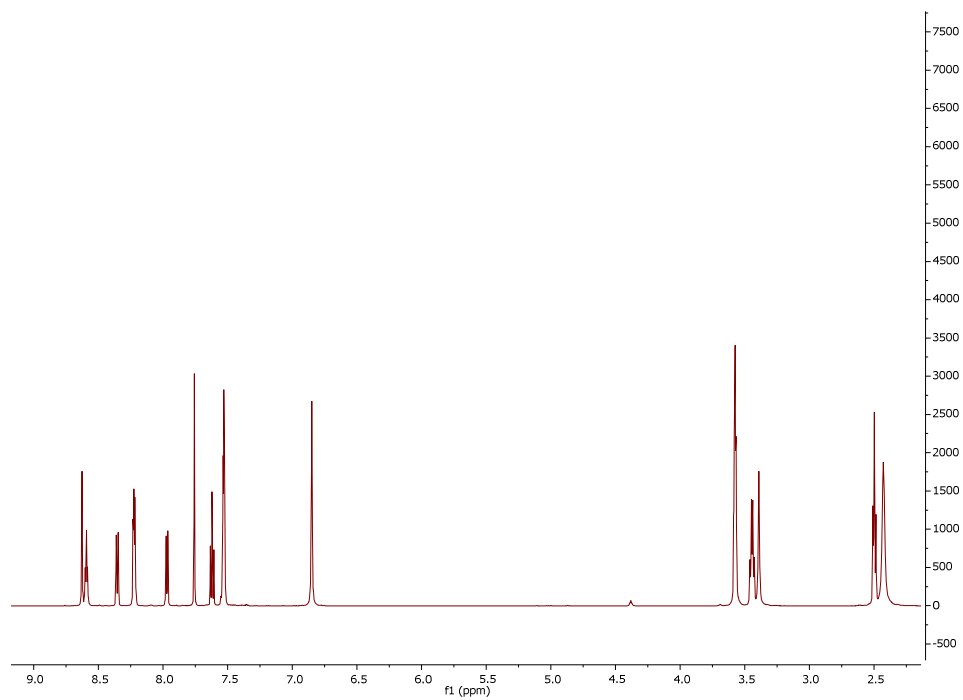
¹³C NMR



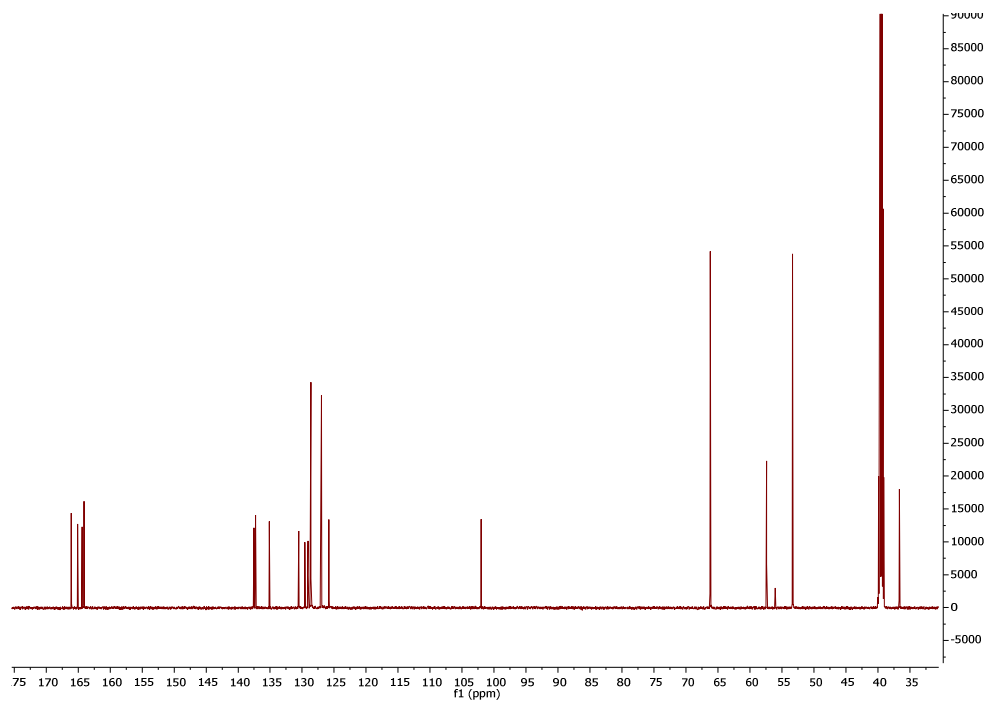
Aminopyrimidines

3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(morpholin-4-yl)ethyl]benzamide (**6a**)

¹H NMR

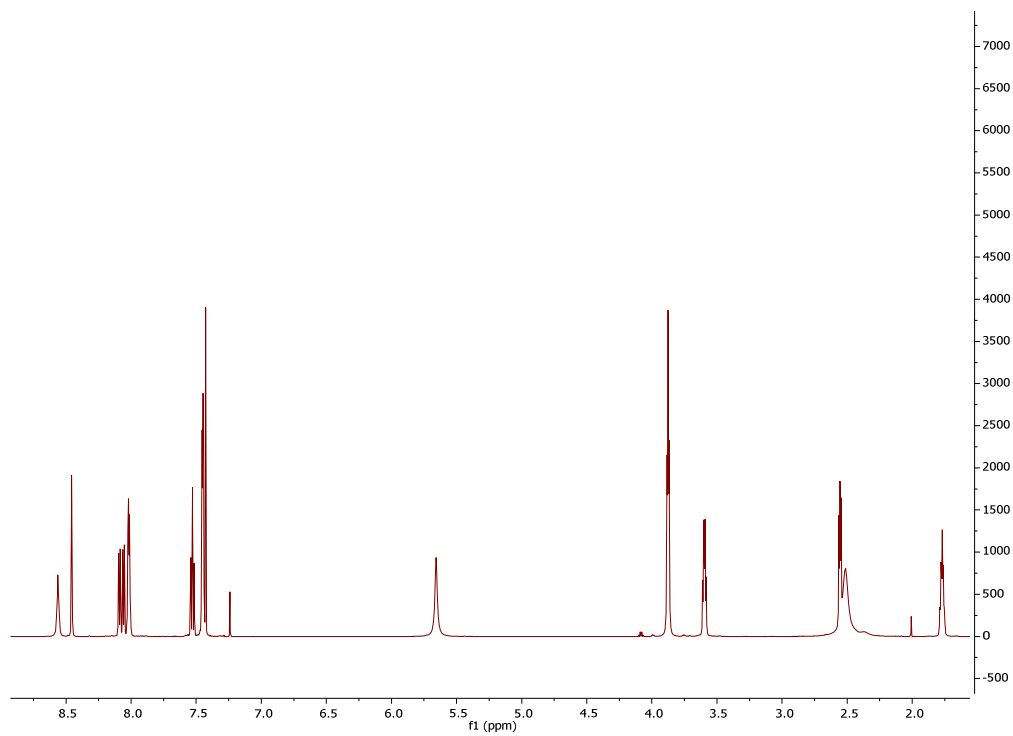


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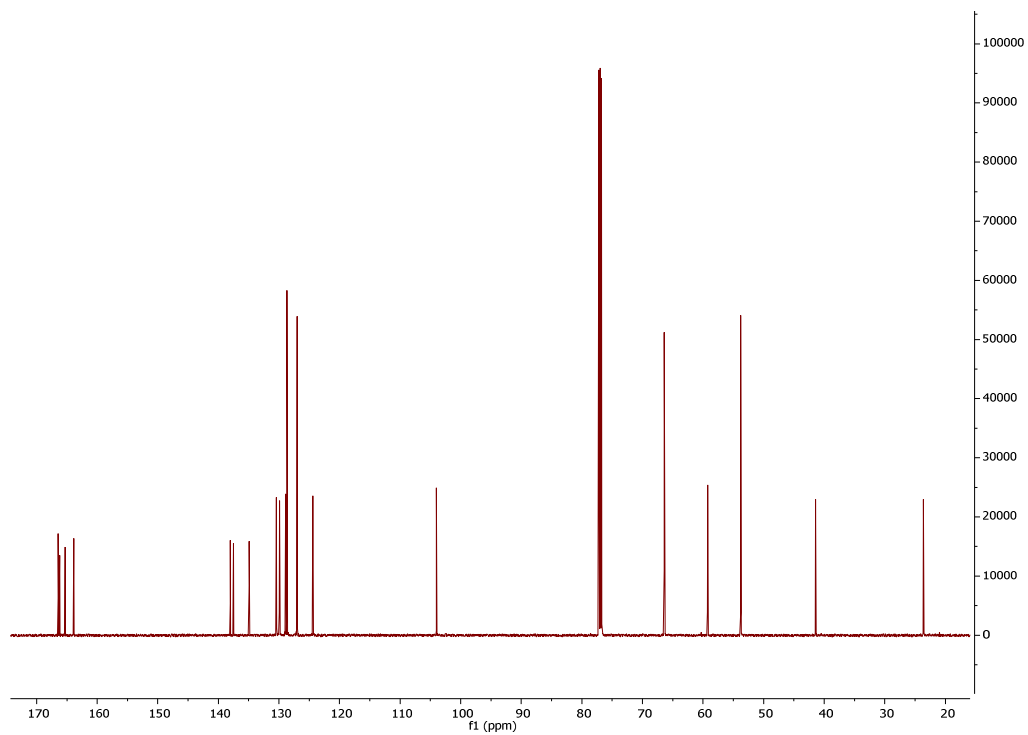


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[3-(morpholin-4-yl)propyl]benzamide (**6b**)

¹H NMR

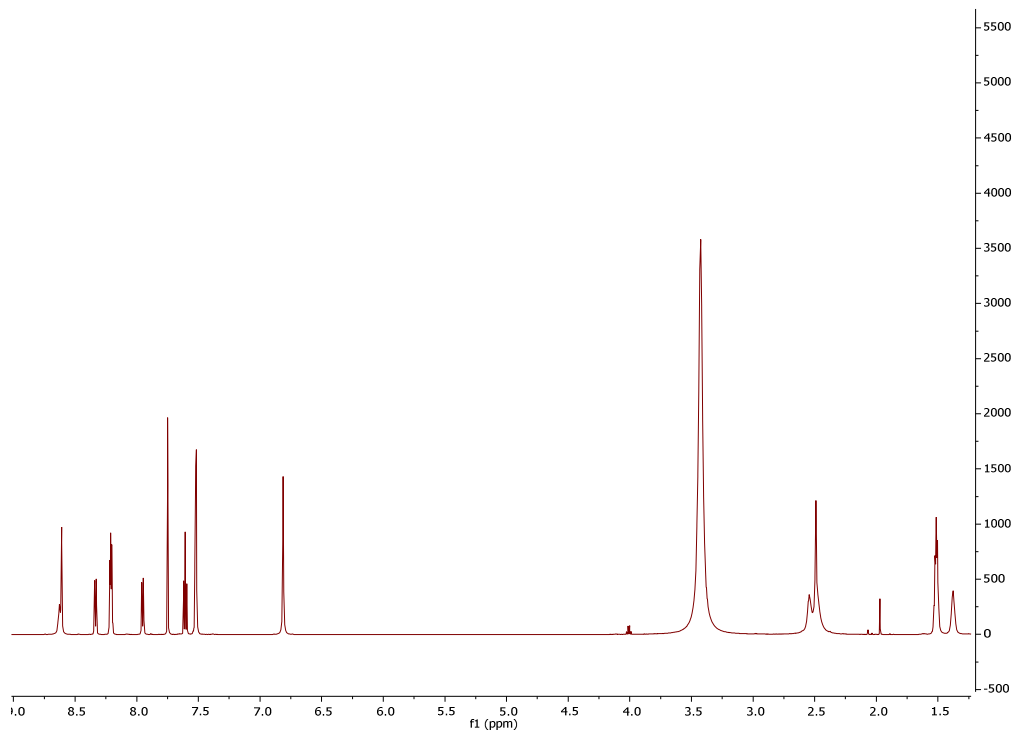


¹³C NMR

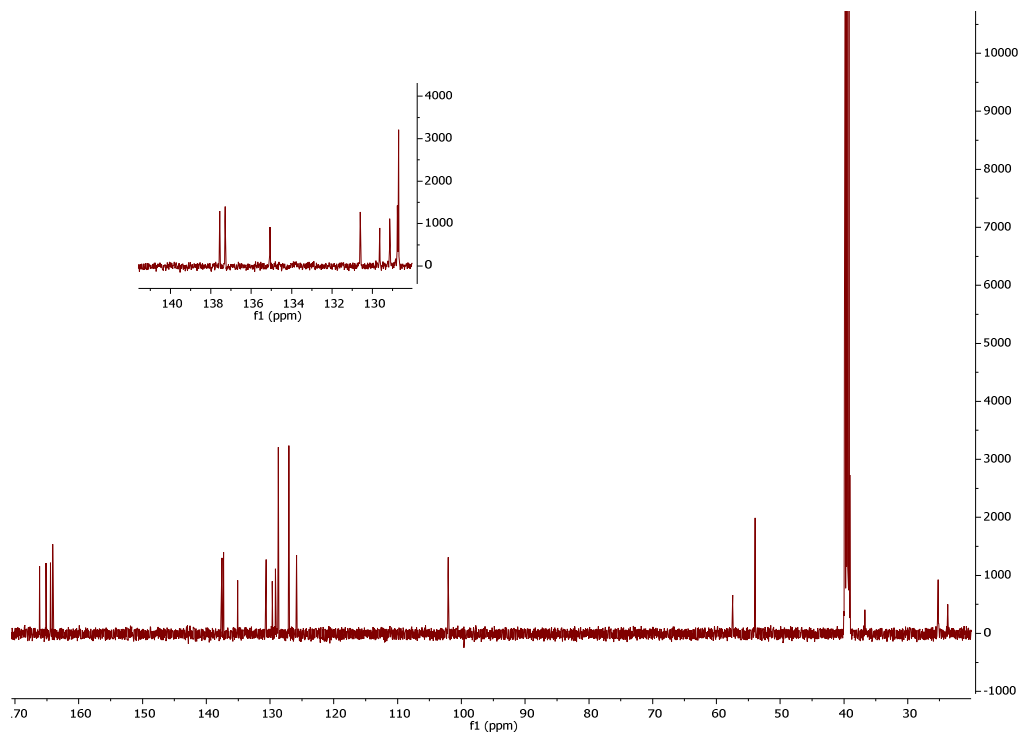


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(piperidin-1-yl)ethyl]benzamide (**6c**)

¹H NMR

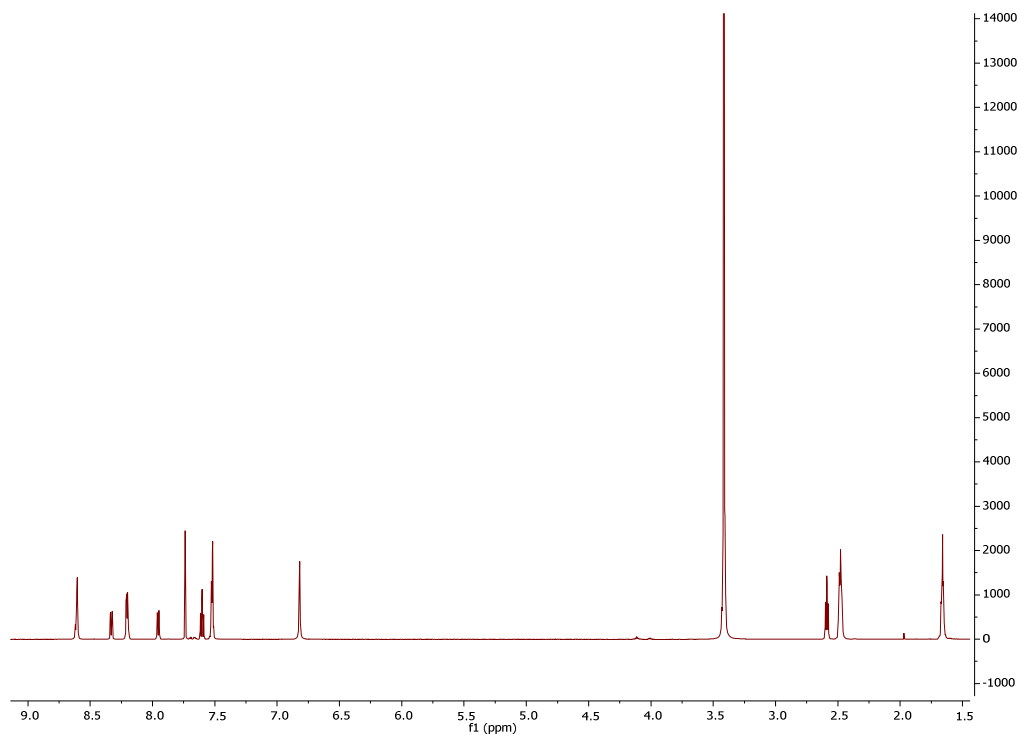


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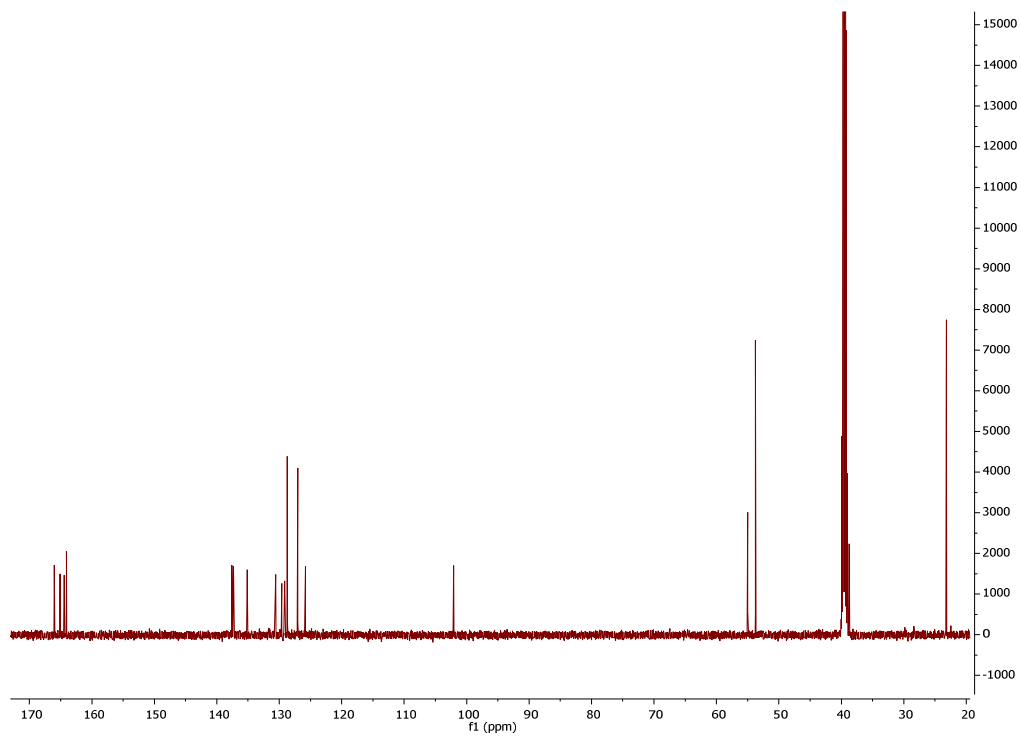


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(pyrrolidin-1-yl)ethyl]benzamide (**6d**)

¹H NMR

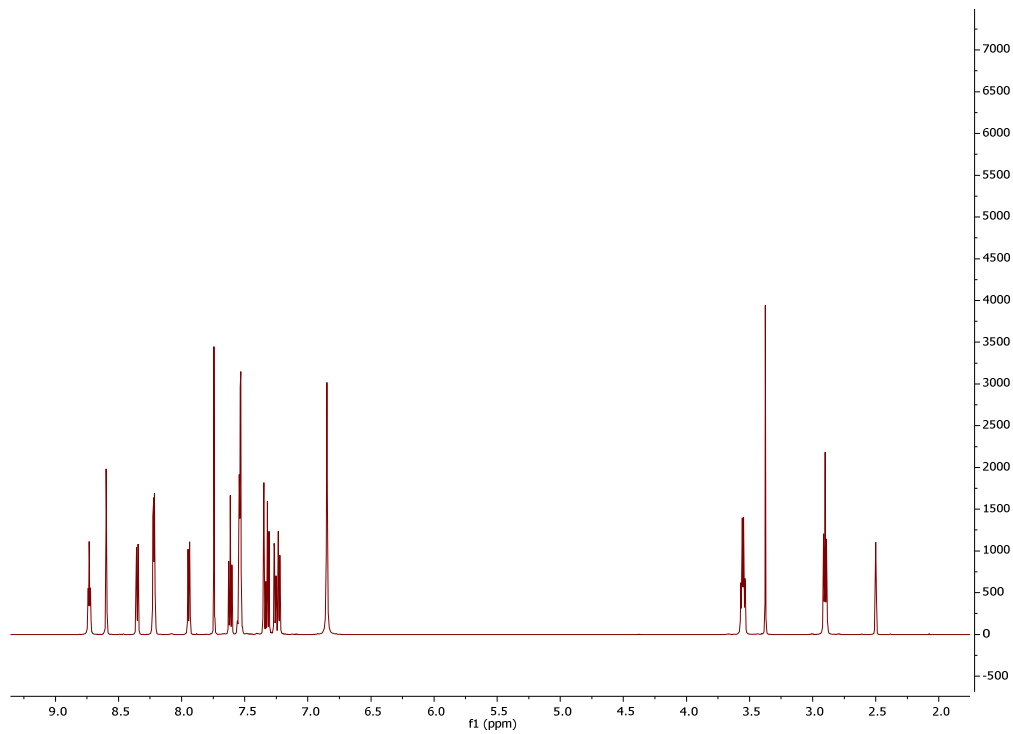


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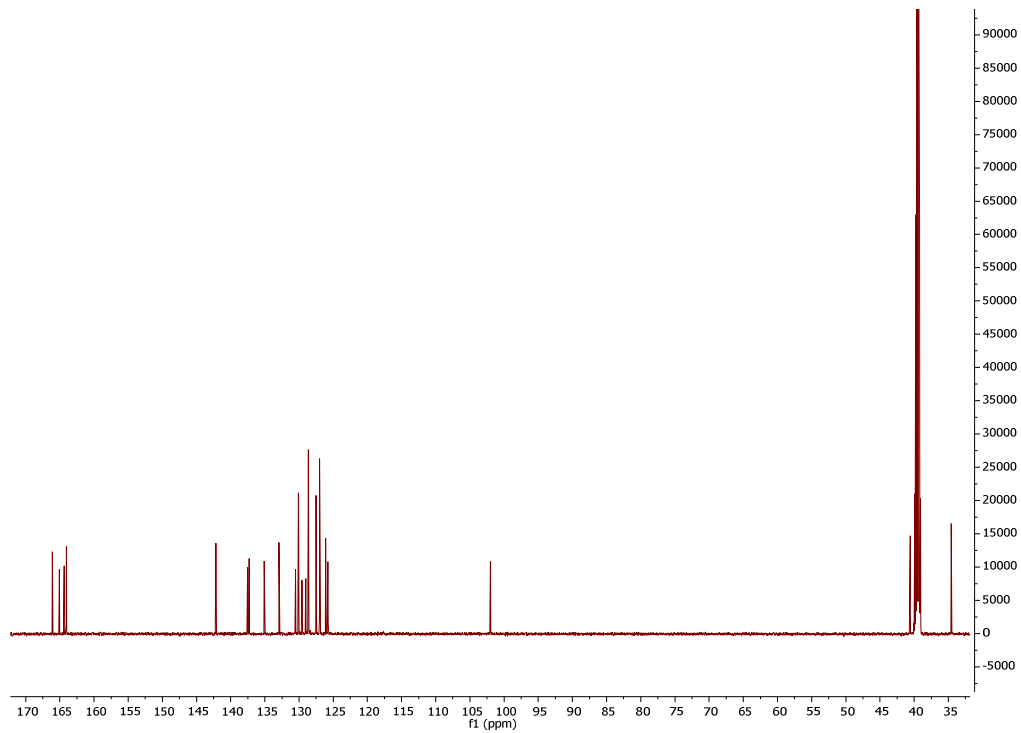


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(3-chlorophenyl)ethyl]benzamide (**6e**)

¹H NMR

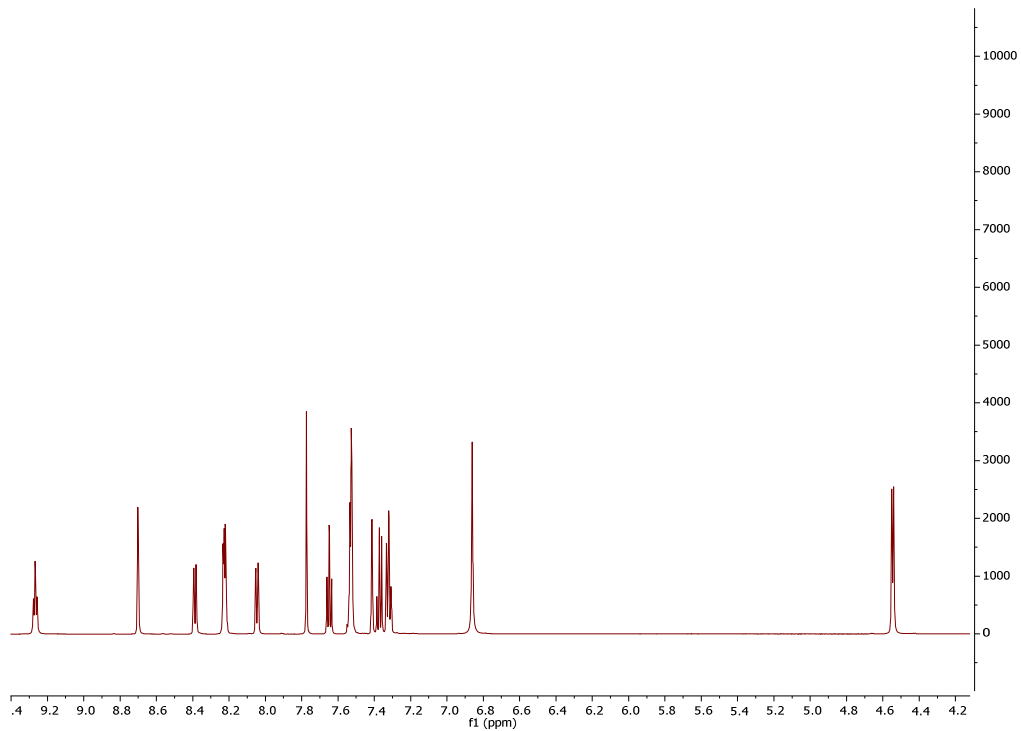


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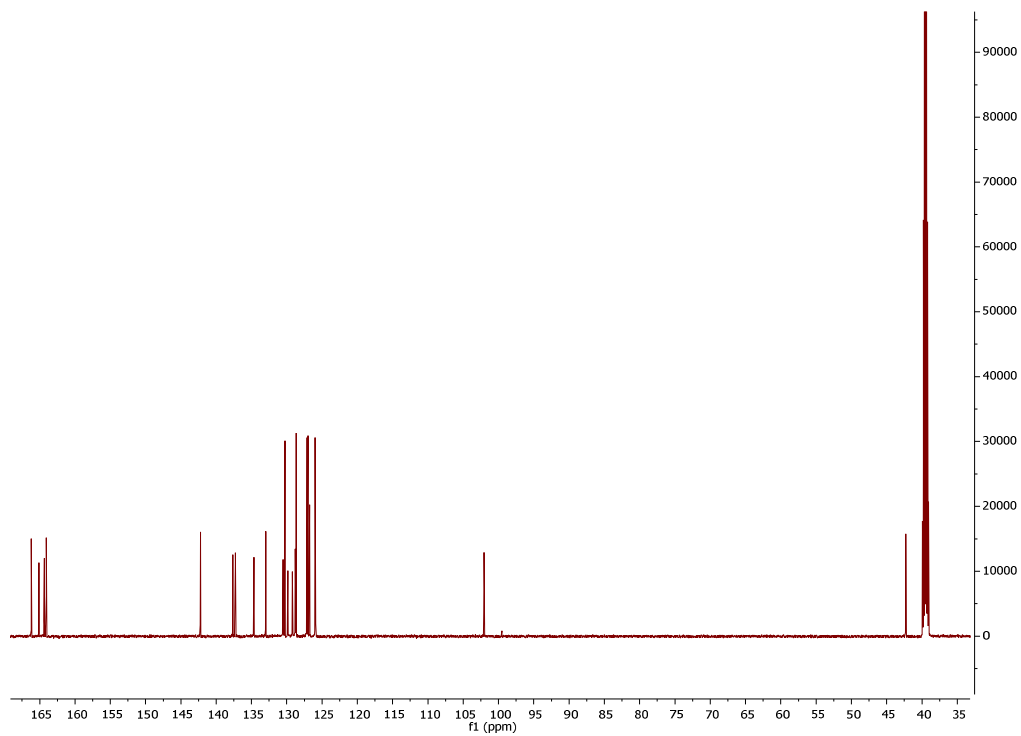


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[(3-chlorophenyl)methyl]benzamide (**6f**)

¹H NMR

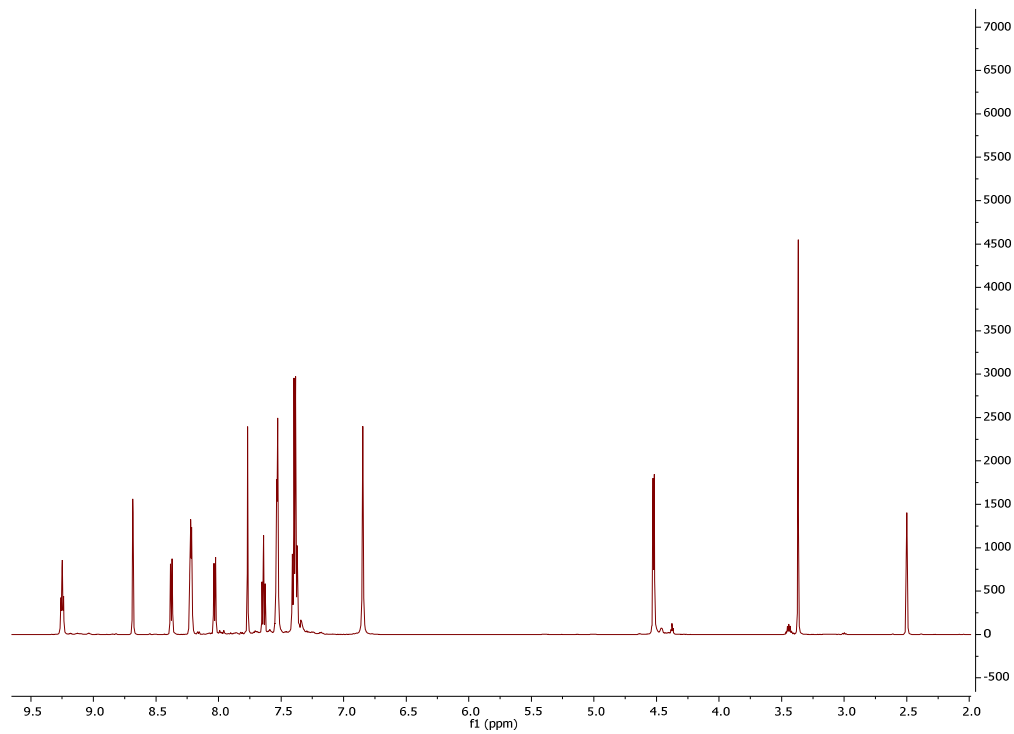


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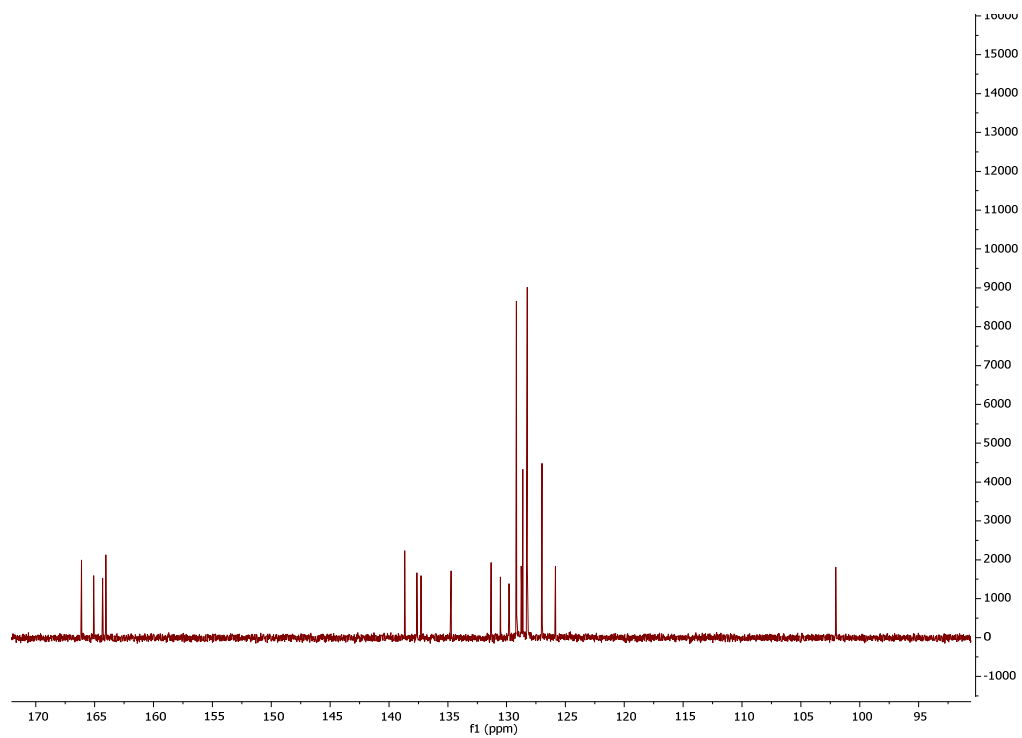


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[(4-chlorophenyl)methyl]benzamide (**6g**)

¹H NMR

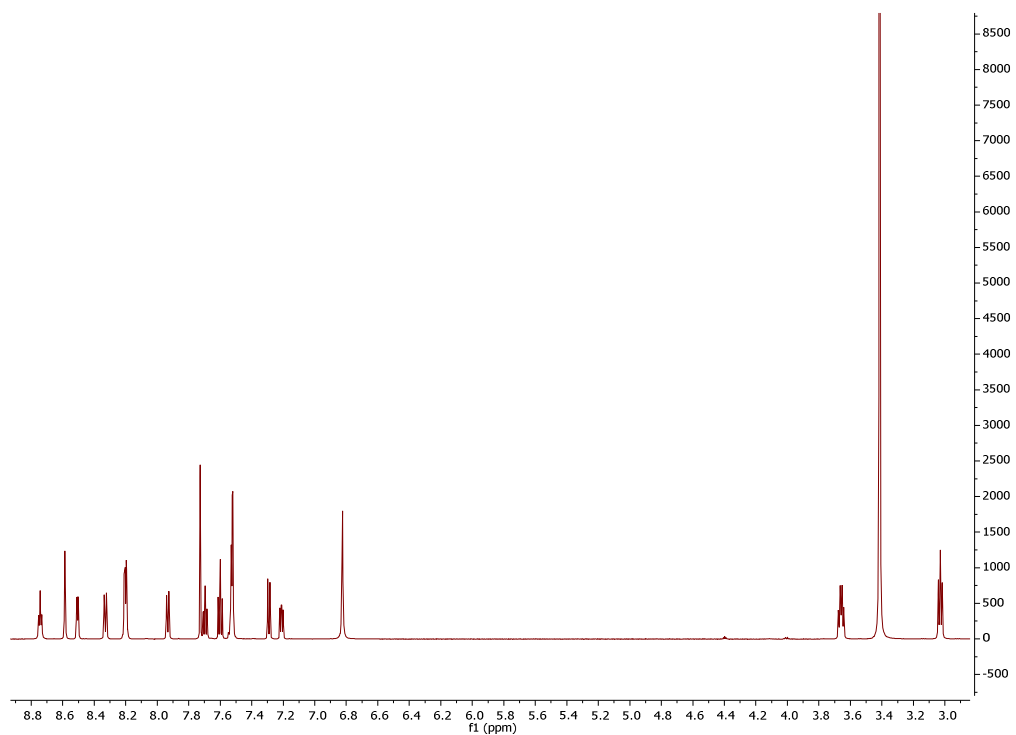


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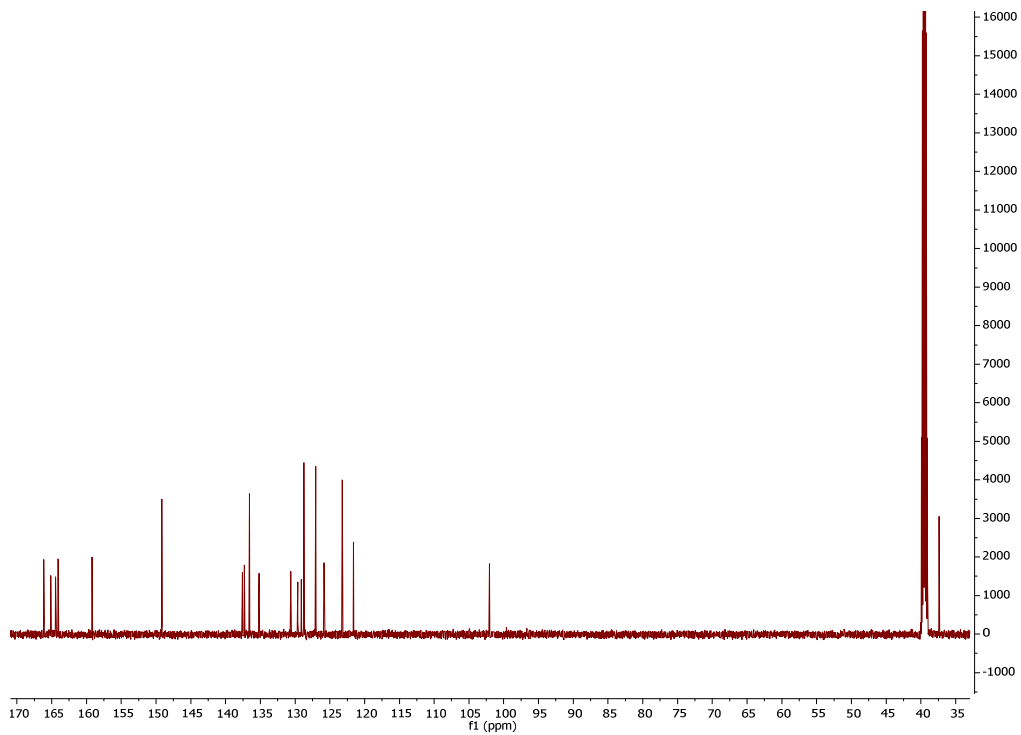


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(pyridin-2-yl)ethyl]benzamide (**6h**)

¹H NMR

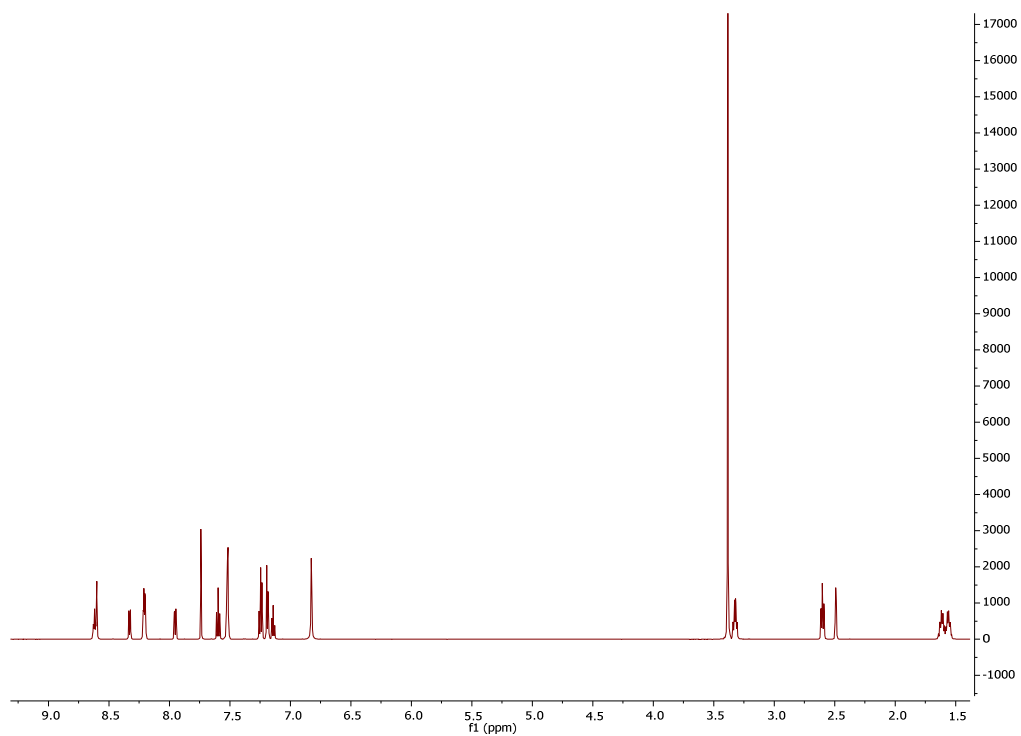


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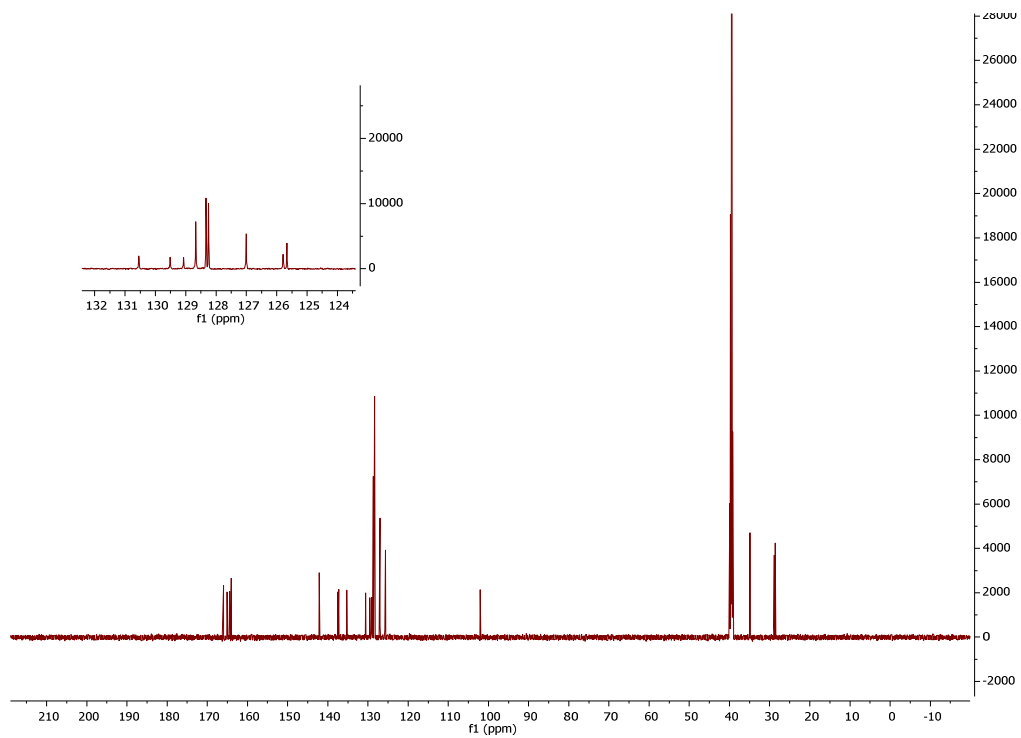


3-(2-amino-6-phenylpyrimidin-4-yl)-N-(4-phenylbutyl)benzamide (**6i**)

¹H NMR

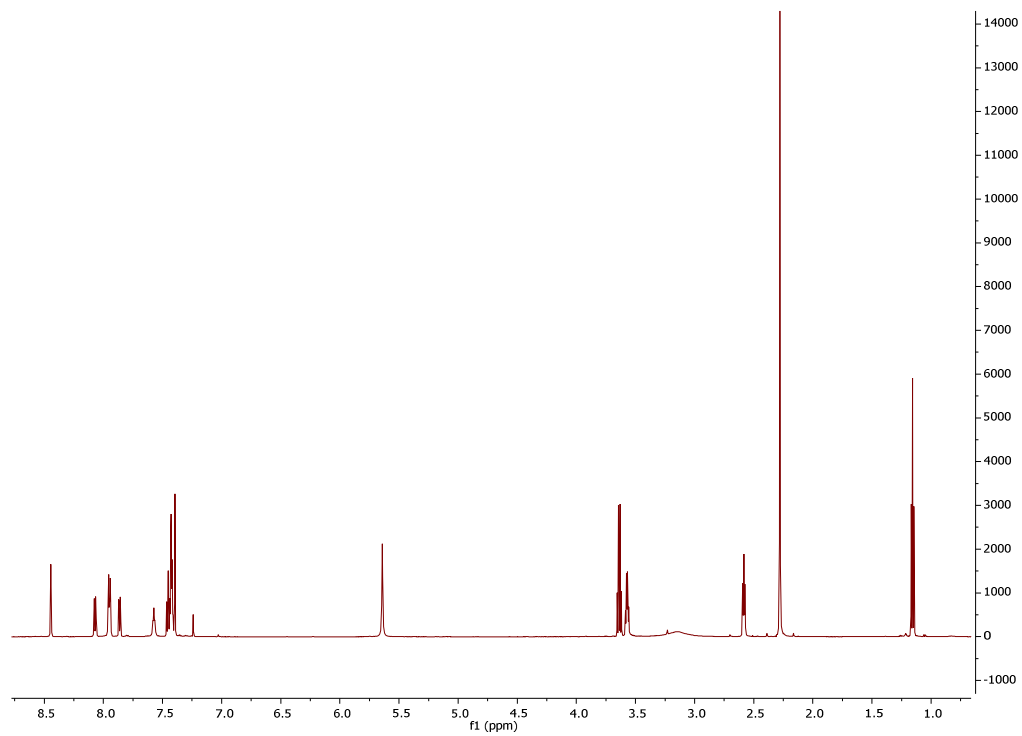


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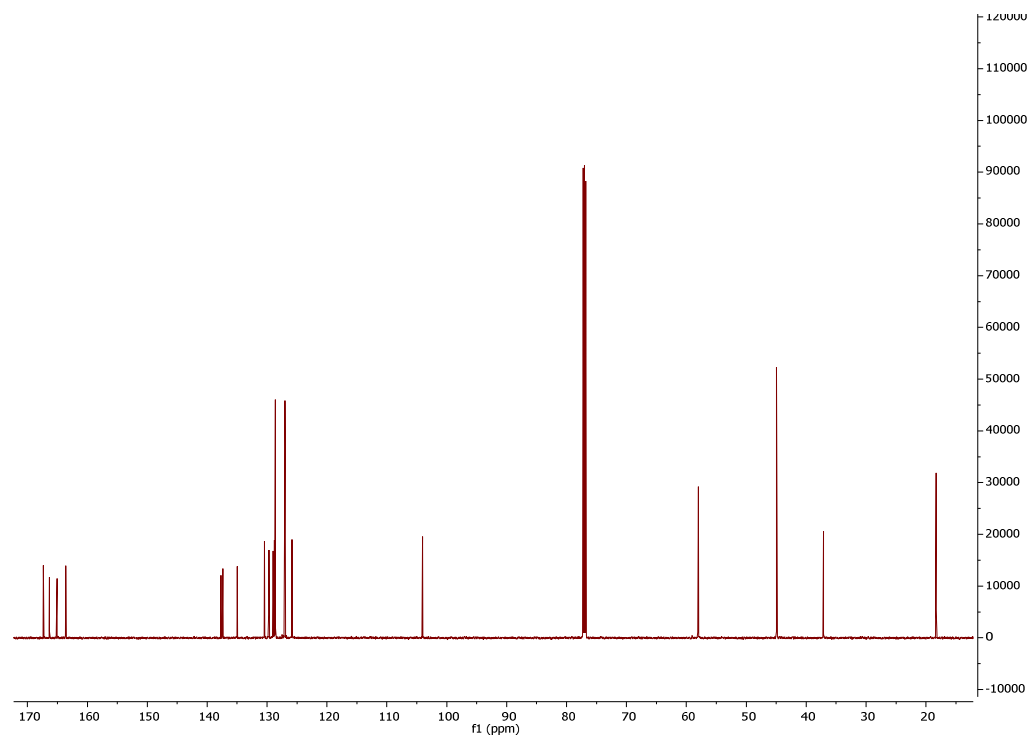


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(dimethylamino)ethyl]benzamide (**6j**)

¹H NMR



¹³C NMR



CHAPTER 5

Article 3

Carbamate substituted 2-amino-4,6-diphenylpyrimidines as adenosine receptor antagonists

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Abstract

A novel series of carbamate substituted 2-amino-4,6-diphenylpyrimidines was evaluated as potential dual adenosine A₁ and A_{2A} receptor antagonists. The majority of the synthesised compounds exhibited promising dual affinities, with A₁K_i values ranging from 0.175 – 10.7 nM and A_{2A}K_i values ranging from 1.58 - 451 nM. The *in vivo* activity illustrated for 3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate (**4c**) is indicative of the potential of these compounds as therapeutic agents in the treatment of Parkinson's Disease, although physicochemical properties may require optimisation.

Keywords: 2-aminopyrimidine; adenosine A₁ and A_{2A} receptor; dual; antagonist.

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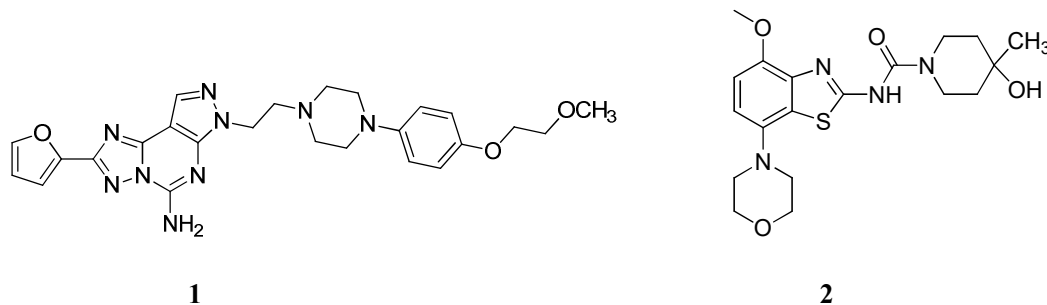
Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease that affects over 1% of the world population. PD is an age related disease, and with life expectancy increasing worldwide, will continue to present a huge social and economic burden in the future.¹ Patients with PD mainly suffer from a progressive loss of motor function, but non-motor symptoms, such as cognitive impairment and depression often occur.² The motor symptoms of the disease can be attributed to the deterioration of dopaminergic neurons in the striatum, resulting in a significant loss of dopamine in this region.³ There is still no cure for PD, but the dopaminergic therapies used clinically are reasonably effective in managing the symptoms during the early stages of the disease. Long-term treatment with dopaminergic therapies however, is associated with several undesirable side effects such as loss of drug efficacy, dyskinesia and depression.⁴

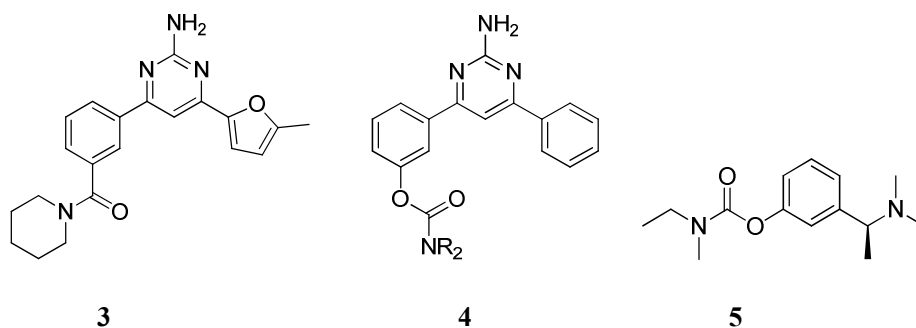
Adenosine is an endogenous ligand that acts as a neurotransmitter in the brain through the activation of its G-protein coupled receptors, namely the A₁, A_{2A}, A_{2B} and A₃ receptors.⁵ Recently, dual targeted antagonism of adenosine A₁ and A_{2A} receptors has emerged as a promising non-dopaminergic alternative for the treatment of neurodegenerative diseases such as Parkinson's disease.^{6,7} The appeal of adenosine A_{2A} receptors as a target in movement disorders is due to their distinct localisation in the striatum as well as their unique integrative action with dopamine D₂ receptors.⁸ Adenosine A_{2A} receptors and dopamine D₂ receptors have a mutual antagonistic interaction, which means that antagonism of adenosine A_{2A} receptors would lead to enhanced D₂ signalling, providing a rationale for their use in the symptomatic treatment of PD.⁹ The use of A_{2A} antagonists adjunctive to current dopaminergic therapy may be beneficial since the dosage of the dopaminergic drugs administered could potentially be lowered.¹⁰ This may reduce the occurrence of dyskinesia and other side effects associated with dopaminergic drugs.¹¹ Furthermore, it has been suggested that A_{2A} antagonism may halt the progression of PD as preclinical evidence exists of its possible neuroprotective benefits.^{12,13} Depression is a co-morbidity that often decreases quality of life in PD patients, especially in the later stages of the disease. A recent study illustrated the antidepressant effect of the known A_{2A} antagonist, KW6002, indicating an additional advantage of A_{2A} antagonism.¹⁴

The brain distribution of the adenosine A₁ receptor on the other hand, is more widespread than that of the A_{2A} receptor, with high levels expressed in the striatum, hippocampus and neocortex.¹⁵ Similar to adenosine A_{2A} antagonism, antagonism of A₁ receptors have been shown to result in activation of motor function in animals,^{16,17} and may thus decrease motor deficiencies experienced in PD. It has also been reported that the antagonism of the A₁ receptor may enhance cognitive ability,¹⁸⁻²¹ and since a decline in cognition is often observed in PD patients over time, A₁ antagonism may thus be advantageous.²² Dual antagonism of A₁ and A_{2A} receptors thus has the potential of addressing the multifactorial nature of PD symptoms with less dopaminergic side-effects than generally experienced with current therapies.

The best known adenosine receptor antagonists are the xanthines, such as caffeine and theophylline, which as a chemical class, have been reviewed extensively.¹⁸ Several heterocyclic compounds have also progressed to clinical trials, and include compounds such as preladenant (**1**) and tozadenant (**2**).^{24,25}



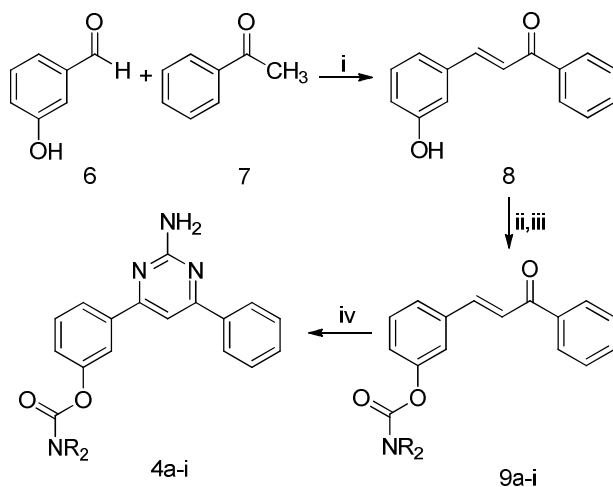
Of particular interest to our group was the fact that the 2-aminopyrimidine motif often occurred in heterocycles with adenosine A_{2A} and/ or A_1 affinity.^{7,25-29} Based on the aforementioned results, we set out to synthesise a small library of 2-aminopyrimidines to investigate the potential of these compounds as dual adenosine A_1 and A_{2A} antagonists. In this preliminary study, we synthesised amide derivative **3** which exhibited high dual affinity ($A_1 K_i = 9.54$ nM; $A_{2A} K_i = 6.34$ nM) and *in vivo* activity.³⁰



In order to further investigate the affinities of this class of compounds for adenosine receptors, we decided to synthesise a series of carbamate substituted 2-amino-4,6-diphenylpyrimidines (**4**). The carbamate moiety is often present in therapeutic agents, such as rivastigmine (**5**), an acetylcholinesterase inhibitor, which is used in the treatment of Alzheimer's disease. This amide-ester hybrid generally displays very good chemical and proteolytic stability and may increase permeability across cellular membranes.³¹ The addition of the extra oxygen in structures such as **4**, alters the position of both the carbonyl and nitrogen groups and has the potential to change the hydrogen bonding between the compound and the receptor binding site, thus changing the affinity and possibly the selectivity of these compounds in comparison with the amide derivatives (**3**). It was also decided to replace the methyl furan substituent on position 4 with a phenyl ring, as this simplified the synthesis. Gratifyingly, preliminary results indicated that this change did not alter affinity to a significant degree (Kleynhans, 2014).

Results and discussion

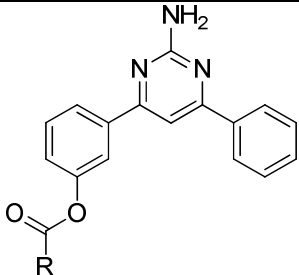
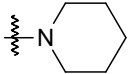
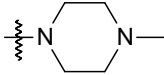
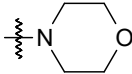
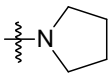

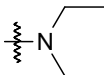
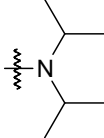
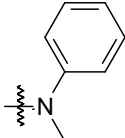
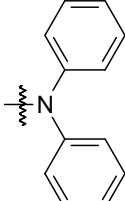
Chemistry



Scheme 1. Synthesis. Reagents and conditions: (i) NaOH, 1 M (2 eq.), MeOH, 90 °C, 5 days (60%); (ii) K₂CO₃ (2 eq.), CH₃CN, rt, 30 min; (iii) Carbamoyl chloride (1.2 eq.), reflux, 90 °C, overnight (70-90%); iv) Guanidine hydrochloride (1.5 eq.), NaH (1.5 eq.), DMF, 110 °C, overnight (15-30%).

Nine carbamate substituted 2-amino-4,6-diphenylpyrimidines (Table 1) were successfully synthesised as indicated in Scheme 1. Firstly, 3-hydroxybenzaldehyde (**6**) was condensed with acetophenone (**7**), yielding chalcone (**8**), which was reacted with commercially available carbamoyl chlorides to obtain carbamates (**9a - i**).³² Cyclisation was carried out with guanidine hydrochloride and sodium hydride in *N,N*-dimethylformamide yielding the desired 2-aminopyrimidines (**4a-i**) in low yields.³³ Initially, three equivalents of sodium hydride were used in the cyclisation step, but this resulted in cleavage of the carbamate group. This problem was overcome by reducing the number of molar equivalents of NaH used in the reaction. The structures of all synthesised compounds (Table 1) were confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry, while purity was assessed by HPLC (see supporting information).

Table 1: Adenosine receptor affinities (K_i) of the synthesised carbamates **4a** – **4i**.

				
Compound	R	A_1^a K_i (nM)	A_{2A}^a K_i (nM)	SI^b (A_{2A}/A_1)
4a		1.95 ± 0.278	8.94 ± 0.259	4.58
4b		2.04 ± 0.002	1.58 ± 0.311	0.775
4c		2.65 ± 0.059	3.50 ± 0.030	1.32
4d		0.835 ± 0.088	24.8 ± 6.61	29.7
4e		2.06 ± 0.135	51.9 ± 3.07	25.2
4f		0.468 ± 0.057	22.8 ± 1.75	48.7
4g		0.175 ± 0.017	12.1 ± 1.35	69.1
4h		1.07 ± 0.079	39.9 ± 4.77	37.3
4i		10.7 ± 0.431	451 ± 143	42.1
°CPA		8.48 ± 0.302		

^d ZM241385			1.20 ± 0.387	
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^aAll values are expressed as the mean ± SEM of duplicate determinations.

^bSelectivity index (A_{2A}/A_1)

^cN⁶-Cyclopentyladenosine, a known adenosine A_1 agonist, used as positive control for A_1 receptor affinity – literature $K_i = 7.9$ nM³⁵

^dA known adenosine A_{2A} receptor antagonist, used as positive control for A_{2A} affinity – literature $K_i = 2$ nM¹⁸

Biological assays

Radioligand binding assays were performed to assess the binding of synthesised compounds to adenosine receptors. The radioligands used were 1,3-[³H]-dipropyl-8-cyclopentylxanthine ([³H]DPCPX) for adenosine A_1 receptors, and [³H]5'-N-ethylcarboxamide-adenosine ([³H]NECA) for adenosine A_{2A} receptors. Striata from male Sprague-Dawley rats were used as receptor source for A_{2A} binding studies, while whole brains were utilised for A_1 binding studies (Ethics number: NWU-0035-10-A5). IC₅₀ values were obtained from sigmoidal dose response curves as generated by the Prism 5 software package (GraphPad) and the K_i values were calculated from the IC₅₀ values using the Cheng-Prusoff equation.³⁴ Results from the receptor binding studies are presented in Table 1.

The results reveal that most of these compounds have potent dual affinity for both receptor subtypes, although affinities are generally higher for the adenosine A_1 receptor compared to the adenosine A_{2A} receptor. Compounds **4a**, **4b** and **4c** are the most promising candidates for dual antagonistic activity with both A_1K_i and $A_{2A}K_i$ values below 10 nM and selectivity indices of 4.6, 0.8 and 1.3, respectively. Compounds **4f** and **4g** in particular exhibit high affinities for the adenosine A_1 receptors, with K_i values of 0.468 and 0.175 nM, respectively. As exemplified by compounds **4a**, **4b** and **4c**, substitution with six-membered saturated cyclic carbamate substituents appears to yield optimal A_{2A} receptor affinity. Interestingly, although compound **4i** with diphenyl substitution still has high affinity for the adenosine A_1 receptor ($K_i = 10.7$ nM), its affinity for the adenosine A_{2A} receptor is comparatively weak with a K_i value of 451 nM. The size of the carbamate substituent therefore appears to affect affinity to a greater degree for A_{2A} receptors, where substitution with groups that are either too small (e.g. **4e**) or too large (e.g. **4h** and **4i**) appears to be detrimental for affinity. By comparison, the A_1 receptors are able to accommodate larger carbamate substituents without loss of binding affinity.

Molecular modelling studies were performed in an attempt to rationalise the results obtained with the radioligand binding assays. The crystal structure of the human A_{2A} receptor crystallised with the known adenosine A_{2A} antagonist ZM241385 (PDB code: 3EML) was used as protein model. All compounds were successfully docked into the binding site using the C-DOCKER function of Discovery studio 3.1 (Accelrys). Hydrophobic (π - π) interactions were observed between the three-membered ring system

and Phe168 for all compounds. Intermolecular hydrogen bonding interactions were also present between the exocyclic amino group of the pyrimidine ring and Glu169 as well as Asn253 (Figure 1) for most derivatives. These interactions are believed to be responsible for anchoring the aminopyrimidine in the binding site and are similar to binding interactions previously determined for other 2-aminopyrimidine antagonists.³⁵ There was another noticeable hydrogen bond interaction between Glu169 and the carbamate carbonyl of most compounds. When ranking the compounds according to C-DOCKER - and C-DOCKER-interaction energies, it was observed that compound **4i** exhibited the least favourable values. This corresponds with the results obtained in the radioligand binding studies, where compound **4i** also exhibited the weakest affinity for the A_{2A} receptor among the compounds synthesised ($K_i = 451$ nM). Visual inspection of the lowest energy pose of compound **4i** reveals that the hydrogen bonding interactions with Glu169 and Asn253 are absent and that it has docked “upside down” in the binding site when compared to the rest of the synthesised compounds (Figure 2A), as well as ZM241385 (Figure 2B). This supports the theory that A_{2A} receptor affinity is influenced by molecular size, with the receptor binding site unable to accommodate this large compound (**4i**). These molecular docking results provide, at least in part, some explanation for the results obtained with the radioligand binding assays.

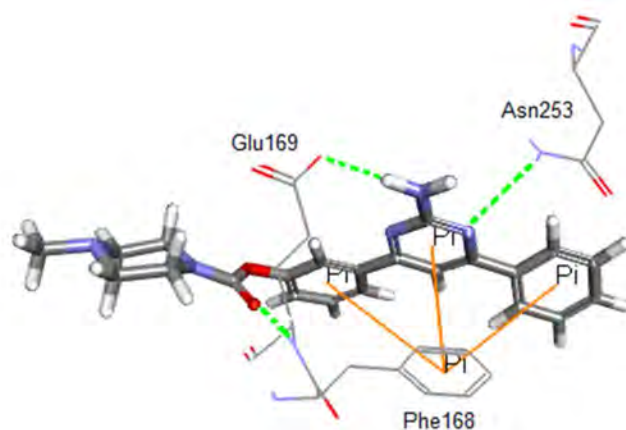


Figure 1. Compound **4b** docked in the binding site of the human A_{2A} receptor. Hydrophobic interactions are indicated in orange while intermolecular hydrogen bonding are indicated in green.

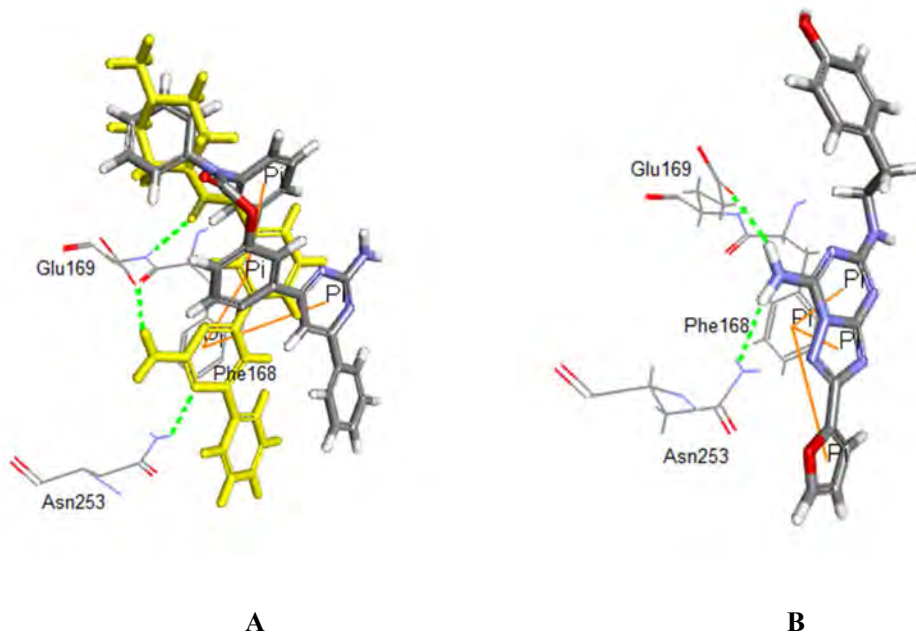


Figure 2A. Compounds **4i** (in colour) and **4b** (in yellow) docked in the binding site of the human A_{2A} receptor. **2B.** ZM241385 bound in the binding site of the human A_{2A} receptor.³⁶ Hydrophobic interactions are indicated in orange while intermolecular hydrogen bonding are indicated in green.

The most promising dual affinity compounds (**4b** and **4c**) were subsequently selected for *in vivo* screening, using the reversal of haloperidol induced catalepsy with the standard bar test as an indication of the potential of these compounds to act as antagonists at A_{2A} receptors.¹⁶ Drug naïve Sprague-Dawley rats were divided into three groups each receiving intraperitoneal (i.p) injections of haloperidol (5.0 mg/kg) to induce catalepsy. Thirty minutes later the animals from group 1 received DMSO (control group) and the other two groups received 0.4 and 2 mg/kg of the test compound, respectively. Catalepsy was measured 60 minutes after the first injection.

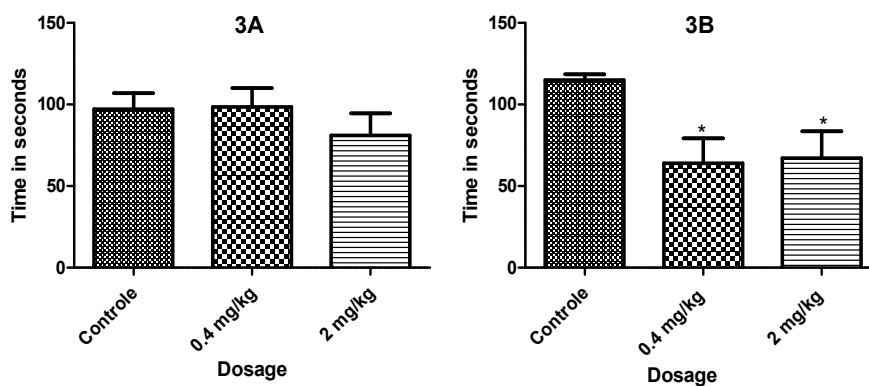


Figure 3A. Graph illustrating no significant reduction of catalepsy for both concentrations of compound **4b**. **3B.** Graph illustrating a significant attenuation of catalepsy with both concentrations of compound **4c**. (* indicates

significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [F (2, 24) = 3.97 (p = 0.032) followed by Dunnet's post test with p = 0.03 – 0.05).

The results obtained after i.p administration of the two selected compounds **4b** and **4c** are shown in Figure 3. Disappointingly, compound **4b** with the highest dual affinity *in vitro*, did not reverse catalepsy and appeared to be inactive *in vivo* (Figure 3A). Contrastingly, after i.p administration of compound **4c**, a significant reduction in catalepsy was observed when compared to the control group (Figure 3B), which is indicative of adenosine A_{2A} antagonism. It is postulated that the negative results obtained with compound **4b** are due to unfavourable physicochemical properties, such as poor water solubility, resulting in the precipitation of the compound upon i.p. injection.

To obtain support for this theory, solubility and log D values were determined for compound **4b** in order to assess its suitability as a drug candidate. The octanol-buffer partition coefficient was determined with the shake flask method, while water solubility was determined by shaking an excessive amount of **4b** in water at 37 °C. For the Log D study potassium phosphate buffer (pH 7.4) was selected for the hydrophilic phase and *n*-octanol for the hydrophobic phase. Results are shown in Table 2.

Table 2. The Log D and solubility of compound **4b**

Log D	Solubility
4.03 +/- 0.197	0.22 +/- 0.072 µM

It is generally accepted that the ideal log D value for a drug should be between 1-3,³⁷ thus allowing solubility in blood plasma while at the same time ensuring that the compound is lipophilic enough to cross the blood-brain-barrier. A Log D value of 4.03 is thus rather lipophilic, and this combined with the low aqueous solubility are probably responsible for the lack of observed activity *in vivo*.

Conclusion

This study provides evidence that carbamate substitution of the 2-amino-4,6-diphenylpyrimidine scaffold results in compounds with potent dual affinity for both adenosine A₁ and A_{2A} receptors. Size of the substituent appears to affect adenosine A_{2A} affinity to a larger extent than A₁ affinity. Diphenyl substitution on the carbamate nitrogen in particular appears to be detrimental, partly due to a loss of important binding interactions as illustrated with molecular docking studies. The physicochemical properties of these compounds would have to be optimised in order to improve *in vivo* activity and applicability as therapeutic agents in the treatment of PD.

Acknowledgements

We gratefully acknowledge the assistance of Dr. Johan Jordaan and Mr. André Joubert of the SASOL Centre for Chemistry, North-West University for recording of NMR and mass spectra as well as Prof Jan du Preez for HPLC analyses. We are also thankful to Ms Madelein Geldenhuys for her assistance with the radioligand binding studies. The financial assistance of the North-West University, Medical Research Council (MRC) and the National Research Foundation (UID 76308) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at.....

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Supplementary material

1. Materials and instrumentation
2. Biological evaluation
3. Physicochemical properties
4. Molecular modelling
5. Synthesis
6. Physical data
7. NMR spectra

1. Materials and instrumentation

Starting materials and other reagents were purchased from Sigma-Aldrich and used without any further purification. Deuterated solvents for nuclear magnetic resonance (NMR) spectroscopy were obtained from Merck while solvents used during synthesis and chromatography were purchased from Associated Chemical Enterprises (ACE).

Thin layer chromatography (TLC):

Precoated Kieselgel 60 F254 plates (Merck) were used to monitor reactions. Detection was done by UV light at a wavelength of 254 nm.

Melting points:

The melting points of all compounds were determined using a Buchi B-545 apparatus, and are uncorrected.

Mass spectrometry (MS):

The mass spectra for all compounds were obtained with a Bruker MicrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode.

Nuclear magnetic resonance (NMR) spectroscopy:

Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively. Samples were dissolved in either deuterated chloroform (CDCl_3) or deuterated dimethylsulfoxide ($\text{DMSO-}d_6$). Reported ^1H NMR spectroscopic data indicate the chemical shift (δ) in ppm, the integration (e.g. 1H), the multiplicity and the coupling constant (J) in Hz. The following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets) t (triplet), br t (broad triplet), q (quartet), p (pentet/quintet) or m (multiplet). Chemical shifts are referenced to the residual solvent signal ($\text{DMSO-}d_6$: 2.5 and 39.5 ppm for ^1H and ^{13}C respectively; CDCl_3 7.26 and 77.0 ppm for

¹H and ¹³C respectively). For most compounds there are equivalent carbons and some carbon signals therefore represent more than one carbon.

High performance liquid chromatography (HPLC):

To determine the purity of the synthesised compounds, HPLC analyses were conducted with an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector. For the chromatography HPLC grade acetonitrile (Merck) and Milli-Q water (Millipore) was used. A Venusil XBP C18 column (4.60 × 150 mm, 5 µm) was used with 30% acetonitrile and 70% MilliQ water as the initial mobile phase at a flow rate of 1 ml/min. At the start of each HPLC run, a solvent gradient program was initiated. The concentration of acetonitrile in the mobile phase was linearly increased up to 85% over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. The test compound was injected (20 µl, 1 mM) into the HPLC system and the eluent was monitored at wavelengths of 210, 254 and 300 nm.

2. Biological evaluation

In vitro

Tissue preparation for binding studies

Radioligand binding studies were carried out as reported in literature^{1,2,3} The Animal Research Ethics Committee of the North-West University (NWU-0035-10-A5) approved the collection of animal tissue required for these assays. Adult male Sprague-Dawley rats were obtained from the Vivarium of the North-West University, Potchefstroom campus. Striata (for the adenosine A_{2A} assays) and whole brains (for the adenosine A₁ assay) were dissected on ice and immediately snap frozen with liquid nitrogen and then stored at -70 °C until required. The frozen striata as well as the whole brain tissue were suspended in ice-cold 50 mM Tris buffer (pH 7.7 at 25 °C) and homogenised using a Polytron PT-10 homogeniser (Brinkman) to yield final suspensions of 1 g/5 ml, which were aliquoted and stored at -70 °C until required. Test compounds were dissolved (10 mM) and further diluted in DMSO, with the final concentration of DMSO in the incubations being 1%. All pipette tips as well as the 4 ml polypropylene tubes used for the incubations were coated with Sigmacote®. The incubations were prepared using 50 mM Tris buffer (pH 7.7 at 25 °C). For the adenosine A_{2A} assay, the final volume of the incubations was 1 ml and each incubation contained test compound (0-100 µM), membrane suspension yielding ~10 mg of original tissue weight of rat striata, 10 mM MgCl₂, 0.2 units of adenosine deaminase, 50 nM CPA and 4 nM [³H]NECA. The MgCl₂ (A_{2A} assay) and adenosine deaminase (A_{2A} and A₁ assays) were firstly added to the membrane suspension, and this mixture was subsequently added to the incubations. The order of addition was test compound, membrane suspension, CPA and [³H]NECA. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 µM replacing the test compound). For the adenosine A₁ assay, the final volume of the incubations was also 1 ml and each incubation contained test compound (0-100 µM), membrane suspension yielding 5

mg of original tissue weight of rat whole brain, 0.1 units of adenosine deaminase and 0.1 nM [³H]DPCPX. The order of addition was test compound, membrane suspension and [³H]DPCPX. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 μM replacing the test compound). After incubation for 1 h (with vortexing after 30 minutes), the incubations were rapidly filtered through Whatman® GF/B 25 filters (25 mm diameter) fitted on a Hoffeler vacuum system. The damp filters were placed into scintillation vials and scintillation fluid (4 ml) was added. The vials were shaken thoroughly and left for 2 h. A Packard Tri-CARB 2100 TR scintillation counter was used to count the radioactivity retained on the filters. Specific binding was defined as total binding minus non-specific binding, and was expressed as counts per minute (CPM).

In vivo

Catalepsy test

The protocol for the *in vivo* studies was approved by the Animal Research Ethics Committee of the North-West University (NWU-0035-10-A5). Male Sprague-Dawley rats (240 -300 g) were used for these assays. Animals had free access to standard laboratory food and water. The assays were performed in the morning between 8:00 and 13:00 in a temperature controlled room. Animals were drug naive and only used once. Haloperidol induced catalepsy was measured with the standard bar test, in a Perspex chamber (length, 23 cm; width, 10.5 cm; height, 9 cm) with a horizontal plastic bar (diameter, 1 cm; length, 10.5 cm) fixed 7 cm from the back of the box, and 9 cm above the floor.

Thirty male (n = 30) Sprague-Dawley rats were divided into 3 groups, (control, 0.4 and 2 mg/kg groups) each group containing 10 rats. Haloperidol 5.0 mg/kg was injected intraperitoneally (i.p) to induce catalepsy. Thirty minutes later, the animals in the control group received i.p injections of DMSO and the animals in the other two groups received i.p injections of the corresponding concentration of test compound. Catalepsy was measured 60 minutes after the haloperidol injections by placing the rats in the Perspex box with their front paws on the horizontal bar. The time the animal maintained its position on the bar was recorded up to 120 seconds of cataleptic time. To compare between the control group and the compound treated groups a 1 way ANOVA test followed by Dunnett's multiple comparison's test was performed. A probability of $p < 0.05$ was used to declare statistically significant differences.

3. Physico-chemical properties

Lipophilicity (logD).

The octanol-buffer/water partition coefficient (D) was measured according to the shake flask method. n-Octanol was selected for the hydrophobic phase while potassium phosphate buffer (pH 7.4) was selected for the hydrophilic phase. Firstly, the potassium phosphate buffer and n-octanol were mutually saturated by occasionally shaking a 50:50 mixture of the two and allowing it to settle overnight. After the separation of the 2 phases (saturated octanol and saturated buffer phase) 640 μl saturated octanol,

800 μ l saturated buffer and 160 μ l of a [10 mM] compound solution in octanol were added to a 2ml micro-tube to yield a final analyte concentration of 1mM. The tube was shaken for 5 min by hand and centrifuged for 10 min at 4000 g. The n-octanol phase was diluted 60-fold with 100% n-octanol and the absorbance of the resulting solution recorded at a maximal absorbance wavelength of 340 nm. The buffer/water phase was diluted into HPLC vials containing 50% deionised water (MilliQ, Millipore, Billerica, USA) and 50% acetonitrile. This was then analysed by HPLC (same method as above). By using the molar extinction coefficients recorded in n-octanol, the concentrations of the test compound in saturated n-octanol and saturated buffer/water phases were determined. These assays were performed in triplicate and the partition coefficients reported as the mean \pm SD of the log D values.

Solubility:

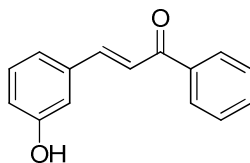
Water (1 ml) was added to 3 microtubes containing \sim 4.5 mg of test compound. Tubes were then placed in a shaking water bath for 24 hours at 37 °C. Afterwards the tubes were centrifuged at 16000 g for 10 minutes. The supernatant was filtered through a syringe filter into HPLC vials. The concentration of dissolved compound was determined by HPLC as described above.

4. Molecular modelling

Windows based Accelrys[®] Discovery Studio (DS 3.1) was used to carry out the molecular docking studies. The crystal structure of the adenosine A_{2A} receptor co-crystallised with the known A_{2A} antagonist ZM241385, was obtained from the Protein data bank (PDB – code: 3EML). To correct errors and prepare the receptor the ‘Clean protein’ function was used. The receptor was typed with the CHARMM forcefield and the ‘Calculate Protein Ionization and Residue pK function was selected to optimise the ionisation state of residues. The protein was then minimized with CHARMM while a fixed atom constraint was applied to the backbone. The minimisation was carried out using the Generalised Born approximation with Molecular Volume (GBMV) as a solvent model. The existing ligand was selected to define a binding sphere (radius of 5Å) and removed afterwards for docking. Ligands were drawn in ChemWindow, copied and pasted in Discovery Studio. These ligands, which include ZM241385 (for docking validation) were prepared for docking with the ‘Prepare Ligands’ function and docked using the C-DOCKER protocol (10 Random conformations, Momany-Rone as Ligand partial charge). Orientation, C-DOCKER and C-DOCKER_INTERACTION energies of the ten different conformers of each ligand were considered and the best conformation for each ligand selected. An *in situ* ligand minimisation was then performed on the selected conformers and the binding energies were calculated afterwards.

5. Synthesis

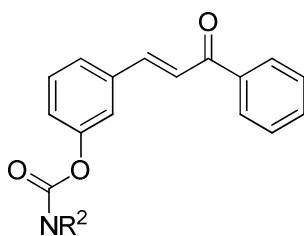
General procedure for the synthesis of (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (8)



8

A solution of 4% (w/v) sodium hydroxide in water (49 mmol) was added to a suspension of 3-hydroxy benzaldehyde (6) (24.5 mmol) and acetophenone (7) (24.5 mmol) in methanol (300 ml). The mixture was refluxed at 90 °C for 5 days. The reaction was subsequently concentrated and equal volumes of water and ethyl acetate added. The water phase was extracted twice with ethyl acetate and the organic layers were combined, concentrated *in vacuo* and the resulting solid recrystallised from ethanol.

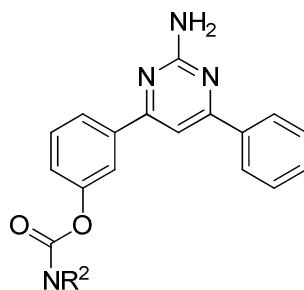
General procedure for the synthesis of carbamates (9a-i)



9a - i

(2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (8) (12 mmol) was added to stirred suspension of 100 ml acetonitrile and potassium carbonate (24 mmol). After stirring for 30 minutes, the carbamate (14.4 mmol) was added to the reaction mixture. The reaction was refluxed overnight at 90 °C and left to cool down to room temperature. Water (~ 100 ml) was added to the reaction until a precipitate formed. The resulting solid was filtered and recrystallised from ethanol. In cases where no precipitate formed, the solvent was evaporated and the product extracted with chloroform.

General procedure for the synthesis of aminopyrimidines (4a-i)

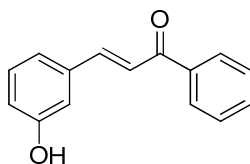


4a - i

Guanidine hydrochloride (4.61 mmol) was dissolved in a small amount of DMF (20 ml) and the required carbamate intermediate (**9a – i**) (3.07 mmol) and sodium hydride (4.61 mmol) added to the reaction. The reaction mixture was heated (90 °C) for 24 hours under nitrogen, allowed to cool down and then diluted with equal volumes of ethyl acetate and water. The aqueous phase was extracted with ethyl acetate and the organic layers combined. All traces of DMF were removed by washing the combined organic layers several times with water. The organic layer was concentrated *in vacuo* and the crude product was purified with column chromatography [dichloromethane: methanol (98:2)] and recrystallised from ethanol.

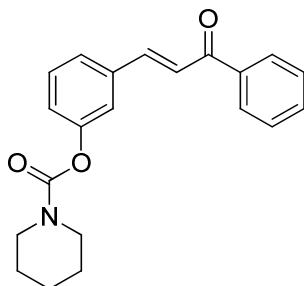
6. Physical data

(*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**8**)



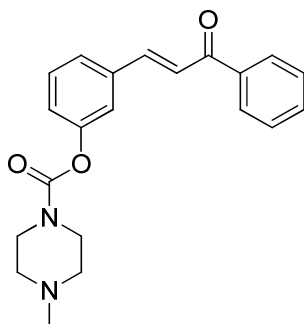
The title compound was prepared from 3-hydroxybenzaldehyde and acetophenone in a yield of 59%: mp 88.4 – 89.2 °C (ethyl acetate), pale yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.68 (s, 1H), 8.17 – 8.12 (m, 2H), 7.84 (d, *J* = 15.6 Hz, 1H), 7.72 – 7.62 (m, 2H), 7.56 (br t, *J* = 7.7 Hz, 2H), 7.32 (br d, *J* = 7.8 Hz, 1H), 7.29 – 7.23 (m, 2H), 6.90 (ddd, *J* = 8.0, 2.5, 1.0 Hz, 1H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.2, 157.8, 144.3, 137.6, 135.9, 133.1, 129.9, 128.8, 128.5, 121.9, 119.9, 117.9, 115.3. APCI-HRMS *m/z*: calcd for C₁₅H₁₃O₂ (M + H)⁺, 225.0910, found 225.0910.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl piperidine-1-carboxylate (**9a**)



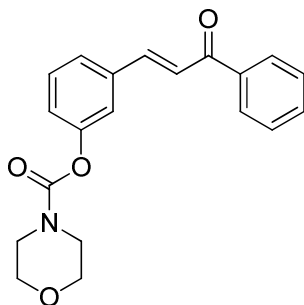
The title compound was prepared from (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and piperidine-1-carbonyl chloride in a yield of 75%: mp 143.3 – 143.6 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.04 – 7.99 (m, 2H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.61 – 7.55 (m, 1H), 7.54 – 7.47 (m, 3H), 7.47 – 7.38 (m, 3H), 7.17 (ddd, *J* = 8.0, 2.4, 1.1 Hz, 1H), 3.62 (br s, 2H), 3.52 (br s, 2H), 1.67 – 1.61 (m, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 153.4, 151.9, 143.9, 138.0, 136.1, 132.8, 129.7, 128.6, 128.5, 125.6, 123.9, 122.6, 121.1, 45.5, 45.1, 25.8, 25.4, 24.2. APCI-HRMS *m/z*: calcd for C₂₁H₂₂NO₃ (M + H)⁺, 336.1594, found 336.1594.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl 4-methylpiperazine-1-carboxylate (**9b**)



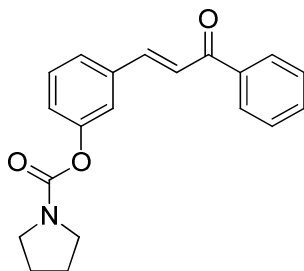
The title compound was prepared from (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and 4-methylpiperazine-1-carbonyl chloride in a yield of 78%: mp 123.5 – 124.8 °C (ethanol), yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.03 – 7.98 (m, 2H), 7.76 (d, *J* = 15.7 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.54 – 7.38 (m, 6H), 7.16 (ddd, *J* = 8.1, 2.4, 1.1 Hz, 1H), 3.70 (t, *J* = 4.9 Hz, 2H), 3.60 (t, *J* = 4.9 Hz, 2H), 2.49 – 2.43 (m, 4H), 2.34 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 190.2, 153.3, 151.7, 143.8, 137.9, 136.2, 132.8, 129.7, 128.5, 128.4, 125.8, 123.8, 122.7, 121.0, 54.6, 54.5, 46.1, 44.4, 43.8. APCI-HRMS *m/z*: calcd for C₂₁H₂₃N₂O₃ (M + H)⁺, 351.1703, found 351.1687.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl morpholine-4-carboxylate (**9c**)



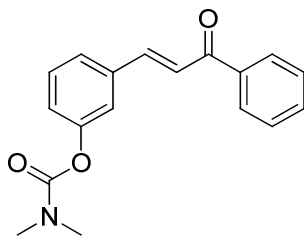
The title compound was prepared from (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and morpholine-4-carbonyl chloride in a yield of 78%: mp 142.2 – 143.1 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, DMSO-*d*6) δ 8.20 – 8.15 (m, 2H), 7.98 (d, *J* = 15.6 Hz, 1H), 7.79 – 7.65 (m, 4H), 7.58 (t, *J* = 7.7 Hz, 2H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.23 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H), 3.70 – 3.57 (m, 8H). ¹³C NMR (151 MHz, DMSO-*d*6) δ 189.2, 153.0, 151.7, 143.1, 137.5, 136.2, 133.4, 129.9, 128.9, 128.7, 126.7, 124.4, 122.9, 121.6, 65.9, 44.7, 43.9. APCI-HRMS *m/z*: calcd for C₂₀H₂₀NO₄ (M + H)⁺, 338.1387, found 338.1357.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl pyrrolidine-1-carboxylate (**9d**)



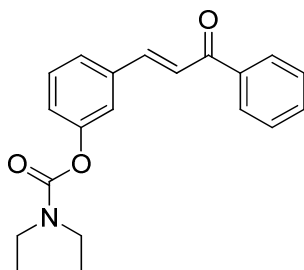
The title compound was prepared from (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and pyrrolidine-1-carbonyl chloride in a yield of 65%: mp 93.1 – 93.8 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.03 – 7.98 (m, 2H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.54 – 7.46 (m, 3H), 7.47 – 7.42 (m, 2H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.19 (ddd, *J* = 8.0, 2.2, 1.2 Hz, 1H), 3.57 (t, *J* = 6.7 Hz, 2H), 3.49 (t, *J* = 6.7 Hz, 2H), 2.01 – 1.88 (m, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 152.7, 151.8, 143.9, 138.0, 136.1, 132.8, 129.6, 128.5, 128.4, 125.5, 123.9, 122.6, 121.0, 46.4, 46.3, 25.7, 24.9. APCI-HRMS *m/z*: calcd for C₂₀H₂₀NO₃ (M + H)⁺, 322.1438, found 322.1434.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-dimethylcarbamate (**9e**)



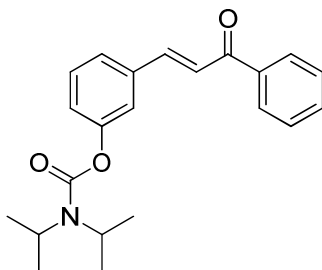
The title compound was prepared from (*2E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and *N,N*-dimethylcarbamoyl chloride in a yield of 92%: mp 88.7 – 89.4 °C (ethanol), light yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.04 – 7.99 (m, 2H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.61 – 7.53 (m, 1H), 7.55 – 7.47 (m, 2H), 7.49 – 7.36 (m, 2H), 7.17 (ddd, *J* = 8.1, 2.4, 1.1 Hz, 1H), 3.12 (s, 3H), 3.03 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 154.6, 151.9, 143.9, 138.0, 136.2, 132.8, 129.7, 128.6, 128.5, 125.7, 123.9, 122.7, 121.1, 36.7, 36.4. APCI-HRMS *m/z*: calcd for C₁₈H₁₈NO₃(*M* + H)⁺, 296.1281, found 296.1272.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-diethylcarbamate (**9f**)



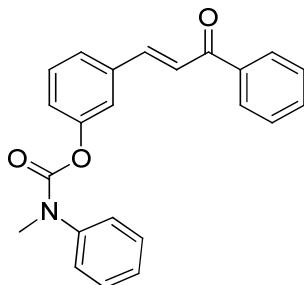
The title compound was prepared from (*2E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and *N,N*-diethylcarbamoyl chloride in a yield of 78%: mp 51.3 – 53.0 °C (ethanol), light yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.21 – 8.16 (m, 2H), 7.99 (d, *J* = 15.7 Hz, 1H), 7.78 – 7.65 (m, 4H), 7.58 (t, *J* = 7.7 Hz, 2H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.21 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H), 3.43 (q, *J* = 7.2 Hz, 2H), 3.32 (q, *J* = 7.1 Hz, 2H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.13 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.1, 153.3, 151.7, 143.1, 137.4, 136.1, 133.3, 129.7, 128.8, 128.6, 126.4, 124.4, 122.8, 121.6, 41.8, 41.5, 14.2, 13.3. APCI-HRMS *m/z*: calcd for C₂₀H₂₂NO₃(*M* + H)⁺, 324.1594, found 324.1591.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-bis(propan-2-yl)carbamate (**9g**)



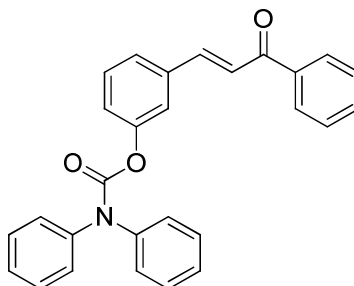
The title compound was prepared from (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and *N,N*-bis(propan-2-yl)carbamoyl chloride in a yield of 74%: mp 109.6 – 110.8 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.04 – 7.99 (m, 2H), 7.78 (d, *J* = 15.7 Hz, 1H), 7.61 – 7.55 (m, 1H), 7.55 – 7.47 (m, 3H), 7.47 – 7.43 (m, 1H), 7.43 – 7.37 (m, 2H), 7.18 (ddd, *J* = 8.1, 2.4, 1.2 Hz, 1H), 4.11 (brs, 1H), 3.97 (brs, 1H), 1.44 – 1.22 (m, 12H). ¹³C NMR (151 MHz, CDCl₃) δ 190.4, 153.5, 151.7, 144.0, 138.0, 136.2, 132.8, 129.7, 128.6, 128.5, 125.4, 124.0, 122.7, 121.2, 47.0, 46.1, 21.5, 20.4. APCI-HRMS *m/z*: calcd for C₂₂H₂₆NO₃ (*M* + *H*)⁺, 352.1907, found 352.1905.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N*-methyl-*N*-phenylcarbamate (**9h**)



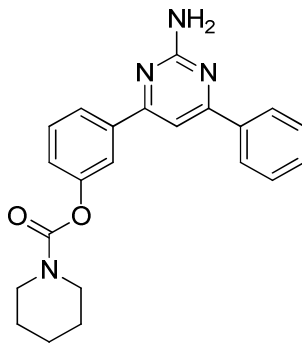
The title compound was prepared from (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and *N*-methyl-*N*-phenylcarbamoyl chloride in a yield of 84%: mp 108.7 – 111.3 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.04 – 7.99 (m, 2H), 7.76 (d, *J* = 15.7 Hz, 1H), 7.62 – 7.56 (m, 1H), 7.53 – 7.47 (m, 3H), 7.48 – 7.36 (m, 7H), 7.29 (brs, 1H), 7.17 (brs, 1H), 3.44 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 153.6, 151.7, 143.8, 142.6, 137.9, 136.2, 132.8, 129.7, 129.1, 128.6, 128.5, 126.8, 126.1, 125.8, 123.7, 122.8, 120.9, 38.3. APCI-HRMS *m/z*: calcd for C₂₃H₂₀NO₃ (*M* + *H*)⁺, 358.1438, found 358.1432.

3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-diphenylcarbamate (**9i**)



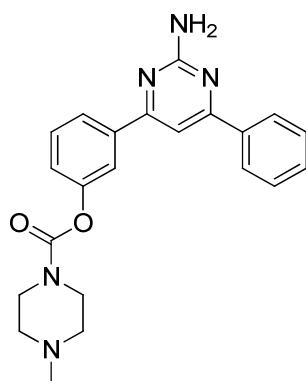
The title compound was prepared from (2*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one and *N,N*-diphenylcarbamoyl chloride in a yield of 75%: mp 141.8 – 142.3 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.05 – 8.00 (m, 2H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.62 – 7.56 (m, 1H), 7.55 – 7.36 (m, 14H), 7.31 – 7.24 (m, 2H), 7.22 (ddd, *J* = 8.1, 2.4, 1.1 Hz, 1H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 152.8, 151.5, 143.7, 142.0, 137.9, 136.2, 132.8, 129.7, 129.1, 128.6, 128.5, 126.8, 126.6, 126.0, 123.5, 122.8, 120.7. APCI-HRMS *m/z*: calcd for C₂₈H₂₂NO₃(*M* + *H*)⁺, 420.1594, found 420.1589.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl piperidine-1-carboxylate (**4a**)



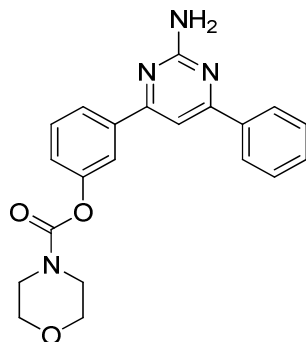
The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl piperidine-1-carboxylate in a yield of 12%: mp 142.9 – 143.8 °C (ethanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 8.00 (m, 2H), 7.87 (br d, *J* = 7.8 Hz, 1H), 7.83 (t, *J* = 2.0 Hz, 1H), 7.52 – 7.44 (m, 4H), 7.42 (s, 1H), 7.28 – 7.22 (m, 1H), 5.51 (s, 2H), 3.63 (br s, 2H), 3.54 (br s, 2H), 1.65 (br s, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 166.3, 165.2, 163.6, 153.5, 151.9, 139.0, 137.6, 130.4, 129.4, 128.7, 127.1, 123.8, 123.7, 120.6, 104.2, 45.5, 45.1, 25.8, 25.4, 24.2. APCI-HRMS *m/z*: calcd for C₂₂H₂₃N₄O₂ (*M* + *H*)⁺, 375.1816, found 375.1816; Purity (HPLC): 99%.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl 4-methylpiperazine-1-carboxylate (**4b**)



The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl 4-methylpiperazine-1-carboxylate in a yield of 20%: mp 173.3 – 174.2 °C (ethanol), white crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 8.00 (m, 2H), 7.88 (ddd, *J* = 7.8, 1.7, 1.0 Hz, 1H), 7.83 (br t, *J* = 2.0 Hz, 1H), 7.52 – 7.45 (m, 4H), 7.43 (s, 1H), 7.25 (ddd, *J* = 8.1, 2.4, 1.0 Hz, 1H), 5.42 (s, 2H), 3.72 (brs, 2H), 3.62 (brs, 2H), 2.53 – 2.39 (m, 4H), 2.34 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 166.3, 165.1, 163.6, 153.4, 151.7, 139.1, 137.6, 130.4, 129.5, 128.7, 127.1, 123.9, 123.7, 120.5, 104.2, 54.7, 54.5, 46.1, 44.4, 43.9. APCI-HRMS *m/z*: calcd for C₂₂H₂₄N₅O₂ (M + H)⁺, 390.1925, found 390.1887; Purity (HPLC): 99%.

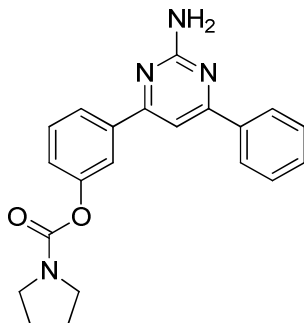
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate (**4c**)



The title compound was prepared from 3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl morpholine-4-carboxylate in a yield of 35%: mp 176.9 – 177.7 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 8.00 (m, 2H), 7.88 (br d, *J* = 7.8 Hz, 1H), 7.85 (t, *J* = 2.0 Hz, 1H), 7.52 – 7.45 (m, 4H), 7.42 (s, 1H), 7.27 – 7.22 (m, 1H), 5.56 (s, 2H), 3.78 – 3.66 (m, 6H), 3.60 (s, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 166.3, 165.0, 163.6, 153.5, 151.6, 139.2, 137.5, 130.4, 129.5, 128.7, 127.0, 124.0, 123.6,

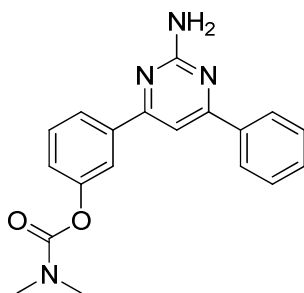
120.5, 104.1, 66.5, 66.4, 44.8, 44.1. APCI-HRMS m/z: calcd for C₂₁H₂₁N₄O₃(M + H)⁺, 377.1608, found 377.1584; Purity (HPLC): 99%.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl pyrrolidine-1-carboxylate (**4d**)



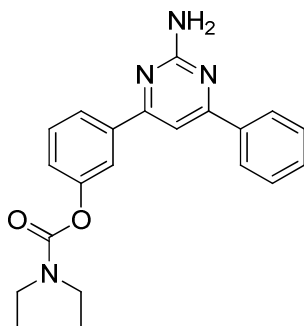
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl pyrrolidine-1-carboxylate in a yield of 10%: mp 154.3 – 156.1 °C (ethanol), pale yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 8.00 (m, 2H), 7.90 – 7.85 (m, 2H), 7.51 – 7.44 (m, 4H), 7.42 (s, 1H), 7.28 (ddd, *J* = 8.1, 2.3, 1.1 Hz, 1H), 5.54 (s, 2H), 3.59 (t, *J* = 6.7 Hz, 2H), 3.52 (t, *J* = 6.7 Hz, 2H), 2.00 – 1.87 (m, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 166.2, 165.2, 163.6, 152.9, 151.8, 139.0, 137.6, 130.3, 129.4, 128.6, 127.1, 123.7, 123.7, 120.5, 104.1, 46.4, 46.3, 25.7, 24.9. APCI-HRMS m/z: calcd for C₂₁H₂₁N₄O₂(M + H)⁺, 361.1659, found 361.1651; Purity (HPLC): 100%.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-dimethylcarbamate (**4e**)



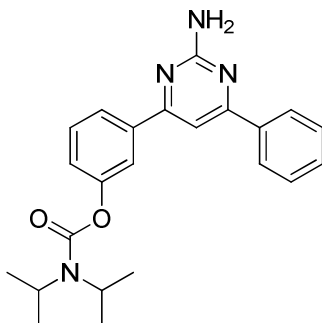
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-dimethylcarbamate in a yield of 7%: mp 135.1 – 137.2 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 8.00 (m, 2H), 7.88 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.84 (t, *J* = 2.0 Hz, 1H), 7.52 – 7.44 (m, 4H), 7.42 (s, 1H), 7.28 – 7.23 (m, 1H), 5.54 (s, 2H), 3.12 (s, 3H), 3.04 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 166.2, 165.2, 163.6, 154.7, 151.9, 139.1, 137.6, 130.4, 129.5, 128.6, 127.1, 123.8, 123.7, 120.5, 104.1, 36.7, 36.4. APCI-HRMS m/z: calcd for C₁₉H₁₉N₄O₂(M + H)⁺, 335.1503, found 335.1498; Purity (HPLC): 99%.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-diethylcarbamate (**4f**)



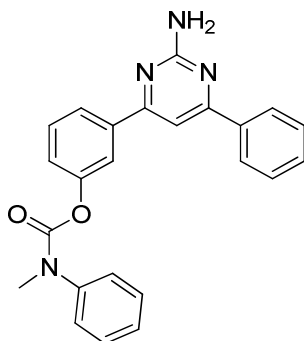
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-diethylcarbamate in a yield of 15%: mp 127.6 – 128.1 °C (ethanol), orange crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 8.01 (m, 2H), 7.87 (br d, *J* = 7.9 Hz, 1H), 7.84 (t, *J* = 2.0 Hz, 1H), 7.52 – 7.45 (m, 4H), 7.43 (s, 1H), 7.29 – 7.24 (m, 2H), 5.54 (s, 2H), 3.47 (q, *J* = 7.1 Hz, 2H), 3.42 (q, *J* = 7.1 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 166.3, 165.3, 163.6, 154.0, 151.9, 139.1, 137.6, 130.4, 129.4, 128.7, 127.1, 123.8, 123.7, 120.6, 104.2, 42.2, 41.9, 14.2, 13.3. APCI-HRMS *m/z*: calcd for C₂₁H₂₃N₄O₂ (M + H)⁺, 363.1816, found 363.1820; Purity (HPLC): 99%.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-bis(propan-2-yl)carbamate (**4g**)



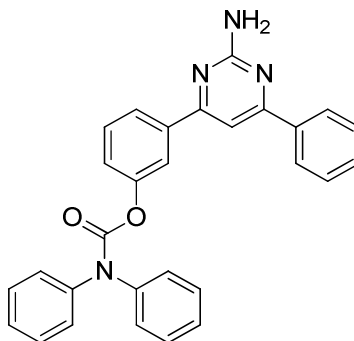
The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-bis(propan-2-yl)carbamate in a yield of 22%: mp 169.6 – 171.9 °C (ethanol), light orange crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.08 – 8.01 (m, 2H), 7.86 (br d, *J* = 7.9 Hz, 1H), 7.83 (t, *J* = 2.0 Hz, 1H), 7.52 – 7.45 (m, 4H), 7.43 (s, 1H), 7.29 – 7.23 (m, 1H), 5.57 (s, 2H), 4.14 (br s, 1H), 3.99 (br s, 1H), 1.37 (br s, 6H), 1.31 (br s, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 166.2, 165.3, 163.6, 153.6, 151.7, 139.1, 137.6, 130.4, 129.4, 128.7, 127.1, 123.9, 123.7, 120.6, 104.2, 46.9, 46.1, 21.5, 20.4. APCI-HRMS *m/z*: calcd for C₂₃H₂₇N₄O₂ (M + H)⁺, 391.2129, found 391.2129; Purity (HPLC): 99%.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N*-methyl-*N*-phenylcarbamate (**4h**)



The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N*-methyl-*N*-phenylcarbamate in a yield of 6%: mp 151.9 – 152.9 °C (ethanol), white solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.19 – 8.14 (m, 2H), 8.05 (br d, *J* = 7.8 Hz, 1H), 7.93 (br s, 1H), 7.66 (s, 1H), 7.55 – 7.34 (m, 8H), 7.25 (t, *J* = 7.3 Hz, 2H), 6.68 (s, 2H), 3.31 (s, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 165.7, 164.4, 164.3, 153.7, 151.9, 143.1, 139.0, 137.5, 131.2, 130.3, 129.6, 129.2, 127.5, 127.1, 126.4, 124.5, 124.5, 120.7, 102.5, 38.5. APCI-HRMS *m/z*: calcd for C₂₄H₂₁N₄O₂ (M + H)⁺, 397.1659, found 397.1644; Purity (HPLC): 100%.

3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-diphenylcarbamate (**4i**)

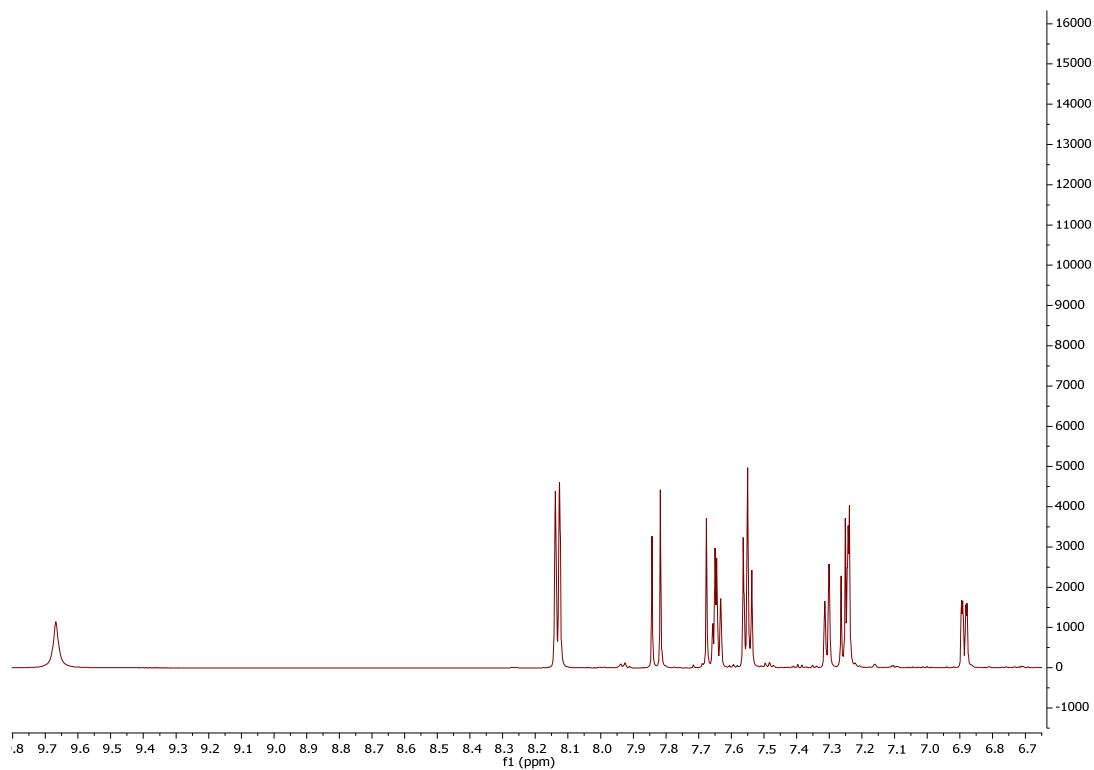


The title compound was prepared from 3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-diphenylcarbamate in a yield of 18%: mp 172.4 – 173.9 °C (ethanol), pale yellow crystals. ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.25 – 8.20 (m, 2H), 8.12 (br d, *J* = 7.9 Hz, 1H), 8.03 (br s, 1H), 7.74 (s, 1H), 7.59 – 7.39 (m, 12H), 7.37 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 2H), 6.81 (s, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 165.1, 164.0, 163.7, 152.5, 151.2, 142.2, 138.8, 137.2, 130.5, 129.6, 129.2, 128.6, 127.2, 127.0, 126.7, 124.3, 123.9, 120.2, 101.9. APCI-HRMS *m/z*: calcd for C₂₉H₂₃N₄O₂ (M + H)⁺, 459.1816, found 459.1786; Purity (HPLC): 99%

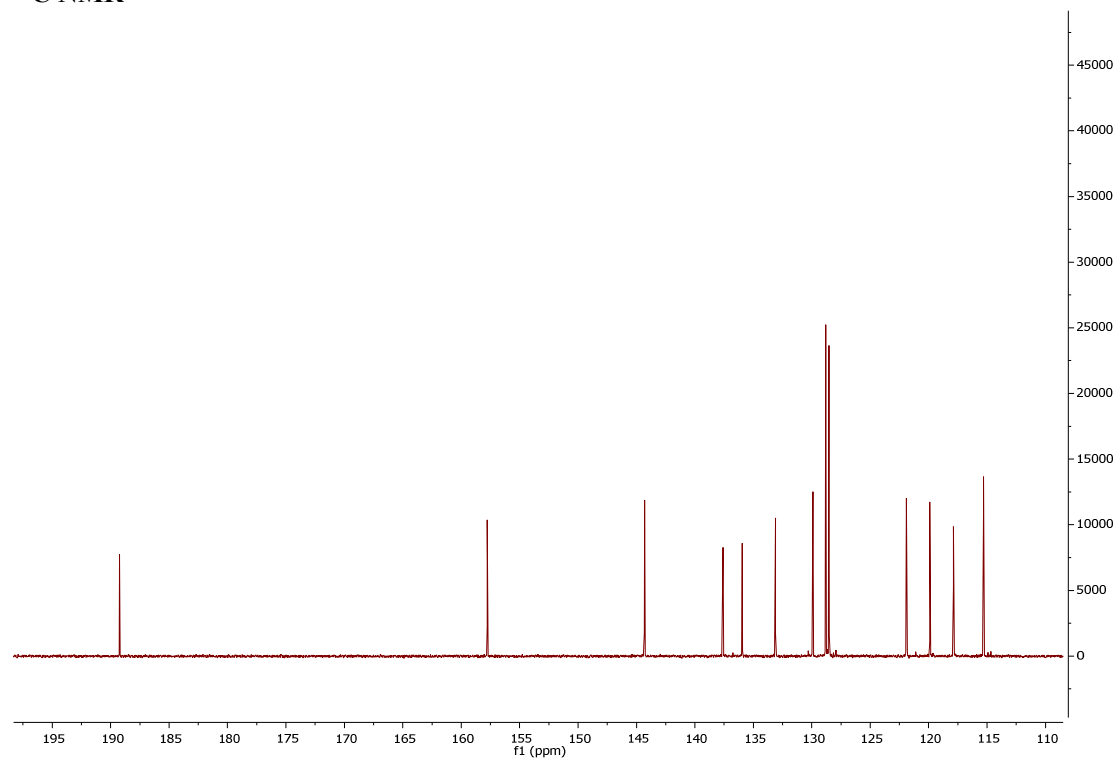
NMR spectra

(*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**8**)

¹H NMR

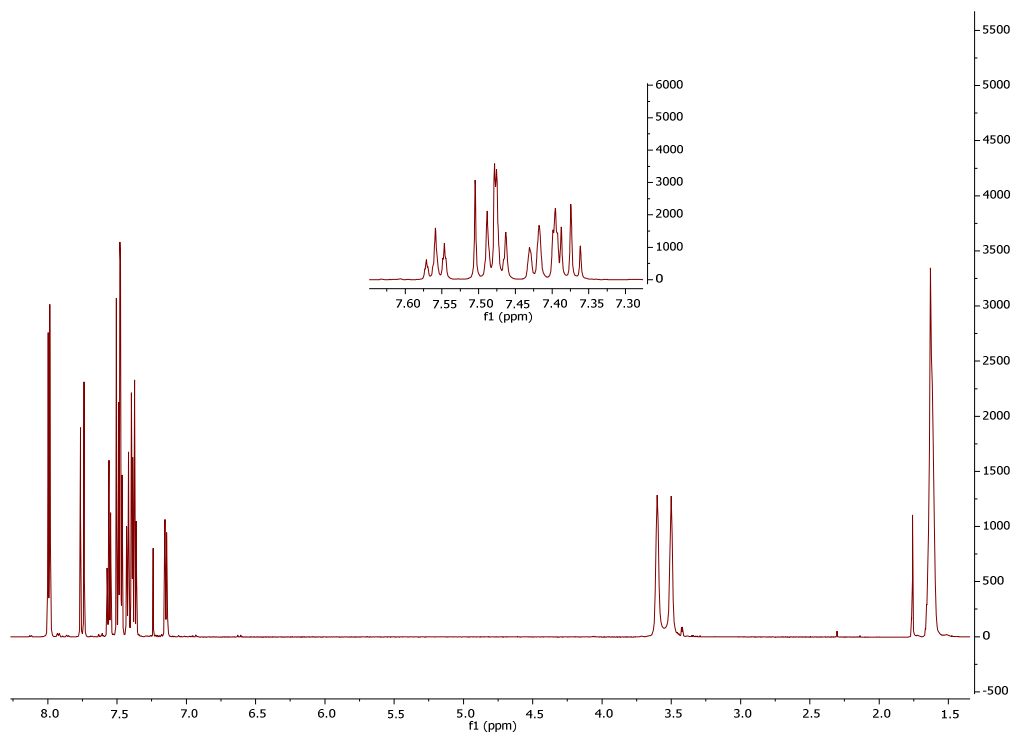


¹³C NMR

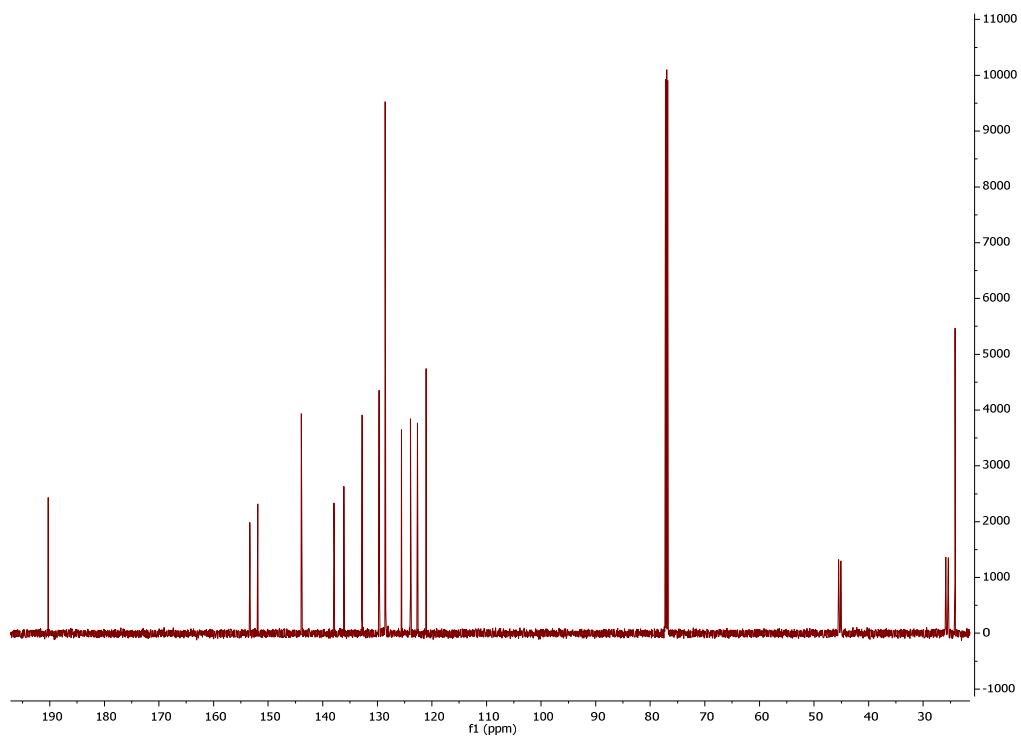


3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl piperidine-1-carboxylate (**9a**)

¹H NMR

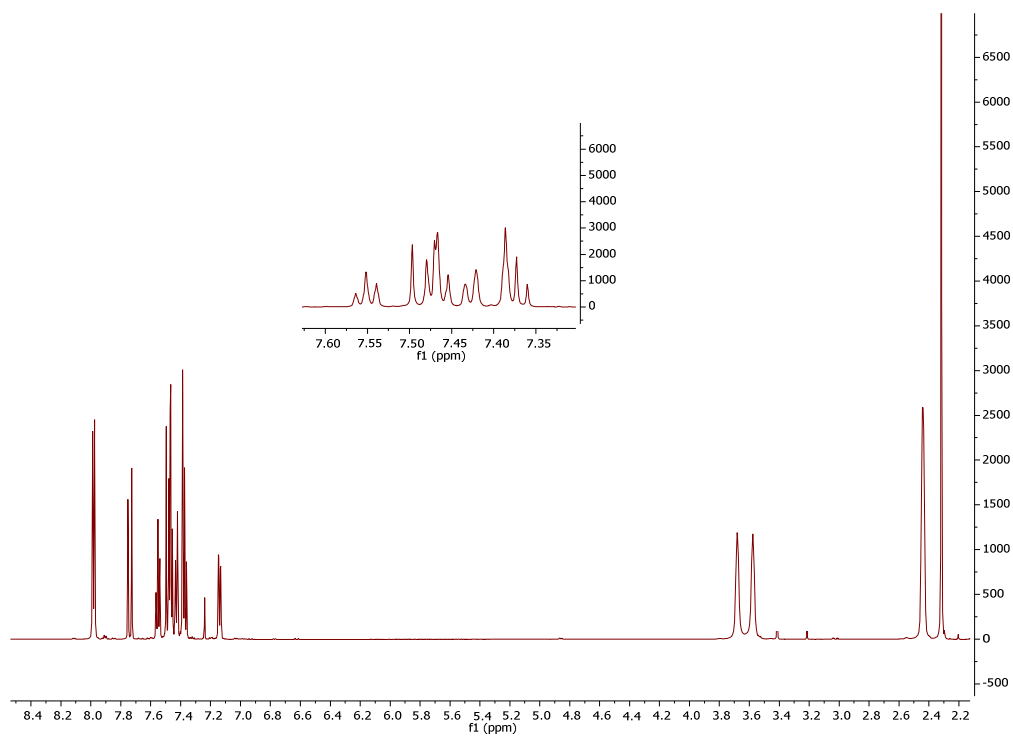


¹³C NMR

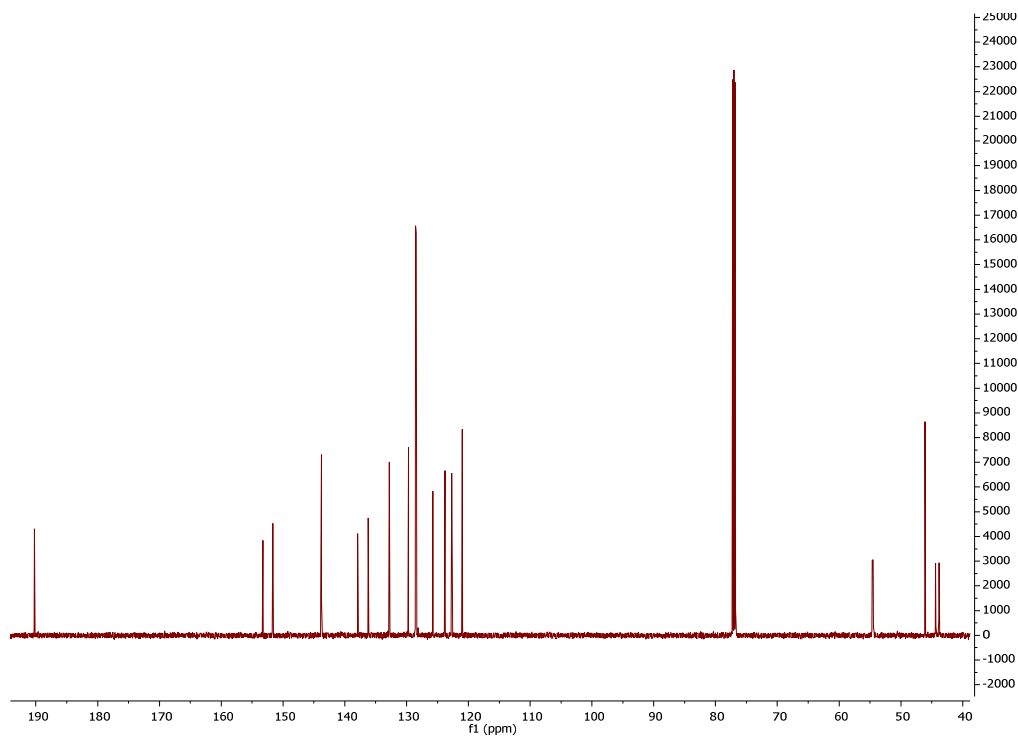


3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl 4-methylpiperazine-1-carboxylate (**9b**)

¹H NMR

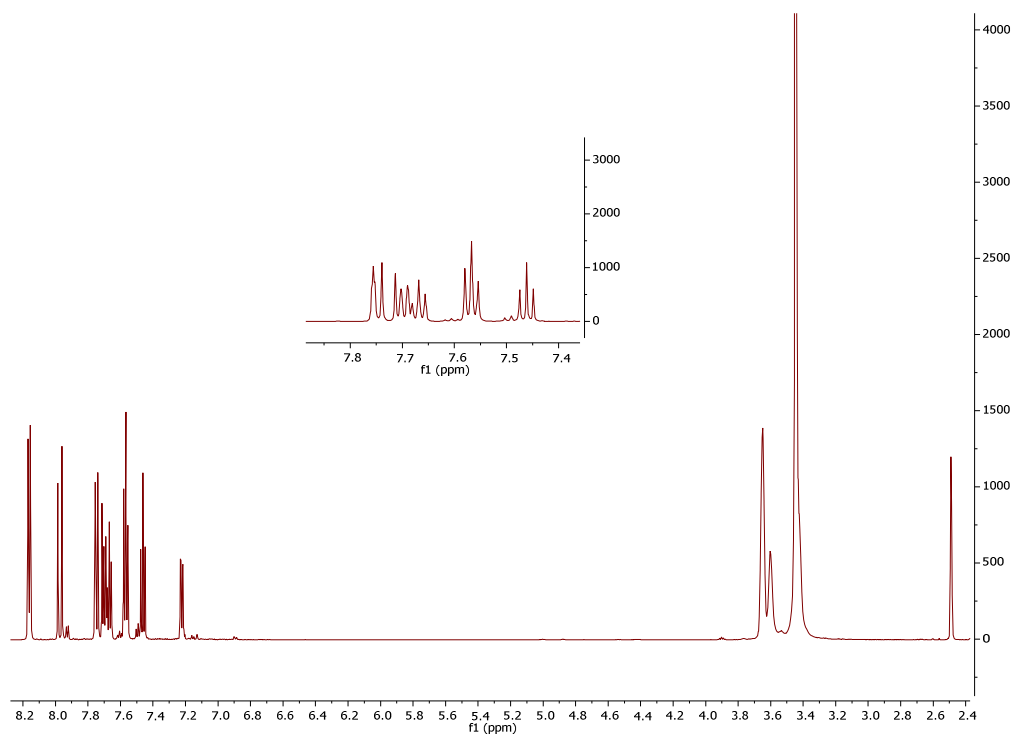


¹³C NMR

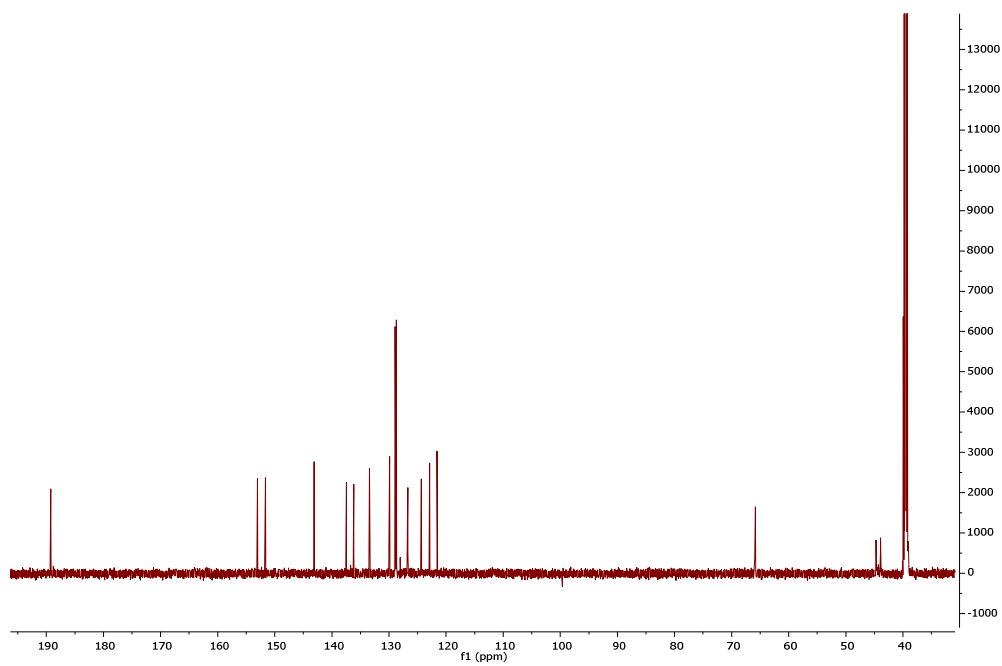


3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl morpholine-4-carboxylate (**9c**)

¹H NMR

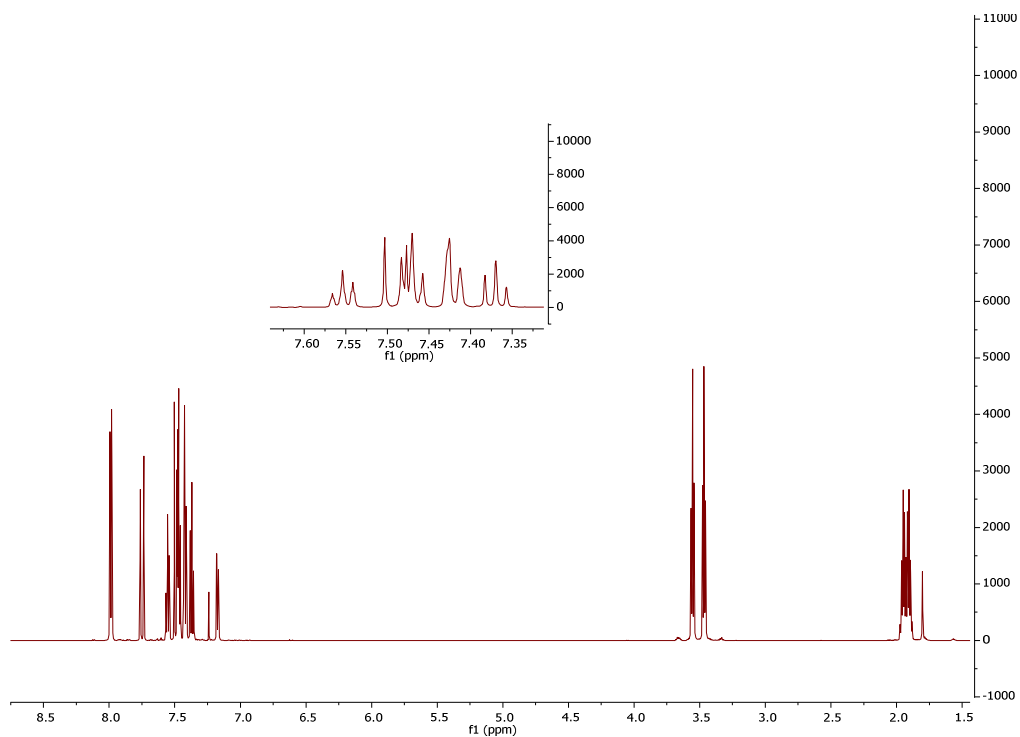


¹³C NMR

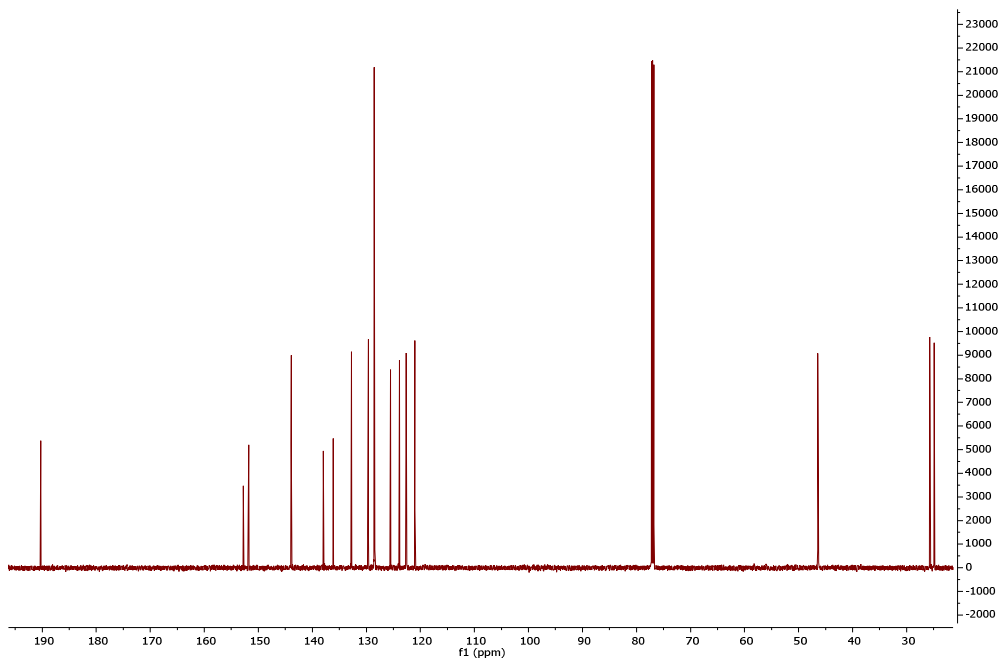


3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl pyrrolidine-1-carboxylate (**9d**)

¹H NMR

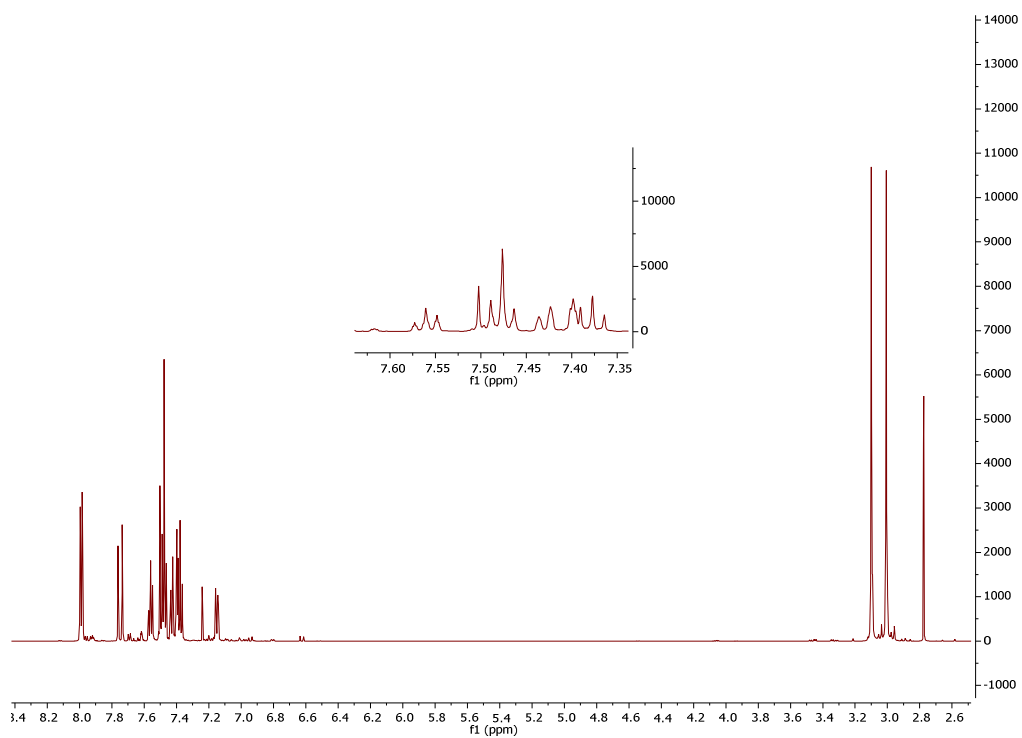


¹³C NMR

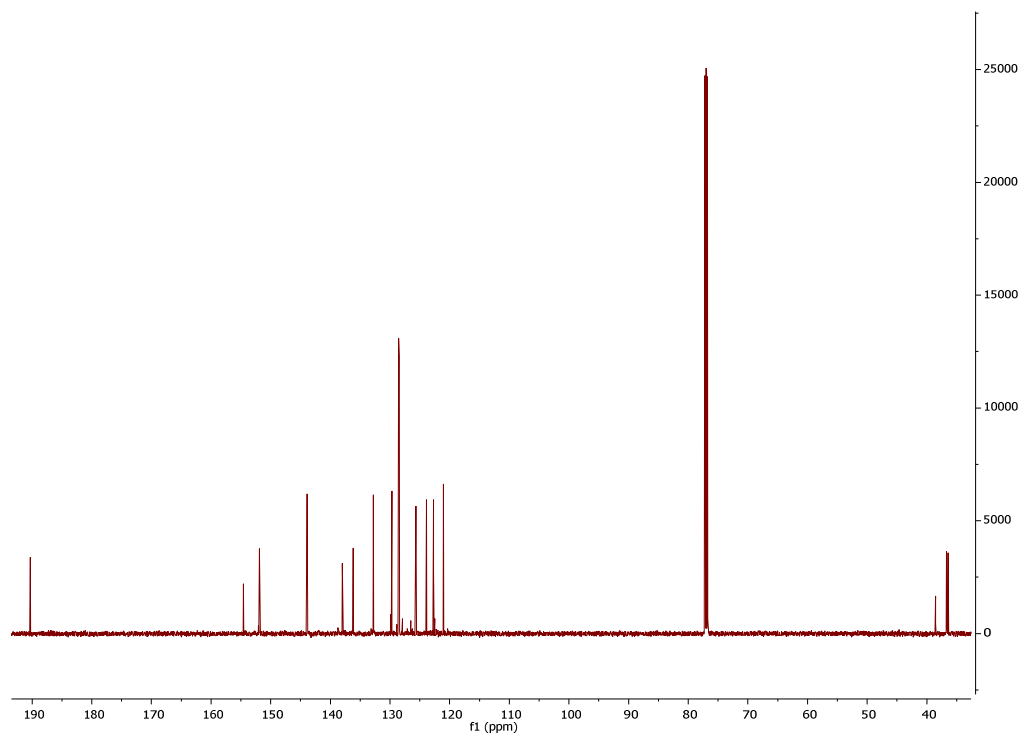


3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl N,N-dimethylcarbamate (**9e**)

¹H NMR

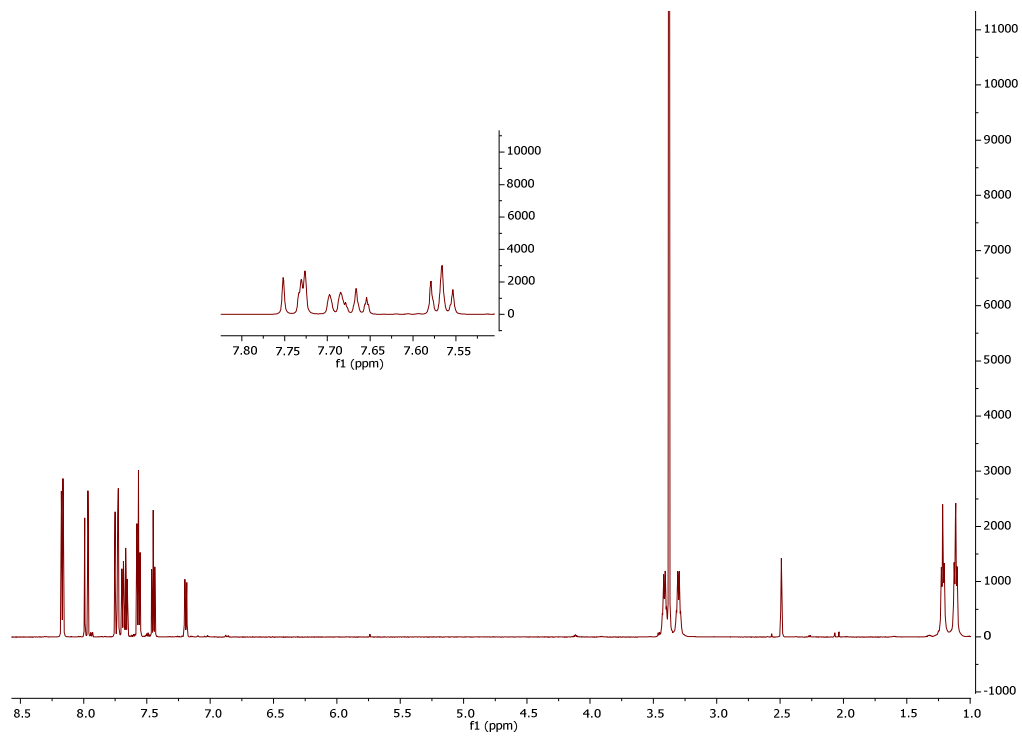


¹³C NMR

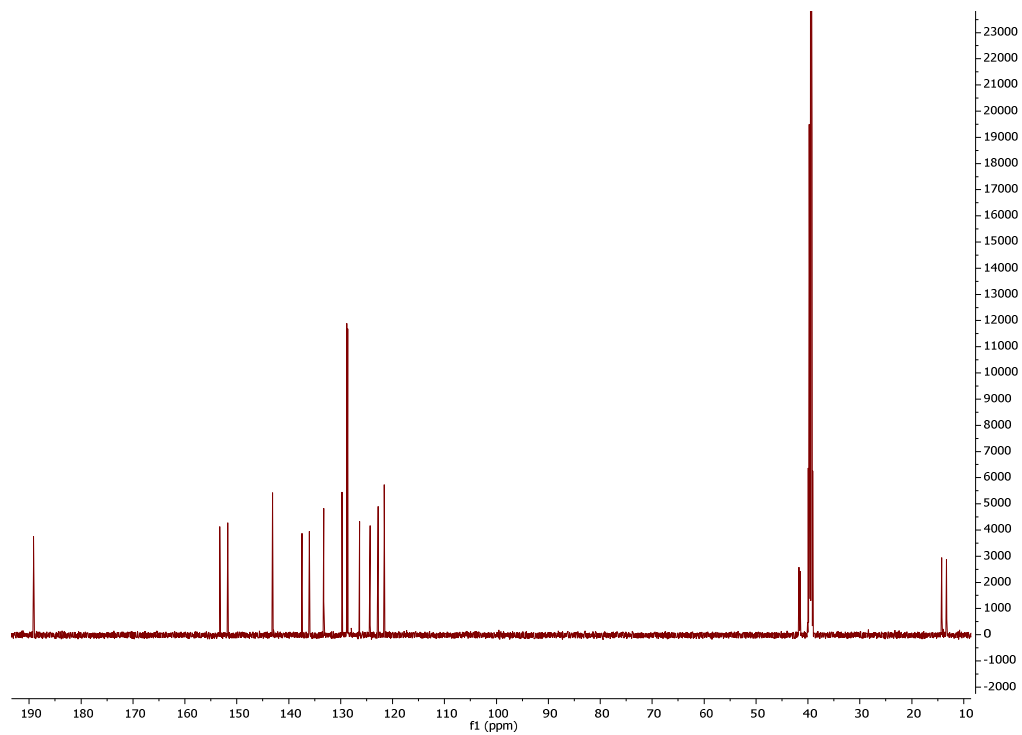


3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl N,N-diethylcarbamate (**9f**)

¹H NMR

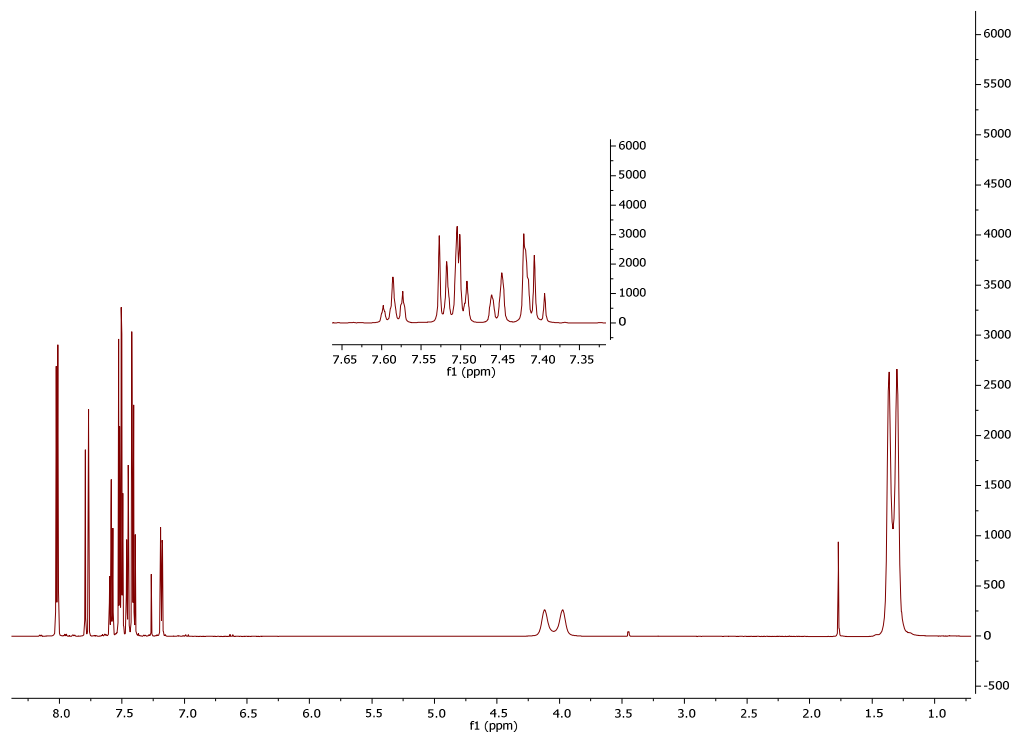


¹³C NMR

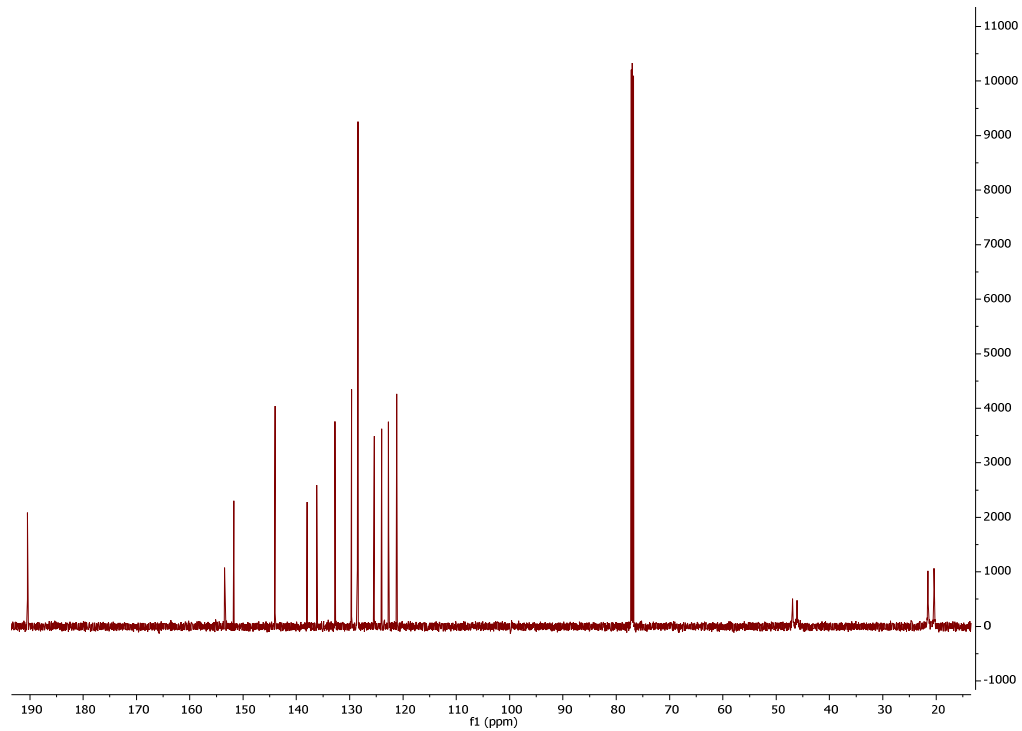


3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl N,N-bis(propan-2-yl)carbamate (**9g**)

¹H NMR

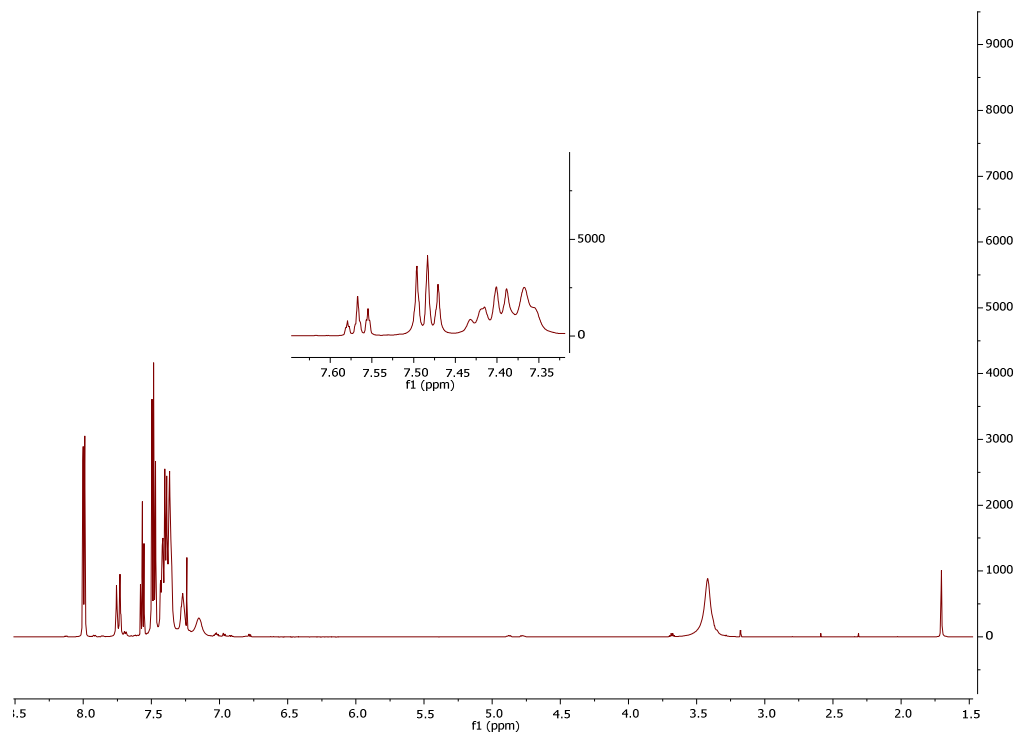


¹³C NMR

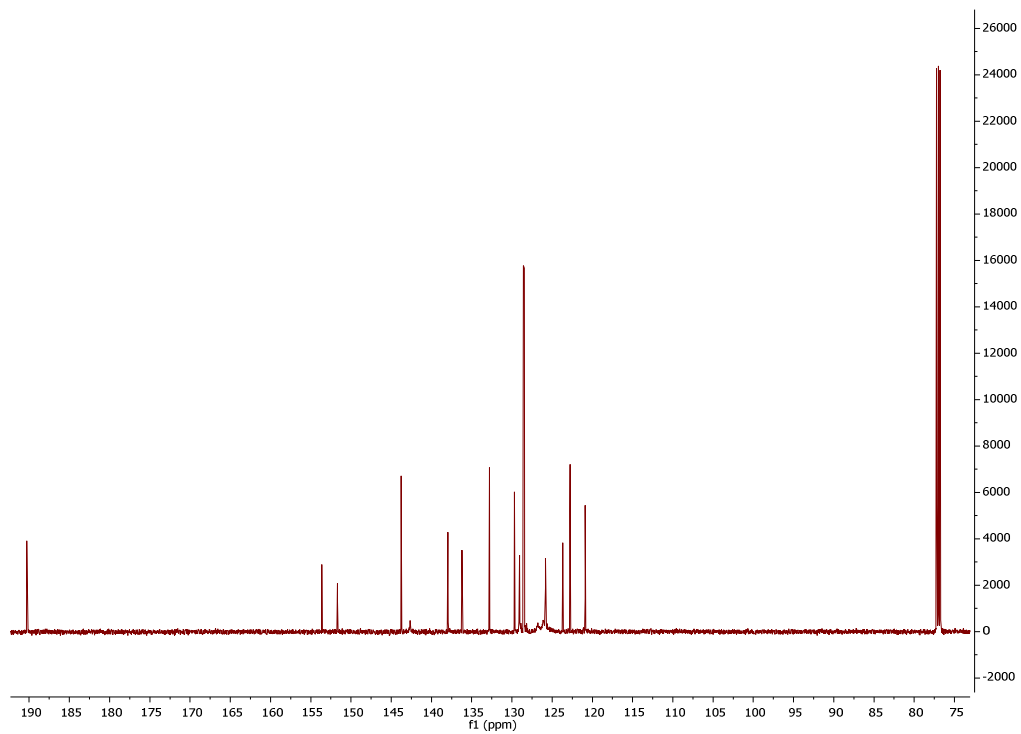


3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl N-methyl-N-phenylcarbamate (**9h**)

¹H NMR

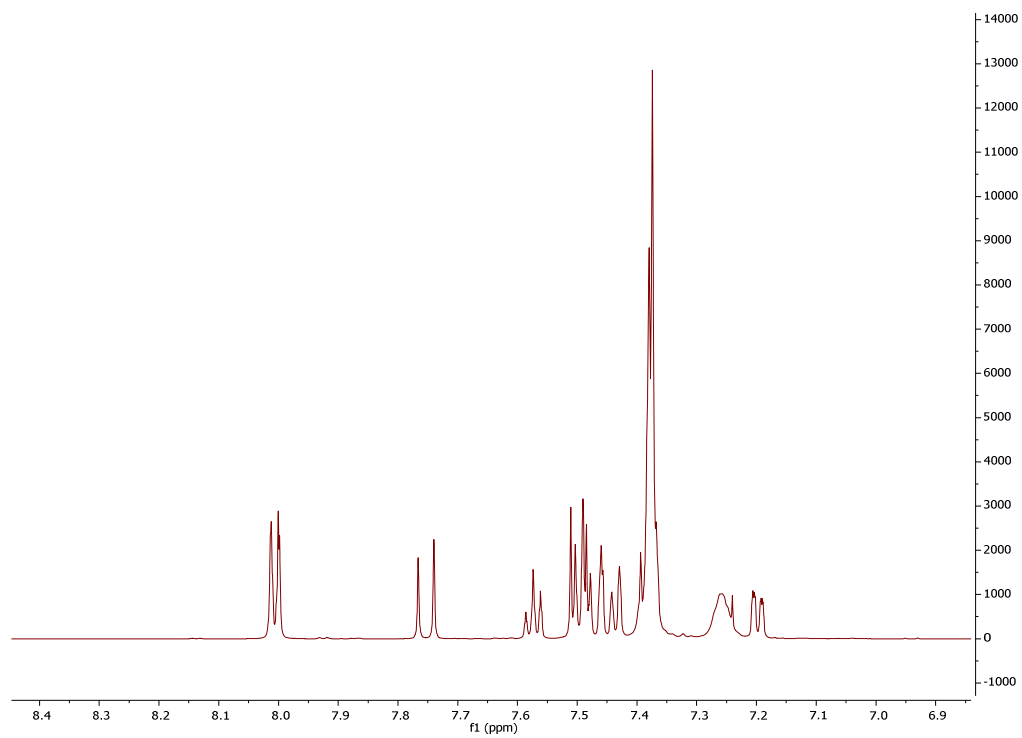


¹³C NMR

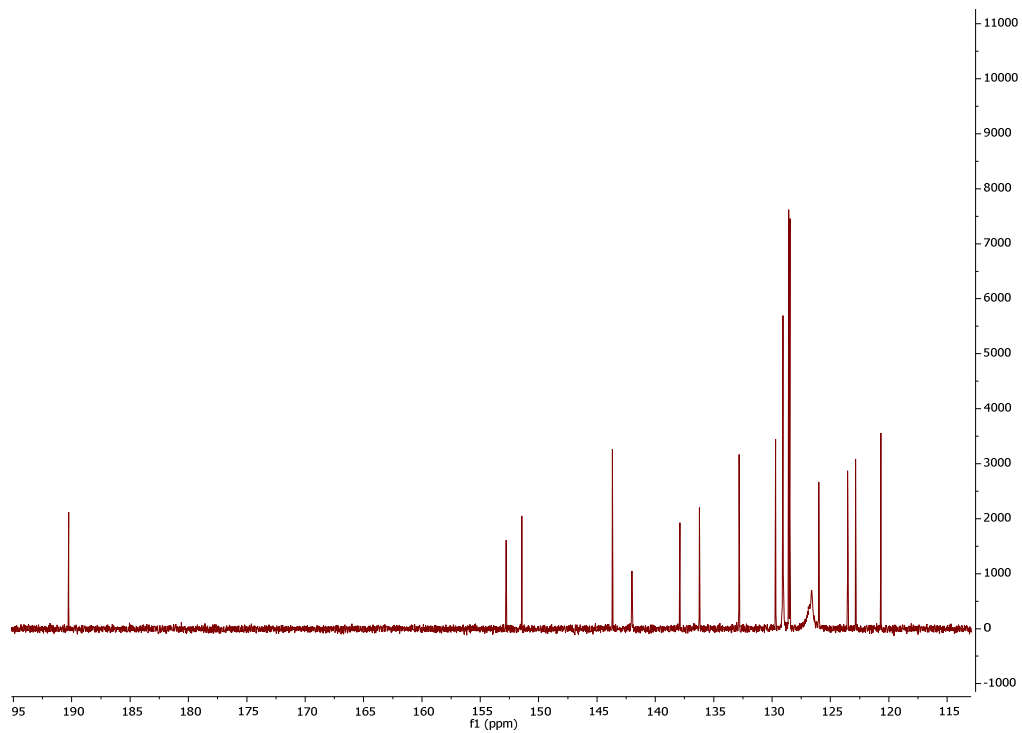


3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl N,N-diphenylcarbamate (**9i**)

¹H NMR

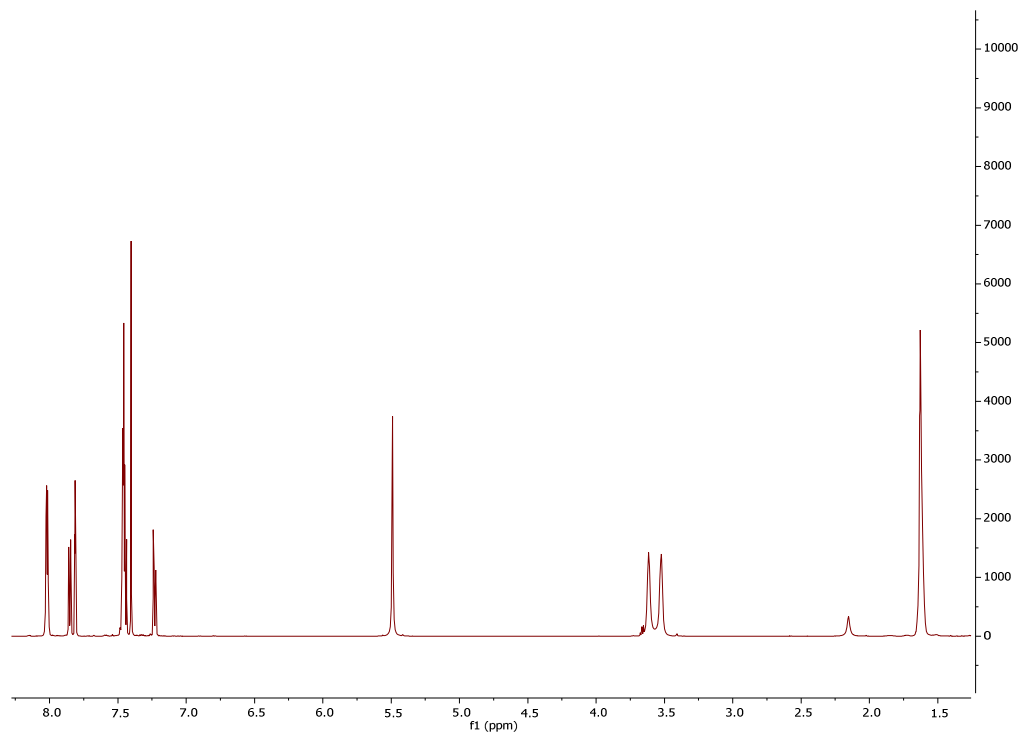


¹³C NMR

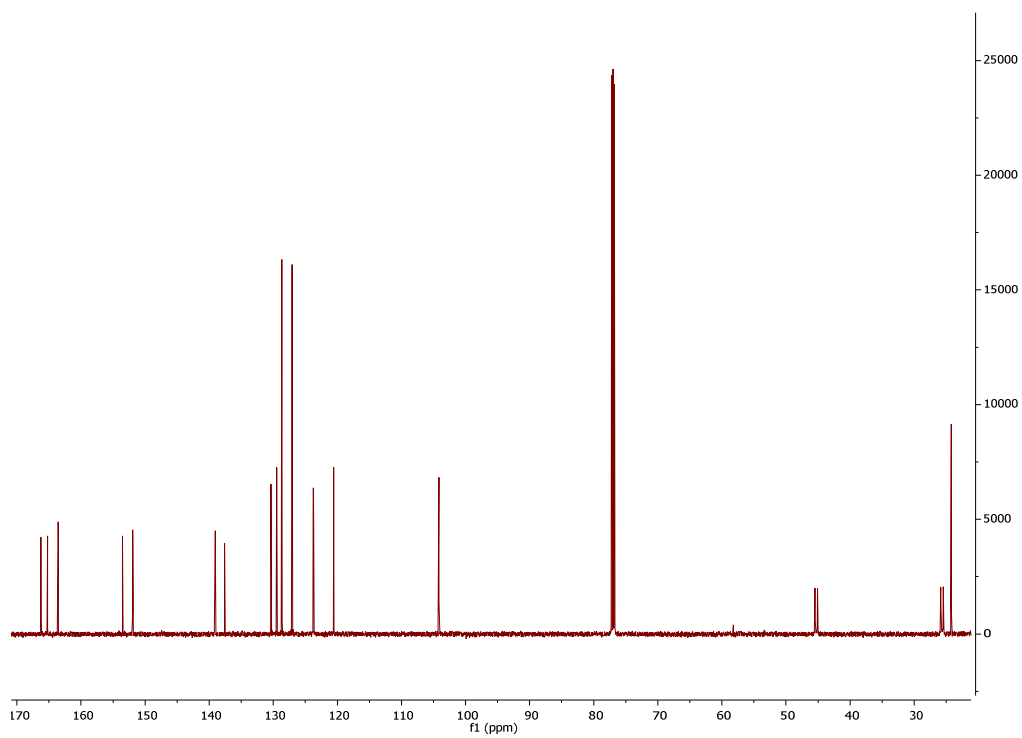


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl piperidine-1-carboxylate (**4a**)

¹H NMR

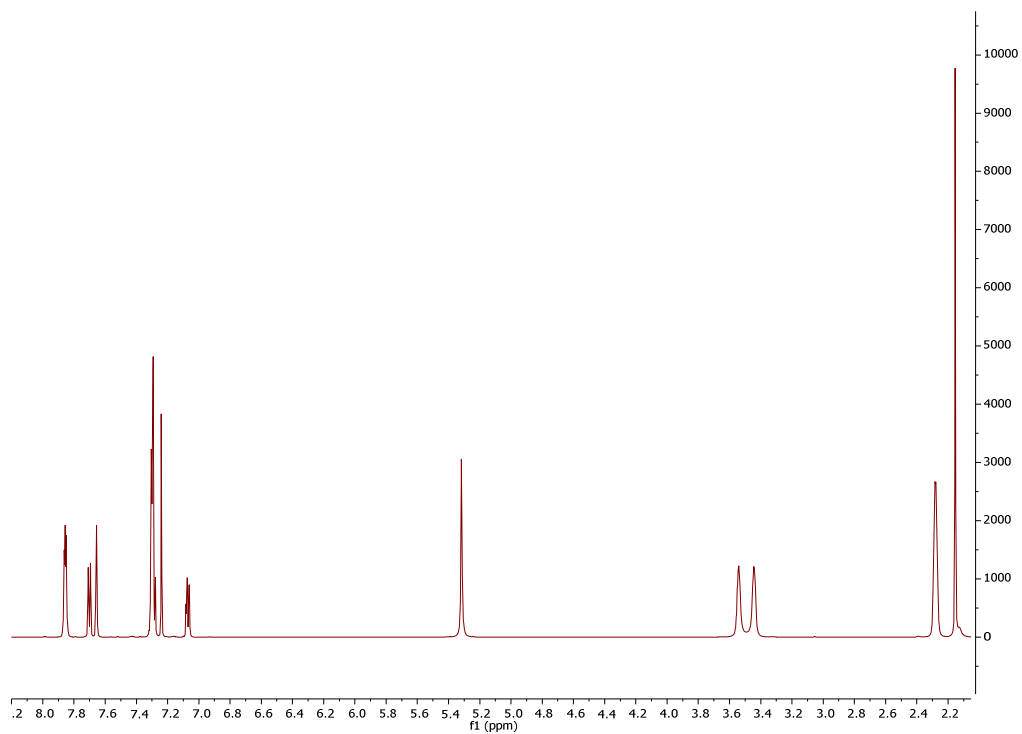


¹³C NMR

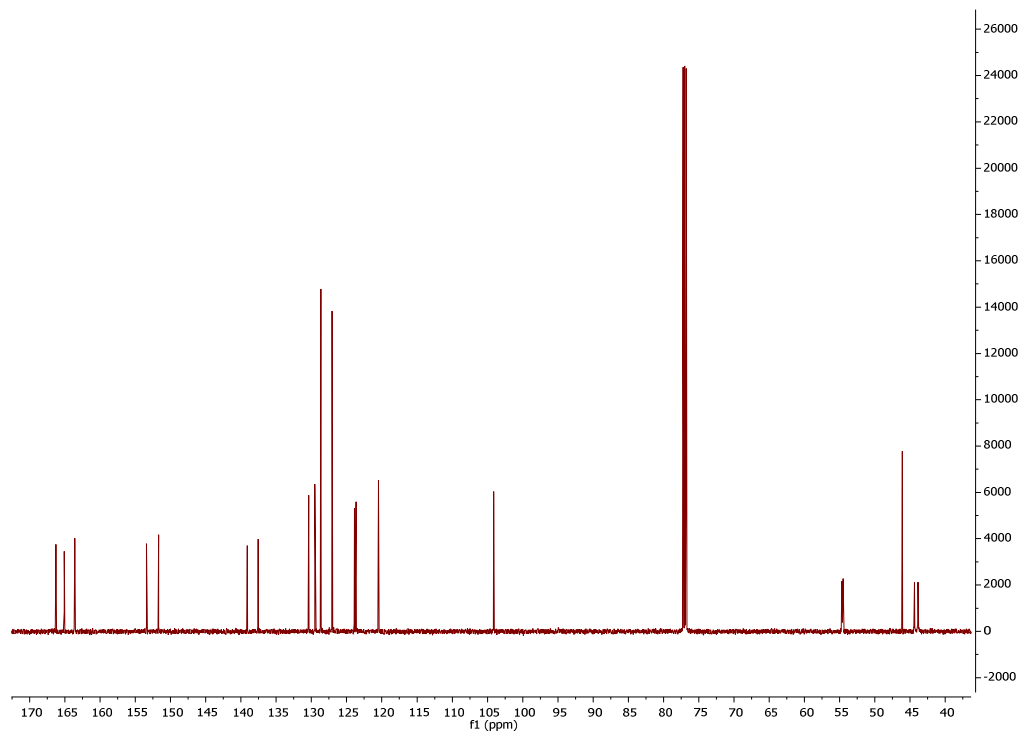


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl 4-methylpiperazine-1-carboxylate (**4b**)

¹H NMR

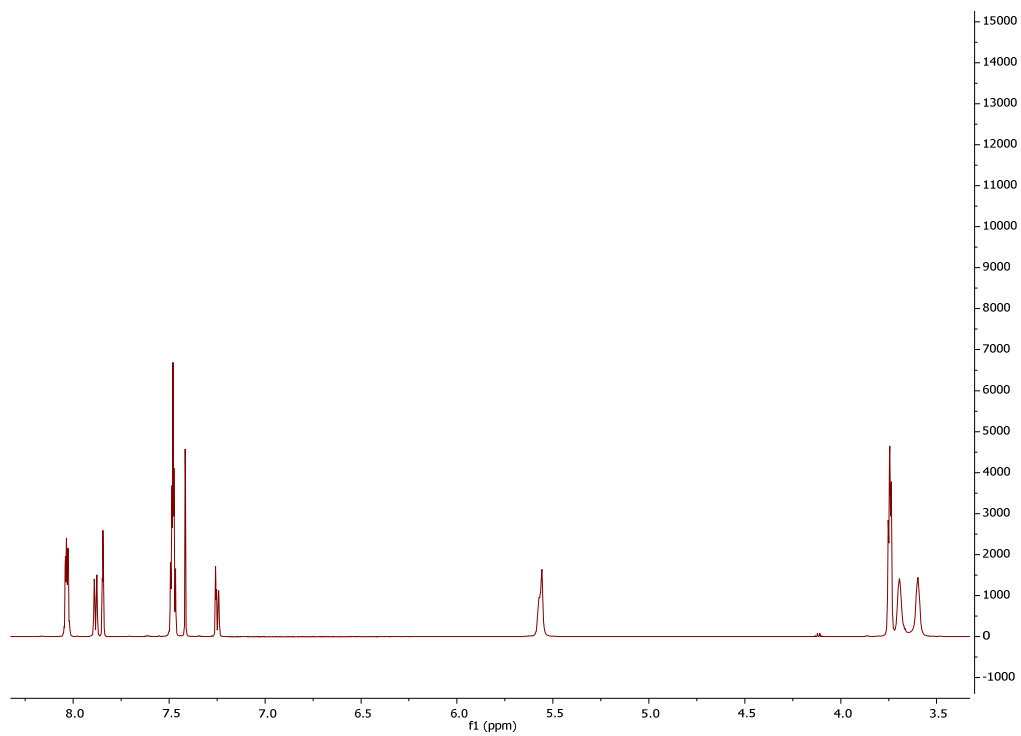


¹³C NMR

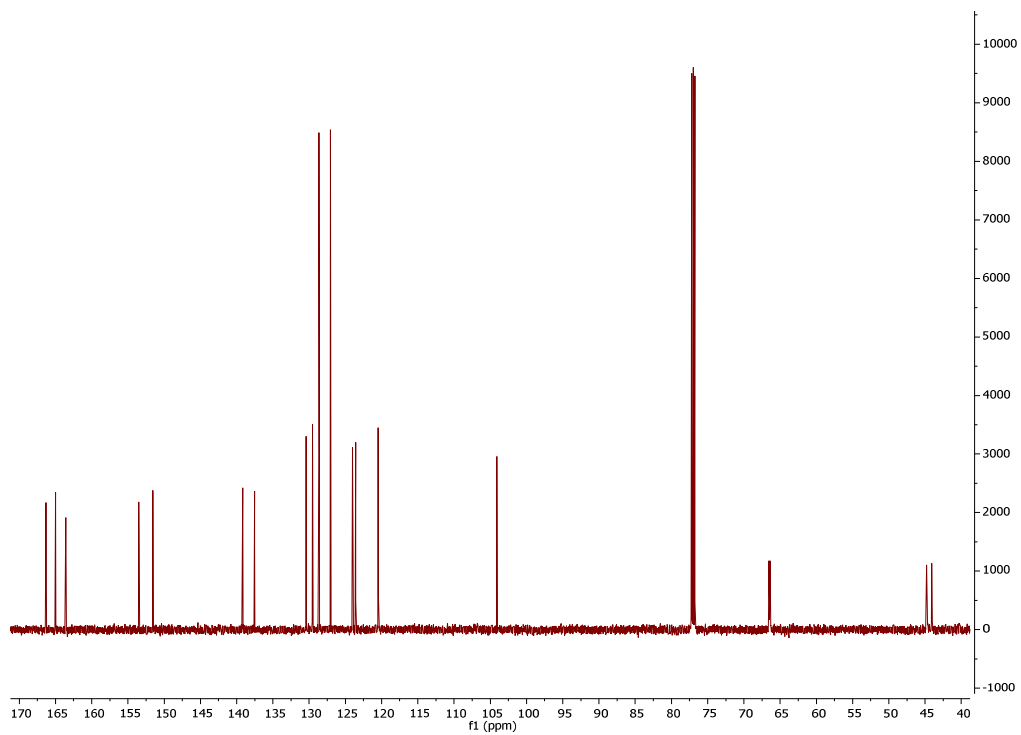


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate (**4c**)

¹H NMR

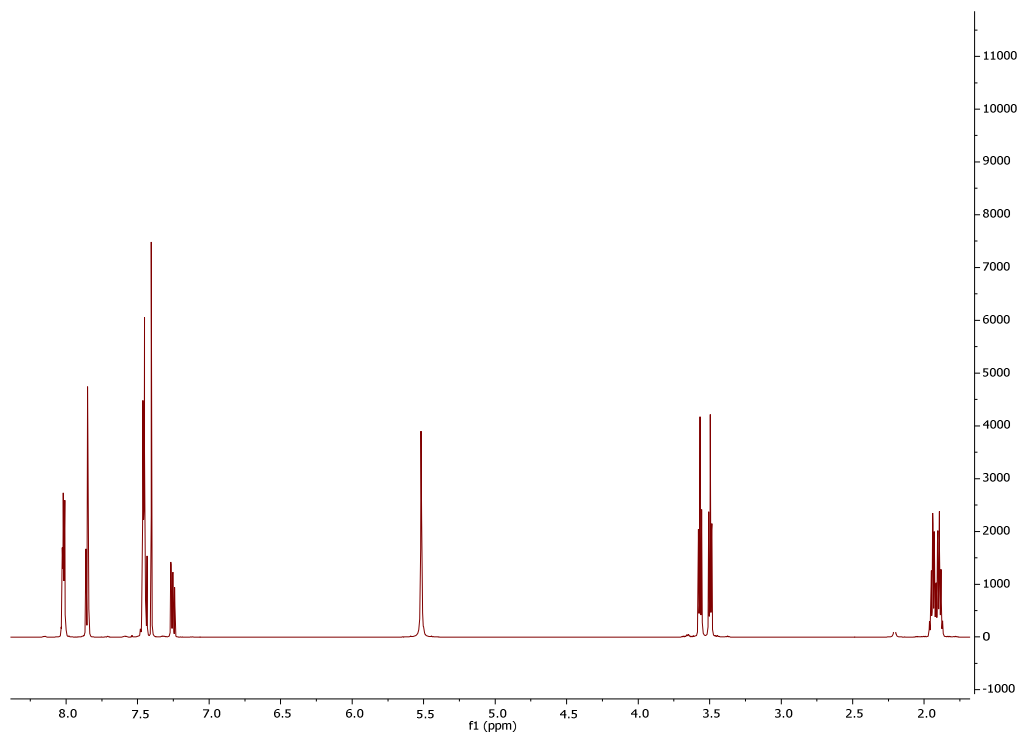


¹³C NMR

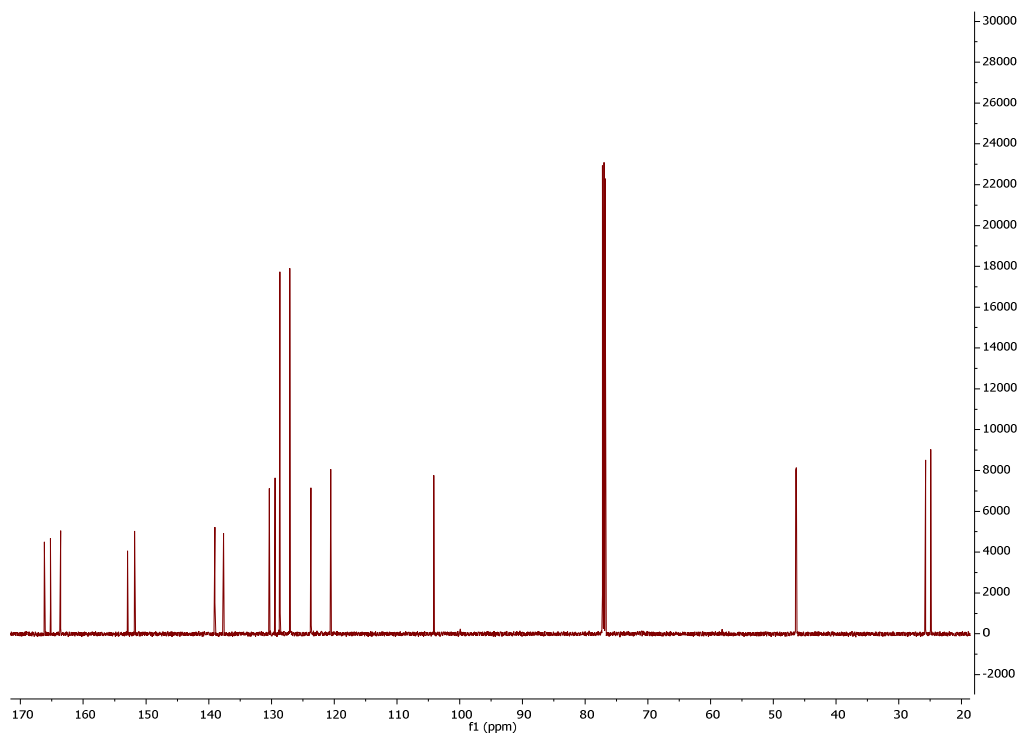


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl pyrrolidine-1-carboxylate (**4d**)

¹H NMR

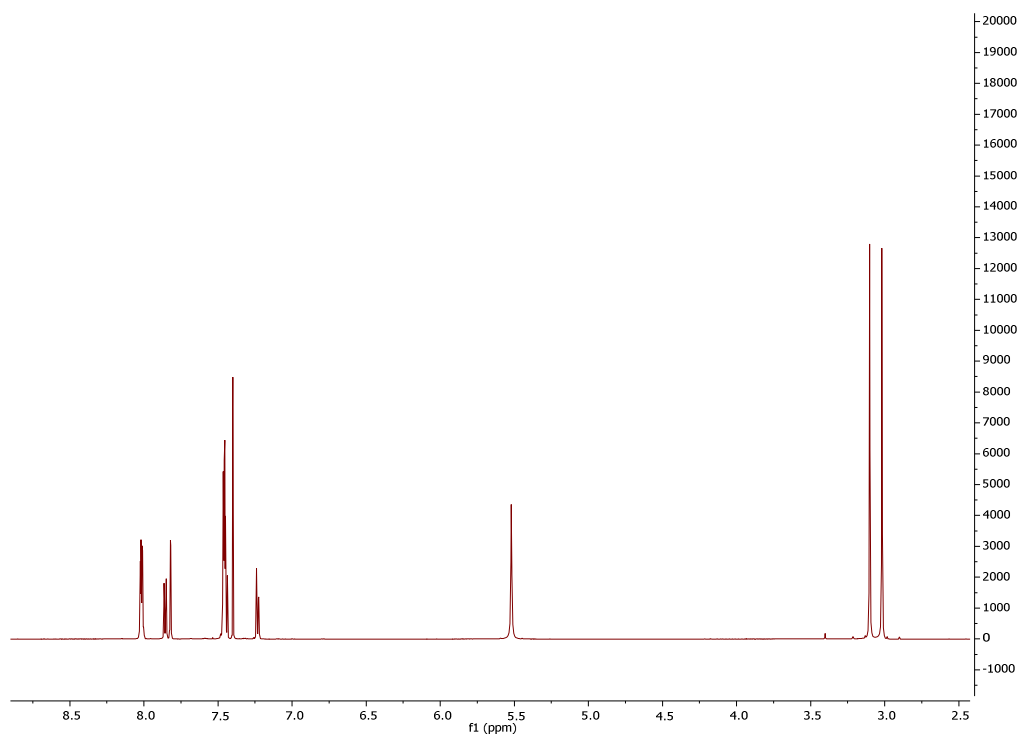


¹³C NMR

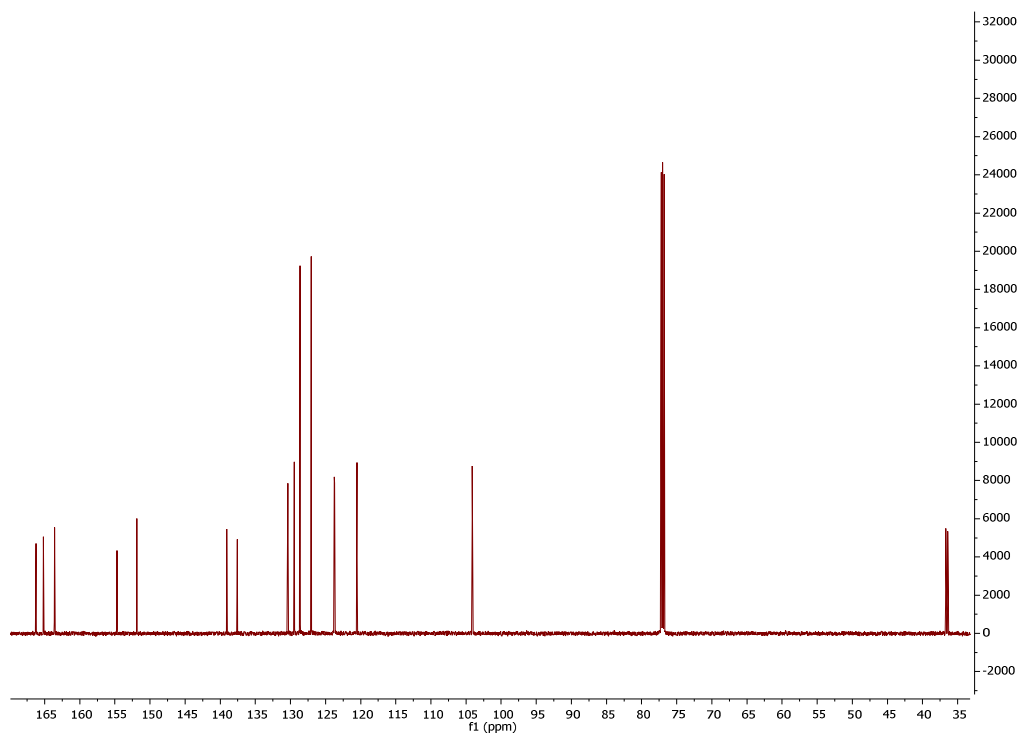


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl N,N-dimethylcarbamate (**4e**)

¹H NMR

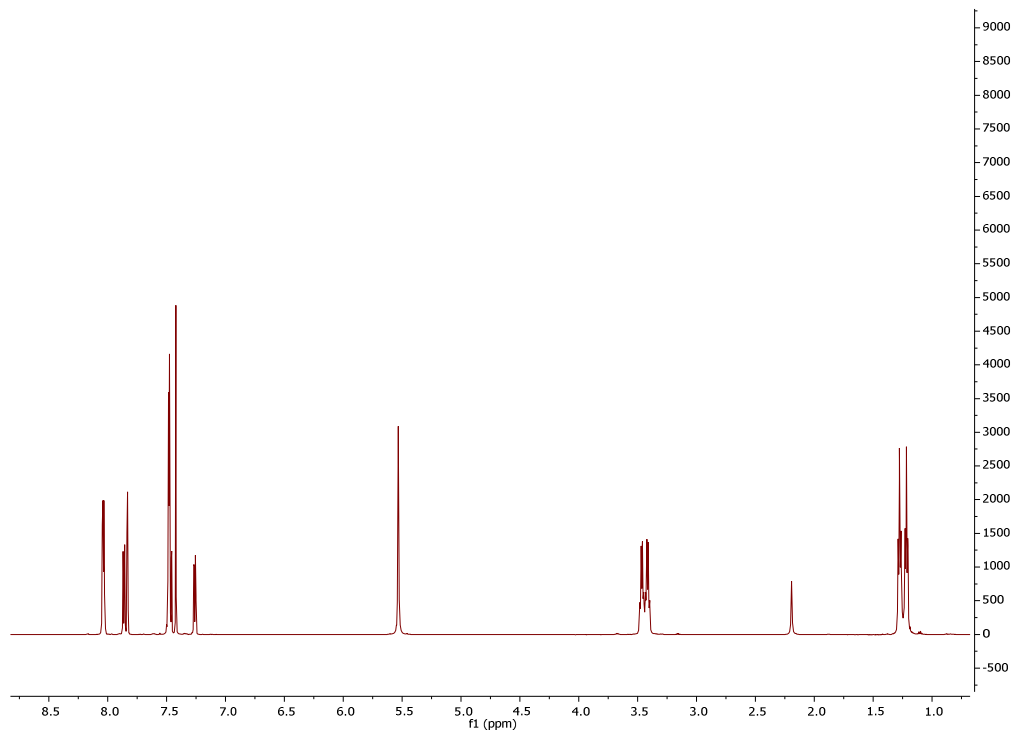


¹³C NMR

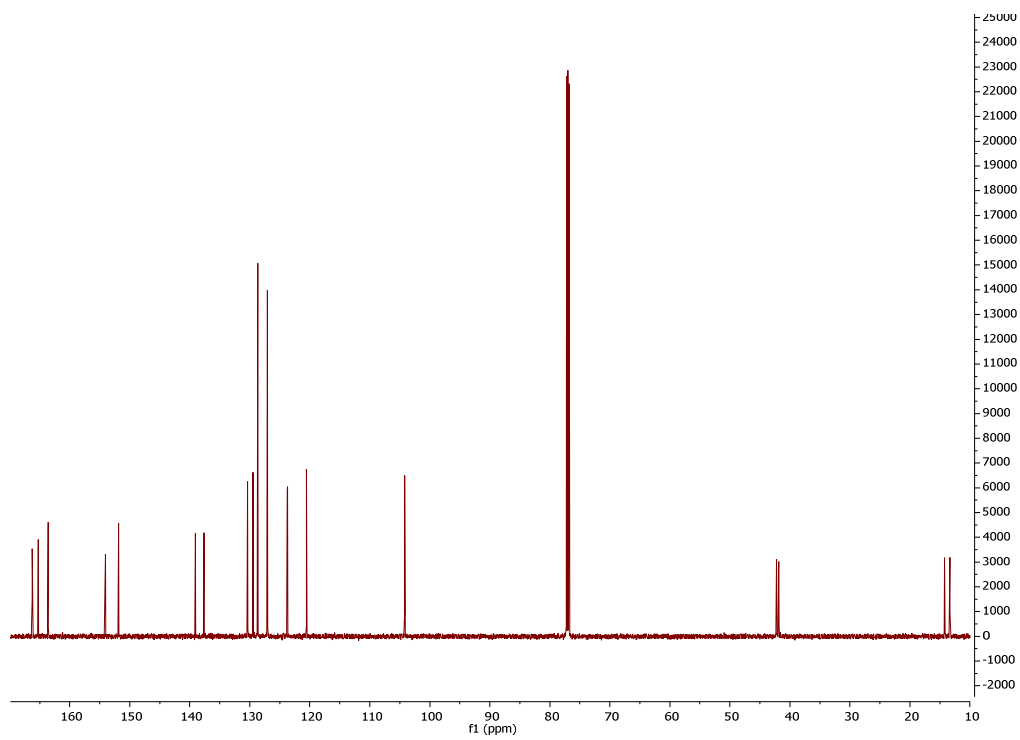


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl N,N-diethylcarbamate (**4f**)

¹H NMR

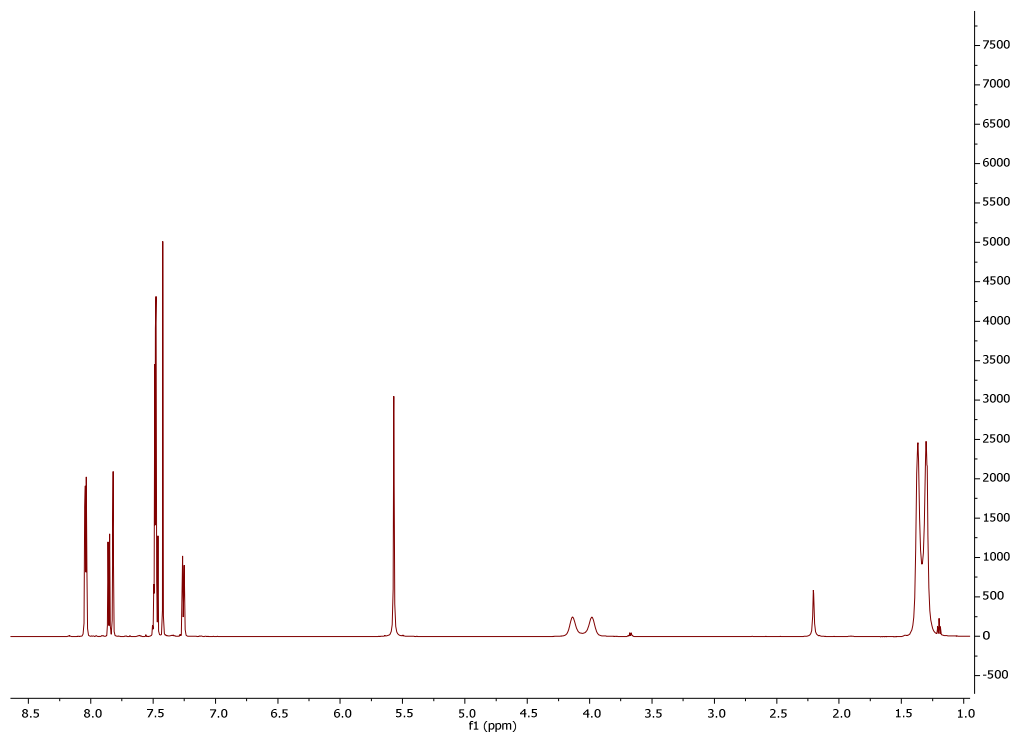


¹³C NMR

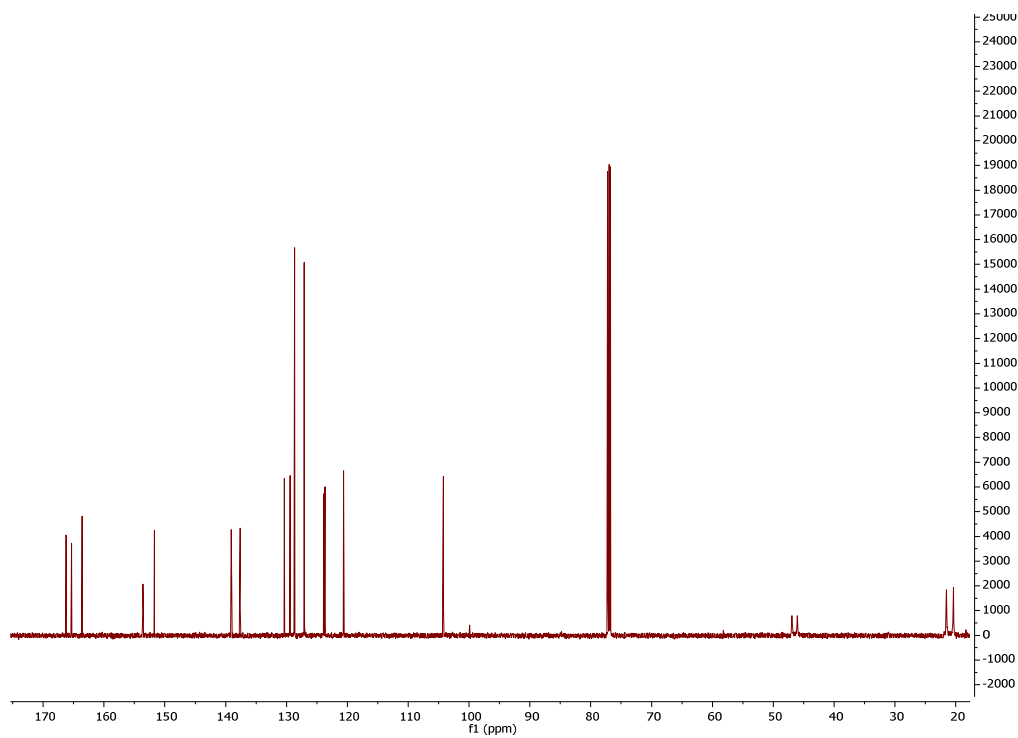


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl N,N-bis(propan-2-yl)carbamate (**4g**)

¹H NMR

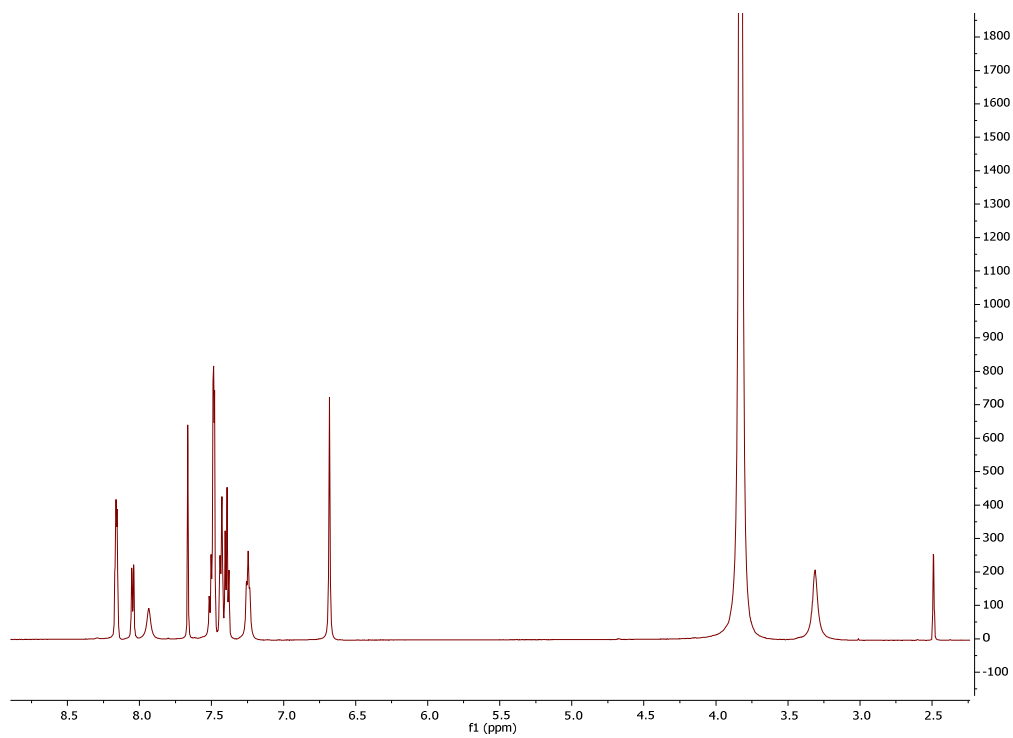


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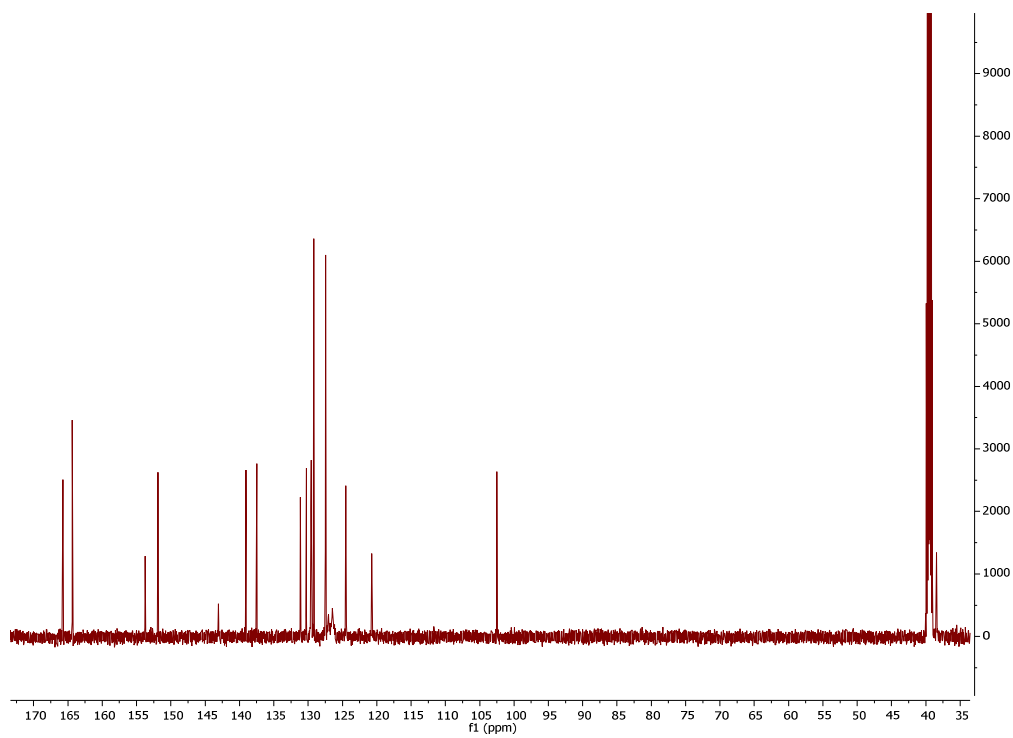


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl N-methyl-N-phenylcarbamate (**4h**)

¹H NMR

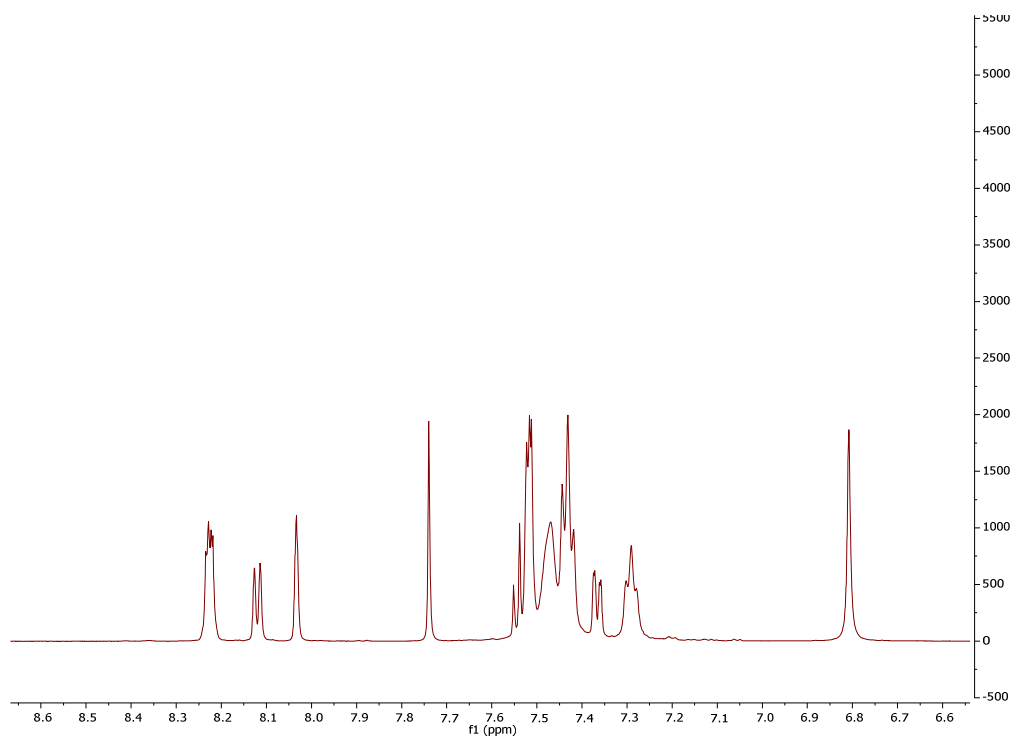


¹³C NMR

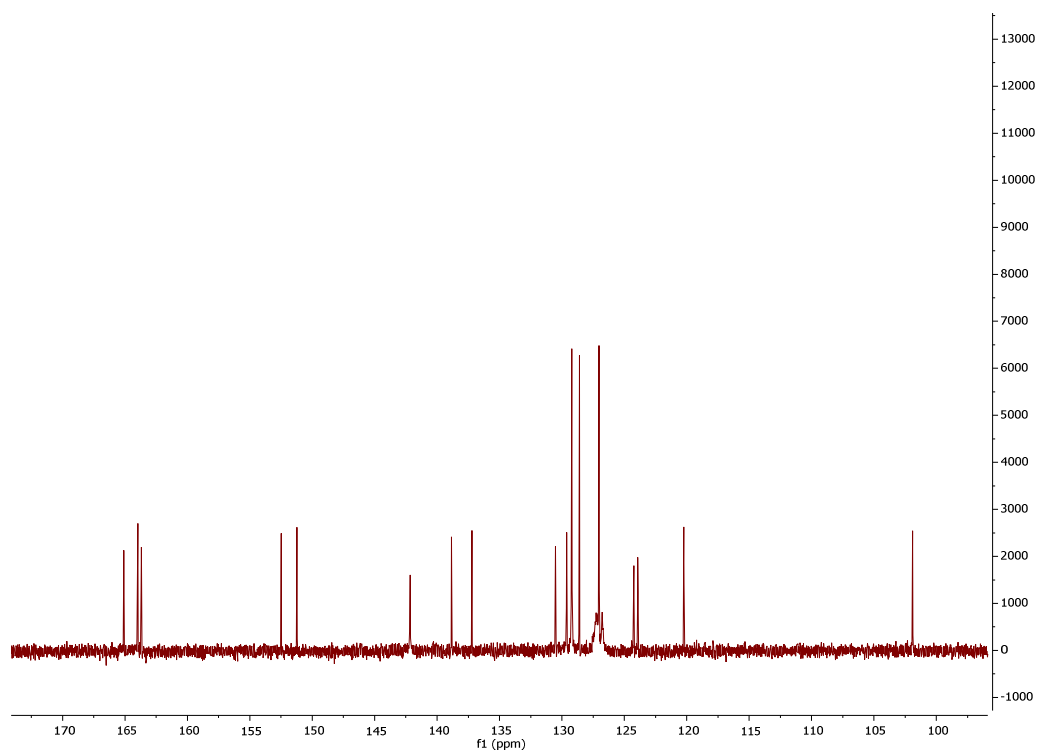


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl N,N-diphenylcarbamate (**4i**)

¹H NMR



¹³C NMR



CHAPTER 6

Article 4

Synthesis and evaluation of novel ether derivatives of 2-amino-4,6-diphenylpyrimidine as potential dual adenosine A₁ and A_{2A} antagonists

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Abstract

A novel series of ether substituted 2-amino-4,6-diphenylpyrimidines were evaluated as potential dual adenosine A₁ and A_{2A} receptor antagonists. The synthesised ether derivatives were mostly selective towards the adenosine A₁ receptor, exhibiting potent A₁ affinities with A₁K_i values ranging from 5.66 – 48.8 nM. Moderate adenosine A_{2A} affinities were recorded with A_{2A}K_i values ranging from 47.0 – 352 nM. Compound **3g** (4-phenyl-6-[3-(3-phenylpropoxy)phenyl]pyrimidin-2-amine), the only compound with a carbonyl group in its side chain, exhibited the most promising dual affinity in this series, with an A₁K_i of 22.2 nM and an A_{2A}K_i of 47.0 nM.

Keywords: 2-aminopyrimidine; ether, adenosine A₁ and A_{2A} receptor; dual; antagonist.

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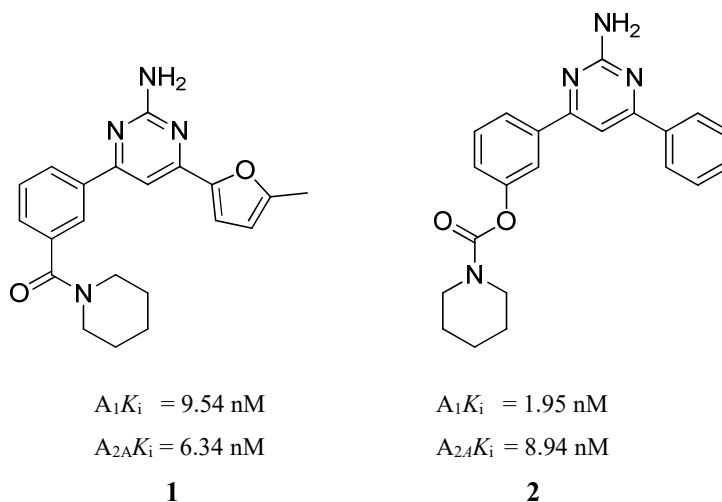
Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease that affects the lives of millions of people worldwide.¹ The primary manifestation of PD is decreased motor function, however, patients may also develop several non-motor symptoms such as depression and experience a decline in cognitive

function.^{2,3} Existing drug therapies suffer from a number of shortcomings such as the inability to halt the progression of the disease, fading of effectivity over time, as well as numerous undesirable side effects that affect quality of life.⁴ There is thus an urgent need for the development of alternatives to current therapies and non-dopaminergic targets for the disease are particularly appealing, especially if these are able to address non-motor symptoms and provide neuroprotection. In recent years, antagonists of the adenosine A_{2A} receptor have received attention as promising non-dopaminergic alternatives.⁵

Several preclinical studies have illustrated that independent antagonism of the adenosine A₁ and A_{2A} receptors has the ability to improve the motor symptoms associated with PD.⁶⁻⁸ Antagonism of both these receptors may also be beneficial in the treatment of non-motor symptoms that usually develop in the more progressive stages of the disease. For example, antidepressant effects have recently been reported for the A_{2A} antagonist, KW6002,⁹ while antagonism of the A₁ receptor on the other hand, is linked to an increase in cognition.¹⁰⁻¹³ Another exciting advantage of A_{2A} antagonism is the possibility of neuroprotection.^{14,15} Antagonism of adenosine A₁ and A_{2A} receptors may thus not only improve motor and non-motor symptoms of PD, but may also be able to slow the progression of the disease.

Several 2-aminopyrimidines with potent dual adenosine A₁ and A_{2A} affinities were recently identified in our laboratories (**1**, **2**).¹⁶⁻¹⁸ These 2-aminopyrimidines were substituted with various amide (e.g. **1**) and carbamate (e.g. **2**) substituents.



Molecular docking studies conducted previously in our laboratories, indicated that the carbonyl group is important for binding, and particularly for adenosine A_{2A} receptor affinity, as intermolecular interactions were frequently observed between this group and residues (such as Tyr271) in the receptor binding site (Figure 1).¹⁶⁻¹⁸

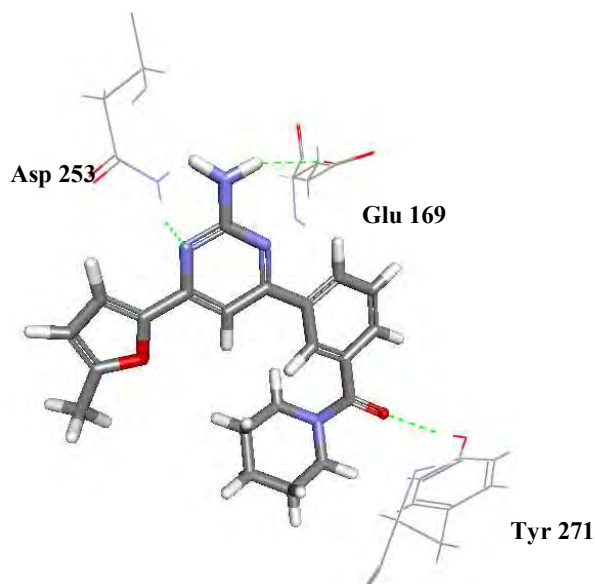
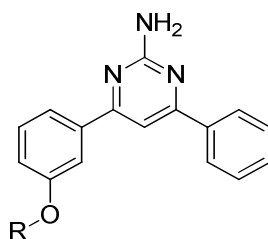


Figure 1: Illustration of the intermolecular hydrogen bond between the amide carbonyl of compound **1** and Tyr271.^{16,18}

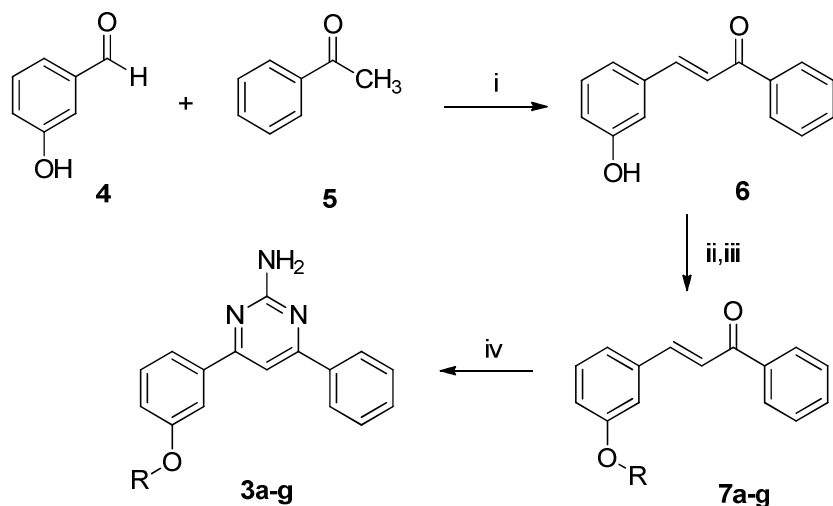
The aim of this study was therefore to further investigate the importance of the carbonyl group by replacing the amide and carbamate side chains with an ether substituent (e.g. **3**). It was also hypothesised that the more flexible ether could possibly bend and rotate to a larger extent than the more rigid analogues, allowing an improved fit in the binding sites of the target receptors, resulting in an increase in binding affinity.



3

Results and discussion:

A series of ether substituted 2-amino-4,6-diphenylpyrimidines was successfully synthesised as illustrated in Scheme 1. Condensation of 3-hydroxybenzaldehyde (**4**) with acetophenone (**5**), resulted in chalcone (**6**), which was reacted with commercially available chlorides to obtain the ether intermediates (**7a - g**).¹⁹ Cyclisation was carried out with guanidine hydrochloride and sodium hydride in *N,N*-dimethylformamide yielding the desired 2-aminopyrimidines (**3a - g**) in low yields.²⁰ The structures of all synthesised compounds (Table 1) were confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry (see supporting information).

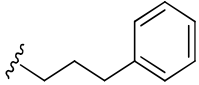
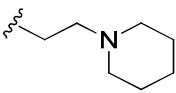
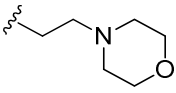
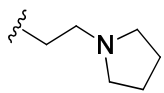
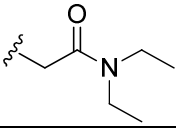


Scheme 1: Synthesis. Reagents and conditions: (i) NaOH, 1 M (2 eq.), MeOH, 90 °C, 5 days (60%); (ii) K₂CO₃ (2 eq.), CH₃CN, rt, 30 min; (iii) RCl (1.2 eq.), reflux at 90 °C, 24 h (60-70%); (iv) Guanidine hydrochloride (1.5 eq.), NaH (1.5 eq.), DMF, 110 °C, 24 h (6-33%).

To determine affinities for the adenosine receptors, radioligand binding assays were performed using the radioligands 1,3-[³H]-dipropyl-8-cyclopentylxanthine ([³H]DPCPX) for binding to A₁ receptors, while the non-selective adenosine antagonist, [³H]5'-*N*-ethylcarboxamide-adenosine ([³H]NECA) was used in the presence of *N*⁶-cyclopentyladenosine (CPA), for binding to the A_{2A} receptors.^{21,22} The affinities of the synthesised compounds are expressed as the dissociation constants (*K_i*, nM) for both the adenosine A₁ and A_{2A} receptors as indicated in Table 1.

Table 1: Adenosine receptor affinities (*K_i*) of the synthesised ethers **3a** – **3g**.

Compound	R	A ₁ ^a <i>K_i</i> (nM)	A _{2A} ^a <i>K_i</i> (nM)	SI ^b
3a		5.66 ± 1.01	326 ± 71.3	58
3b		7.31 ± 0.69	77.8 ± 1.49	11

3c		48.8 ± 7.64	352 ± 24.4	7.2
3d		11.7 ± 1.48	194 ± 3.00	17
3e		16.2 ± 4.43	133 ± 14.3	8.2
3f		7.80 ± 2.98	307 ± 7.48	39
3g		22.2 ± 3.93	47.0 ± 3.00	2.1
^c CPA		8.48 ± 0.30		
^d ZM241385			1.20 ± 0.39	

^aAll values are expressed as the mean ± SD of triplicate determinations.

^bSelectivity index ($A_{2A}K_i/A_1K_i$)

^cN⁶-Cyclopentyladenosine, a known adenosine A₁ agonist, used as reference for A₁ receptor affinity – literature $K_i = 7.9 \text{ nM}^{23}$

^dA known adenosine A_{2A} receptor antagonist, used as positive reference for A_{2A} receptor affinity – literature $K_i = 2 \text{ nM}^{24}$

The ether substituted 2-amino-4,6-diphenylpyrimidines were mostly selective for the A₁ receptor and exhibited A₁K_i values that ranged from 5.66 - 48.8 nM. For the compounds with the 2 carbon linker in the side chain (**3b**, **3d**, **3e** and **3f**) similar A₁ affinities were obtained, however morpholine substitution appears to be least favourable, possibly indicating that a more hydrophobic terminal ring is preferable. Interestingly, addition of a carbonyl to the side chain and opening of the terminal ring (**3g**) also appears to affect adenosine A₁ affinity negatively. Comparison of the A₁ affinities of compound **3c**, with the weakest A₁ receptor affinity ($K_i = 48.8 \text{ nM}$), to **3a** ($K_i = 5.66 \text{ nM}$) and **3b** ($K_i = 7.31 \text{ nM}$) reveals that the longer carbon side chain linker (**3c**) is detrimental for A₁ receptor affinity. In fact, compound **3a**, with the shortest side chain, has the highest A₁ receptor affinity ($K_i = 5.66 \text{ nM}$), which further suggests that a shorter, hydrophobic side chain is better accommodated by the A₁ receptor binding site.

By comparison, adenosine A_{2A} affinities were generally weaker with A_{2A}K_i values ranging from 47.0 – 352 nM. When comparing the adenosine A_{2A} receptor affinities within the series of compounds, it is evident that ether substitution is less favourable than amide (e.g. **1**) or carbamate (e.g. **2**) substitution

(these results can be directly compared since these compounds were evaluated under identical experimental conditions).^{16,17} This is probably due to the absence of the carbonyl group which was previously shown to be important for adenosine A_{2A} receptor affinity. This theory was further supported by the finding that compound **3g**, which is the only compound from this series containing a carbonyl group in the side chain, exhibited the highest A_{2A} affinity ($K_i = 47.0$ nM) of the series. However, it also appears that the position of the carbonyl in the side chain is important as the A_{2A} affinity of this compound is weaker than those observed for the previously synthesised amides and carbamates.^{16,17}

Additional molecular modelling studies were subsequently performed in order to rationalise the results obtained from the radioligand binding assays. The synthesised compounds were docked into a model of the binding site of the crystallised adenosine A_{2A} receptor (PDB code: 3EML) using the CDOCKER function of Discovery Studio 3.1. Visual inspection of the docked ether derivatives revealed that these compounds undergo several of the interactions previously observed for other 2-aminopyrimidines, which are most likely responsible for anchoring these compounds in the binding site.¹⁶ These include π - π interactions with Phe168 and intermolecular hydrogen bonding between the exocyclic amino group of the pyrimidine ring and Glu169 as well as Asn253 (Figure 2A). However, since most of these compounds do not have a carbonyl group in their side chains, no interaction is observed with Tyr271 as previously reported for related amides (Figure 2A).¹⁶ However, compound **3g** is an exception, since it is the only compound from the current series that exhibits an additional hydrogen bond interaction between the carbonyl oxygen and Tyr271 (Figure 2B). This additional interaction provides, at least in part, an explanation for the superior adenosine A_{2A} affinity observed for **3g** in the radioligand binding assays. It can therefore be concluded that the interaction between the carbonyl and Tyr271 is important and that loss of this interaction leads to a decrease in adenosine A_{2A} receptor affinity.

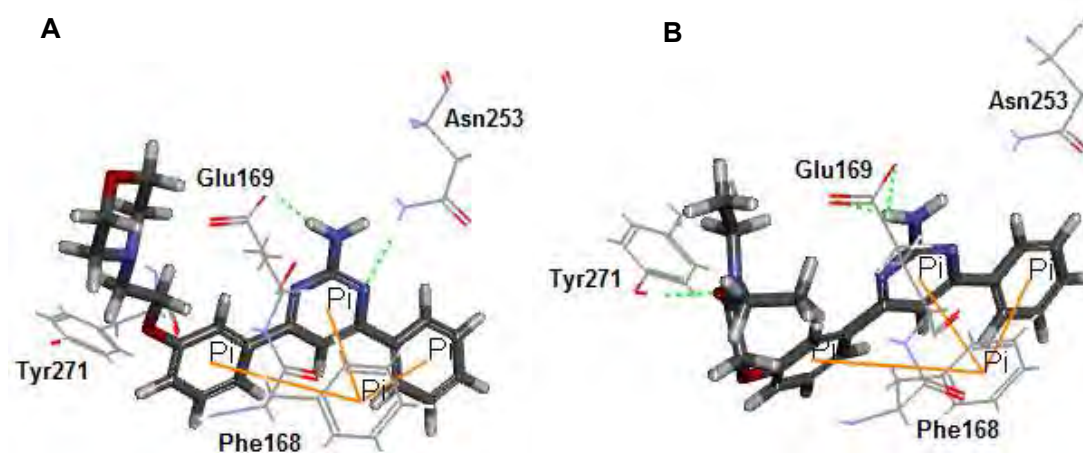


Figure 2: Compound **3e** (A) and **3g** (B) docked in the binding site of the human A_{2A} receptor. π - π Interactions are indicated in orange while intermolecular hydrogen bond interactions are indicated in green.

Conclusion

The ether substituted 2-amino-4,6-diphenylpyrimidines synthesised in this study exhibited some selectivity for the A₁ receptor, over the A_{2A} receptor. The moderate A_{2A} affinities observed for these compounds are most likely due to the absence of a carbonyl group in the side chain, which was supported by the results obtained from molecular docking studies. These particular compounds, being A₁ receptor selective, could therefore be investigated for application in alternative disease states.

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Supplementary material

1. Materials and instrumentation
2. Synthesis
3. Physical data
4. NMR Spectra

1. Materials and instrumentation

All chemicals were purchased from Sigma-Aldrich and used without any further purification. Solvents for synthesis and chromatography were obtained from Associated Chemical Enterprises while deuterated solvents for nuclear magnetic resonance (NMR) spectroscopy were purchased from Merck.

Thin layer chromatography (TLC):

Precoated Kieselgel 60 F254 plates (Merck) were used to monitor reactions. Detection was done by UV light at a wavelength of 254 nm.

Melting points:

The melting points of all compounds were determined using a Buchi B-545 apparatus, and are uncorrected.

Mass spectrometry (MS):

The mass spectra for all compounds were obtained with a Bruker micrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode.

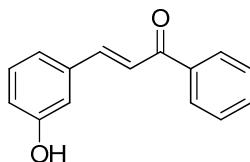
Nuclear magnetic resonance (NMR) spectroscopy:

A BrukerAvance III 600 spectrometer was used to record proton (^1H) and carbon (^{13}C) NMR spectra at frequencies of 600 MHz and 151 MHz, respectively. Samples were dissolved in either deuterated dimethylsulfoxide (DMSO-*d*6) or deuterated chloroform (CDCl_3). Reported ^1H NMR spectroscopic data indicate the chemical shift (δ) in ppm, the integration (e.g. 1H), the multiplicity and the coupling constant (*J*) in Hz. The following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), br t (broad triplet), q (quartet), p (pentet/quintet) or m (multiplet). Chemical shifts are referenced to the residual

solvent signal (DMSO-*d*₆: 2.5 and 39.5 ppm for ¹H and ¹³C respectively; CDCl₃ 7.26 and 77.0 ppm for ¹H and ¹³C respectively).

2. Synthesis

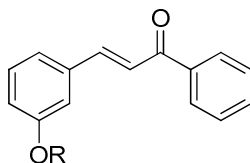
General procedure for the synthesis of (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (6)



6

A solution of sodium hydroxide (4% w/v; 49 mmol) in water, was added dropwise to a suspension of acetophenone (5) (24.5 mmol) and benzaldehyde (4) (24.5 mmol) in 300 ml methanol. The reaction mixture was refluxed at 90 °C for 5 days. Afterwards the reaction was concentrated *in vacuo* and equal volumes of ethyl acetate and water were added. The layers were separated and the water phase was extracted twice with ethyl acetate. The organic layers were combined, concentrated *in vacuo* and the subsequent solid was recrystallised from ethanol.

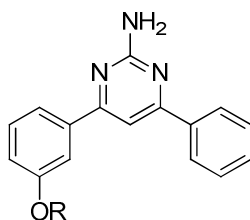
General procedure for the synthesis of ethers (7a-g)



7a - g

(2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (6) (11.1 mmol) was added to a stirred suspension of 100 ml acetonitrile and potassium carbonate (22.2 mmol). After stirring for 30 minutes, the alkyl chloride (13.3 mmol) was added to the mixture. The reaction was refluxed overnight at 90 °C, cooled to room temperature and water (~ 100 ml) was added until a precipitate formed. The resulting solid was filtered and recrystallised from ethanol. In cases where no precipitate formed, the solvent was evaporated and the product extracted with chloroform.

General procedure for the synthesis of aminopyrimidines (3a-g)



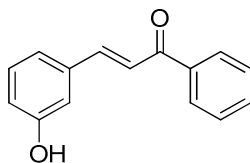
3a – g

Guanidine hydrochloride (4.61 mmol) was suspended in a small amount of DMF (20 ml) and the required ether intermediate (**7a – g**) (3.07 mmol) and sodium hydride (4.61 mmol) were added to the reaction mixture. The reaction was heated (90 °C) overnight under nitrogen, allowed to cool down and subsequently diluted with equal volumes of water and ethyl acetate. The aqueous phase was extracted with ethyl acetate and the organic layers combined. All traces of DMF were removed by washing the combined organic layers four to five times with water. The organic layer was concentrated *in vacuo* and the crude product purified by silica gel column chromatography [dichloromethane: methanol (98:2)]. The resulting solid was recrystallised from ethanol.

3. Physical data

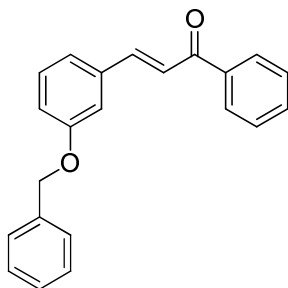
Chalcones:

(*2E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**)



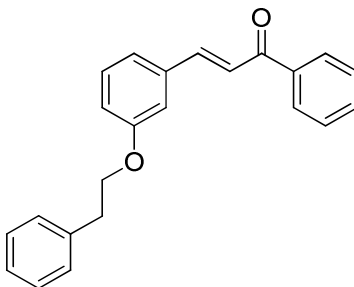
The title compound was prepared from 3-hydroxybenzaldehyde and acetophenone in a yield of 59%: mp 88.4 – 89.2 °C (ethyl acetate), pale yellow solid. ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.68 (s, 1H), 8.17 – 8.12 (m, 2H), 7.84 (d, *J* = 15.6 Hz, 1H), 7.72 – 7.62 (m, 2H), 7.56 (br t, *J* = 7.7 Hz, 2H), 7.32 (br d, *J* = 7.8 Hz, 1H), 7.29 – 7.23 (m, 2H), 6.90 (ddd, *J* = 8.0, 2.5, 1.0 Hz, 1H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 189.2, 157.8, 144.3, 137.6, 135.9, 133.1, 129.9, 128.8, 128.5, 121.9, 119.9, 117.9, 115.3. APCI-HRMS *m/z*: calcd for C₁₅H₁₃O₂ (M + H)⁺, 225.0910, found 225.0910.

(2E)-3-[3-(benzyloxy)phenyl]-1-phenylprop-2-en-1-one (**7a**)



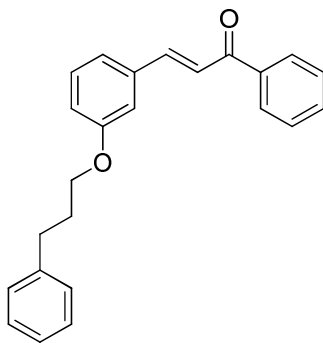
The title compound was prepared from (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**) and benzyl chloride in a yield of 80%: mp 88.4 – 89.2 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.94 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 15.7 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.46 – 7.39 (m, 3H), 7.38 (br d, *J* = 8.3 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.30 – 7.23 (m, 2H), 7.20 – 7.15 (m, 2H), 6.96 (dd, *J* = 8.2, 2.4 Hz, 1H), 5.03 (s, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 190.4, 159.1, 144.6, 138.1, 136.6, 136.2, 132.8, 129.9, 128.61 (2C), 128.58 (2C), 128.5 (2C), 128.1, 127.5 (2C), 122.3, 121.4, 117.0, 114.4, 70.1. APCI-HRMS *m/z*: calcd for C₂₂H₁₉O₂ (M + H)⁺, 315.1380, found 315.1354.

(2E)-1-phenyl-3-[3-(2-phenylethoxy)phenyl]prop-2-en-1-one (**7b**)



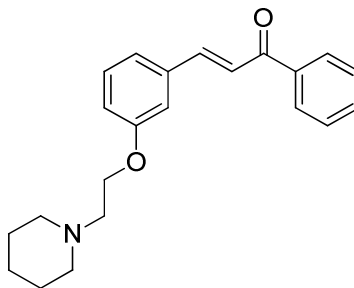
The title compound was prepared from (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**) and (2-chloroethyl)benzene in a yield of 61%: light yellow gum. ¹H NMR (600 MHz, CDCl₃) δ 8.04 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 15.7 Hz, 1H), 7.63 – 7.55 (m, 1H), 7.55 – 7.49 (m, 3H), 7.38 – 7.30 (m, 5H), 7.30 – 7.22 (m, 2H), 7.18 (br s, 1H), 6.98 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.24 (t, *J* = 7.0 Hz, 2H), 3.15 (t, *J* = 7.0 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 190.4, 159.1, 144.7, 138.1, 138.0, 136.2, 132.7, 129.9, 129.0 (2C), 128.6 (2C), 128.48 (2C), 128.45 (2C), 126.5, 122.2, 121.3, 116.8, 113.9, 68.7, 35.7. APCI-HRMS *m/z*: calcd for C₂₃H₂₁O₂ (M + H)⁺, 329.1536, found 329.1534.

(2E)-1-phenyl-3-[3-(3-phenylpropoxy)phenyl]prop-2-en-1-one (**7c**)



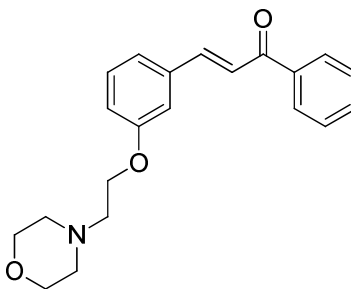
The title compound was prepared from (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**) and (3-chloropropyl)benzene in a yield of 39%: mp 68.0 – 69.5 °C (ethanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 8.02 (m, 2H), 7.79 (d, *J* = 15.7 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.56 – 7.50 (m, 3H), 7.37 – 7.29 (m, 3H), 7.27 – 7.21 (m, 4H), 7.17 (t, *J* = 2.0 Hz, 1H), 6.97 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.02 (t, *J* = 6.3 Hz, 2H), 2.85 (t, *J* = 7.6 Hz, 2H), 2.19 – 2.11 (m, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 190.5, 159.3, 144.7, 141.3, 138.1, 136.2, 132.7, 129.9, 128.6 (2C), 128.5 (4C), 128.4 (2C), 125.9, 122.2, 121.1, 116.8, 114.0, 66.9, 32.1, 30.7. APCI-HRMS *m/z*: calcd for C₂₄H₂₃O₂ (M + H)⁺, 343.1693, found 343.1682.

(2E)-1-phenyl-3-{3-[2-(piperidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (**7d**)



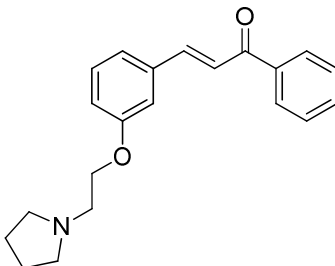
The title compound was prepared from (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**) and 1-(2-chloroethyl)piperidine in a yield of 69%: orange gum. ¹H NMR (600 MHz, CDCl₃) δ 8.01 (br d, *J* = 8.1 Hz, 2H), 7.76 (d, *J* = 15.6 Hz, 1H), 7.61 – 7.53 (m, 1H), 7.53 – 7.47 (m, 3H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.25 – 7.15 (m, 2H), 6.96 (dd, *J* = 8.2, 2.5 Hz, 1H), 4.15 (t, *J* = 6.0 Hz, 2H), 2.81 (t, *J* = 6.0 Hz, 2H), 2.54 (br s, 4H), 1.65 – 1.59 (m, 4H), 1.48 – 1.42 (m, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 190.4, 159.1, 144.7, 138.1, 136.1, 132.7, 129.9, 128.6 (2C), 128.4 (2C), 122.2, 121.3, 116.8, 113.9, 65.9, 57.8, 55.0 (2C), 25.7 (2C), 24.0. APCI-HRMS *m/z*: calcd for C₂₂H₂₆NO₂ (M + H)⁺, 336.1958, found 336.1959.

(2E)-3-{3-[2-(morpholin-4-yl)ethoxy]phenyl}-1-phenylprop-2-en-1-one (7e)



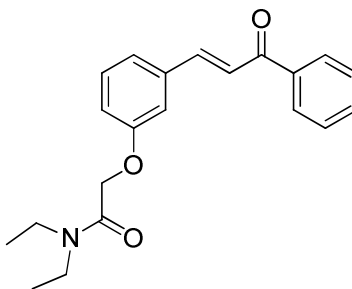
The title compound was prepared from (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**) and 4-(2-chloroethyl)morpholine in a yield of 67%: amber gum. ¹H NMR (600 MHz, CDCl₃) δ 8.03 – 7.98 (m, 2H), 7.75 (d, *J* = 15.7 Hz, 1H), 7.60 – 7.56 (m, 1H), 7.53 – 7.47 (m, 3H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.23 (br d, *J* = 7.6 Hz, 1H), 7.17 (t, *J* = 2.0 Hz, 1H), 6.96 (dd, *J* = 8.3, 2.6 Hz, 1H), 4.15 (t, *J* = 5.7 Hz, 2H), 3.76 – 3.68 (m, 4H), 2.82 (t, *J* = 5.7 Hz, 2H), 2.65 – 2.56 (m, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 190.4, 159.0, 144.6, 138.0, 136.2, 132.8, 129.9, 128.6 (2C), 128.4 (2C), 122.3, 121.3, 116.8, 114.0, 66.8 (2C), 65.8, 57.5, 54.0 (2C). APCI-HRMS *m/z*: calcd for C₂₁H₂₄NO₃ (M + H)⁺, 338.1751, found 338.1729.

(2E)-1-phenyl-3-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (7f)



The title compound was prepared from (2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**) and 1-(2-chloroethyl)pyrrolidine in a yield of 38%: amber gum. ¹H NMR (600 MHz, CDCl₃) δ 8.00 (br d, *J* = 8.2 Hz, 2H), 7.75 (d, *J* = 15.7 Hz, 1H), 7.60 – 7.54 (m, 1H), 7.54 – 7.46 (m, 3H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.24 – 7.16 (m, 2H), 6.96 (dd, *J* = 8.2, 2.5 Hz, 1H), 4.17 (t, *J* = 5.8 Hz, 2H), 2.96 (t, *J* = 5.8 Hz, 2H), 2.72 – 2.64 (m, 4H), 1.82 (p, *J* = 3.2 Hz, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 190.4, 159.0, 144.6, 138.0, 136.1, 132.7, 129.9, 128.5 (2C), 128.4 (2C), 122.2, 121.3, 116.8, 113.9, 66.8, 54.9, 54.6 (2C), 23.4 (2C). APCI-HRMS *m/z*: calcd for C₂₁H₂₄NO₂ (M + H)⁺, 322.1802, found 322.1782.

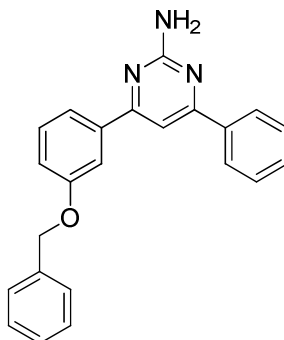
N,N-diethyl-2-{3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenoxy}acetamide (**7g**)



The title compound was prepared from (*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**) and 2-chloro-*N,N*-diethylacetamide in a yield of 99%: light yellow gum. ¹H NMR (600 MHz, CDCl₃) δ 8.02 – 7.97 (m, 2H), 7.73 (d, *J* = 15.7 Hz, 1H), 7.60 – 7.53 (m, 1H), 7.53 – 7.45 (m, 3H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.28 – 7.19 (m, 2H), 6.99 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.71 (s, 2H), 3.46 – 3.23 (m, 4H), 1.21 (t, *J* = 7.1 Hz, 3H), 1.13 (t, *J* = 6.7 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 190.3, 166.5, 158.4, 144.3, 138.0, 136.3, 132.7, 129.9, 128.5 (2C), 128.4 (2C), 122.4, 121.7, 116.7, 114.3, 67.3, 41.5, 40.3, 14.3, 12.7. APCI-HRMS *m/z*: calcd for C₂₁H₂₄NO₃ (M + H)⁺, 338.1751, found 338.1749.

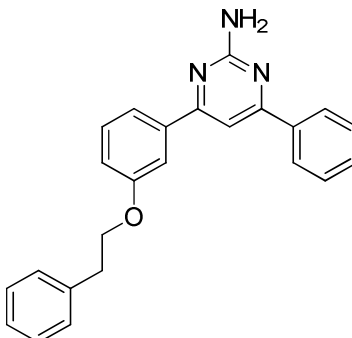
Aminopyrimidines:

4-[3-(benzyloxy)phenyl]-6-phenylpyrimidin-2-amine (**3a**)



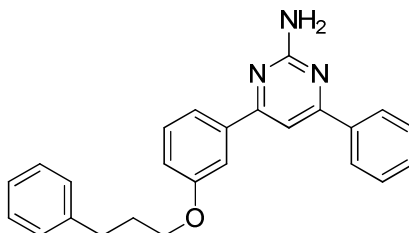
The title compound was prepared from (*E*)-3-[3-(benzyloxy)phenyl]-1-phenylprop-2-en-1-one (**7a**) in a yield of 6%: mp 106.6 – 107.3 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.09 – 8.03 (m, 2H), 7.75 (dd, *J* = 2.6, 1.6 Hz, 1H), 7.65 (dt, *J* = 7.8, 1.1 Hz, 1H), 7.55 – 7.46 (m, 5H), 7.45 – 7.38 (m, 4H), 7.39 – 7.32 (m, 1H), 7.12 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 5.43 (s, 2H), 5.16 (s, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 166.2, 165.9, 163.6, 159.1, 139.2, 137.7, 136.8, 130.4, 129.8, 128.7 (2C), 128.6 (2C), 128.0, 127.5 (2C), 127.1 (2C), 119.7, 117.0, 113.4, 104.3, 70.1. APCI-HRMS *m/z*: calcd for C₂₃H₂₀N₃O (M + H)⁺, 354.1601, found 354.1567.

4-phenyl-6-[3-(2-phenylethoxy)phenyl]pyrimidin-2-amine (**3b**)



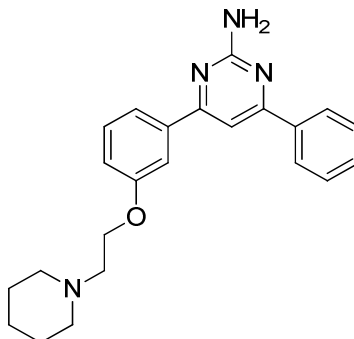
The title compound was prepared from (*2E*)-1-phenyl-3-[3-(2-phenylethoxy)phenyl]prop-2-en-1-one (**7b**) in a yield of 8%: mp 123.4 – 124.9 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.00 – 7.93 (m, 2H), 7.56 – 7.50 (m, 2H), 7.44 – 7.36 (m, 3H), 7.33 (s, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.27 – 7.21 (m, 4H), 7.20 – 7.13 (m, 1H), 6.94 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 5.33 (br s, 2H), 4.18 (t, *J* = 7.1 Hz, 2H), 3.05 (t, *J* = 7.1 Hz, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 166.2, 165.9, 163.6, 159.2, 139.1, 138.1, 137.7, 130.4, 129.7, 129.0 (2C), 128.7 (2C), 128.5 (2C), 127.1 (2C), 126.5, 119.5, 116.9, 112.9, 104.3, 68.7, 35.8. APCI-HRMS *m/z*: calcd for C₂₄H₂₂N₃O (M + H)⁺, 368.1757, found 368.1729.

4-phenyl-6-[3-(3-phenylpropoxy)phenyl]pyrimidin-2-amine (**3c**)



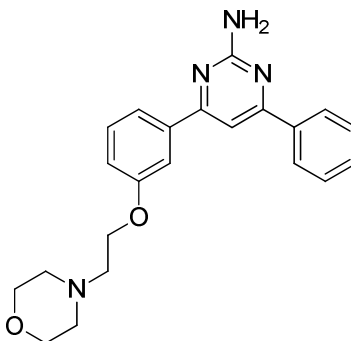
The title compound was prepared from (*2E*)-1-phenyl-3-[3-(3-phenylpropoxy)phenyl]prop-2-en-1-one (**7c**) in a yield of 33%: mp 121.0 – 122.6 °C (ethanol), light yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.10 – 8.05 (m, 2H), 7.67 – 7.61 (m, 2H), 7.53 – 7.49 (m, 3H), 7.46 (s, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.29 – 7.19 (m, 3H), 7.04 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H), 5.44 (s, 2H), 4.07 (t, *J* = 6.2 Hz, 2H), 2.89 – 2.83 (m, 2H), 2.20 – 2.12 (m, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 166.2, 166.0, 163.6, 159.4, 141.4, 139.1, 137.7, 130.4, 129.7, 128.7 (2C), 128.5 (2C), 128.4 (2C), 127.1 (2C), 125.9, 119.4, 116.7, 113.0, 104.3, 66.9, 32.1, 30.8. APCI-HRMS *m/z*: calcd for C₂₅H₂₄N₃O (M + H)⁺, 382.1914, found 382.1876.

4-phenyl-6-{3-[2-(piperidin-1-yl)ethoxy]phenyl}pyrimidin-2-amine (**3d**)



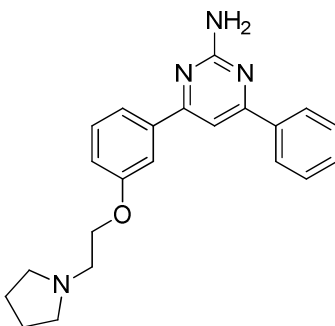
The title compound was prepared from (*2E*)-1-phenyl-3-{3-[2-(piperidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (**7d**) in a yield of 31%: orange gum. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 8.06 – 8.01 (m, 2H), 7.65 – 7.58 (m, 2H), 7.51 – 7.45 (m, 3H), 7.42 (s, 1H), 7.37 (t, $J = 7.9$ Hz, 1H), 7.02 (dd, $J = 8.2, 2.5$ Hz, 1H), 5.40 (s, 2H), 4.20 (t, $J = 6.0$ Hz, 2H), 2.81 (t, $J = 6.0$ Hz, 2H), 2.53 (s, 4H), 1.66 – 1.57 (m, 4H), 1.47 – 1.41 (m, 2H). $^{13}\text{C NMR}$ (151 MHz, CDCl_3) δ 166.1, 165.9, 163.5, 159.1, 139.1, 137.7, 130.4, 129.7, 128.7 (2C), 127.0 (2C), 119.5, 116.8, 113.0, 104.3, 65.9, 57.9, 55.0 (2C), 25.8 (2C), 24.1. APCI-HRMS m/z : calcd for $\text{C}_{23}\text{H}_{27}\text{N}_4\text{O}$ ($\text{M} + \text{H}$) $^+$, 375.2179, found 375.2155. *Acetone in NMR sample, due to acetone in NMR tube.

4-{3-[2-(morpholin-4-yl)ethoxy]phenyl}-6-phenylpyrimidin-2-amine (**3e**)



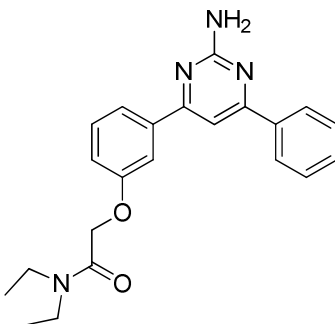
The title compound was prepared from (*2E*)-3-{3-[2-(morpholin-4-yl)ethoxy]phenyl}-1-phenylprop-2-en-1-one (**7e**) in a yield of 19%: mp 104.4 – 106.1 °C (ethanol), light yellow crystals. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 8.06 – 8.01 (m, 2H), 7.67 – 7.59 (m, 2H), 7.52 – 7.46 (m, 3H), 7.42 (s, 1H), 7.38 (t, $J = 7.9$ Hz, 1H), 7.03 (dd, $J = 8.2, 2.6$ Hz, 1H), 5.46 (br s, 2H), 4.20 (t, $J = 5.7$ Hz, 2H), 3.74 (t, $J = 4.7$ Hz, 4H), 2.83 (t, $J = 5.7, 1.5$ Hz, 2H), 2.59 (br s, 4H). $^{13}\text{C NMR}$ (151 MHz, CDCl_3) δ 166.2, 165.8, 163.6, 159.1, 139.1, 137.6, 130.4, 129.7, 128.7 (2C), 127.0 (2C), 119.6, 116.8, 113.0, 104.3, 66.9 (2C), 65.8, 57.6, 54.0 (2C). APCI-HRMS m/z : calcd for $\text{C}_{22}\text{H}_{25}\text{N}_4\text{O}_2$ ($\text{M} + \text{H}$) $^+$, 377.1972, found 377.1986.

4-phenyl-6-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}pyrimidin-2-amine (**3f**)



The title compound was prepared from (*2E*)-1-phenyl-3-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (**7f**) in a yield of 30%: amber paste. ¹H NMR (600 MHz, CDCl₃) δ 8.05 – 8.00 (m, 2H), 7.64 (br t, *J* = 2.1 Hz, 1H), 7.60 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.51 – 7.45 (m, 3H), 7.41 (s, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.03 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 5.43 (s, 2H), 4.27 (t, *J* = 5.7 Hz, 2H), 3.05 (t, *J* = 5.7 Hz, 2H), 2.81 (t, *J* = 5.4 Hz, 4H), 1.87 (p, *J* = 3.6 Hz, 4H). ¹³C NMR (151 MHz, CDCl₃) δ 166.2, 165.8, 163.5, 158.8, 139.1, 137.6, 130.4, 129.7, 128.7 (2C), 127.0 (2C), 119.7, 116.8, 113.0, 104.3, 66.2, 54.7, 54.6 (2C), 23.3 (2C). APCI-HRMS *m/z*: calcd for C₂₂H₂₅N₄O (M + H)⁺, 361.2023, found 361.2018. * Methanol in sample.

2-[3-(2-amino-6-phenylpyrimidin-4-yl)phenoxy]-*N,N*-diethylacetamide (**3g**)



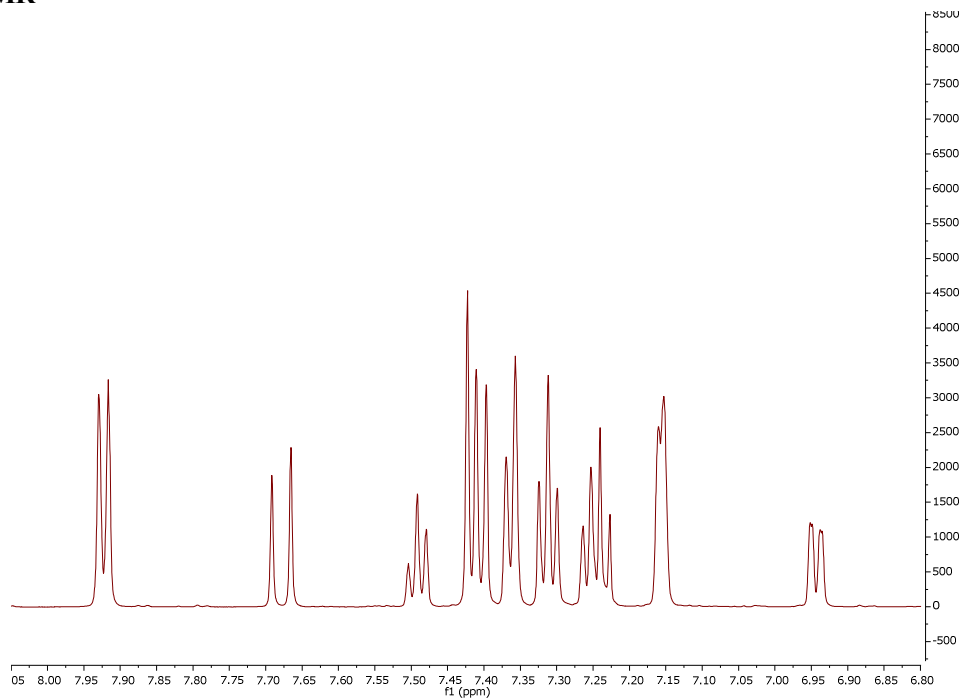
The title compound was prepared from *N,N*-diethyl-2-{3-[(*1E*)-3-oxo-3-phenylprop-1-en-1-yl]phenoxy}acetamide (**7g**) in a yield of 28%: mp 91.6 – 92.8 °C (ethanol), orange solid. ¹H NMR (600 MHz, CDCl₃) δ 8.06 – 8.00 (m, 2H), 7.69 – 7.61 (m, 2H), 7.51 – 7.45 (m, 3H), 7.43 – 7.36 (m, 2H), 7.08 (ddd, *J* = 8.2, 2.7, 0.9 Hz, 1H), 5.39 (s, 2H), 4.75 (s, 2H), 3.40 (dq, *J* = 12.6, 7.1 Hz, 4H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.14 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 166.7, 166.2, 165.7, 163.5, 158.5, 139.2, 137.6, 130.4, 129.8, 128.7 (2C), 127.0 (2C), 120.2, 116.9, 113.2, 104.3, 67.4, 41.5, 40.3, 14.3, 12.8. APCI-HRMS *m/z*: calcd for C₂₂H₂₅N₄O₂ (M + H)⁺, 377.1972, found 377.1945. * Acetone in NMR sample, due to acetone in NMR tube.

4. NMR Spectra

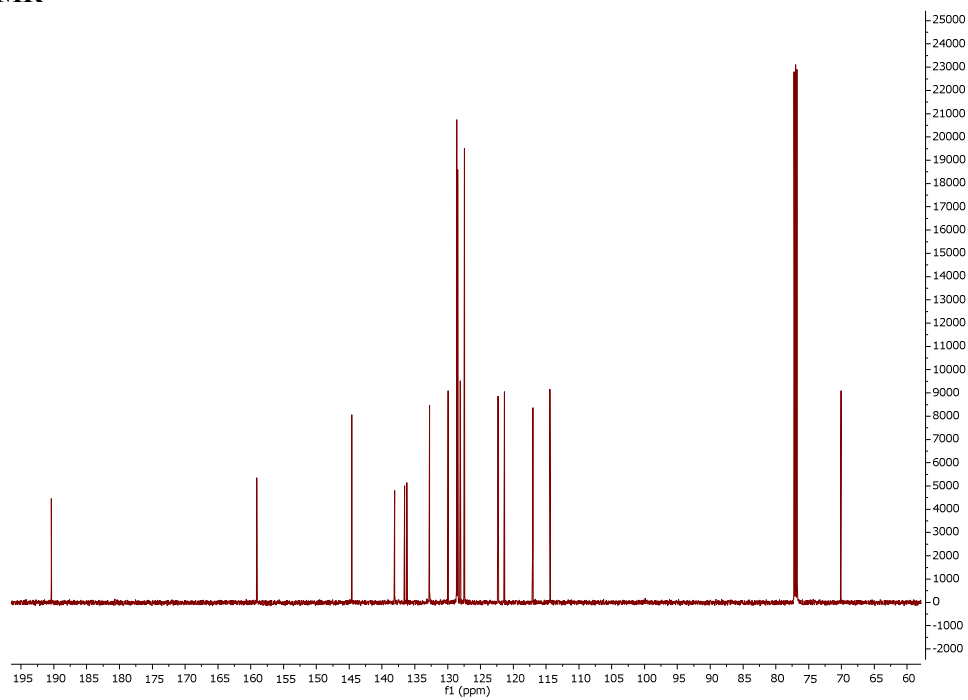
Chalcones:

(2*E*)-3-[3-(benzyloxy)phenyl]-1-phenylprop-2-en-1-one (**7a**)

¹H NMR

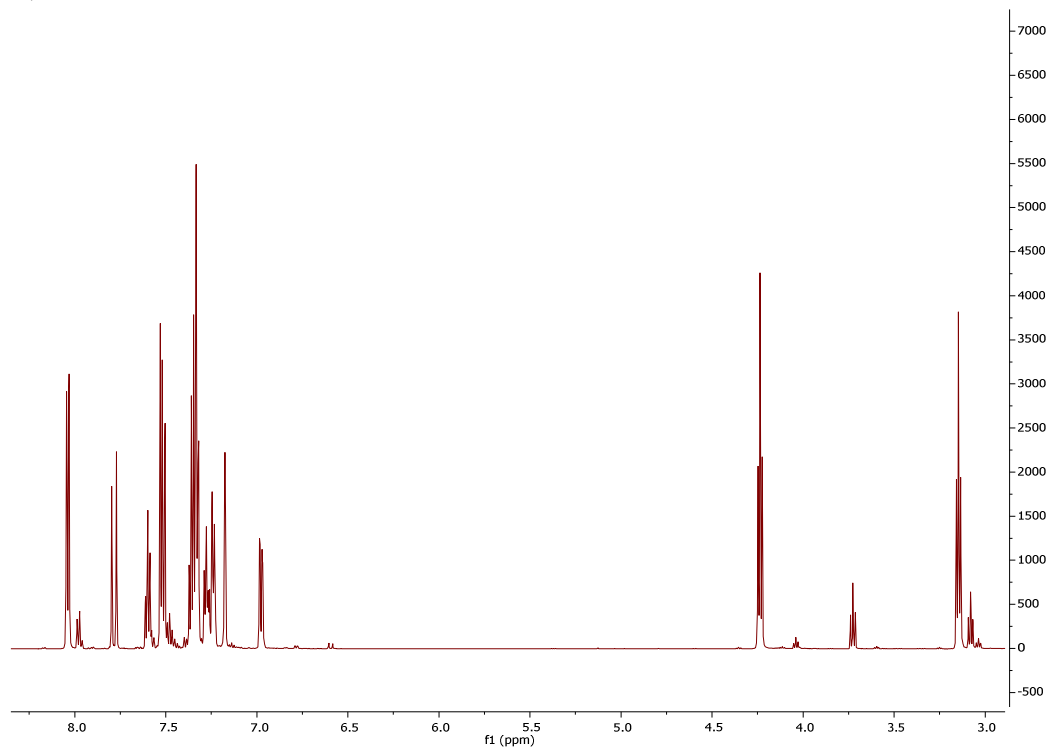


¹³C NMR

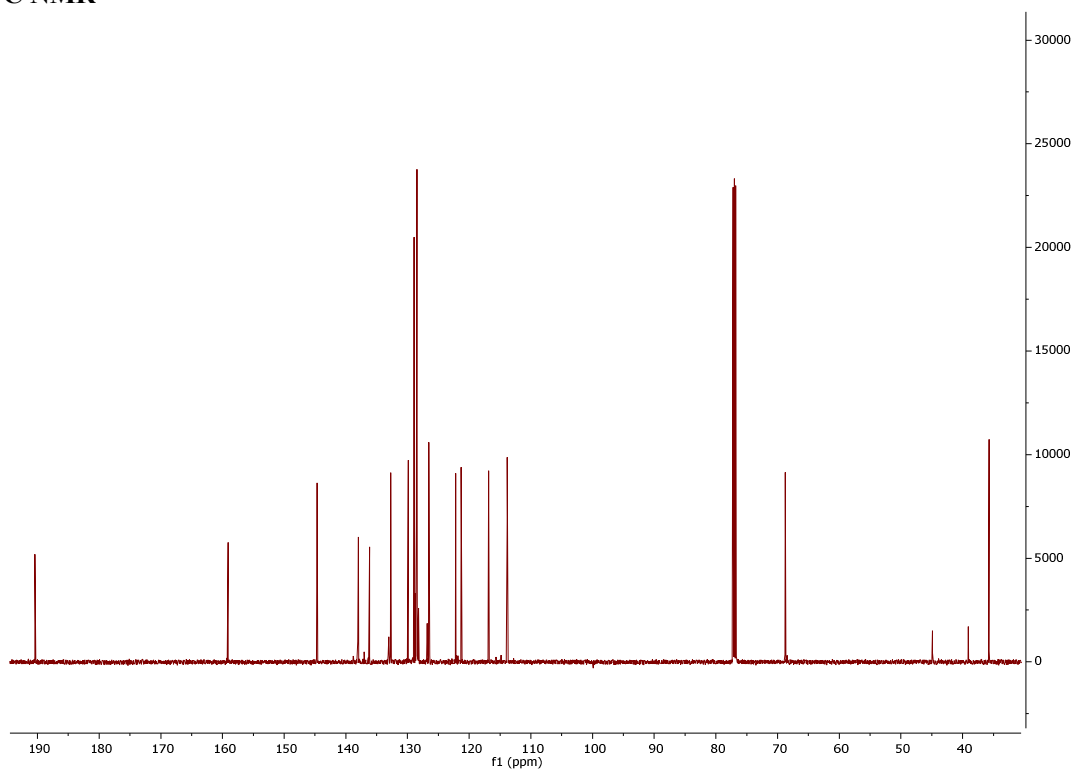


(2E)-1-phenyl-3-[3-(2-phenylethoxy)phenyl]prop-2-en-1-one (7b)

¹H NMR

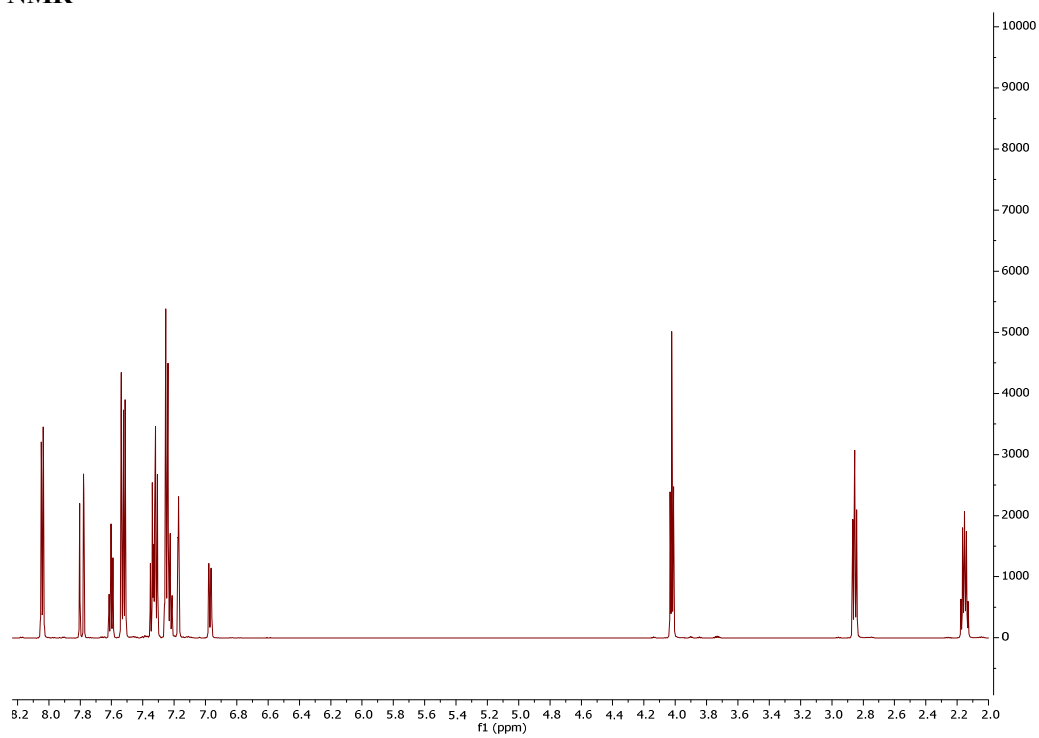


¹³C NMR

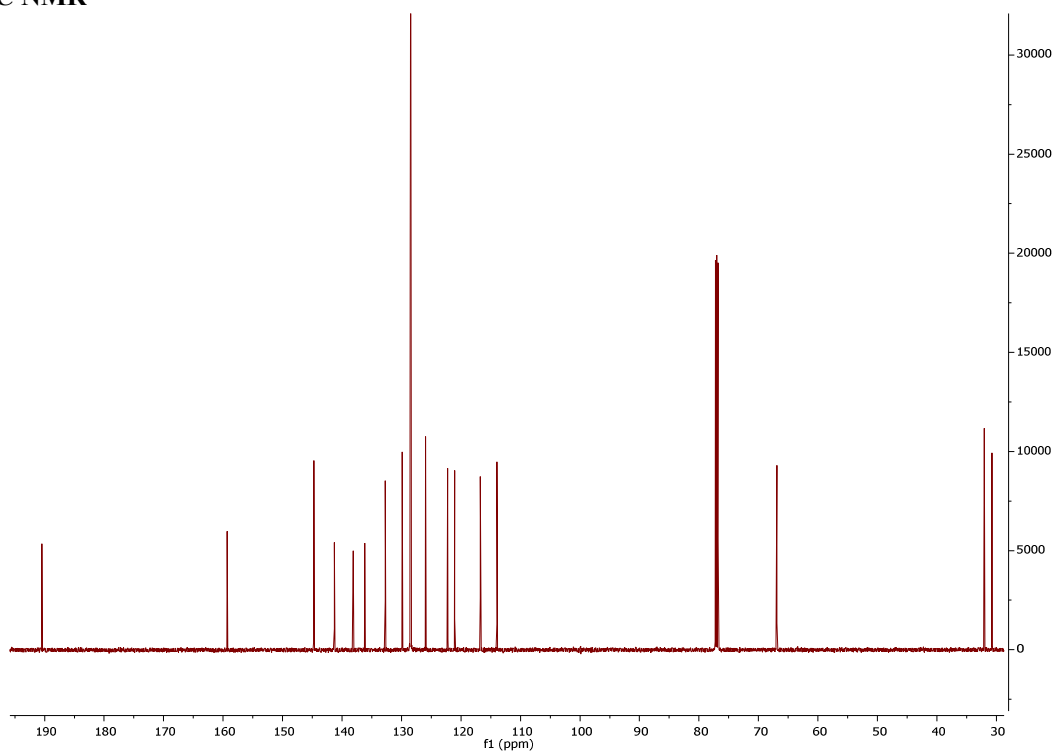


(2E)-1-phenyl-3-[3-(3-phenylpropoxy)phenyl]prop-2-en-1-one (7c)

¹H NMR

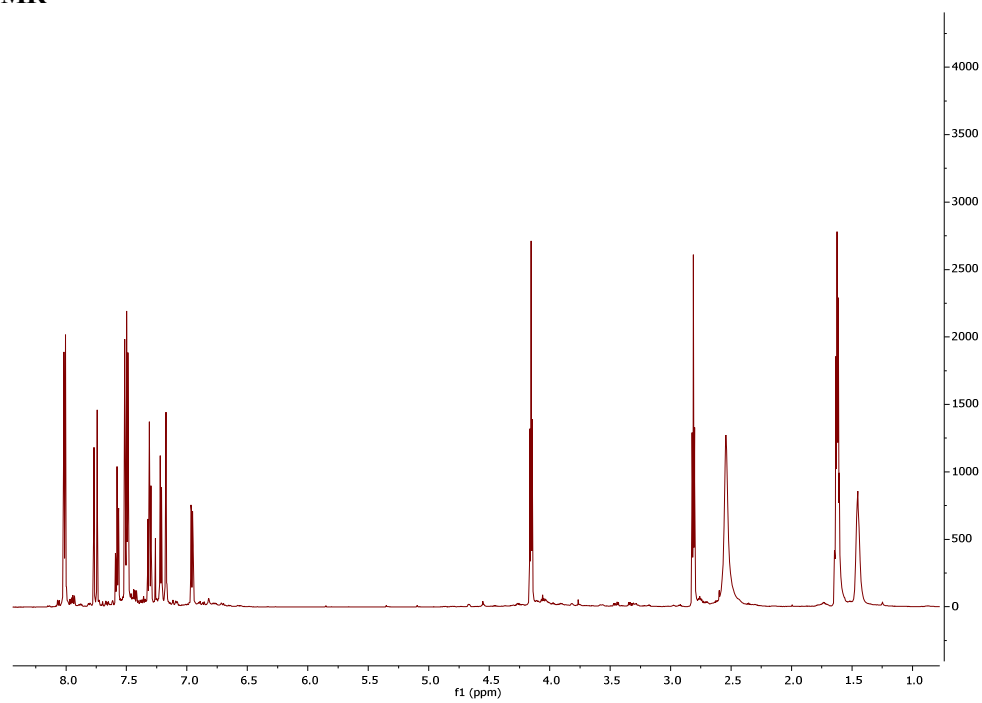


¹³C NMR

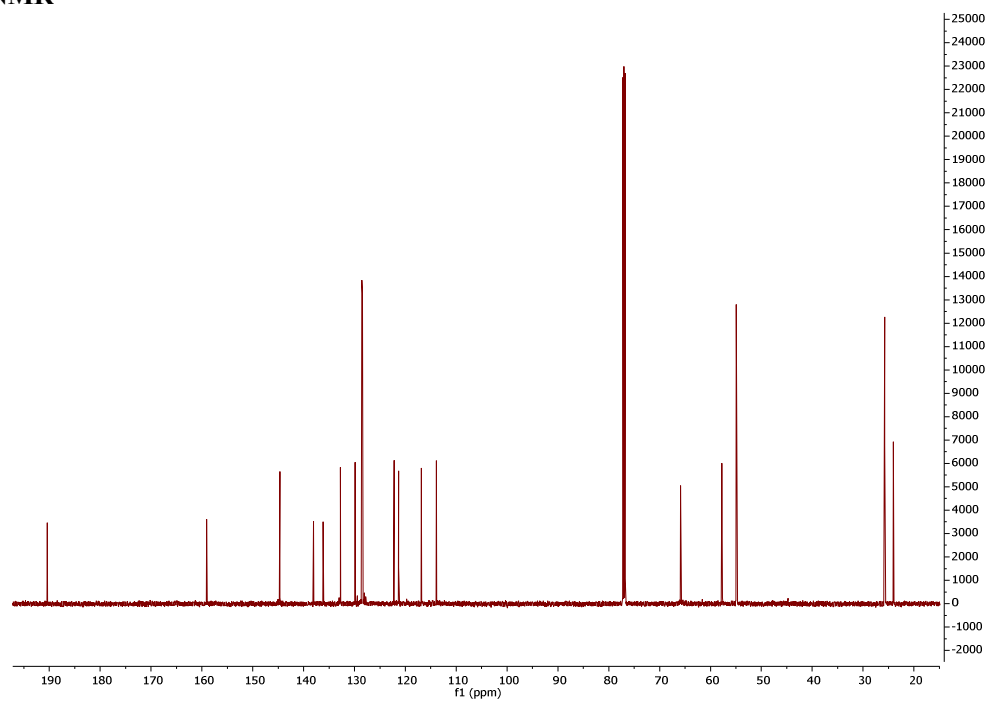


(2E)-1-phenyl-3-{3-[2-(piperidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (7d)

¹H NMR

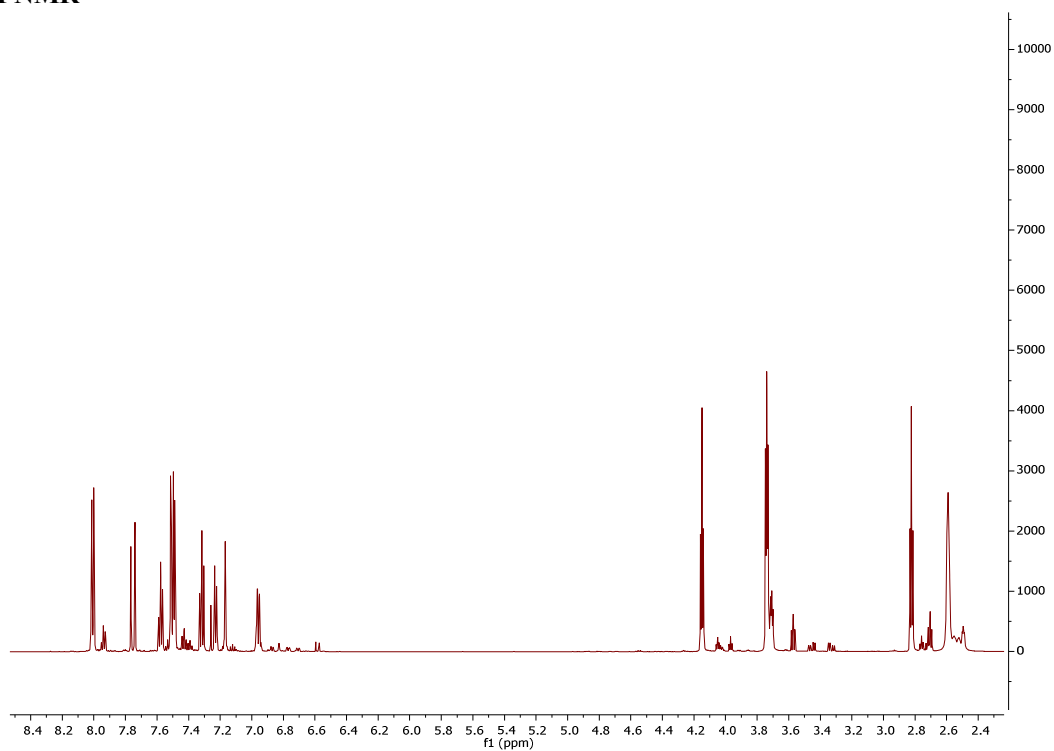


¹³C NMR

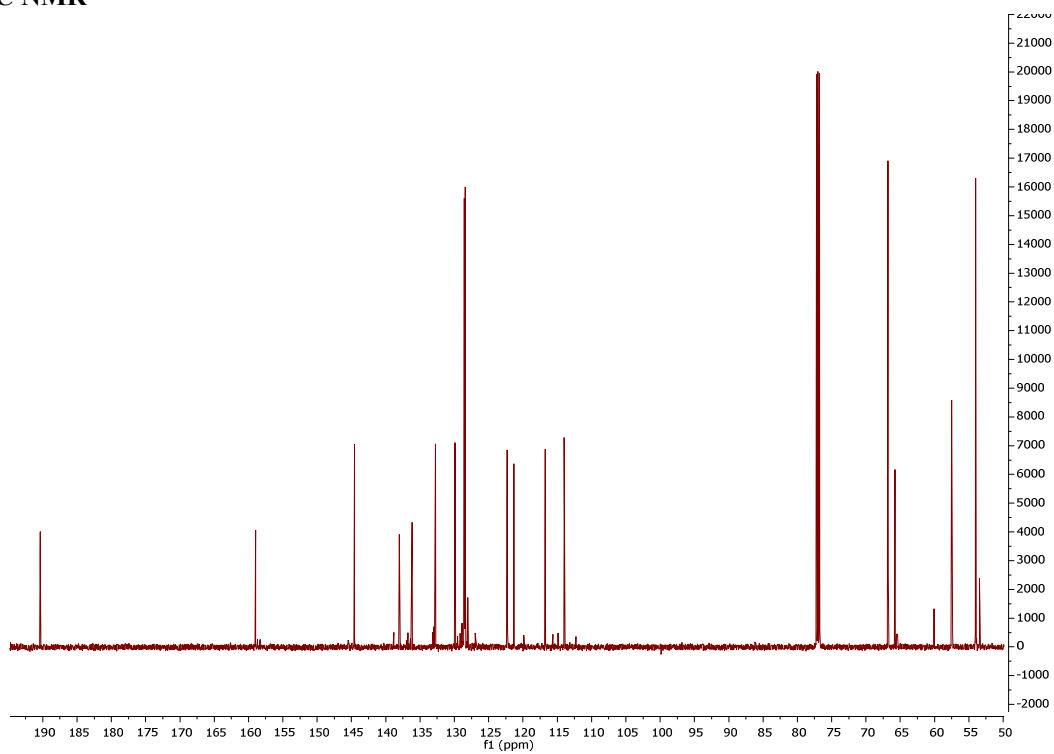


(2E)-3-{3-[2-(morpholin-4-yl)ethoxy]phenyl}-1-phenylprop-2-en-1-one (7e)

¹H NMR

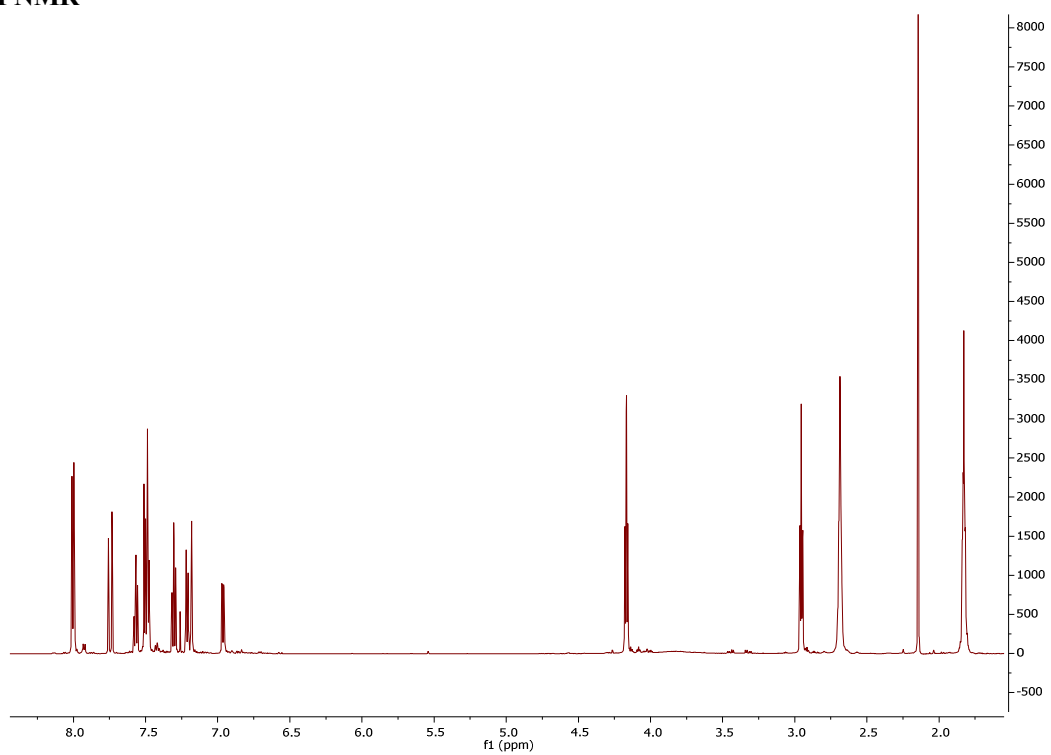


¹³C NMR

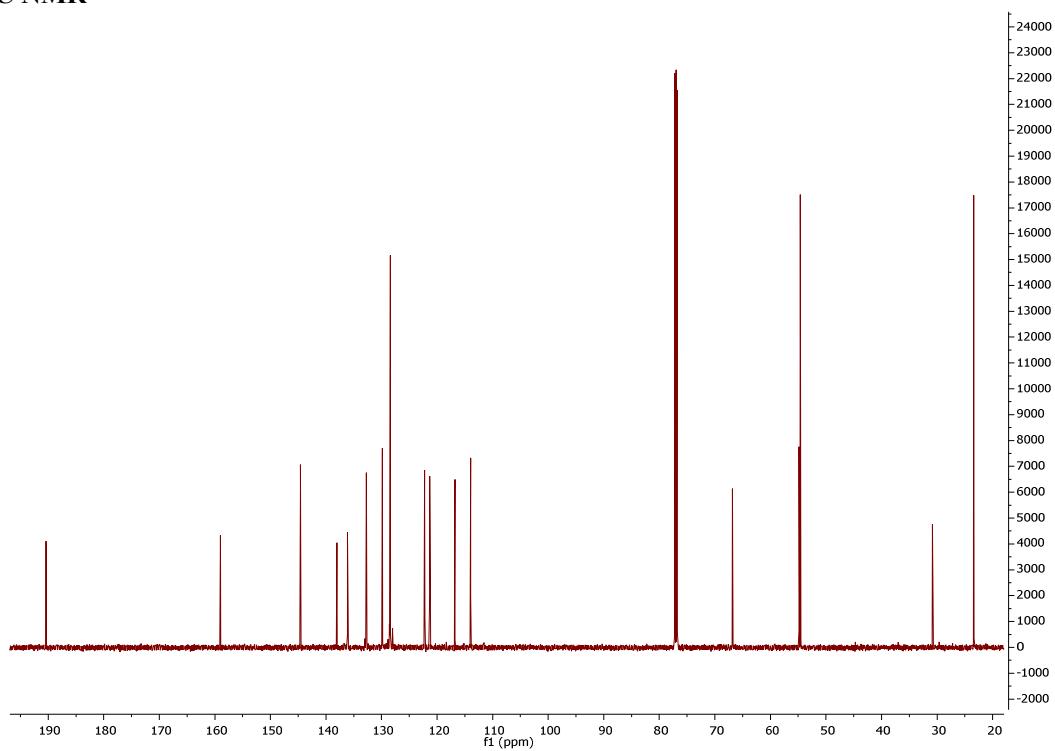


(2E)-1-phenyl-3-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (7f)

¹H NMR

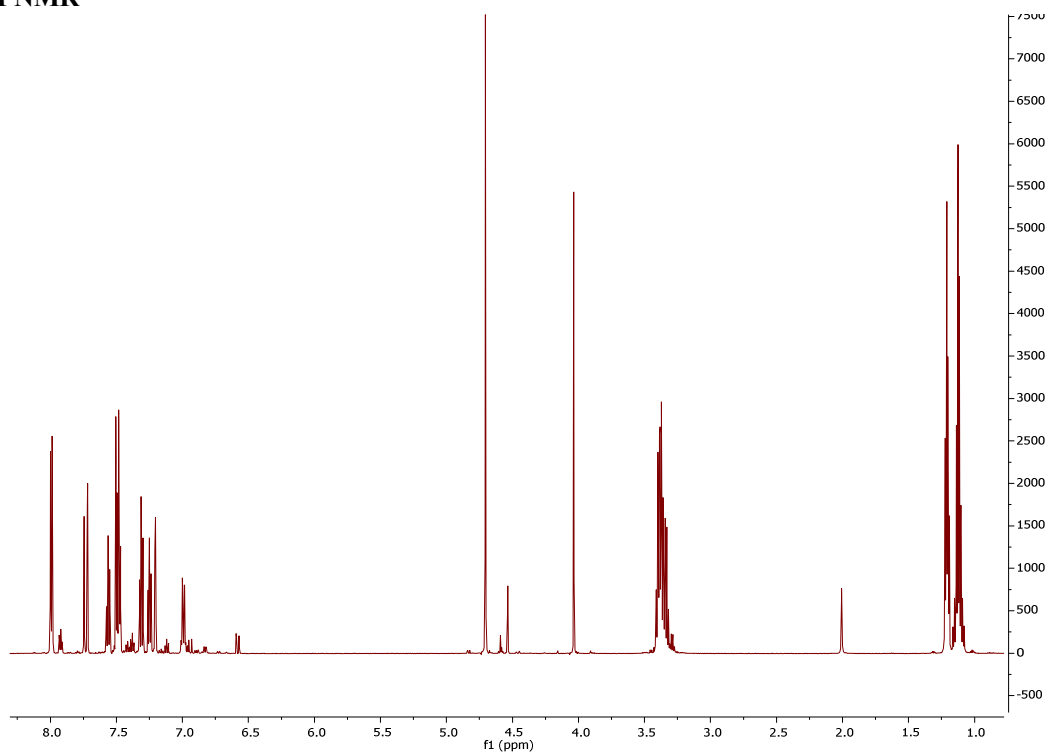


¹³C NMR

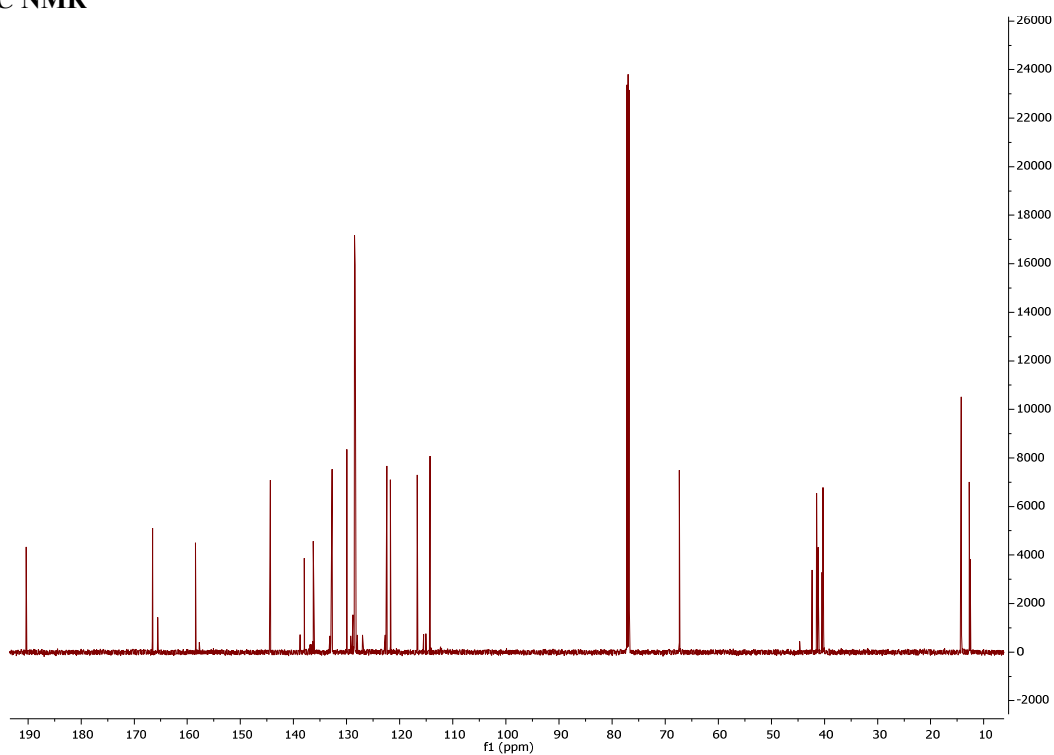


N,N-diethyl-2-{3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenoxy}acetamide (**7g**)

¹H NMR



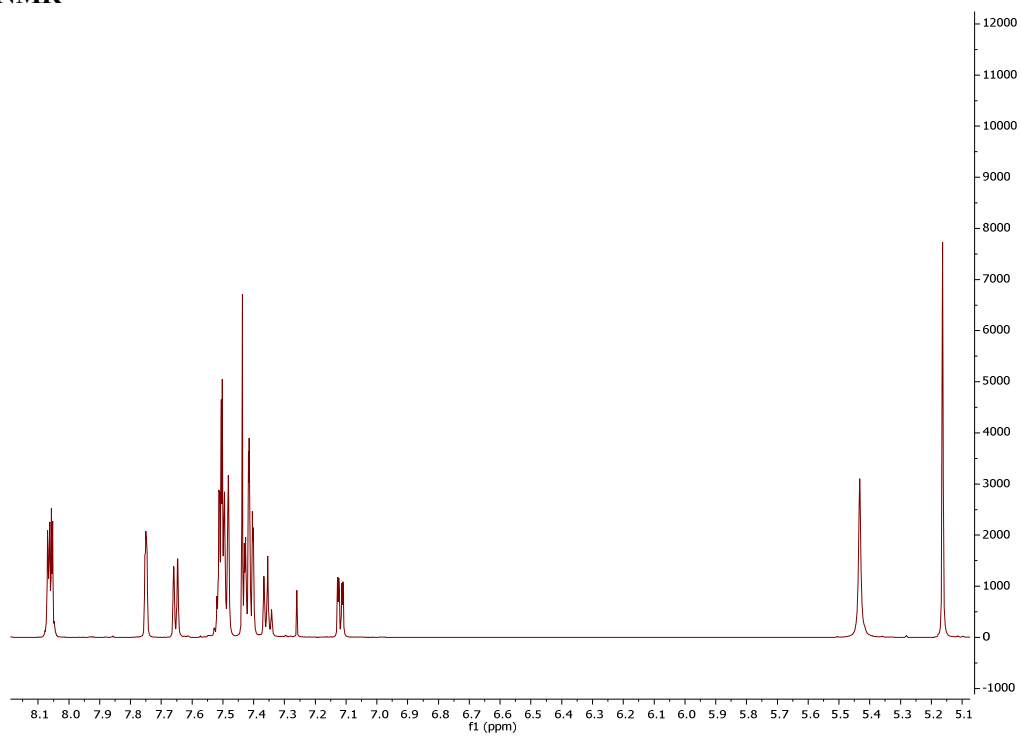
¹³C NMR



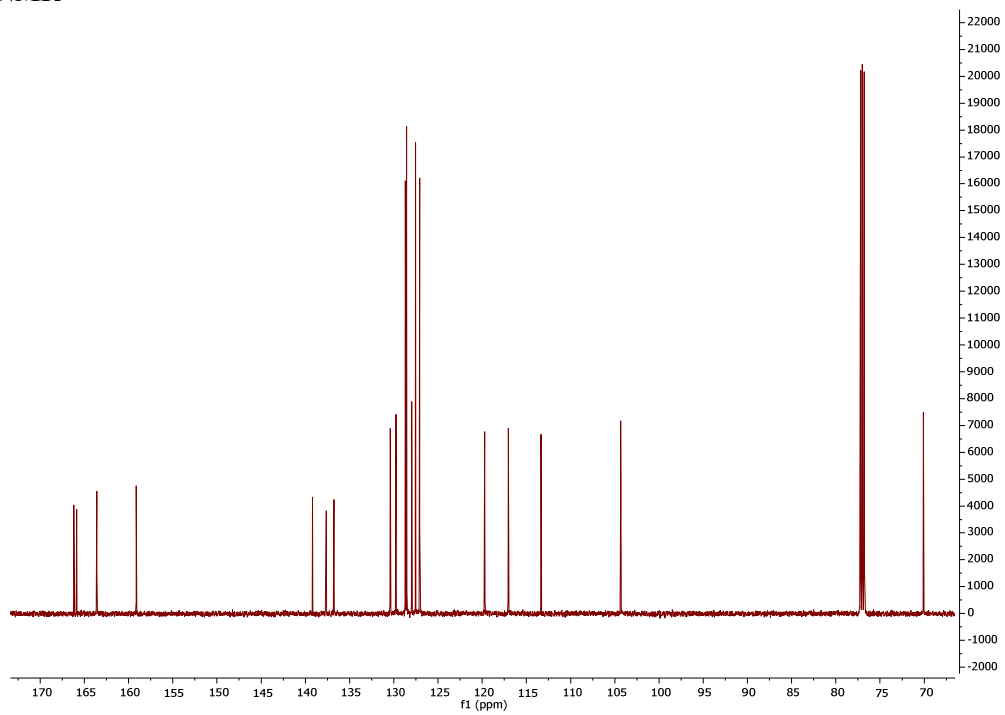
Aminopyrimidines

4-[3-(benzyloxy)phenyl]-6-phenylpyrimidin-2-amine (**3a**)

¹H NMR

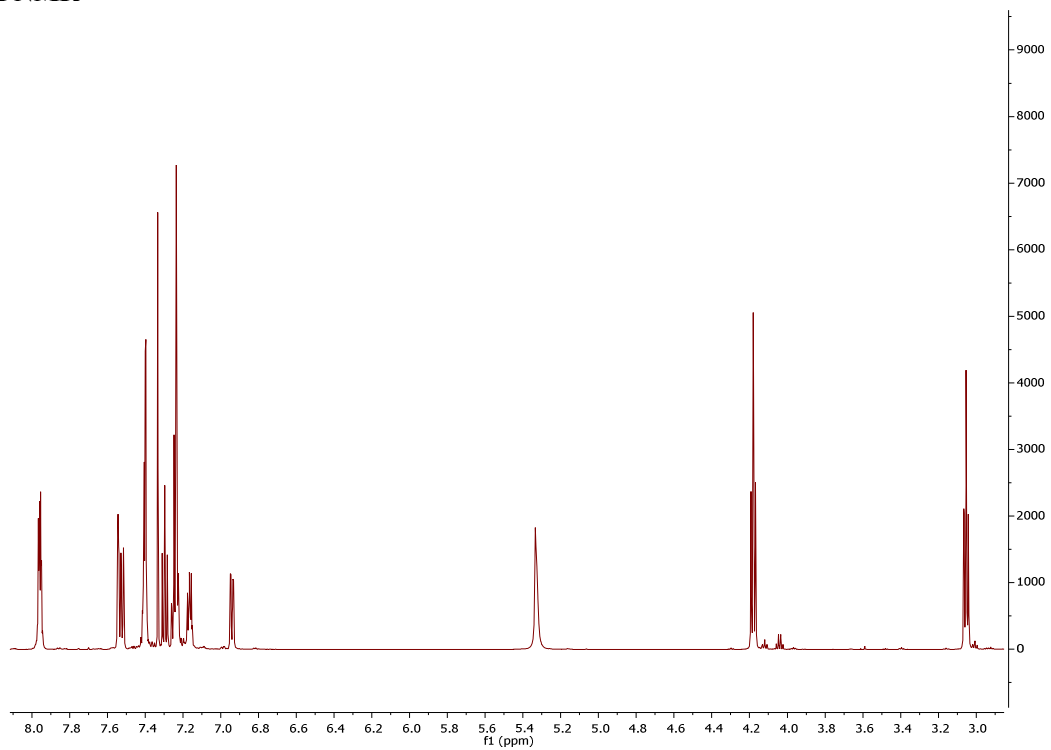


¹³C NMR

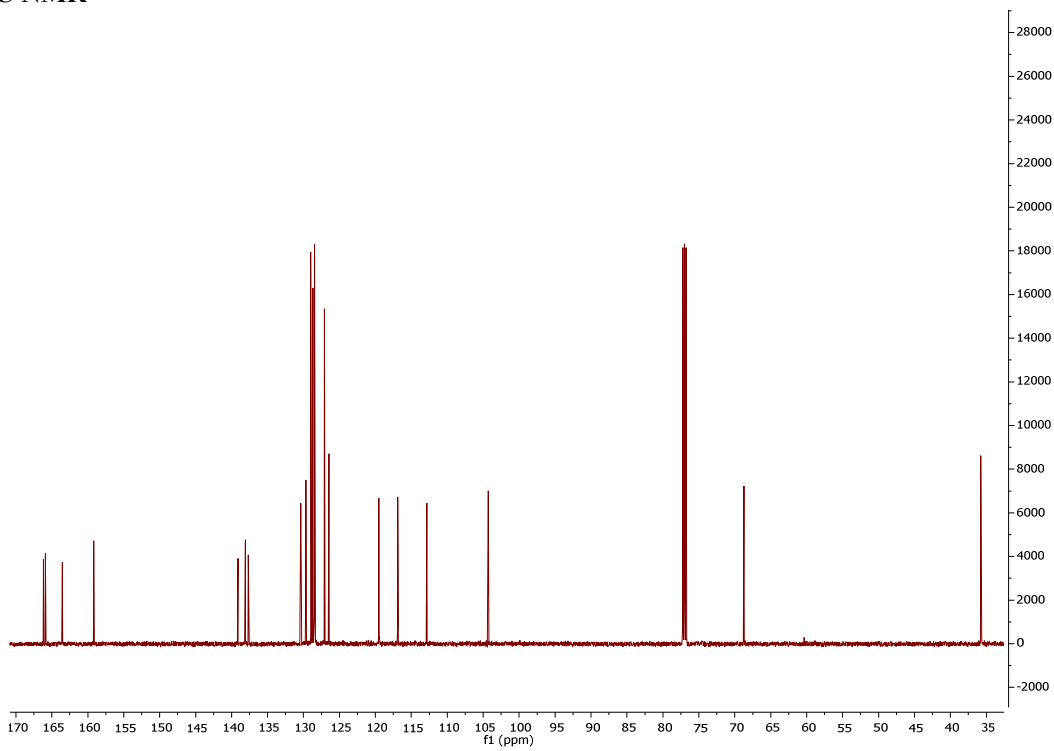


4-phenyl-6-[3-(2-phenylethoxy)phenyl]pyrimidin-2-amine (**3b**)

¹H NMR

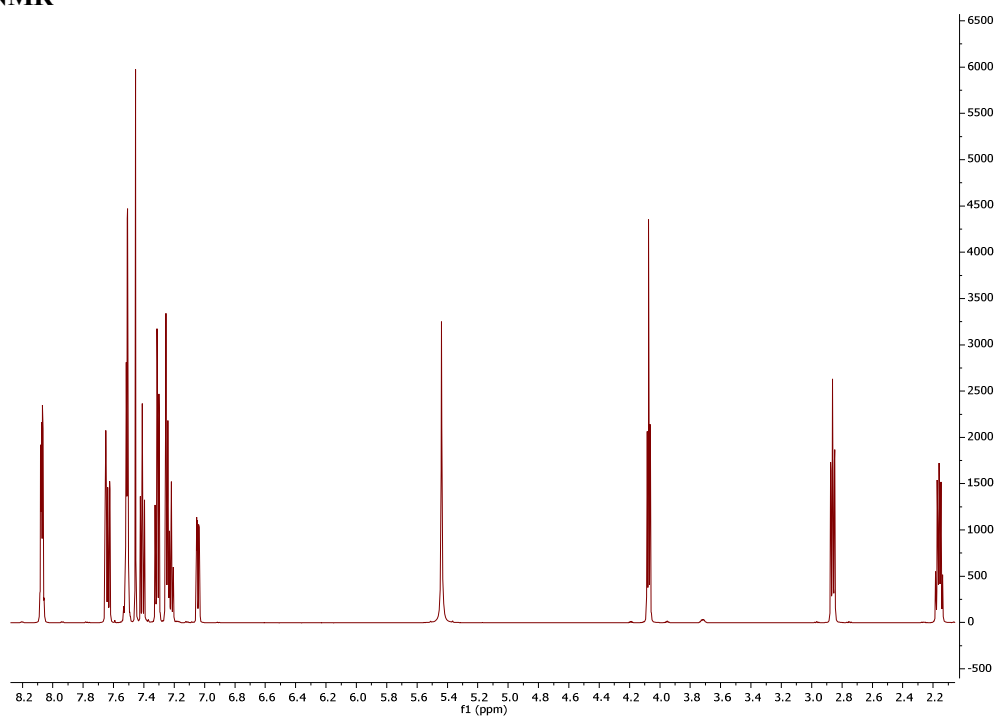


¹³C NMR

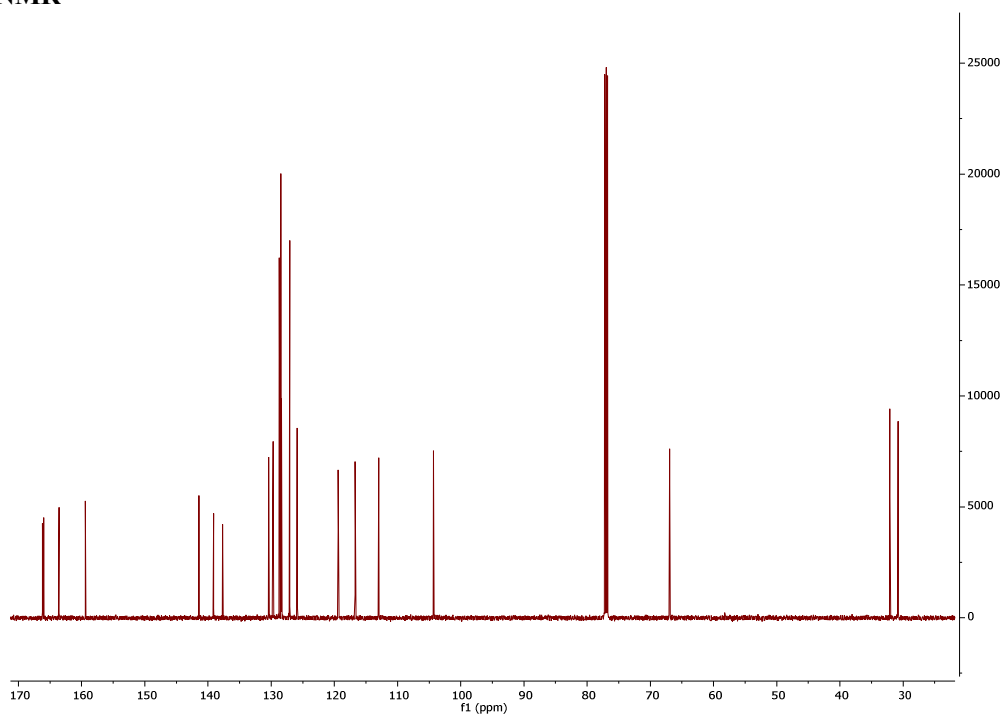


4-phenyl-6-[3-(3-phenylpropoxy)phenyl]pyrimidin-2-amine (3c)

¹H NMR

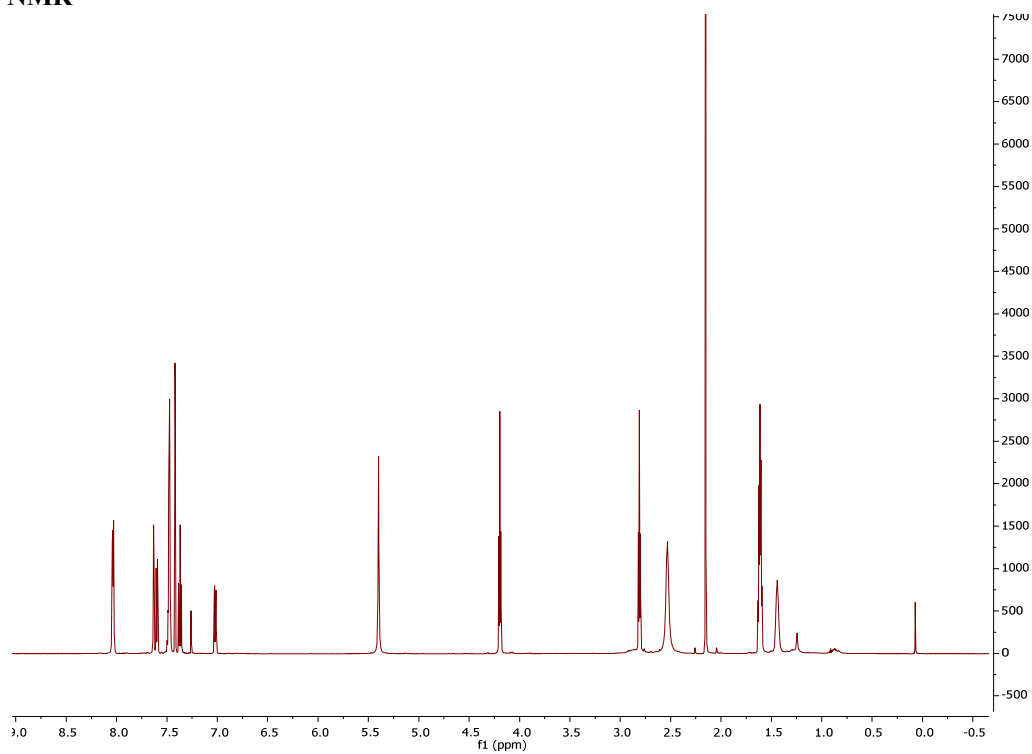


¹³C NMR

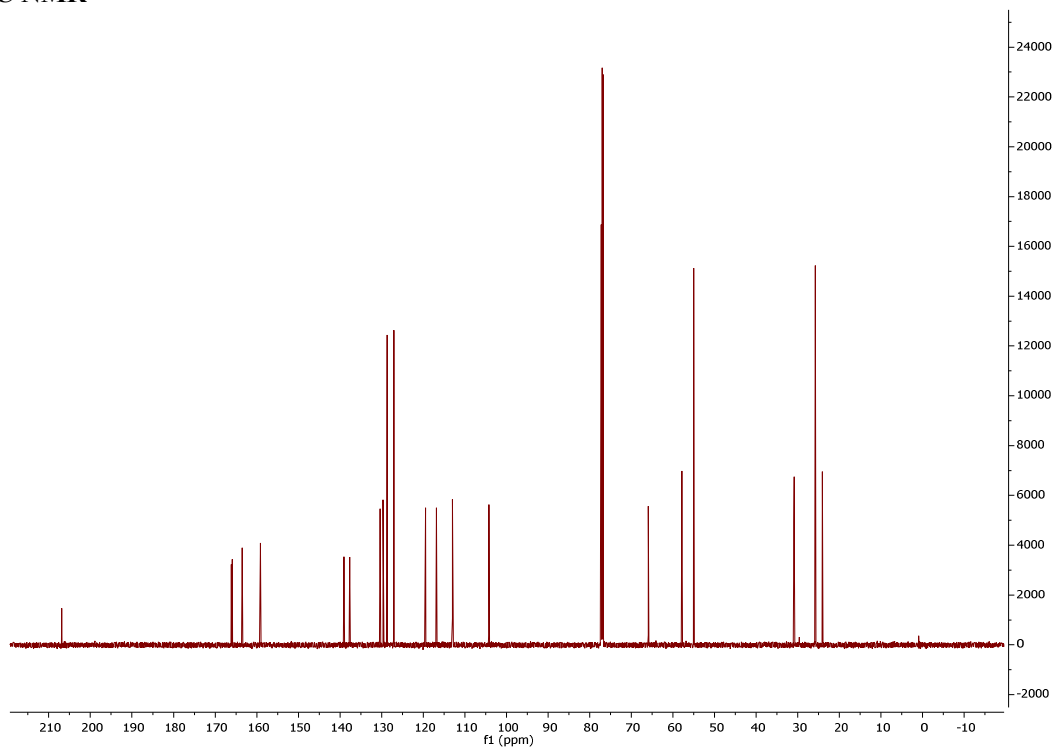


4-phenyl-6-{3-[2-(piperidin-1-yl)ethoxy]phenyl} pyrimidin-2-amine (**3d**)

¹H NMR

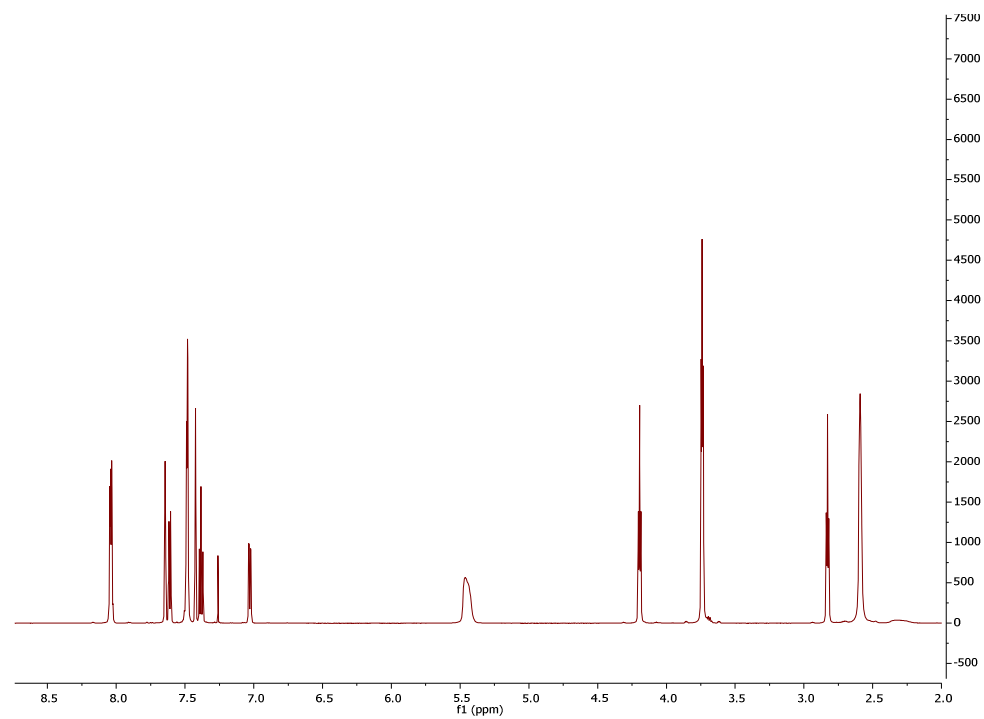


¹³C NMR

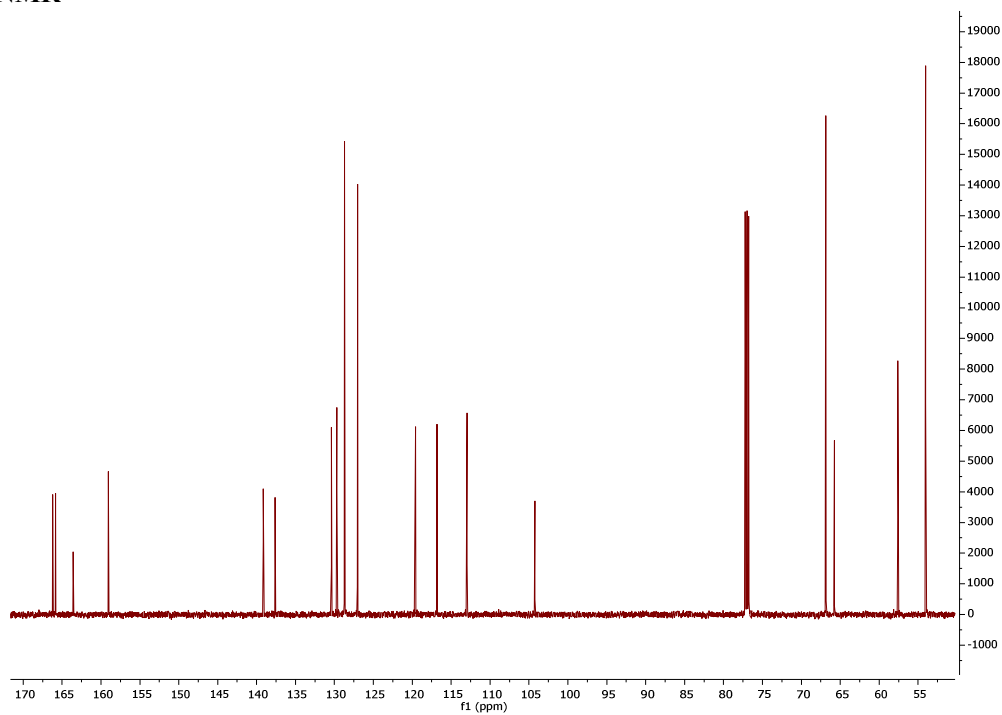


4-{3-[2-(morpholin-4-yl)ethoxy]phenyl}-6-phenylpyrimidin-2-amine (**3e**)

¹H NMR

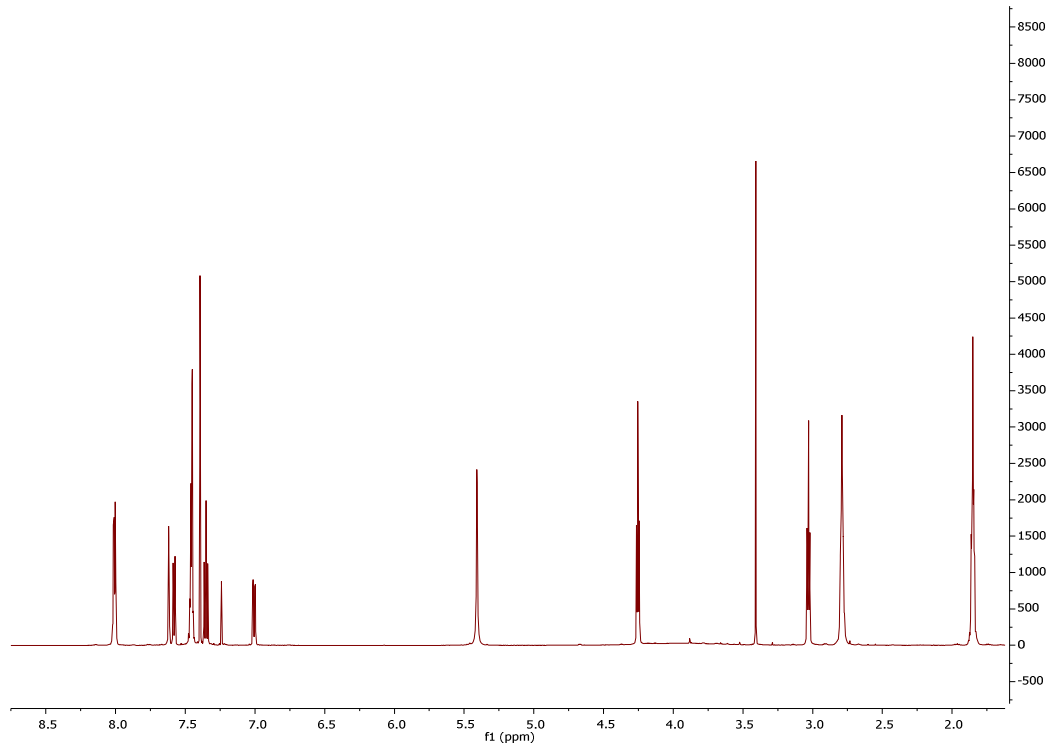


¹³C NMR

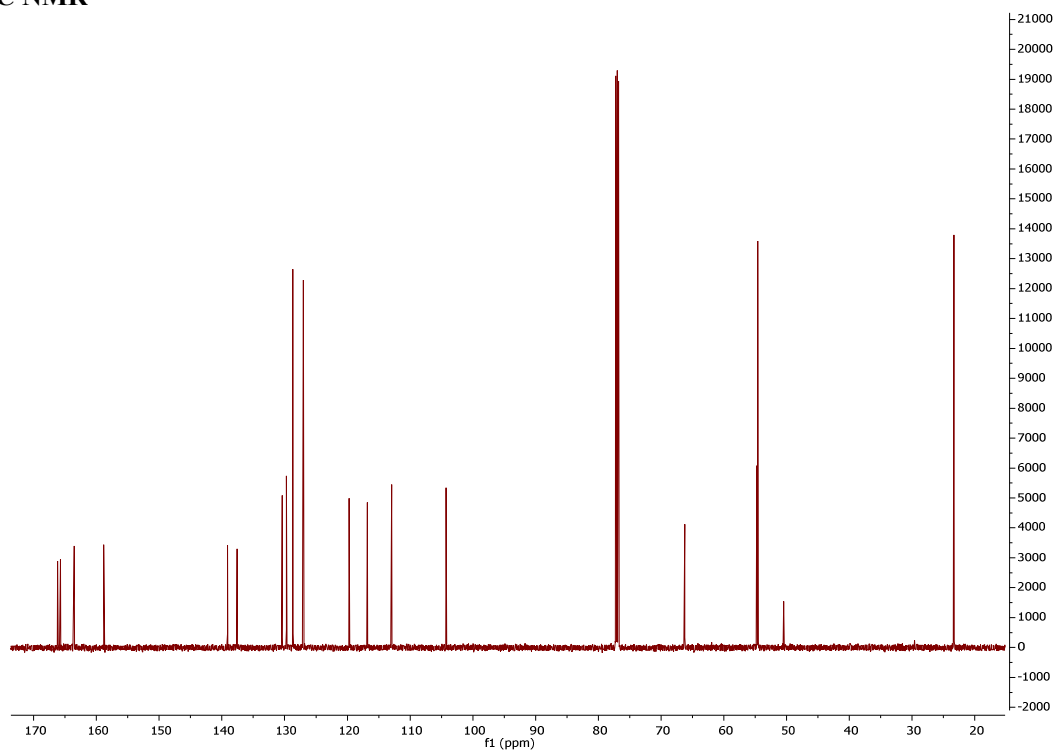


4-phenyl-6-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}pyrimidin-2-amine (**3f**)

¹H NMR

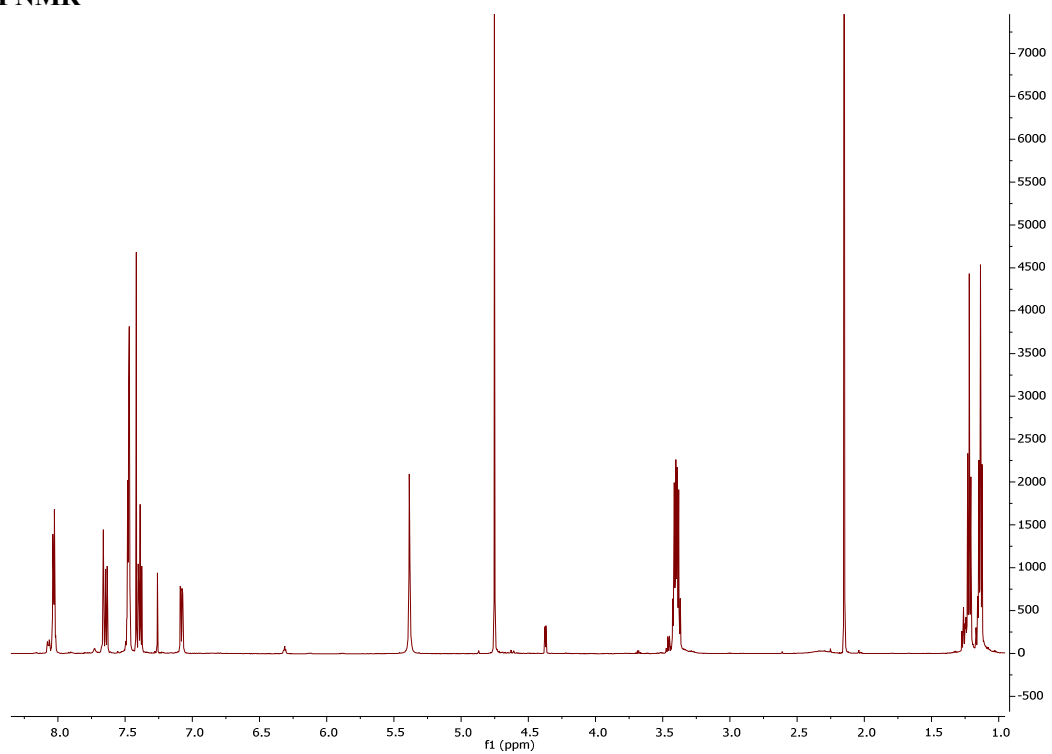


¹³C NMR

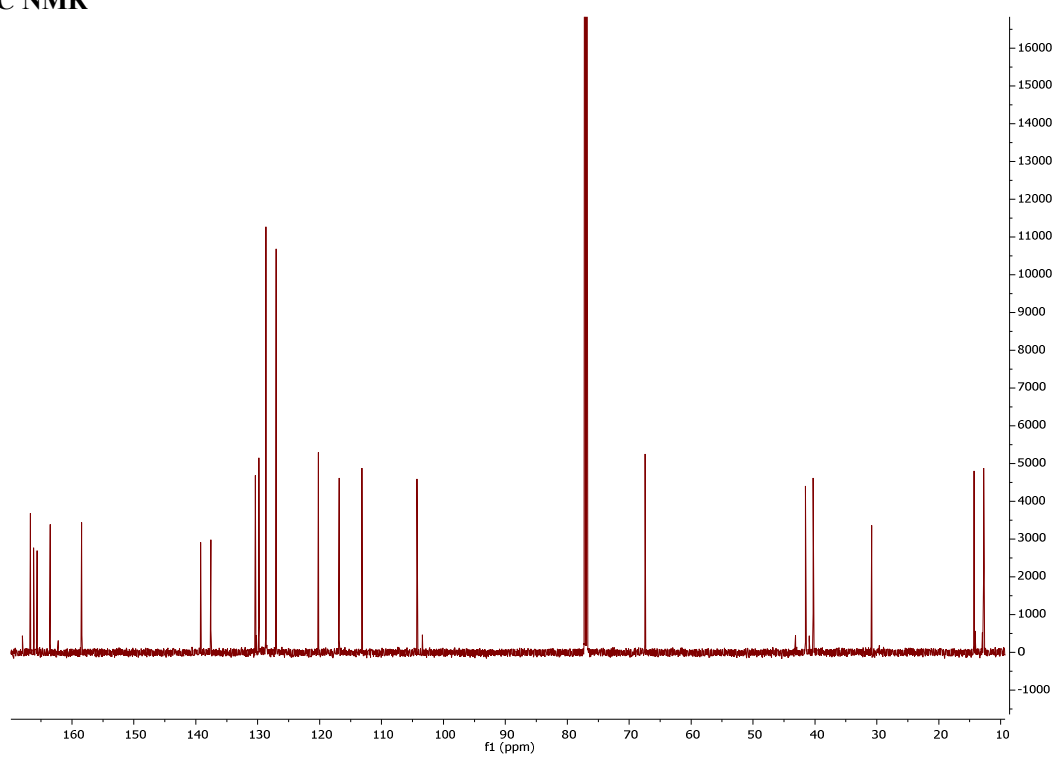


2-[3-(2-amino-6-phenylpyrimidin-4-yl)phenoxy]-*N,N*-diethylacetamide (**3g**)

¹H NMR



¹³C NMR



CHAPTER 7

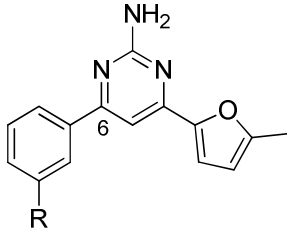
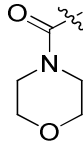
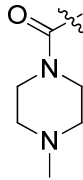
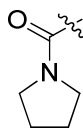
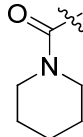
Conclusion

The 2-aminopyrimidine chemotype is a privileged scaffold for antagonism of adenosine receptors as this motif frequently occurs in compounds that exhibit potent adenosine A_{2A} and/or adenosine A_1 affinity. These compounds, which act on a non-dopaminergic target, have the potential to improve the motor and non-motor symptoms of Parkinson's disease, and could, in addition, also inhibit further neurodegeneration, thus preventing the progress of this debilitating disease.

The main objectives of this study were as follows: Firstly, the adenosine A_1 affinities of a series of 2-aminopyrimidines synthesised in a preceding study needed to be determined, and preliminary cytotoxicity data had to be obtained. Secondly, the structure-activity relationships of the promising 2-amino-4,6-diphenylpyrimidine scaffold required further investigation, and would be done by synthesising three novel series of amide, carbamate and ether substituted 2-amino-4,6-diphenylpyrimidines. These objectives were successfully met and culminated in the drafting of four manuscripts for publication.

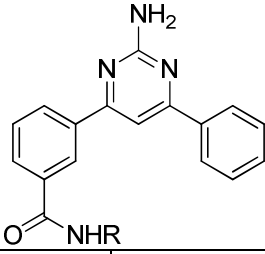
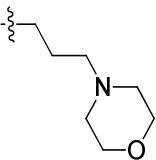
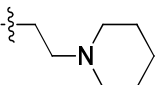
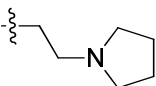
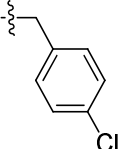
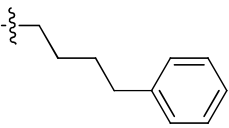
The first article (Robinson *et al.*, *European Journal of Medicinal Chemistry* **2015**, 104: 177-188) describes the potential of 2-aminopyrimidines as dual adenosine A_1 and A_{2A} antagonists and consists of a combination of work done in the preceding MSc study, as well as work done during the current PhD study. In the preceding MSc study, two series of 2-aminopyrimidine derivatives were synthesised and their affinities for adenosine A_{2A} receptors determined (see appendix 1). The amide series showed promising affinities with $A_{2A}K_i$ values ranging from 6 nM - 3 μ M (e.g. **7.1** – **7.4**). In addition, *in vivo* activity was illustrated for two of the amide derivatives, which was indicative of adenosine A_{2A} antagonism. Molecular docking results furthermore indicated that interactions observed between the synthesised compounds and residues in the binding site, were similar to interactions previously observed for structurally related adenosine A_{2A} antagonists. This provided further confirmation of the viability of this scaffold in the design of adenosine A_{2A} receptor antagonists. In the present study, the biological data of these derivatives were expanded by evaluating their potential as adenosine A_1 receptor antagonists. The amide substituted series exhibited promising affinities for the A_1 receptor and 4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**7.4**) was identified as a compound with potent dual affinity with an $A_{2A}K_i$ value of 6.34 nM and an A_1K_i value of 9.54 nM. Cell viability assays further indicated that the amide series exhibited low cytotoxicity. These results provided proof of concept and formed a basis for further exploration of these compounds as dual adenosine A_{2A} and A_1 antagonists in the current study.

Table 7.1:

					
Compound	R	A_1K_i (nM) \pm SEM	$A_{2A}K_i$ (nM) \pm SEM	Cell viability	
				1 μ M	10 μ M
7.1		52.12 \pm 16.86 (n=4)	29.32 \pm 0.9779	99	82
7.2		135.6 \pm 1.878	16.28 \pm 2.181	107	77
7.3		36.78 \pm 5.424	58.22 \pm 19.94	83	79
7.4		9.54 \pm 1.338	6.344 \pm 0.5317	115	68

In the second article (submitted to *Bioorganic and Medicinal Chemistry Letters*), alternative amide substitution of the 2-amino-4,6-diphenylpyrimidine core was investigated. The methyl furan substituent at the 4-position of the pyrimidine ring in the original series of compounds (Table 7.1) was replaced with a phenyl ring as this simplified the synthesis. Furthermore, results from a related study indicated that this change did not alter affinity to a large degree. The main aim was to investigate the effect of elongation of the amide side chain on adenosine A_1 and A_{2A} affinities. Therefore, ten amide substituted 2-amino-4,6-diphenylpyrimidines were synthesised. These derivatives (Table 7.2) generally showed improved dual affinities, when compared to related derivatives of the previous series (Table 7.1). K_i values for this series ranged from 5.42 - 25.2 nM and 3.37 - 106.5 nM for A_1 and A_{2A} receptors, respectively.

Table 7.2:

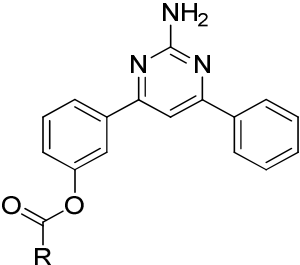
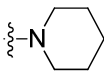
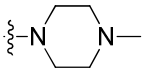
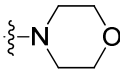
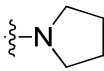

			
Compound	R	A ₁ K _i (nM)	A _{2A} K _i (nM)
7.5		7.53 ± 2.11	3.37 ± 1.63
7.6		6.00 ± 1.11	45.5 ± 3.64
7.7		6.93 ± 0.85	25.2 ± 1.30
7.8		5.42 ± 0.60	9.09 ± 0.839
7.9		20.0 ± 1.77	1.67 ± 0.73

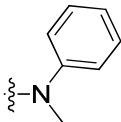
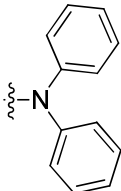
Although no clear structure-activity relationships could be derived regarding A₁ affinities, *p*-chlorobenzyl substitution (**7.8**) was most favourable (A₁K_i = 5.42 nM). The difference between the adenosine A₁ affinities of the compounds from this series are however, reasonably small, (ranging from 5.42 nM to 25.2 nM) and it would thus appear that the adenosine A₁ receptor is capable of accommodating a variety of substituents and different chain lengths (depending on the terminal substituent) almost equally well. On the other hand, the A_{2A} receptor affinity was noticeably influenced by the length of the side chain as compounds **7.9** (K_i = 1.67 nM) and **7.5** (K_i = 3.37 nM) with the longest side chains had the best affinities. The best dual affinity compound from this series (**7.5**), was selected for *in vivo* screening. Compound **7.5** attenuated catalepsy in rats as previously observed for other adenosine A_{2A} antagonists, and provides evidence of *in vivo* efficacy of this compound as an antagonist. These results are also indicative of bio-availability. Molecular docking results did not provide clarification regarding the differences in affinities observed between individual compounds, but

interactions similar to those observed for related 2-aminopyrimidines were observed, confirming their ability to fit into the binding site.

The third article describes the synthesis and evaluation of a novel series of carbamate substituted 2-amino-4,6-diphenylpyrimidines as dual adenosine A₁ and A_{2A} receptor antagonists (e.g. compounds in Table 7.3). The carbamate moiety was incorporated since it is often found in therapeutic agents such as the acetylcholinesterase inhibitor rivastigmine, used as therapy for Alzheimer's disease. Gratifyingly, most of the synthesised carbamates illustrated potent dual affinity for both receptor subtypes with A₁K_i values ranging from 0.175 nM - 10 nM and A_{2A}K_i values ranging from 1.58 nM – 451 nM. In fact, the most potent dual affinity derivatives from this PhD were from this series of compounds, and it is clear that carbamate substitution is preferable over both amide and ether substitution (Table 7.3 vs Tables 7.1, 7.2 and 7.4). Substitution with six-membered saturated cyclic carbamate substituents appears to yield optimal A_{2A} receptor affinity (7.10, 7.11, 7.12). The size of the carbamate substituent further affects affinity to a greater degree for A_{2A} receptors, where substitution with groups that are either too small (e.g. 7.14) or too large (e.g. 7.15 and 7.16) appears to be detrimental for affinity. By comparison, the A₁ receptors are able to accommodate larger carbamate substituents without loss of binding affinity.

Table 7.3:

			
Compound	R	A ₁ K _i (nM)	A _{2A} K _i (nM)
7.10		1.95 ± 0.278	8.94 ± 0.259
7.11		2.04 ± 0.002	1.58 ± 0.311
7.12		2.65 ± 0.059	3.50 ± 0.030
7.13		0.835 ± 0.088	24.8 ± 6.61
7.14		2.06 ± 0.135	51.9 ± 3.07

7.15		1.07 ± 0.079	39.9 ± 4.77
7.16		10.7 ± 0.431	451 ± 143

These results were partially explained by molecular docking studies. Interestingly, in contrast to interactions observed for the previously synthesised amide derivatives, a hydrogen bond was observed between Glu169 (Figure A) and the carbonyl group instead of Tyr271 (Figure B) as observed for the amide derivatives. It is thus postulated that this particular interaction plays an important role in the potent affinities observed for these compounds.

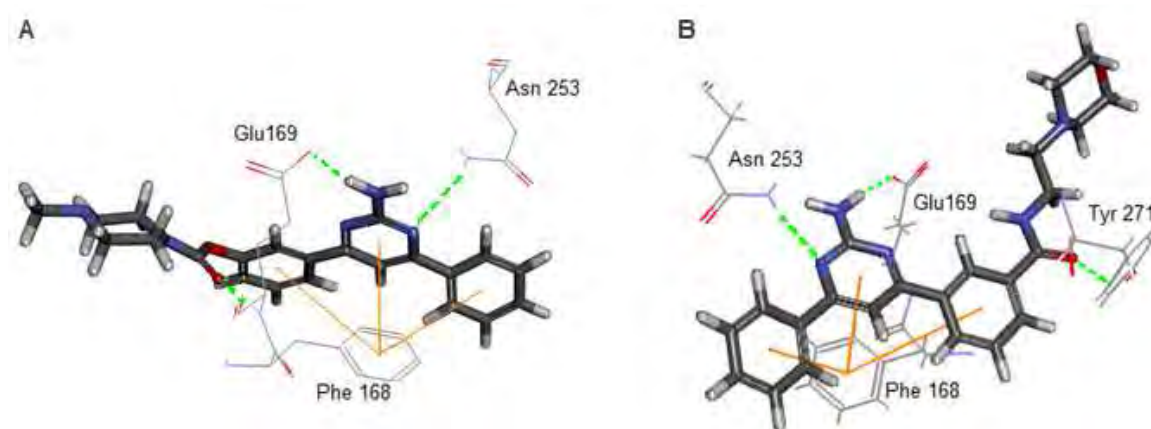


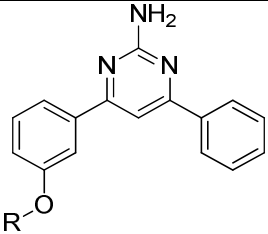
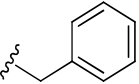
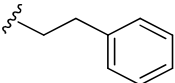
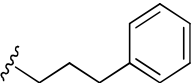
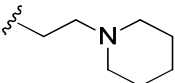
Figure 7.1: A) Example of amide derivative **7.2** and B) carbamate derivative **7.12** docked in adenosine A_{2A} receptor binding pocket. Orange lines illustrate hydrophobic interactions. Green lines illustrate intermolecular hydrogen bonding.

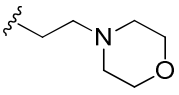
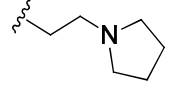
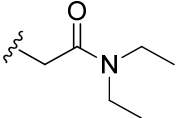
Furthermore, compound **7.16**, with the lowest A_{2A} affinity, also exhibited the least favourable C-DOCKER – as well as C-DOCKER-interaction energies. In addition, it was observed that compound **7.16** is docked “upside down” in the binding site when compared to the rest of the synthesised compounds, resulting in the absence of several important interactions observed for other structurally related A_{2A} antagonists. The most promising dual affinity candidates, compounds **7.11** and **7.12** were subsequently selected for *in vivo* screening. Catalepsy was attenuated after administration of compound **7.12**, providing evidence of bio-availability and indicating that this compound is indeed an antagonist. Disappointingly, compound **7.11**, with the highest dual affinity, did not reverse catalepsy and appeared to be inactive *in vivo*, possibly due to unfavourable physicochemical properties, such as poor water solubility. The solubility and log D of compound **7.11** was thus subsequently determined and a Log D

value of 4.03 and an aqueous solubility of 0.22 μM were obtained. This compound is therefore probably too lipophilic and most likely precipitates after i.p. injection, and this combined with the low aqueous solubility, are probably responsible for the lack of observed activity *in vivo*. Although compounds from this series were thus highly active *in vitro*, optimisation of physicochemical properties may be required in future studies.

For the fourth article, novel ether substituted 2-amino-4,6-diphenylpyrimidines were evaluated as dual adenosine A_1 and A_{2A} antagonists. The main objective was to determine the importance of the carbonyl group present in the amide and carbamate derivatives, since the molecular docking studies showed that an interaction between this group and a residue in the binding site (Tyr271 or Glu169) is usually associated with good affinity. It was also hypothesised that the more flexible ether side chain would be able to rotate to a larger degree than the more rigid derivatives, allowing them to fit better in the binding sites of the A_1 and A_{2A} receptors, resulting in improved affinities for both receptor subtypes. However, results obtained in radioligand studies provided evidence that ether substitution is less favourable than related amide- (Table 7.1 and 7.2) or carbamate- (Table 7.3) substitution, particularly for adenosine A_{2A} affinity, since adenosine A_{2A} affinities were only moderate ($A_{2A}K_i$ values ranging from 47.04 – 351.6 nM) (Table 7.4).

Table 7.4:

				
Compound	R	A_1 K_i (nM)	A_{2A} K_i (nM)	SI
7.17		5.658 ± 1.005	326.8 ± 71.25	58
7.18		7.305 ± 0.691	77.81 ± 1.488	11
7.19		48.84 ± 7.643	351.6 ± 24.35	7.2
7.20		11.65 ± 1.482	193.5 ± 2.998	17

7.21		16.19 ± 4.426	133.4 ± 14.34	8.2
7.22		7.798 ± 2.984	306.9 ± 7.478	39
7.23		22.21 ± 3.929	47.04 ± 2.996	2.1

The importance of the carbonyl group for adenosine A_{2A} affinity was thus confirmed. In contrast, these compounds still exhibited potent A₁K_i values that ranged from 5.658 - 48.84 nM (Table 7.4). Most of these derivatives were also selective towards the A₁ receptor, indicating that the increase in flexibility of the side chain did not significantly alter binding to the binding site of the A₁ receptor. However, compound **7.17**, with the shortest side chain, has the best A₁ receptor affinity ($K_i = 5.658$ nM) which suggests that short, hydrophobic side chains are preferable for A₁ affinity. Molecular modelling studies were again performed in order to obtain some explanation for these results. The interactions observed for the 2-amino-4,6-diphenylpyrimidine scaffold were similar to those observed for the amide and carbamate derivatives. However, since the carbonyl group was absent in the side chains of most of these derivatives, no additional interactions were observed with either Glu169 or Tyr271, which confirms that this additional interaction results in improved adenosine A_{2A} affinity. Further evidence for this is provided by the observation that an additional hydrogen bonding interaction was observed for compound **7.23**, which is the only compound with a carbonyl group in its side chain that also exhibited the best A_{2A} affinity.

In conclusion, 26 novel 2-amino-4,6-diphenylpyrimidines and their chalcone intermediates were synthesised for the first time during this study. The aminopyrimidines were evaluated for their potential as dual adenosine A₁ and A_{2A} antagonists and several derivatives with potent dual affinity were identified. Although carbamate substitution resulted in the best *in vitro* results, bio-availability of these compounds could be problematic (further investigation in this regard is required) and amide substitution therefore seems preferable, as this resulted in high dual affinity and *in vivo* activity. Ether substitution on the other hand improves adenosine A₁ selectivity and could find application in alternative diseases. These results were compiled in four journal articles, of which one has been published (*European Journal of Medicinal Chemistry*), one has been submitted (*Bioorganic and Medicinal Chemistry Letters*) and two which are ready for submission to *Chemical, Biology and Drug Design* and *Bioorganic and Medicinal Chemistry Letters*, respectively. This study provided evidence of the potential of 2-

aminopyrimidines to be utilised in the treatment of the multiple symptoms as experienced in Parkinson's disease.

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Table 8.1: Compounds synthesised in the preceding M.Sc. study

Compound	R ¹	R ²	R ³	Compound	R ¹	R ²	R ³
8a	H	-Cl		8j	H		
8b	H	-Cl		8k	H		
8c	H	-Cl		8l	H		
8d	H	-OCH ₃		8m	H		
8e	H	-OCH ₃		8n	H		
8f	-OCH ₃	-OCH ₃					
8g	-Cl	H					
8h	F	H					



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Research paper

2-Aminopyrimidines as dual adenosine A₁/A_{2A} antagonists



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ABSTRACT

In this study thirteen 2-aminopyrimidine derivatives were synthesised and screened as potential antagonists of adenosine A₁ and A_{2A} receptors in order to further investigate the structure activity relationships of this class of compounds. 4-(5-Methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (**8m**) was identified as a compound with high affinities for both receptors, with an A_{2A}K_i value of 6.34 nM and an A₁K_i value of 9.54 nM. The effect of selected compounds on the viability of cultured cells was assessed and preliminary results indicate low cytotoxicity. *In vivo* efficacy at A_{2A} receptors was illustrated for compounds **8k** and **8m** since these compounds attenuated haloperidol-induced catalepsy in rats. A molecular docking study revealed that the interactions between the synthesised compounds and the adenosine A_{2A} binding site most likely involve Phe168 and Asn253, interactions which are similar for structurally related adenosine A_{2A} receptor antagonists.

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1. Introduction

Parkinson's disease is a complex, chronic neurodegenerative disorder, mainly characterised by a decline in motor function, but also associated with non-motor manifestations such as cognitive deficits (e.g. dementia) and neuropsychiatric symptoms, such as depression [1]. Management of the non-motor symptoms in particular is challenging and represents an important unmet medical need [2–4]. The neuropathology of the disease is hallmarked by the selective degeneration of dopaminergic neurons of the nigrostriatal pathway and the resulting deficiency of dopamine in the basal ganglia [5]. However, the importance of other neurotransmitter systems and organs in Parkinson's disease pathogenesis are increasingly recognised [6–8].

Nevertheless, current treatments are still focused on enhancing dopaminergic neurotransmission and include the use of levodopa, (which is still the gold standard nearly 50 years since its introduction), dopamine agonists, catechol-*O*-methyltransferase and monoamine oxidase inhibitors. Since the chronic nature of the disease requires long-term use of medication, the occurrence of side-effects is unavoidable, and levodopa especially is associated

with the development of debilitating dyskinesias [9,10]. Limited progress has been made in altering the progress of neurodegeneration, and to date, no agent has been established as neuroprotective or disease modifying [11,12]. Non-dopaminergic targets for the disease are thus particularly appealing; especially if these would also improve non-motor symptoms and provide neuroprotection [13]. In recent years, antagonists of the adenosine A_{2A} receptor in particular has received attention as a promising non-dopaminergic alternative [3, 4, 14–17] and several of these agents such as istradefylline (KW-6002), preladenant and tozadenant have been investigated clinically with promising results. Istradefylline for example has been approved for use as adjunctive treatment for Parkinson's disease in Japan [18–22].

Adenosine receptors are G-protein coupled receptors and consist of four subtypes, namely A₁, A_{2A}, A₃ and A_{2B} [23,24]. Of particular importance to Parkinson's disease are adenosine A_{2A} receptors which are concentrated in the indirect striatopallidal GABAergic pathway. This pathway also expresses the D₂ dopamine receptor and enkephalin [25–27]. Antagonism of A_{2A} receptors potentiate dopamine mediated responses and partly restores the imbalance between the hypoactive direct striatonigral and hyperactive indirect striatopallidal pathways that develops in Parkinson's disease, thus relieving motor symptoms [14,16,28,29]. Both epidemiological and experimental data have shown that adenosine A_{2A} antagonists exert a neuroprotective effect [16]. Furthermore, it has

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been reported that the adenosine A_{2A} antagonist KW-6002, exhibit antidepressant properties in animal models of depression [30,31] and that adenosine A_{2A} antagonists have potential in the management of dyskinesias [29,32]. This illustrates the promise of these agents as multifactorial non-dopaminergic treatment of Parkinson's disease.

Adenosine A_1 receptors on the other hand, are expressed throughout the brain, including the cortex, hippocampus and striatum [23]. Since A_1 receptor antagonism also results in motor activation in animals [33,34] it has been suggested that dual antagonism of A_1 and A_{2A} receptors would act synergistically in improving motor deficits in Parkinson's disease [4]. Furthermore, since the A_1 receptor also occurs in systems that are important for cognitive function, adenosine A_1 antagonism may improve cognitive deficits experienced in Parkinson's disease, as illustrated in animals [35–37]. Several dual adenosine A_1/A_{2A} antagonists have in fact been investigated in animals and has not only shown effectiveness in improving motor disabilities [4,38–43], but has also illustrated effectiveness in enhancing cognition [38]. In summary, dual adenosine A_1 and A_{2A} antagonists would thus not only treat the motor symptoms of Parkinson's disease and potentially be neuroprotective, but may also improve non-motor symptoms.

The 2-aminopyrimidine motif frequently occurs in compounds (e.g. 1–6) that exhibit potent adenosine A_{2A} and/or adenosine A_1 affinity (Fig. 1) and indicates that this scaffold is privileged for antagonism of these receptors [4,15,17,40–48]. Of particular relevance to this paper are the findings of Van Veldhoven, Matasi and Shook and co-workers [40–42,46,47], who synthesised a number of 2-aminopyrimidines (e.g. 3), indenopyrimidones (e.g. 4, 5) and

indenopyrimidines (e.g. 6), respectively.

Our research group has been interested in the design, synthesis and evaluation of heterocycles as antagonists of adenosine receptors. Based on the aforementioned findings, the aim of the present study was to explore the necessity of the methylene bridge as present in the indenopyrimidine or indenopyrimidone scaffolds previously synthesised [40–42,46] and to further investigate the structure–activity relationships of the 2-aminopyrimidine scaffold for dual antagonism of adenosine A_1 and A_{2A} receptors. We thus set out to synthesise firstly, a set of 2-aminopyrimidines substituted with simple electron withdrawing and donating groups (8a–h), and secondly an amide substituted series (8j–n), related to the indenopyrimidones (e.g. 5) as synthesised by Shook and co-workers [41]. We report herein their affinities for adenosine A_{2A} and A_1 receptors, the results of docking selected compounds into the adenosine A_{2A} receptor's binding site, as well as the *in vivo* activities of selected compounds.

2. Chemistry

As shown in Scheme 1, the 2-aminopyrimidines were readily synthesised in two or three steps, albeit in low yields. Firstly, a Claisen–Schmidt condensation, using commercially available ketones and aldehydes under basic conditions [49] yielded the desired intermediate chalcones (7a–i). For the amide derivatives (8j–n), the condensation reaction was followed by an amide coupling reaction mediated by 1,1'-carbonyldiimidazole (CDI) resulting in chalcones 7j–n. All chalcones were cyclised with guanidine hydrochloride in the presence of sodium hydride [50] to

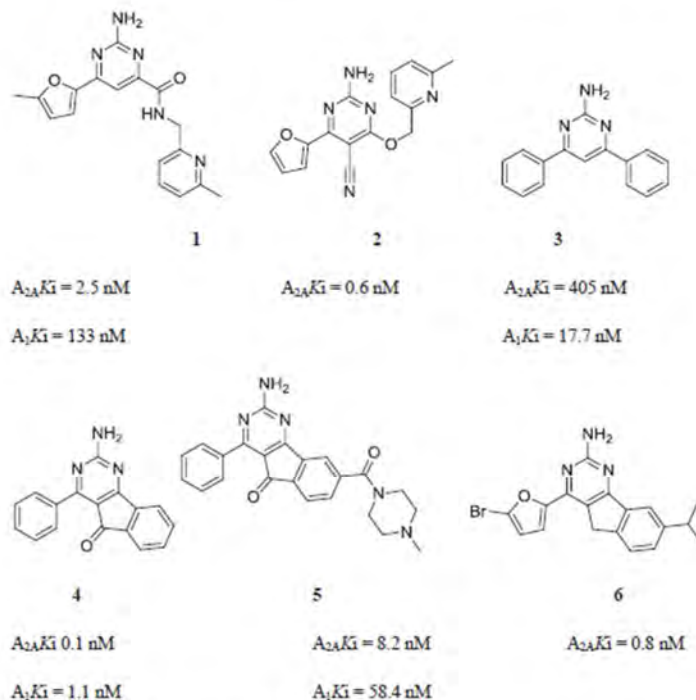
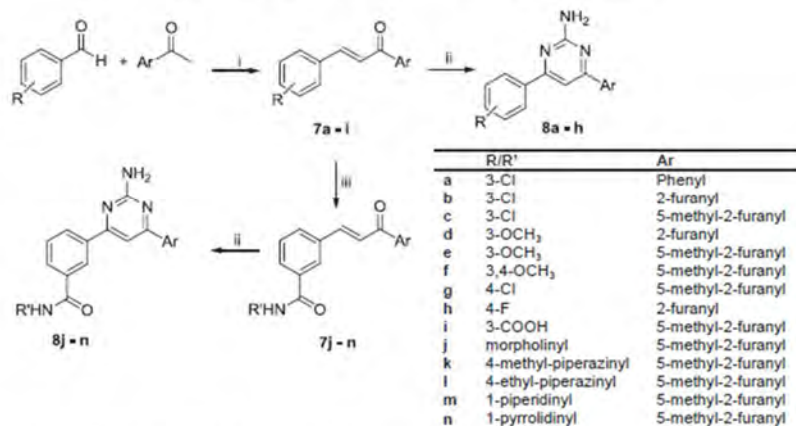


Fig. 1. Adenosine A_{2A} antagonists containing the 2-aminopyrimidine moiety.



Scheme 1. Synthesis of 2-aminopyrimidine derivatives **8**. Reagents and Conditions: (i) NaOH, EtOH/MeOH, rt, 3 h; (ii) Guanidine hydrochloride, NaH, DMF, 110 °C, 24 h (iii) CDI, CH₂Cl₂, NHR, rt, 5 h.

yield the desired 2-aminopyrimidines (**8a – n**), since the use of sodium hydroxide in ethanol resulted in complicated mixtures of products.

3. Results and discussion

The affinities of the 2-aminopyrimidines for the adenosine A_{2A} and A₁ receptors were determined by radioligand binding and are expressed as the receptor-ligand dissociation constants (K_i, nM) (Tables 1 and 2). Adenosine A_{2A} receptor affinity was determined using the non-selective adenosine antagonist, [³H]5'-N-ethylcarboxamide-adenosine ([³H]NECA) in the presence of N⁶-cyclopentyladenosine (CPA), and A₁ receptor affinity was determined using 1,3-[³H]-dipropyl-8-cyclopentylxanthine ([³H]DPCPX) [51–53].

The 2-aminopyrimidines of series 1 (**8a – h**) exhibited moderate to weak affinities for the adenosine A_{2A} receptor and moderate to good affinities for A₁ receptors with A_{2A}K_i values ranging from approximately 3 μM (**8f**, **8g**) to approximately 250 nM (**8b**, **8d**, **8h**) and A₁K_i values ranging from 23 nM (**8a**) to 650 nM (**8f**). These compounds all have higher affinities for the adenosine A₁ receptor than for the adenosine A_{2A} receptor, with compound **8a** being the most selective (selectivity index of 42). For adenosine A_{2A} receptor affinity, the replacement of the phenyl substituent (R¹) with either a furan or methylfuran group, resulted in improved affinity (compound **8a** vs. compounds **8b** and **8c**), whereas the opposite was true for A₁ affinity, where methylfuran substitution in particular proved to be detrimental. When the affinities of compounds **8b** and **8c**, and compounds **8d** and **8e** are compared, it is clear that methyl substitution of the furan ring results in decreased affinity, especially for the adenosine A₁ receptor. Based on these results, it thus appears that for R³, furan substitution is preferable for A_{2A} affinity, while phenyl substitution is optimal for A₁ receptor affinity. These results are in agreement with literature since the preference of the adenosine A_{2A} receptor for furan substitution is well documented [e.g. 46]. The affinity is apparently not significantly affected by the electronic effects of the substituent on the phenyl ring (R²) as compounds **8b** and **8d** (A_{2A}K_i values approx. 250 nM, A₁K_i values 40–60 nM) as well as compounds **8c** and **8e** (A_{2A}K_i values approx. 400 nM, A₁K_i values 130–140 nM) had similar affinities for A_{2A} and

A₁ receptors, respectively.

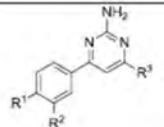








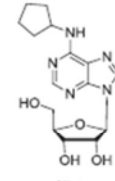
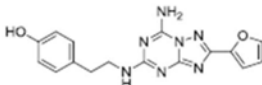
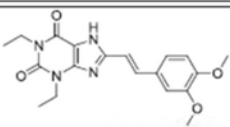
On the other hand, the position of the substituent on the phenyl ring seems to have a significant effect on both A₁ and A_{2A} affinity, as substitution on the *meta* position (**8c**, R¹ = H, R² = Cl) is superior to substitution on the *para* position (**8g**, R¹ = Cl, R² = H). A similar observation is made when the affinities of compounds **8e** and **8f** are compared, where addition of a second methoxy group, in the *para* position, results in much weaker A₁ and A_{2A} affinity. However, since only a limited number of derivatives have been synthesised, these structure–activity relationships should be seen as preliminary.

For compounds **8j – n**, the 5-methyl-2-furanyl group was selected as the R³ substituent, since it was synthetically easier to work with than furan and is also less likely to present with metabolic liabilities [40]. Gratifyingly, this amide series showed improved adenosine A_{2A} affinity, while still retaining good A₁ affinity. The most promising candidate was compound **8m**, with dual affinity for both A_{2A} and A₁ receptors with K_i values of 6.34 nM and 9.54 nM, respectively. For adenosine A_{2A} affinity, the amine groups yielded the following order of affinity: piperidine > methylpiperazine > morpholine = ethyl piperazine > pyrrolidine. Piperidine substitution was also most advantageous for A₁ affinity, while methylpiperazine substitution was least favourable. The affinities of this series of compounds are quite similar to those reported for related arylindolopyrimidones [41], and indicate that the presence of the five-membered indenyl ring (e.g. as present in **5** and **6**) is not an absolute requirement for dual affinity.

To gain an indication of potential cytotoxicity of the amide derivatives, the effect of these compounds on the viability of cultured HeLa cells was measured. For this purpose, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay was used [54]. Cell viability was generally above 60% when exposed for 24 h to either 1 μM or 10 μM of test compound (Table 3). These concentrations are almost 1000-fold higher than the reported K_i values of these compounds. Cytotoxicity at the doses required to obtain adenosine A₁ and A_{2A} affinity should thus not be problematic.

Selected representatives **8m** (the compound with best dual affinity, A_{2A}K_i = 6.34 nM, A₁K_i = 9.54 nM) and **8k** (A_{2A}K_i = 16.3 nM), were subjected to *in vivo* studies to determine the effectiveness of

Table 1
Adenosine receptor affinities (K_i) of compounds **8a**–**h**.

Comp.				A _{2A} K _i (nM)	A ₁ K _i (nM)	SI A _{2A} K _i /A ₁ K _i
	R ¹	R ²	R ³			
8a	-H	-Cl		948 ± 141	22.8 ± 5.51	42
8b	-H	-Cl		245 ± 21.1	39.1 ± 3.64	6.3
8c	-H	-Cl		399 ± 151	129 ± 12.1	3.1
8d	-H	-OCH ₃		249 ± 79.6	61.4 ± 0.435	4.1
8e	-H	-OCH ₃		409 ± 217	145 ± 19.6	2.8
8f	-OCH ₃	-OCH ₃		2778 ± 375	650 ± 63.0	4.3
8g	-Cl	-H		3320 ± 484	434 ± 13.0	7.6
8h	-F	-H		257 ± 36.4	158 ± 12.1	1.6
			 CPA		10.4 ± 1.57	
			 ZM241385	2.88 ± 0.670		
			 KW-6002	11.1 ± 3.97		

^aValues are given as mean ± SEM of duplicate determinations

^bSelectivity index

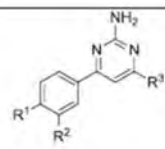










these compounds as dual adenosine A₁/A_{2A} antagonists. For these studies the effect of the test compounds on haloperidol-induced catalepsy in rats was investigated [33].

As depicted in Fig. 2, a significant reduction in catalepsy time was observed for **8m** at all intraperitoneal (ip) administered doses

while a significant reduction in catalepsy time was only observed for the highest two doses (1 and 2 mg/kg) for compound **8k** (Fig. 2a and b).

The results obtained with compounds **8m** and **8k** are thus similar to those obtained with other adenosine A_{2A} antagonists

Table 2
Adenosine receptor affinities (K_i) of compounds **8j** – **n**.

Comp.	R ¹	R ²	R ³			
				A _{2A} K _i (nM) ^a	A ₁ K _i (nM) ^a	SI ^b A _{2A} K _i /A ₁ K _i
8j	-H			29.3 ± 0.978	52.1 ± 16.9 ^c	0.6
8k	-H			16.3 ± 2.18	136 ± 1.88	0.1
8l	-H			30.9 ± 3.10	37.5 ± 0.857	0.8
8m	-H			6.34 ± 0.532	9.54 ± 1.34	0.7
8n	-H			58.2 ± 19.9	36.8 ± 5.42	1.6
		CPA			10.4 ± 1.57	
		ZM241385		2.88 ± 0.670		
		KW-6002		11.1 ± 3.97		

^aValues are given as mean ± SEM of duplicate determinations^bSelectivity index^cn = 4**Table 3**
The percentage viable cells remaining after treatment with amide derivatives (**8j** – **8n**), as compared to untreated cells (100%).

Comp.	% Viable cells	
	1 μM ^a	10 μM ^a
8j	98.6 ± 4.45	81.7 ± 5.97
8k	106.6 ± 9.76	76.8 ± 18.1
8l	65.7 ± 4.95	60.1 ± 0.00
8m	64.3 ± 4.74	61.6 ± 4.46
8n	101 ± 6.22	67.9 ± 7.09

^a Values are given as mean ± SD of triplicate determinations.

where catalepsy is reversed in the presence of A_{2A} antagonists or dual adenosine A_{2A}/A₁ antagonists [33,34,43], and provides evidence of *in vivo* efficacy of these compounds as antagonists.

In order to rationalise the results obtained in the radioligand

binding studies, a docking study was performed using CDOCKER (Discovery Studio 3.1). The synthesised compounds were docked into a model of the binding site of the adenosine A_{2A} receptor (PDB code: 3PWH). Visual inspection of the docked poses with most favourable CDOCKER interaction energy firstly revealed that the three-membered ring system of all derivatives (**8a** – **n**), undergo π–π interactions with Phe168. Hydrogen bonding interactions also occur between the exocyclic amino-group and Asn253 for most derivatives. These interactions most likely anchor the aminopyrimidine in the binding site and are also important binding interactions predicted for other 2-aminopyrimidine antagonists [55] (Figs. 3 and 4). Additionally, hydrogen bonding interactions were also observed with His250, Ser67, Glu169, Phe168 and Ala63 for some compounds. Interestingly, for compounds **8a** – **h** there were two different orientations, one where the C-6 substituent was orientated towards His250 and another where the C-4 substituent

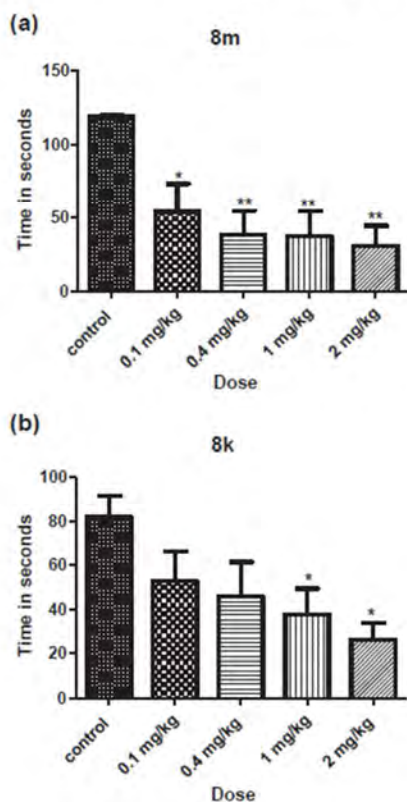


Fig. 2. a. Reduction in haloperidol induced-catalepsy in male Sprague–Dawley rats by compound **8m**. Time to descend from the bar was measured in haloperidol (5 mg/kg, ip) treated rats after ip administration of compound **8m** (0.1, 0.4, 1, 2 mg/kg). Each bar represents average time (\pm SEM) of ($n = 6$) rats in the cataleptic position (*, ** indicate significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [$F(4, 25) = 5.893$; $P < 0.005$] followed by Dunnett's post test with * $P = 0.01$ – 0.05 and ** $P = 0.001$ – 0.01). b. Reduction in haloperidol induced-catalepsy in male Sprague–Dawley rats by compound **8k**. Time to descend from the bar was measured in haloperidol (5 mg/kg, ip) treated rats after ip administration of compound **8k** (0.1, 0.4, 1, 2 mg/kg). Each bar represents average time (\pm SEM) of ($n = 6$) rats in the cataleptic position (* indicates significant differences compared with the haloperidol + vehicle control group as determined by one-way ANOVA [$F(4, 25) = 3.103$; $P < 0.05$] followed by Dunnett's post test with $P = 0.01$ – 0.05).

was orientated towards His250 (Fig. 3). The CDocker interaction energies of these two poses were in all cases very similar. The docking results gave no clear explanation for the observed higher affinity of the amide derivatives (**8j**–**n**) compared to the series 1 compounds (**8a**–**h**). However, when ranked according to CDocker interaction energy, most of the series 2 compounds ranked above the series 1 compounds, except for compound **8f**, which ranked above compound **8n**. It would thus appear that the interaction between the amide derivatives (**8j**–**n**) and the receptor is generally more favourable than for the “smaller” derivatives and provides some explanation for the superior affinity of compounds **8j**–**n**.

4. Conclusion

This series of 2-aminopyrimidines, particularly the amide derivatives (**8j**–**n**), which are related to previously synthesised arylindenoypyrimidines, retain affinity for both adenosine A_1 and A_{2A} receptors. The 2-aminopyrimidine scaffold can thus be optimised and the presence of a five-membered “linker ring” is not an absolute requirement for affinity. *In vivo* activity is indicative of adenosine antagonism rather than agonism and has been illustrated for compounds **8k** and **8m** with $A_{2A}K_i$ values of 16.3 nM and 6.34 nM and A_1K_i values of 136 nM and 9.54 nM, respectively. Docking results indicate that these compounds are predicted to bind in a fashion similar to that illustrated for other compounds of the 2-aminopyrimidine class.

5. Experimental section

5.1. Chemistry

Chemical reagents were purchased from Sigma–Aldrich, and used without further purification. Reactions were routinely monitored on TLC using precoated Kieselgel 60 F254 sheets with ethyl acetate: petroleum ether (1:4) as mobile phase for series 1 (**8a**–**h**) and dichloromethane: methanol (9:1) as mobile phase for series 2 compounds (**8j**–**n**). Melting points were determined using a Buchi B-545 apparatus, and are uncorrected. Mass spectra were obtained on a dual focusing DFS magnetic sector mass spectrometer in EI+ mode. The mass spectrum for compound **7n** was obtained with a Bruker micrOTOF-QII mass spectrometer in atmospheric-pressure chemical ionisation (APCI) mode. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively. Samples were dissolved in either deuteriochloroform (CDCl_3) or deuterated dimethylsulfoxide ($\text{DMSO-}d_6$). ^1H NMR data are reported in parts per million (ppm) and the following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), br d (broad doublet), dd (doublet of doublets), t (triplet), br t (broad triplet), q (quartet), p (pentet/quintet) or m (multiplet). Chemical shifts are referenced to the residual solvent signal (CDCl_3 7.26 and 77.0 ppm for ^1H and ^{13}C respectively; $\text{DMSO-}d_6$: 2.5 and 39.5 ppm for ^1H and ^{13}C , respectively). Assignments were based on data obtained from 1D (^1H , ^{13}C , DEPT) and 2D (HSQC, HMBC, COSY) NMR experiments. HPLC analyses were conducted with an Agilent 1100 HPLC system equipped with a quaternary pump and an Agilent 1100 series diode array detector. A Venusil XBP C18 column (4.60 \times 150 mm, 5 μm) was used with a solvent gradient program (30% acetonitrile and 70% MilliQ water initially) at a flow rate of 1 ml/min. The concentration of acetonitrile in the mobile phase was linearly increased up to 85% over a period of 5 min. Each HPLC run lasted 15 min and a time period of 5 min was allowed for equilibration between runs. The test compound was injected (20 μl , 1 mM) into the HPLC system and the eluent was monitored at a wavelength of 254 nm.

5.1.1. Procedure for the synthesis of 3-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (**7i**)

A solution of 4% (w/v) sodium hydroxide (34 mmol) was added to a suspension of 3-formylbenzoic acid (17 mmol) and 1-(5-methyl-2-furyl)ethanone (17 mmol) in methanol (100 ml). The mixture was stirred at room temperature for 24 h and acidified with concentrated hydrochloric acid to a pH of 1–2. The precipitate that formed was filtered, rinsed with water and recrystallised from methanol to afford **7i**.

5.1.1.1. 3-[(1E)-3-(5-methylfuran-2-yl)-3-oxoprop-1-en-1-yl]benzoic acid (**7i**). Yield 68%; Pale yellow crystals; mp 191.7–194.1 $^\circ\text{C}$

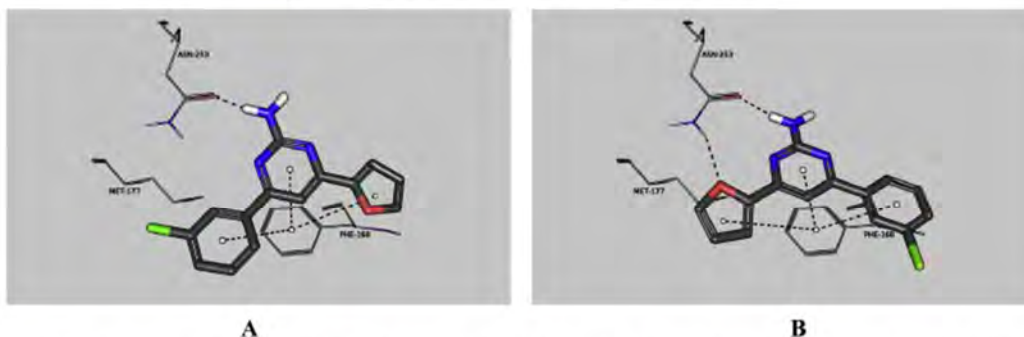


Fig. 3. Illustration of the different orientations of compound **8b** in the binding site of the A_{2A} receptor. Intermolecular hydrogen bond interactions are observed between the exocyclic amino group and Asn253, while $\pi = \pi$ stacking occurs between the three ring systems and Phe168 (A). In orientation B, an additional hydrogen bonding interaction is observed between the furan oxygen and Asn253 (Figure generated using Pymol).

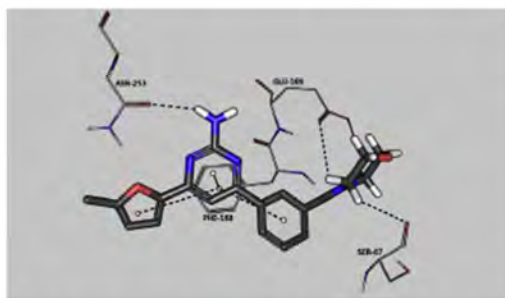


Fig. 4. Docking orientation of compound **8j** in the active site of the adenosine A_{2A} receptor. An intermolecular hydrogen bond interaction is observed between the exocyclic amino group and Asn253, while $\pi = \pi$ stacking occurs between the three ring systems and Phe168. Additional hydrogen bond interactions occur between the morpholine in the side chain and Glu169 and Ser67 (Figure generated using Pymol).

(methanol): $^1\text{H NMR}$ (600 MHz, $\text{DMSO}-d_6$) δ 13.20 (br s, 1H, OH), 8.33 (br s, 1H, H-2'), 8.07 (br d, $J = 7.8$ Hz, 1H, H-6'), 7.98 (dt, $J = 1.4, 7.7$ Hz, 1H, H-4'), 7.82 (d, $J = 3.5$ Hz, 1H, H-3''), 7.75 (d, $J = 16.0$ Hz, 1H, H-7 or H-8), 7.71 (d, $J = 16.0$ Hz, 1H, H-7 or H-8), 7.57 (t, $J = 7.7$ Hz, 1H, H-5'), 6.42 (dd, $J = 1.1, 3.5$ Hz, 1H, H-4''), 2.40 (s, 3H, CH_3). $^{13}\text{C NMR}$ (151 MHz, $\text{DMSO}-d_6$) δ 175.6 (C-1), 167.0 (acid C=O), 158.8 (C-5''), 151.8 (C-2''), 141.2 (C-3), 135.0 (C-1'), 132.8 (C-6'), 131.6 (C-3'), 131.0 (C-4'), 129.2 (C-2', C-5'), 123.1 (C-2), 121.9 (C-3''), 109.6 (C-4''), 13.8 (CH_3). EI-HRMS m/z : calcd for $\text{C}_{15}\text{H}_{12}\text{O}_4$, 256.07356, found 256.07292; Purity (HPLC): 100%.

5.1.2. General procedure for the synthesis of chalcones (**7j** – **7n**)

1,1'-Carbonyldiimidazole (CDI) (7.0 mmol) was added to a suspension of the acid (**7i**) (5.8 mmol) in dichloromethane (70 ml). The reaction mixture was stirred under nitrogen at room temperature for 2 h and the appropriate amine (7.0 mmol) was added. The mixture was then stirred for a further 3 h. The reaction was quenched by the addition of brine and the aqueous phase extracted with dichloromethane (2 \times 20 ml). The combined organic fractions were washed once with saturated sodium hydrogen carbonate and twice with brine. The organic fraction was concentrated (*in vacuo*), purified with column chromatography [dichloromethane: methanol (98:2)] and recrystallised from methanol.

5.1.2.1. (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one (**7j**). The title compound was prepared from (1*E*)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and morpholine in a yield of 54%; mp 149.1–149.9 °C (methanol), pale yellow crystals. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.80 (d, $J = 15.8$ Hz, 1H, H-3), 7.69 (br t, $J = 1.6$ Hz, 1H, H-2'), 7.65 (br d, $J = 1.6, 7.7$ Hz, 1H, H-6'), 7.47–7.36 (m, 3H, H-2, H-5', H-4'), 7.25 (br d, $J = 3.4$ Hz, 1H, H-3''), 6.21 (dd, $J = 1.0, 3.4$ Hz, 1H, H-4''), 3.93–3.31 (m, 8H, 4 \times morpholine CH_2), 2.43 (s, 3H, CH_3). $^{13}\text{C NMR}$ (151 MHz, CDCl_3) δ 176.8 (C-1), 169.6 (amide C=O), 158.4 (C-5'), 152.3 (C-2'), 141.8 (C-3), 136.0 (C-1' or C-3'), 135.4 (C-1' or C-3'), 129.9 (C-6'), 129.1, 128.5 (C-4', C-5''), 126.6 (C-2'), 122.4 (C-2), 119.8 (C-3''), 109.4 (C-4''), 66.8, 48.2, 42.5 (morpholine CH_2). EI-HRMS m/z : calcd for $\text{C}_{19}\text{H}_{19}\text{NO}_4$, 325.13141, found 325.13031; Purity (HPLC): 100%. * In no particular order.

5.1.2.2. (2*E*)-1-(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one (**7k**). The title compound was prepared from (1*E*)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and 1-methylpiperazine in a yield of 67%; mp 131.0–132.2 °C (methanol), yellow crystals. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.79 (d, $J = 15.8$ Hz, 1H, H-3), 7.68 (br t, $J = 1.6$ Hz, 1H, H-2'), 7.63 (dt, $J = 1.6, 7.7$ Hz, 1H, H-6'), 7.45–7.35 (m, 3H, H-4', H-5', H-2), 7.25 (d, $J = 3.5$ Hz, 1H, H-3''), 6.20 (d, $J = 3.5$ Hz, 1H, H-4''), 3.81 (br s, 2H, CONCH_2), 3.44 (br s, 2H, CONCH_2), 2.50–2.27 (m, 10H, 2 \times CH_2NCH_3 , 2 \times CH_3). $^{13}\text{C NMR}$ (151 MHz, CDCl_3) δ 176.8 (C-1), 169.5 (amide C=O), 158.3 (C-5''), 152.3 (C-2''), 141.9 (C-3), 136.5 (C-1' or C-3'), 135.3 (C-1' or C-3'), 129.7 (C-6'), 129.0 (C-4' or C-5'), 128.5 (C-4' or C-5'), 126.6 (C-2'), 122.3 (C-2), 119.8 (C-3''), 109.4 (C-4''), 55.2 (CH_2NCH_3), 54.6 (CH_2NCH_3), 47.6 (CONCH_2), 45.9 (piperazine CH_3), 42.0 (CONCH_2), 14.1 (furan CH_3). EI-HRMS m/z : calcd for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_3$, 338.16304, found 338.16270; Purity (HPLC): 97%.

5.1.2.3. (2*E*)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one (**7l**). The title compound was prepared from (1*E*)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (**7i**) and 1-ethylpiperazine in a yield of 63%; mp 98.5–98.8 °C (methanol), orange solid. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.80 (d, $J = 15.9$ Hz, 1H, H-3), 7.68 (br s, 1H, H-2'), 7.63 (br d, $J = 7.6$ Hz, 1H, H-6'), 7.45–7.36 (m, 3H, H-4', H-5', H-2), 7.25 (d, $J = 3.5$ Hz, 1H, H-3''), 6.21 (d, $J = 3.5$ Hz, 1H, H-4''), 3.81 (br s, 2H, CONCH_2), 3.44 (br s, 2H, CONCH_2), 2.55–2.33 (m, 9H, 2 \times CH_2NCH_3 , NCH_2CH_3 , furan CH_3), 1.08 (t, $J = 7.2$ Hz, 3H, NCH_2CH_3). $^{13}\text{C NMR}$ (151 MHz, CDCl_3) δ 176.8 (C-1), 169.4 (amide C=O), 158.3 (C-5''),

152.3 (C-2'), 141.9 (C-3), 136.5 (C-1' or C-3'), 135.3 (C-1' or C-3'), 129.8 (C-6'), 129.0 (C-4' or C-5'), 128.5 (C-4' or C-5'), 126.6 (C-2'), 122.3 (C-2), 119.8 (C-3'), 109.4 (C-4'), 53.1 (CH₂NCH₂), 52.3 (CH₂NCH₂), 52.2 (NCH₂CH₃), 47.7 (CONCH₂), 42.1 (CONCH₂), 14.1 (furan CH₂), 11.8 (piperazine CH₃). EI-HRMS m/z: calcd for C₂₁H₂₄N₂O₃, 352.17869, found 352.17790; Purity (HPLC): 98%.

5.1.2.4. (2E)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one (7m). The title compound was prepared from (1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (7i) and piperidine in a yield of 78%; mp 144.7–145.1 °C (methanol), pale yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 7.81 (d, J = 15.8 Hz, 1H, H-3), 7.68 (br s, 1H, H-2'), 7.63 (br d, J = 7.8 Hz, 1H, H-6'), 7.45–7.35 (m, 3H, H-4', H-5', H-2), 7.25 (d, 1H, J = 3.4 Hz, H-3'), 6.21 (d, J = 3.4 Hz, 1H, H-4'), 3.71 (s, 2H, CONCH₂), 3.33 (s, 2H, CONCH₂), 2.43 (s, 3H, CH₃), 1.71–1.42 (m, 6H, 3 × piperidine CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 176.9 (C-1), 169.5 (amide C=O), 158.3 (C-5'), 152.3 (C-2'), 142.1 (C-3), 137.2 (C-3'), 135.2 (C-1'), 129.5 (C-6'), 128.9 (C-4' or C-5'), 128.3 (C-4' or C-5'), 126.4 (C-2'), 122.2 (C-2), 119.8 (C-3'), 109.4 (C-4'), 48.7 (CONCH₂), 43.1 (CONCH₂), 26.5, 25.5, 24.5 (3 × piperidine CH₂), 14.2 (CH₃). EI-HRMS m/z: calcd for C₂₀H₂₁NO₃, 323.15214, found 323.15116; Purity (HPLC): 96%.

5.1.2.5. (2E)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one (7n). The title compound was prepared from (1E)-1-(5-methylfuran-2-yl)-3-[3-oxoprop-1-en-1-yl]benzoic acid (7i) and pyrrolidine in a yield of 74%; mp 120.8–122.6 °C (methanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.82–7.76 (m, 2H, H-2', H-3), 7.62 (br d, J = 7.7 Hz, 1H, H-6'), 7.49 (br d, J = 7.7 Hz, 1H, H-4'), 7.44–7.37 (m, 2H, H-5', H-2), 7.24 (d, J = 3.5 Hz, 1H, H-3'), 6.2 (d, J = 3.5 Hz, 1H, H-4'), 3.63 (t, J = 7.1 Hz, 2H, CONCH₂), 3.41 (t, J = 6.6 Hz, 2H, CONCH₂), 2.41 (s, 3H, CH₃), 1.95 (p, J = 6.9 Hz, 2H, CH₂CH₂CH₂), 1.86 (p, J = 6.7 Hz, 2H, CH₂CH₂CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 176.9 (C-1), 168.9 (amide C=O), 158.3 (C-5'), 152.3 (C-2'), 142.1 (C-3), 137.8 (C-3'), 135.0 (C-1'), 129.9 (C-6'), 128.8 (C-4' or C-5'), 128.6 (C-4' or C-5'), 126.6 (C-2'), 122.1 (C-2), 119.8 (C-3'), 109.4 (C-4'), 49.5 (CONCH₂), 46.2 (CONCH₂), 26.3 (CH₂CH₂CH₂), 24.3 (CH₂CH₂CH₂), 14.1 (CH₃). APIC-HRMS m/z: calcd for C₁₉H₁₉NO₃ (M + H)⁺, 310.1438, found 310.1448; Purity (HPLC): 93%.

5.1.3. General procedure for the synthesis of 2-aminopyrimidines

Guanidine hydrochloride (4.6 mmol) was dissolved in a small amount of DMF (15 ml), and the appropriate chalcone (3.1 mmol) and sodium hydride (9.2 mmol) were added while stirring. The reaction mixture was heated (110 °C) for 24 h under nitrogen, allowed to cool to room temperature and then diluted with equal volumes of ethyl acetate and water. The aqueous phase was extracted with ethyl acetate (twice) and the organic layers were combined. All traces of DMF were removed by washing the combined organic layers with water (4 times). The organic layer was concentrated *in vacuo* and the crude product was purified with column chromatography [petroleum ether: ethyl acetate (4:1)] and recrystallised from ethanol.

5.1.3.1. 4-(3-chlorophenyl)-6-phenylpyrimidin-2-amine (8a). The title compound was prepared from (2E)-3-(3-chlorophenyl)-1-phenylprop-2-en-1-one (7a) in a yield of 15%; mp 128.6–131.4 °C (ethanol), (lit. 132–133 °C) [56] white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.05 (m, 3H, Ar-H), 7.92 (dt, J = 1.5, 7.6 Hz, 1H, H-4'/6'), 7.53–7.39 (m, 6H, Ar-H, H-5), 5.42 (s, 2H, NH₂). ¹³C NMR (151 MHz, CDCl₃) δ 166.5, 164.64, 163.6 (C-2, C-4, C-6), 139.5, 137.4, 134.8 (C-1', C-1'', C-3'), 130.6, 130.3, 130.0, 128.8 (2C), 127.2, 127.1 (2C), 125.1 (Ar-C), 104.1 (C-5). EI-HRMS m/z: calcd for C₁₆H₁₂ClN₂, 281.07198, found 281.07167; Purity (HPLC): 99%.* In no particular order.

5.1.3.2. 4-(3-chlorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (8b). The title compound was prepared from (2E)-3-(3-chlorophenyl)-1-(furan-2-yl)prop-2-en-1-one (7b) in a yield of 22%; mp 144.1–144.4 °C (ethanol), light yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.05 (br t, J = 1.9 Hz, 1H, H-2'), 7.91 (dt, J = 1.45, 7.6 Hz, 1H, H-6'), 7.59 (dd, J = 0.8, 1.8 Hz, 1H, H-5'), 7.42 (ddd, J = 8.0, 2.1, 1.2 Hz, 1H, H-4'), 7.38 (br t, J = 7.8 Hz, 1H, H-5'), 7.34 (s, 1H, H-5), 7.20 (dd, J = 0.8, 3.5 Hz, 1H, H-3'), 6.56 (dd, J = 1.8, 3.5 Hz, 1H, H-4'), 5.41 (s, 2H, NH₂). ¹³C NMR (151 MHz, CDCl₃) δ 164.6 (C-2 or C-4), 163.4 (C-2 or C-4), 157.24 (C-6), 152.0 (C-2'), 144.6 (C-5'), 139.3 (C-1'), 134.8 (C-3'), 130.4 (C-4' or C-5'), 129.9 (C-4' or C-5'), 127.2 (C-2'), 125.1 (C-6'), 112.3 (C-3' or C-4'), 111.80 (C-3' or C-4'), 101.94 (C-5). EI-HRMS m/z: calcd for C₁₄H₁₀ClN₂O, 271.05124, found 271.05049; Purity (HPLC): 98%.

5.1.3.3. 4-(3-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (8c). The title compound was prepared from (2E)-3-(3-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7c) in a yield of 45%; mp 170.5–172.1 °C (ethanol), yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.05 (br s, 1H, H-2'), 7.92 (d, J = 7.6 Hz, 1H, H-6'), 7.46–7.37 (m, 2H, H-4', H-5'), 7.31 (s, 1H, H-5), 7.11 (br s, 1H, H-3'), 6.17 (br s, 1H, H-4'), 5.26 (s, 2H, NH₂), 2.43 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 164.3 (C-2 or C-4), 163.3 (C-2 or C-4), 157.3 (C-6), 155.4 (C-5'), 150.5 (C-2'), 139.4 (C-1'), 134.8 (C-3'), 130.3 (C-4' or C-5'), 129.9 (C-4' or C-5'), 127.2 (C-2'), 125.1 (C-6'), 113.3 (C-3'), 108.8 (C-4'), 101.6 (C-5), 14.0 (CH₃). EI-HRMS m/z: calcd for C₁₅H₁₂ON₂Cl, 285.06690, found 285.06556; Purity (HPLC): 100%.

5.1.3.4. 4-(3-methoxyphenyl)-6-(furan-2-yl)pyrimidin-2-amine (8d). The title compound was prepared from (2E)-1-(furan-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (7d) in a yield of 19%; mp 113.8–116.8 °C (ethanol), dark brown crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.64–7.57 (m, 3H, H-2', H-4', H-5'), 7.40–7.37 (m, 2H, H-5, H-5'), 7.19 (dd, J = 0.82, 3.4 Hz, 1H, H-3'), 7.03 (ddd, J = 0.96, 2.59, 8.1 Hz, 1H, H-6'), 6.55 (dd, J = 1.7, 3.4 Hz, 1H, H-4'), 5.41 (s, 2H, NH₂), 3.89 (s, 3H, OCH₃). ¹³C NMR (151 MHz, CDCl₃) δ 165.92 (C-4), 163.35 (C-2), 159.96 (C-3'), 156.43 (C-6), 152.15 (C-2'), 144.51 (C-5'), 138.89 (C-1'), 129.68 (C-5'), 119.47 (C-4'), 116.54 (C-6'), 112.21 (C-2'/C-3'/C-4'), 112.21 (C-2'/C-3'/C-4'), 112.07 (C-2'/C-3'/C-4'), 102.18 (C-5), 55.37 (OCH₃). EI-HRMS m/z: calcd for C₁₅H₁₃N₂O₂, 267.10078, found 267.09949; Purity (HPLC): 92%.

5.1.3.5. 4-(3-methoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (8e). The title compound was prepared from (2E)-3-(3-methoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7e) in a yield of 43%; mp 149.0–150.6 °C (ethanol), light orange crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.64–7.58 (m, 2H, H-2', H-4'), 7.38 (br t, J = 7.9 Hz, 1H, H-5'), 7.33 (s, 1H, H-5), 7.09 (d, J = 3.3 Hz, 1H, H-3'), 7.02 (dd, J = 2.6, 8.2 Hz, 1H, H-6'), 6.15 (dd, J = 0.9, 3.3 Hz, 1H, H-4'), 5.44 (s, 2H, NH₂), 3.88 (s, 3H, OCH₃), 2.44 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 165.6 (C-4), 163.4 (C-2), 159.9 (C-3'), 157.0 (C-6), 155.1 (C-5'), 150.6 (C-2'), 139.1 (C-1'), 129.6 (C-5'), 119.4 (C-4'), 116.3 (C-6'), 113.0 (C-3'), 112.1 (C-2'), 108.7 (C-4'), 101.7 (C-2'), 55.4 (OCH₃), 14.0 (CH₃). EI-HRMS m/z: calcd for C₁₆H₁₅N₂O₂, 281.11643, found 281.11556; Purity (HPLC): 100%.

5.1.3.6. 4-(3,4-dimethoxyphenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (8f). The title compound was prepared from (2E)-3-(3,4-dimethoxyphenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7f) in a yield of 27%; mp 167.4–168.1 °C (ethanol), dark yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 7.68 (d, J = 2.0 Hz, 1H, H-2'), 7.61 (dd, J = 2.1, 8.4 Hz, 1H, H-6'), 7.29 (s, 1H, H-5), 7.08 (d, J = 3.3 Hz, 1H, H-3'), 6.93 (d, J = 8.4 Hz, 1H, H-5'), 6.15 (dd, J = 0.8, 3.3 Hz, 1H, H-4'), 5.35 (s, 2H, NH₂), 3.98 (s, 3H, OCH₃), 3.93 (s, 3H, OCH₃), 2.42 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 165.2 (C-4), 163.3 (C-2), 156.7 (C-

6), 155.0 (C-5''), 151.1, 150.6 (C-4' or C-3' and C-2''), 149.0 (C-3' or C-4'), 130.2 (C-1'), 120.1 (C-6'), 112.8 (C-3''), 110.7 (C-5'), 109.8 (C-2'), 108.7 (C-4'), 100.8 (C-5), 56.0 (OCH₃), 55.9 (OCH₃), 14.00 (CH₃). EI-HRMS m/z: calcd for C₁₇H₁₇N₃O₃, 311.12699, found 311.12618; Purity (HPLC): 100%* In no particular order.

5.1.3.7. 4-(4-chlorophenyl)-6-(5-methylfuran-2-yl)pyrimidin-2-amine (8g). The title compound was prepared from (2E)-3-(4-chlorophenyl)-1-(5-methylfuran-2-yl)prop-2-en-1-one (7g) in a yield of 30%; mp 204.6–205.8 °C (ethanol), dark yellow powder. ¹H NMR (600 MHz, DMSO-d₆) δ 8.14 (d, J = 8.6 Hz, 2H, H-2', H-6'), 7.56 (d, J = 8.6 Hz, 2H, H-3', H-5'), 7.39 (s, 1H, H-5), 7.21 (d, J = 3.3 Hz, 1H, H-3''), 6.76 (s, 2H, NH₂), 6.32 (dd, J = 1.0, 3.3 Hz, 1H, H-4''), 2.37 (s, 3H, CH₃). ¹³C NMR (151 MHz, DMSO-d₆) δ 163.9 (C-2 or C-4), 163.1 (C-2 or C-4), 156.7 (C-6), 154.7 (C-5''), 150.4 (C-2''), 136.0 (C-1'), 135.2 (C-4'), 128.8 (C-2', C-6' or C-3', C-5'), 128.6 (C-2', C-6' or C-3', C-5'), 113.3 (C-3''), 108.9 (C-4''), 99.4 (C-5), 13.7 (CH₃). EI-HRMS m/z: calcd for C₁₅H₁₂ClN₃O, 285.06689, found 285.06633; Purity (HPLC): 99%.

5.1.3.8. 4-(4-fluorophenyl)-6-(furan-2-yl)pyrimidin-2-amine (8h). The title compound was prepared from (2E)-1-(4-fluorophenyl)-3-(furan-2-yl)prop-2-en-1-one (7h) in a yield of 41%; mp 162.3–162.5 °C (lit. 170 °C) [57] (ethanol), faded yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.09–8.03 (m, 2H, H-2', H-6'), 7.59 (dd, J = 0.8, 1.8 Hz, 1H, H-5''), 7.37 (s, 1H, H-5), 7.19–7.13 (m, 3H, H-3', H-3'', H-5'), 6.57 (dd, J = 1.8, 3.4 Hz, 1H, H-4''), 5.28 (s, 2H, NH₂). ¹³C NMR (151 MHz, CDCl₃) δ 165.0 (C-2 or C-4), 164.4 (d, J_{C-F} = 250.5 Hz, C-4'), 163.3 (C-2 or C-4), 157.1 (C-6), 152.2 (C-2''), 144.6 (C-5''), 133.5 (d, J_{C-F} = 3.5 Hz, C-1'), 129.1 (d, J_{C-F} = 8.5 Hz, C-2', C-6'), 115.7 (d, J_{C-F} = 21.3 Hz, C-3', C-5'), 112.3 (C-3''), 111.6 (C-4''), 101.7 (C-5). EI-HRMS m/z: calcd for C₁₄H₁₀FN₃O, 255.08079, found 255.07982; Purity (HPLC): 100%.

5.1.3.9. 4-(5-methylfuran-2-yl)-6-[3-(morpholine-4-carbonyl)phenyl]pyrimidin-2-amine (8j). The title compound was prepared from (2E)-1-(5-methylfuran-2-yl)-3-[3-(morpholine-4-carbonyl)phenyl]prop-2-en-1-one (7j) in a yield of 20%; mp 205.9–206.4 °C (methanol), yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.11 (m, 2H, H-2', H-6'), 7.55–7.46 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, J = 3.4 Hz, 1H, H-3''), 6.16 (br d, J = 3.4 Hz, 1H, H-4''), 5.32 (s, 2H, NH₂), 3.87–3.43 (m, 8H, morpholine CH₂), 2.42 (s, 3H, CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 170.0 (C=O), 164.6 (C-2 or C-6), 163.4 (C-2 or C-6), 157.3 (C-4), 155.3 (C-5''), 150.4 (C-2''), 138.1 (C-1'), 135.7 (C-3'), 128.8, 128.7, 128.4, 125.9 (C-2', C-4', C-5', C-6''), 113.2 (C-3''), 108.7 (C-4''), 101.5 (C-5), 66.8, 48.2, 42.6 (morpholine CH₂), 14.0 (CH₃). EI-HRMS m/z: calcd for C₂₀H₂₀N₄O₃, 364.15354, found 364.15292; Purity (HPLC): 92%. * In no particular order.

5.1.3.10. 4-(5-methylfuran-2-yl)-6-[3-(4-methylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (8k). The title compound was prepared from (2E)-1-(5-methylfuran-2-yl)-3-[3-(4-methylpiperazine-1-carbonyl)phenyl]prop-2-en-1-one (7k) in a yield of 57%; mp 194.3–194.4 °C (methanol), cream solid. ¹H NMR (600 MHz, CDCl₃) δ 8.13–8.07 (m, 2H, H-2', H-6'), 7.53–7.45 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, J = 3.4 Hz, 1H, H-3''), 6.15 (br d, J = 3.4 Hz, 1H, H-4''), 5.30 (s, 2H, NH₂), 3.83 (br s, 2H, CONCH₂), 3.46 (br s, 2H, CONCH₂), 2.60–2.19 (m, 10H, 2 × CH₂NCH₃, 2 × CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 169.8 (C=O), 164.7 (C-2 or C-6), 163.4 (C-2 or C-6), 157.3 (C-4), 155.3 (C-5''), 150.5 (C-2''), 138.1 (C-1'), 136.2 (C-3'), 128.8, 128.7, 128.2, 125.9 (C-2', C-4', C-5', C-6''), 113.2 (C-3''), 108.8 (C-4''), 101.6 (C-5), 55.2 (CH₂NCH₃), 54.6 (CH₂NCH₃), 47.7 (CONCH₂), 46.0 (piperazine CH₃), 42.1 (CONCH₂), 14.0 (furan CH₃). EI-HRMS m/z: calcd for C₂₁H₂₃N₅O₂, 377.18518, found 377.18422; Purity (HPLC): 85%. * In no particular order.

5.1.3.11. 4-(5-methylfuran-2-yl)-6-[3-(4-ethylpiperazine-1-carbonyl)phenyl]pyrimidin-2-amine (8l). The title compound was prepared from (2E)-3-[3-(4-ethylpiperazine-1-carbonyl)phenyl]-1-(5-methylfuran-2-yl)prop-2-en-1-one (7l) in a yield of 16%; mp 178.1–178.4 °C (methanol), light orange solid. ¹H NMR (600 MHz, CDCl₃) δ 8.12–8.07 (m, 2H, H-2', H-6'), 7.52–7.44 (m, 2H, H-4', H-5'), 7.34 (s, 1H, H-5), 7.08 (d, J = 3.4 Hz, 1H, H-3''), 6.14 (dd, J = 1.0, 3.4 Hz, 1H, H-4''), 5.38 (s, 2H, NH₂), 3.83 (br s, 2H, CONCH₂), 3.46 (s, 2H, CONCH₂), 2.62–2.21 (m, 9H, 2 × CH₂NCH₃, NCH₂CH₃, furan CH₃), 1.07 (t, J = 7.4 Hz, 3H, NCH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 169.7 (C=O), 164.7 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-5''), 150.4 (C-2''), 138.0 (C-1'), 136.2 (C-3'), 128.7, 128.2, 125.9 (C-2', C-4', C-5', C-6''), 113.1 (C-3''), 108.7 (C-4''), 101.5 (C-5), 53.0 (CH₂NCH₃), 52.3 (CH₂NCH₃), 52.2 (NCH₂CH₃), 47.7 (CONCH₂), 42.1 (CONCH₂), 14.0 (furan CH₃), 11.8 (NCH₂CH₃). EI-HRMS m/z: calcd for C₂₂H₂₅N₅O₂, 391.20083, found 391.20052; Purity (HPLC): 100%. * In no particular order.

5.1.3.12. 4-(5-methylfuran-2-yl)-6-[3-(piperidine-1-carbonyl)phenyl]pyrimidin-2-amine (8m). The title compound was prepared from (2E)-1-(5-methylfuran-2-yl)-3-[3-(piperidine-1-carbonyl)phenyl]prop-2-en-1-one (7m) in a yield of 56%; mp 179.2–180.5 °C (methanol), orange crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.12–8.05 (m, 2H, H-2', H-6'), 7.52–7.44 (m, 2H, H-4', H-5'), 7.35 (s, 1H, H-5), 7.09 (d, J = 3.4 Hz, 1H, H-3''), 6.15 (dd, J = 1.1, 3.4 Hz, 1H, H-4''), 5.35 (s, 2H, NH₂), 3.73 (br s, 2H, CONCH₂), 3.36 (br s, 2H, CONCH₂), 2.42 (s, 3H, CH₃), 1.77–1.39 (m, 6H, 3 × piperidine CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 169.8 (C=O), 164.9 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-5''), 150.4 (C-2''), 137.9 (C-1'), 136.8 (C-3'), 128.7, 128.5, 127.9125.7 (C-2', C-4', C-5', C-6''), 113.1 (C-3''), 108.7 (C-4''), 101.6 (C-5), 48.8 (CONCH₂), 43.1 (CONCH₂), 26.5, 25.5, 24.5 (3 × piperidine CH₂), 14.0 (CH₃). EI-HRMS m/z: calcd for C₂₁H₂₂N₄O₂, 362.17428, found 362.17292; Purity (HPLC): 97%.

5.1.3.13. 4-(5-methylfuran-2-yl)-6-[3-(pyrrolidine-1-carbonyl)phenyl]pyrimidin-2-amine (8n). The title compound was prepared from (2E)-1-(5-methylfuran-2-yl)-3-[3-(pyrrolidine-1-carbonyl)phenyl]prop-2-en-1-one (7n) in a yield of 27%; mp 212.7–213.4 °C (methanol), yellow crystals. ¹H NMR (600 MHz, CDCl₃) δ 8.20 (t, J = 1.8 Hz, 1H, H-2'), 8.10 (dt, J = 1.5, 7.8 Hz, 1H, H-6'), 7.59 (dt, J = 1.4, 7.7 Hz, 1H, H-4''), 7.49 (t, J = 7.7 Hz, 1H, H-5'), 7.36 (s, 1H, H-5), 7.08 (d, J = 3.4 Hz, 1H, H-3''), 6.14 (dd, J = 1.2, 3.3 Hz, 1H, H-4''), 5.37 (s, 2H, NH₂), 3.66 (t, J = 7.0 Hz, 2H, CONCH₂), 3.44 (t, J = 6.7 Hz, 2H, CONCH₂), 2.41 (s, 3H, CH₃), 1.95 (p, J = 7.0 Hz, 2H, CH₂CH₂CH₂), 1.86 (p, J = 6.7 Hz, 2H, CH₂CH₂CH₂). ¹³C NMR (151 MHz, CDCl₃) δ 169.2 (C=O), 164.8 (C-2 or C-6), 163.4 (C-2 or C-6), 157.2 (C-4), 155.2 (C-5''), 150.5 (C-1''), 137.7 (C-1' or C-3'), 137.6 (C-1' or C-3'), 128.8, 128.5, 128.3 (C-4', C-5', C-6''), 125.9 (C-2'), 113.1 (C-3''), 108.7 (C-4''), 101.5 (C-5), 49.6 (CONCH₂), 46.2 (CONCH₂), 26.3 (CH₂CH₂CH₂), 24.4 (CH₂CH₂CH₂), 14.0 (CH₃). EI-HRMS m/z: calcd for C₂₀H₂₀N₄O₂, 348.15863, found 348.15730; Purity (HPLC): 95%. * In no particular order.

5.2. Biological methods

5.2.1. Materials

Adenosine deaminase (type X from calf spleen), N⁶-cyclopentyladenosine (CPA), anhydrous magnesium chloride, Trizma[®] Base, Trizma[®] Hydrochloride, silicone solution (Sigma-cote), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), phosphate-buffered saline (PBS) and isopropanol were obtained from Sigma Aldrich. Dimethyl sulfoxide (DMSO), Whatman[®] GF/B 25 mm diameter filters and formic acid were obtained from Merck. Filtercount scintillation fluid, radioligands, [³H]5'-N-ethylcarboxamide-adenosine ([³H]NECA, 25 Ci/mmol (250 μCi))

and 1,3- ^3H -dipropyl-8-cyclopentylxanthine (^3H]DPCPX, 120 Ci/mmol) were obtained from Separation Scientific SA. Cell culture media (Dulbecco's Modified Eagle Medium (DMEM)), fungizone, trypsin/EDTA, streptomycin, fetal bovine serum and penicillin were obtained from Gibco and Merck. Well-plates (24 and 96) and culture flasks were obtained from Corning. Sterile syringe filters (0.22 μM) were obtained from Pall Corporation Life Sciences.

5.2.2. Radioligand binding studies

5.2.2.1. Tissue preparation for binding studies. Radioligand binding studies were carried out as reported in literature [51–53]. The Animal Research Ethics Committee of the North-West University (NWU-0035-10-A5) approved the collection of animal tissue required for these assays. Adult male Sprague Dawley rats were obtained from the Vivarium of the North-West University, Potchefstroom campus. Striata (for the adenosine A_{2A} assays) and whole brains (for the adenosine A_1 assay) were dissected on ice and immediately snap frozen with liquid nitrogen and then stored at -70°C until required. The frozen striata as well as the whole brain tissue were suspended in ice-cold 50 mM Tris buffer (pH 7.7 at 25°C) and homogenised using a Polytron PT-10 homogeniser (Brinkman) to yield final suspensions of 1 g/5 ml, which were aliquoted and stored at -70°C until required. Test compounds were dissolved (10 mM) and further diluted in DMSO, with the final concentration of DMSO in the incubations being 1%. All pipette tips as well as the 4 ml polypropylene tubes used for the incubations were coated with Sigmacoat[®]. The incubations were prepared using 50 mM Tris buffer (pH 7.7 at 25°C). For the adenosine A_{2A} assay, the final volume of the incubations was 1 ml and each incubation contained test compound (0–100 μM), membrane suspension yielding ~ 10 mg of original tissue weight of rat striata, 10 mM MgCl_2 , 0.2 units of adenosine deaminase, 50 nM CPA and 4 nM ^3H]NECA. The MgCl_2 (A_{2A} assay) and adenosine deaminase (A_{2A} and A_1 assays) were firstly added to the membrane suspension, and this mixture was subsequently added to the incubations. The order of addition was test compound, membrane suspension, CPA and ^3H]NECA. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 μM replacing the test compound). For the adenosine A_1 assay, the final volume of the incubations was also 1 ml and each incubation contained test compound (0–100 μM), membrane suspension yielding 5 mg of original tissue weight of rat whole brain, 0.1 units of adenosine deaminase and 0.1 nM ^3H]DPCPX. The order of addition was test compound, membrane suspension and ^3H]DPCPX. All incubations were carried out in duplicate. Non-specific binding was determined in triplicate in the presence of CPA (100 μM replacing the test compound). After incubation for 1 h (with vortexing after 30 min), the incubations were rapidly filtered through Whatman[®] GF/B 25 filters (25 mm diameter) fitted on a Hoffeler vacuum system. The damp filters were placed into scintillation vials and scintillation fluid (4 ml) was added. The vials were shaken thoroughly and left for 2 h. A Packard Tri-CARB 2100 TR scintillation counter was used to count the radioactivity retained on the filters. Specific binding was defined as total binding minus nonspecific binding, and was expressed as counts per minute (CPM).

5.2.2.2. Data analysis. Using the one site competition model of the Prism 5 software package (GraphPad) the CPM values were plotted against the logarithm of the ligand concentration to give a sigmoidal dose–response curve, from which the IC_{50} values were determined. For adenosine A_1 binding, K_i values were calculated from the IC_{50} values by using the Cheng-Prusoff equation [58], as applicable to radioligand binding assays [51]. K_d , the equilibrium dissociation constant for the radioligand ^3H]DPCPX, was taken as

0.36 nM [52]. Since adenosine A_{2A} binding was performed in the presence of CPA, an adapted version of the Cheng-Prusoff equation was used. The K_d of the radioligand, ^3H]NECA, was taken as 15.3 nM and a K_c value of 685 nM was used for CPA [51]. The binding affinities of the known adenosine A_1 agonist, CPA, and the A_{2A} antagonists, KW-6002 and ZM241385 were also determined as controls. The results of the radioligand binding studies are reported as the mean \pm standard error of the mean (SEM) of duplicate determinations.

5.2.3. MTT cell viability assay

5.2.3.1. Cell culture. HeLa cells were maintained in 250 cm^2 flasks in DMEM media (30 ml) containing 10% fetal bovine serum, 1% penicillin (10,000 units/ml)/streptomycin (10 mg/ml), and fungizone (250 $\mu\text{g}/\text{ml}$). The cells were incubated at 37°C in an atmosphere of 10% CO_2 . The media was replaced once a week and cells were allowed to reach confluency before use in assays.

5.2.3.2. Preparation of compounds. Stock solutions of test compounds were prepared in DMSO (10 mM) and further diluted in DMSO to concentrations of 1 μM and 10 μM . These solutions were filtered via a syringe filter before addition to the cell cultures.

5.2.3.3. MTT assay. Once confluent, cells were detached with 3 ml trypsin/EDTA (0.25%/0.02%) and seeded in 24-well plates at 5,00,000 cells/well. Plates were then incubated for 24 h and the wells subsequently rinsed with 0.5 ml DMEM free from fetal bovine serum. A volume of 0.99 ml DMEM (free from fetal bovine serum) was subsequently added to each well followed by 10 μl of the test compound. In each 24-well plate, wells were reserved as either positive control (100% cell death via lyses with 0.03% formic acid) or negative controls (100% cell viability as a result of no drug treatment). The plates were then incubated at 37°C for a further 24 h where after media was aspirated from each well.

The wells were then washed twice with 0.5 ml/well PBS and 200 μl of 0.5% MTT (prepared in PBS) was added to each well. The well plates were incubated at 37°C for 2 h in the dark, where after the residual MTT was aspirated and 250 μl isopropanol was added to dissolve the formed formazan crystals. The well-plates were then incubated at room temperature for 5 min to allow dissolution of the blue formazan crystals, where after 100 μl of the isopropanol solution of each well plate was transferred to a corresponding well in a 96-well plate. The absorbance was measured spectrophotometrically at 560 nm (using a Labsystems Multiscan RC UV/V spectrophotometer), with the absorbance of the negative control signifying 100% viability and the absorbance of the positive control signifying 0% viability. The effects of the test compounds were evaluated in triplicate and the residual cell viabilities reported as the mean \pm SD of the percentage viable cells compared to the negative control (100%).

5.2.4. In vivo assays

5.2.4.1. Animals. Sprague–Dawley rats were given free access to standard laboratory food and water until the required weight was obtained (240 g – 300 g). All efforts were made to minimise animal suffering as experiments were carried out in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the North-West University ethical committee (NWU-00035-10-A5).

5.2.4.2. Compounds. To induce catalepsy a dose of 5 mg/kg haloperidol (Serenace Injection; 5 mg/ml) was administered intraperitoneally (ip) [33]. A vehicle solution was prepared by mixing a 1:1:4 solution of DMSO, Tween 80 and saline. Compounds (**8k** and **8m**) were dissolved in a required amount of vehicle solution to yield

concentrations of 0.1, 0.4, 1 and 2 mg/ml. A suitable volume of these solutions was injected depending on the weight of the rat, resulting in final dose concentrations of 0.1, 0.4, 1 and 2 mg/kg. All injections were administered ip.

5.2.4.3. Catalepsy test. The experiments were carried out between 8:00 and 15:30 in a lit room with a controlled temperature. All the rats were drug naïve and were only used once. Haloperidol-induced catalepsy was measured with the standard bar test, in a Perspex chamber (length, 23 cm; width, 10.5 cm; height, 9 cm) with a horizontal plastic bar (diameter, 1 cm; length, 10.5 cm) fixed at 9 cm above the floor, and at 7 cm from the back of the box.

Animals were divided into 5 groups, each group containing 6 rats. The 5 groups were treated with 0, 0.1, 0.4, 1 and 2 mg/kg of the test compound, respectively. All rats received ip injections of haloperidol (5.0 mg/kg) to induce catalepsy. 30 Min. Later, the rats in each group received ip injections of compound. The vehicle solution was administered to rats in the control group. Catalepsy was measured 60 min after the haloperidol injections by placing the rats in the Perspex box with their front paws on the horizontal bar. Catalepsy was measured as the time the animal maintained its position on the bar. Time was recorded until one or both of the rat's front paws were removed from the bar, or up to 120 s.

The results of the animal experiments are reported as the mean \pm standard error of the mean (SEM). Data were analysed by means of one-way analysis of variance (ANOVA) across all groups, and were subsequently subjected to Dunnett's post-test to determine if statistical differences exist between mean values. A *p* value < 0.05 is judged as being statistically significant. These analyses were carried out with the Prism software package.

5.3. Molecular docking

Molecular docking studies were carried out with the Windows based Accelrys® Discovery Studio 3.1 software. The crystal structure of the adenosine A_{2A} receptor [Protein Data Bank (PDB) code 3PWH] co-crystallised with the known A_{2A} antagonist ZM241385 was used. This receptor was prepared with the 'Clean protein' function to correct problems such as incomplete amino acid side chains and typed with the CHARMM forcefield. A fixed atom constraint was applied to the backbone and a minimisation was then carried out using the Generalised Born approximation with Molecular Volume (GBMV) as the solvent model to obtain a receptor at energetic minimum. A binding sphere with a radius of 5 Å was defined using the existing ligand (ZM241385) before it was removed from the receptor. Selected inhibitors were cleaned and prepared for docking with the 'Prepare ligand' protocol to correct valences and remove duplicates whereafter ligands were visually inspected and remaining errors corrected. The CDOCKER protocol was used for the docking of ligands. The orientations, CDOCKER and CDOCKER interaction energies of the ten different conformers of each ligand were considered and the best conformation for each ligand selected. An *in situ* ligand minimisation was then performed on the selected conformers and minimised conformers visually inspected and compared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2015.09.035>.

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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Tetrahedron Journal for Research at the Interface of Chemistry and Biology

AUTHOR INFORMATION PACK

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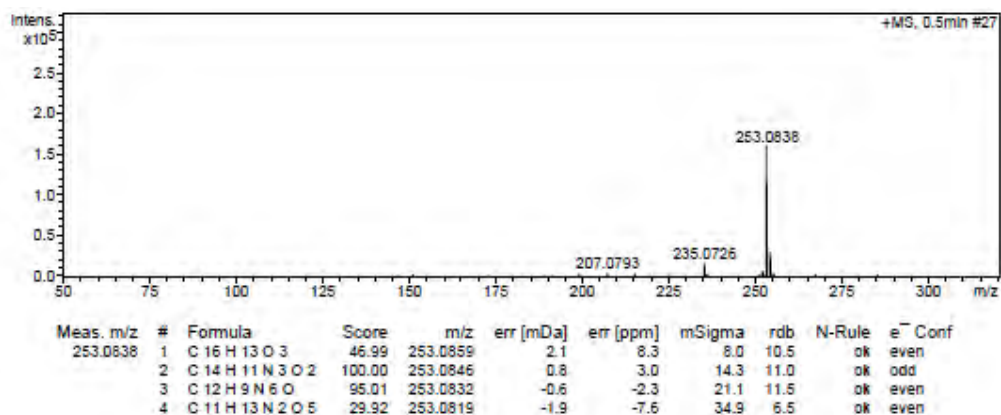
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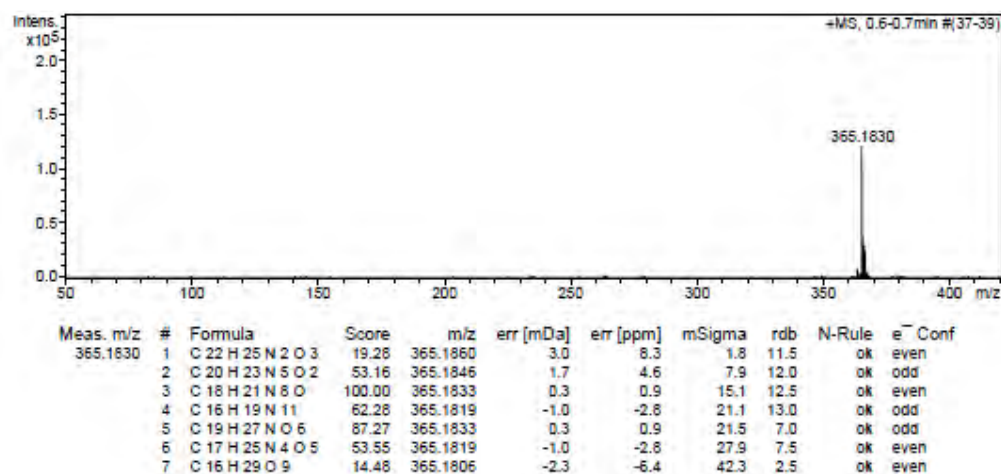
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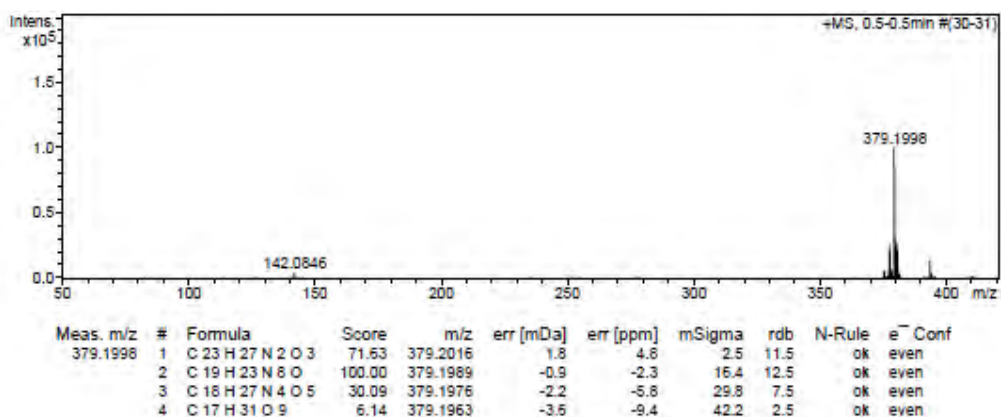
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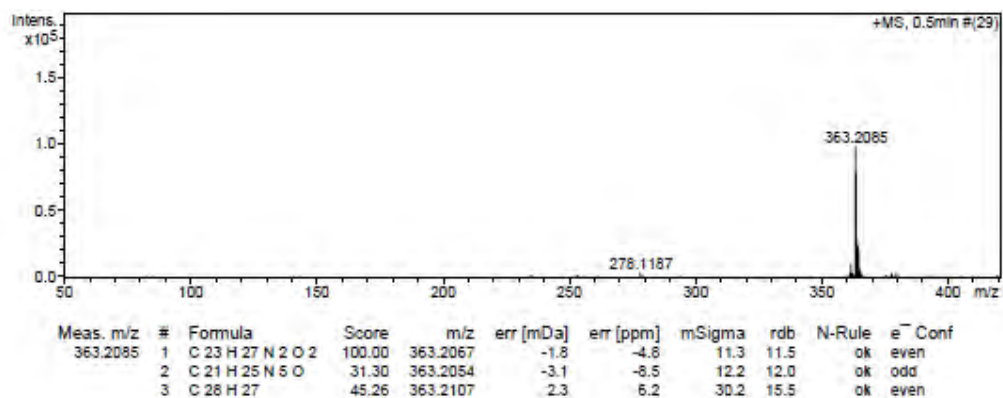
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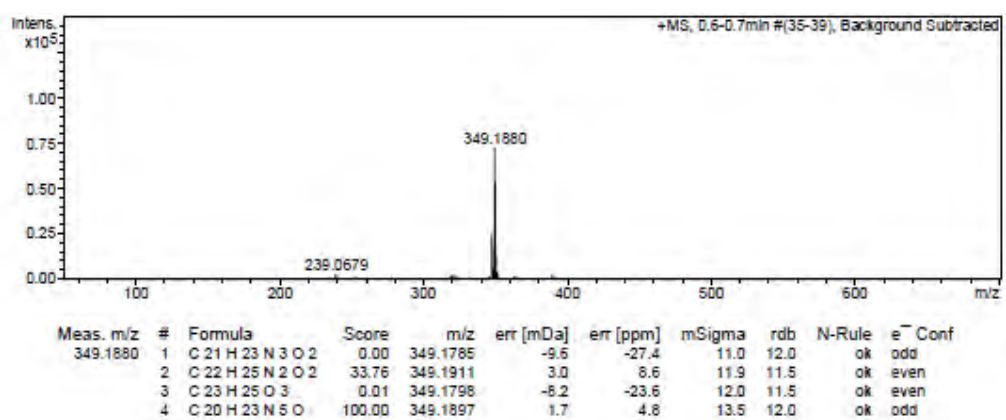
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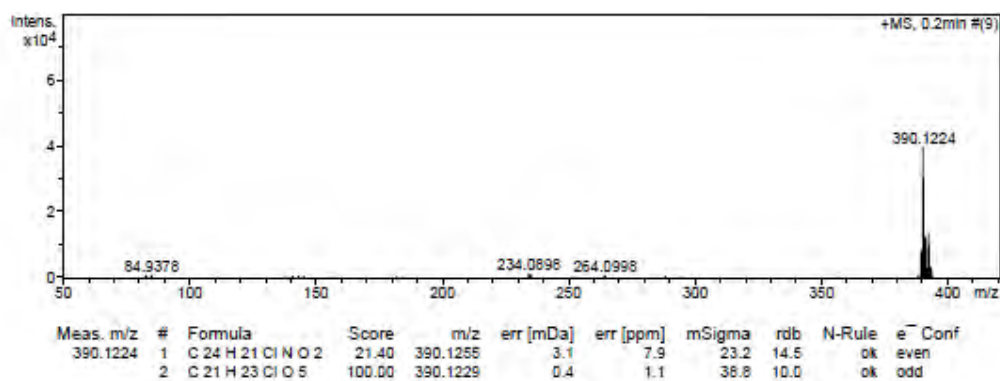
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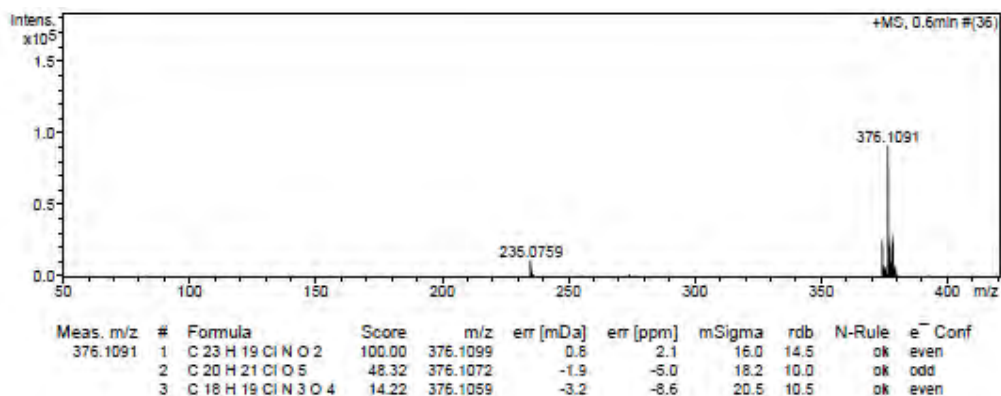
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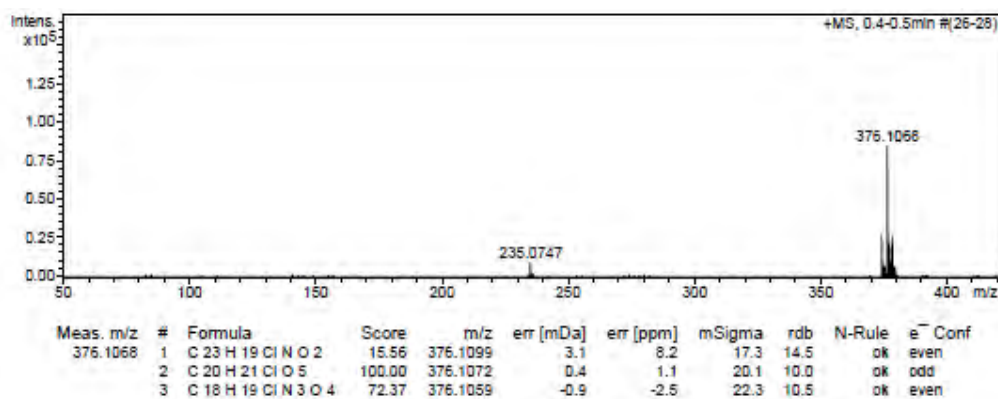
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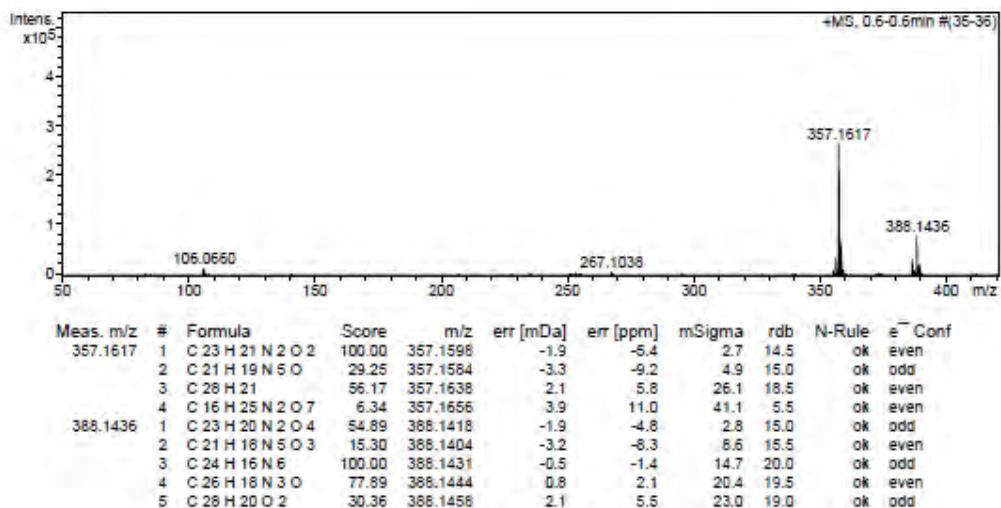
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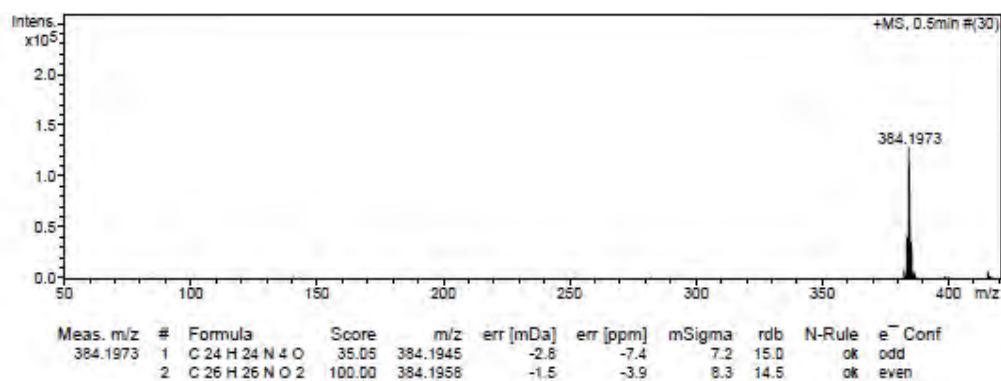
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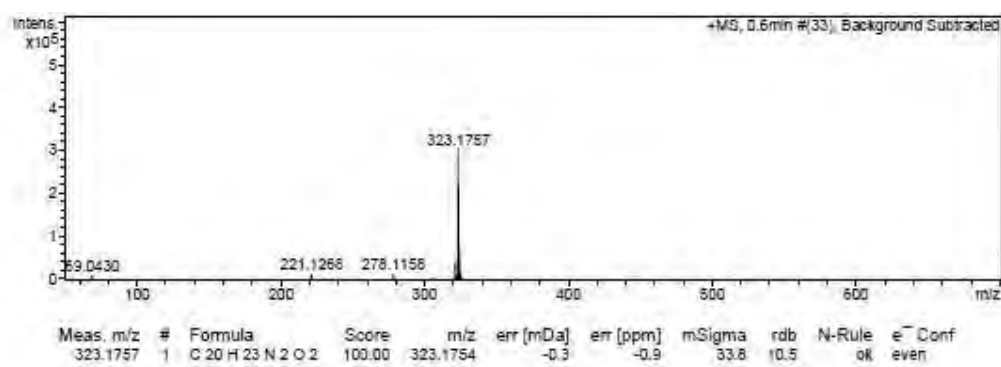
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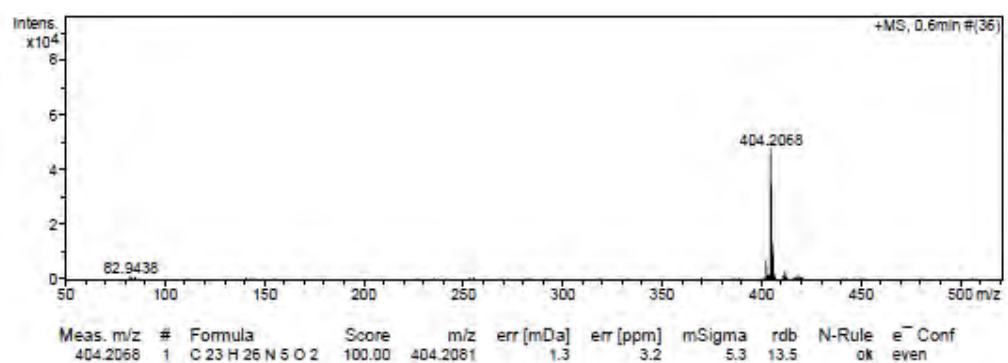
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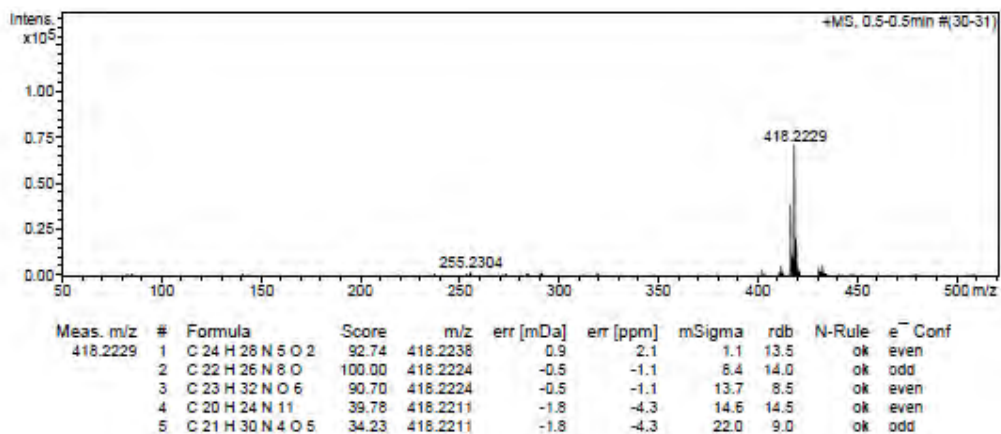
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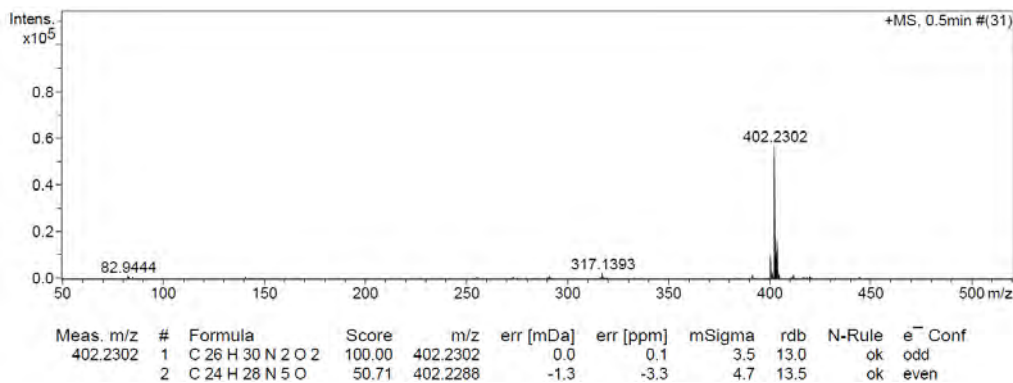
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(morpholin-4-yl)ethyl]benzamide (**6a**)



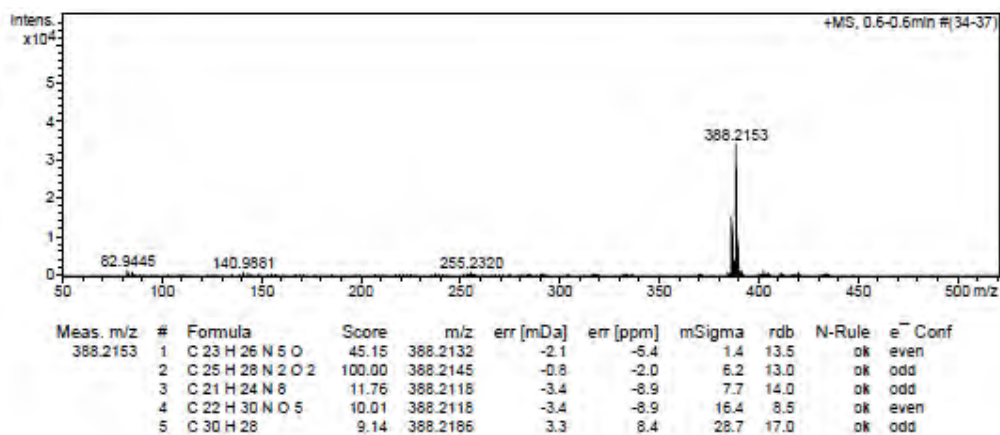
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[3-(morpholin-4-yl)propyl]benzamide (**6b**)



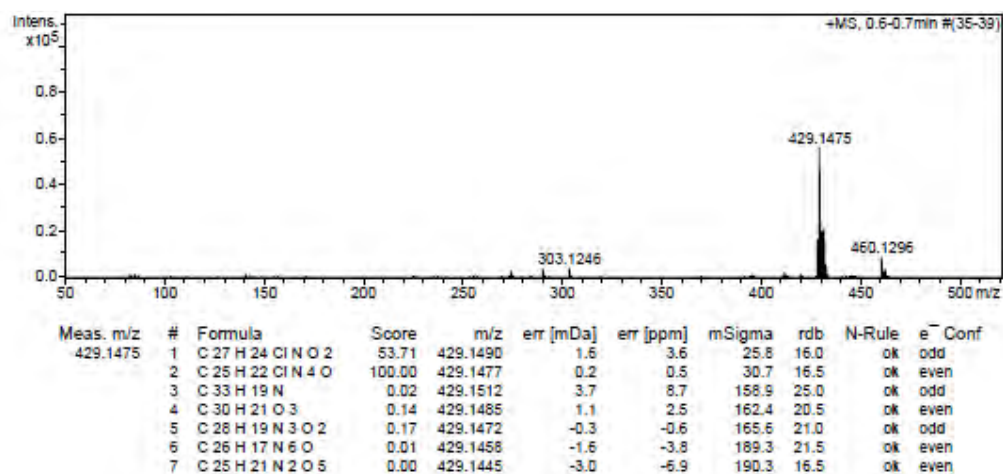
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(piperidin-1-yl)ethyl]benzamide (**6c**)



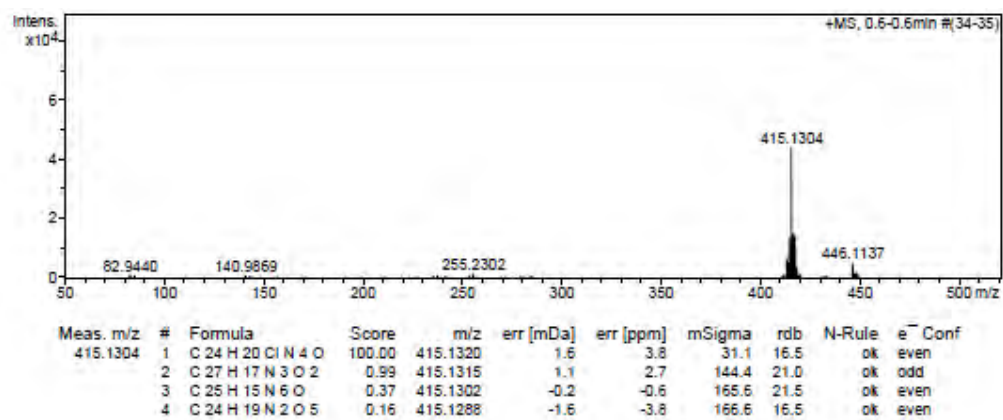
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(pyrrolidin-1-yl)ethyl]benzamide (**6d**)



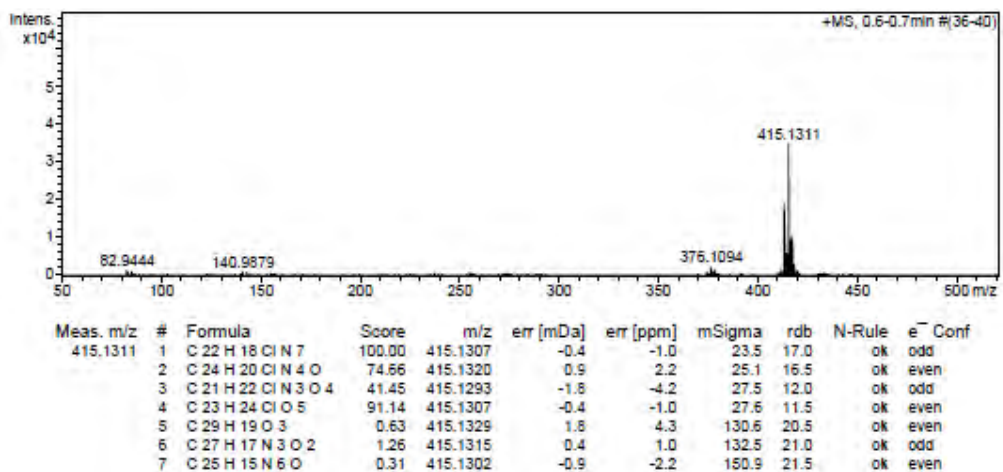
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(3-chlorophenyl)ethyl]benzamide (**6e**)



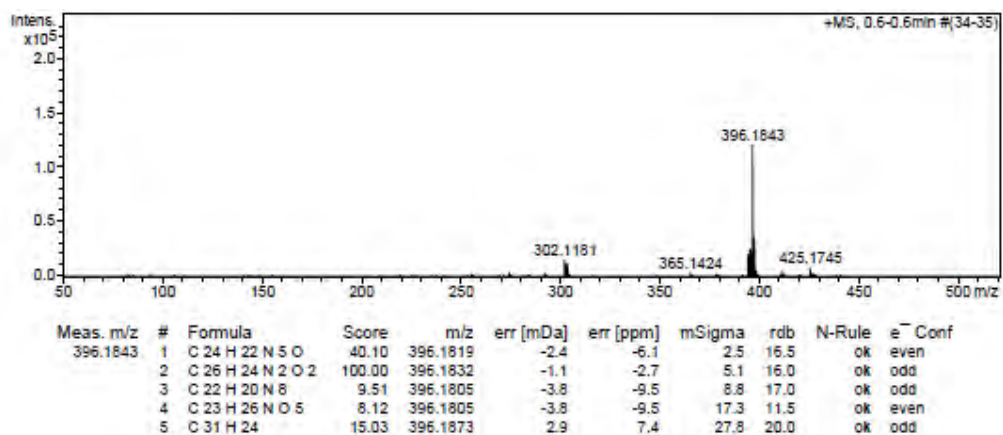
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[(3-chlorophenyl)methyl]benzamide (**6f**)



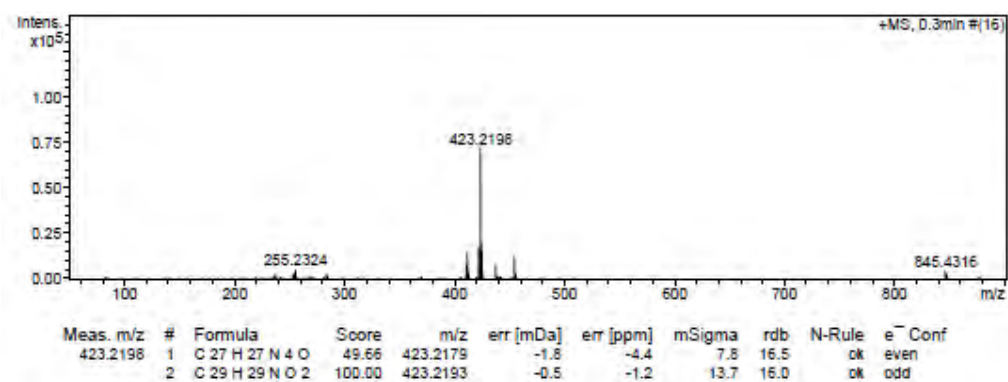
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[(4-chlorophenyl)methyl]benzamide (**6g**)



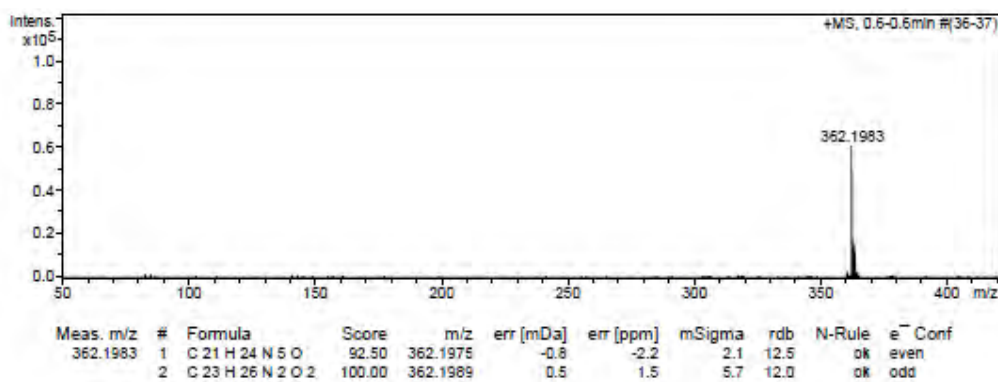
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(pyridin-2-yl)ethyl]benzamide (**6h**)



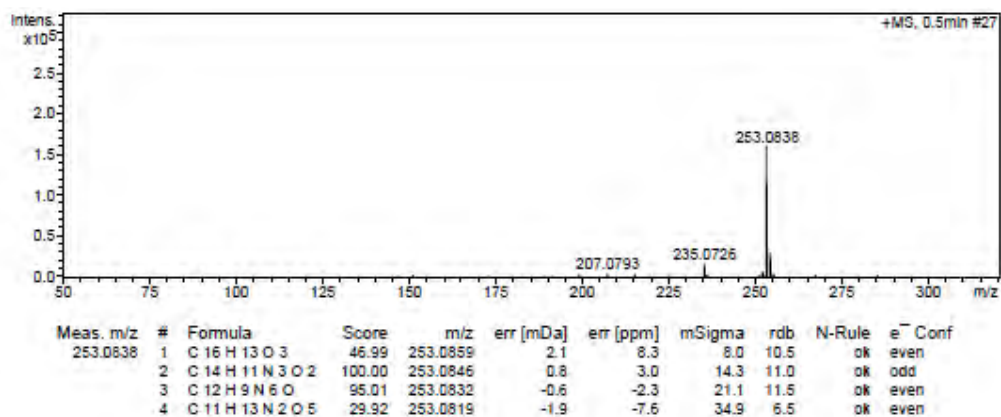
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-(4-phenylbutyl)benzamide (**6i**)



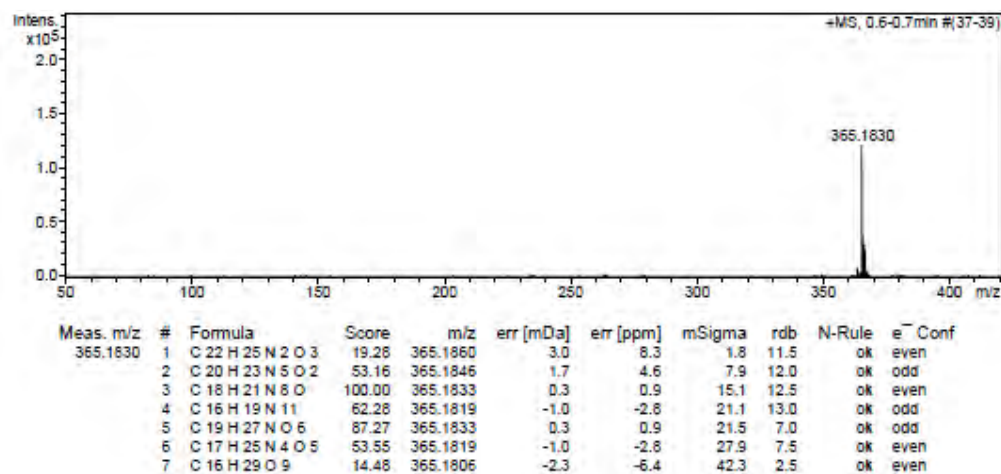
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(dimethylamino)ethyl]benzamide (**6j**)



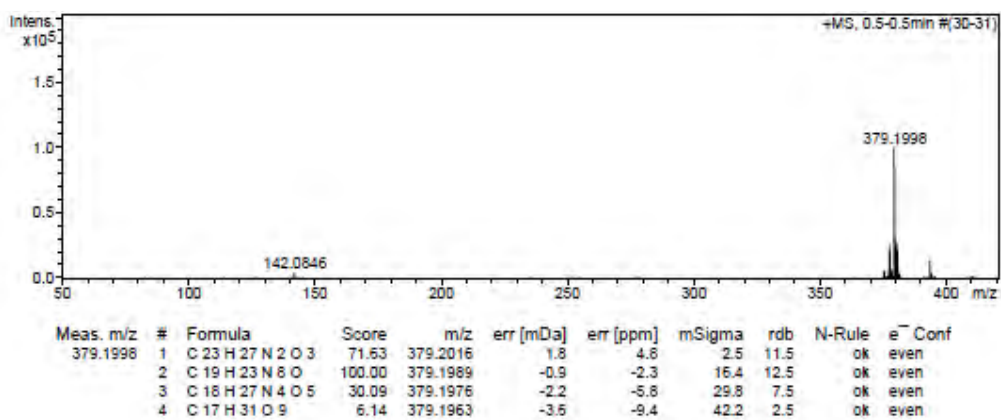
3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzoic acid (**4**)



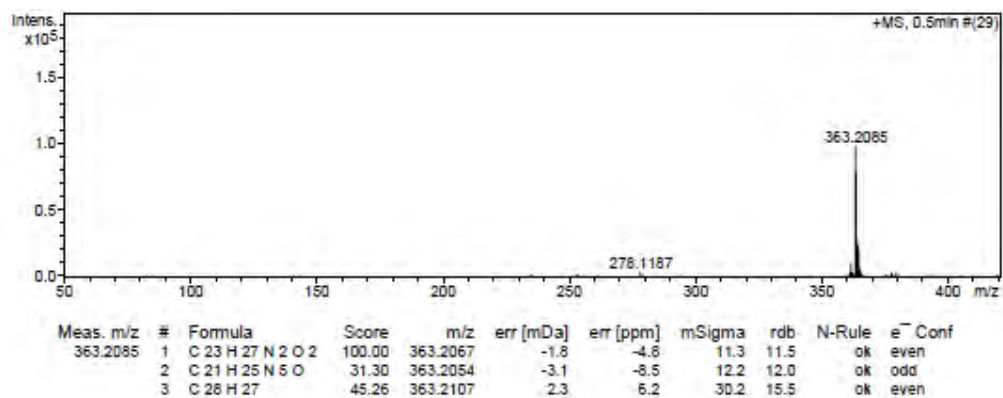
N-[2-(morpholin-4-yl)ethyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5a**)



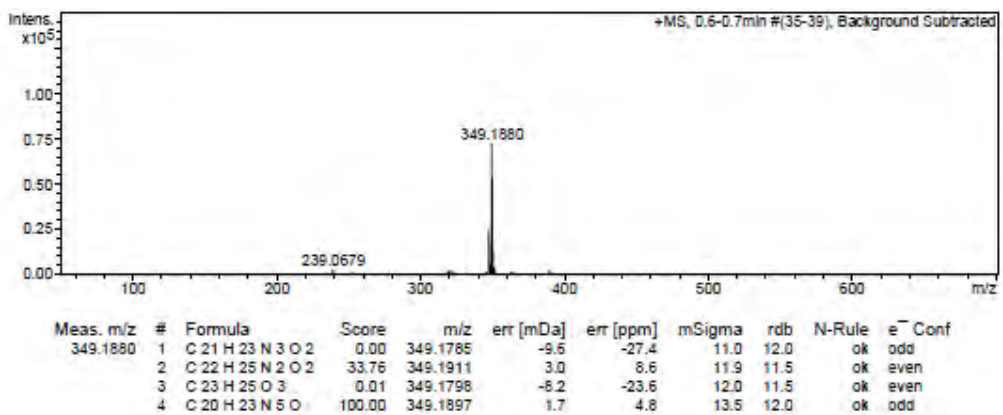
N-[3-(morpholin-4-yl)propyl]-3-[(*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5b**)



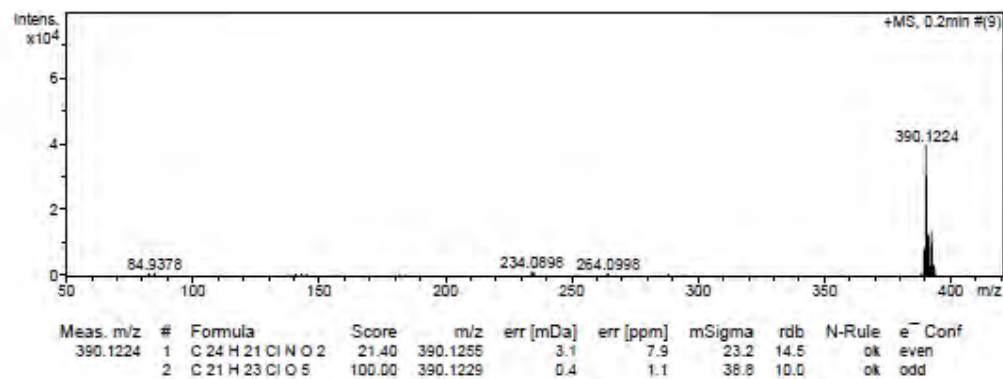
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]-N-[2-(piperidin-1-yl)ethyl]benzamide (**5c**)



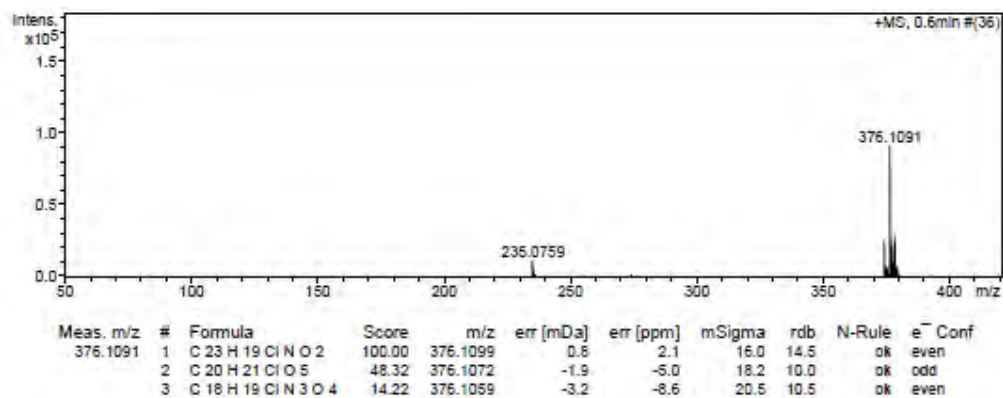
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]-N-[2-(pyrrolidin-1-yl)ethyl]benzamide (**5d**)



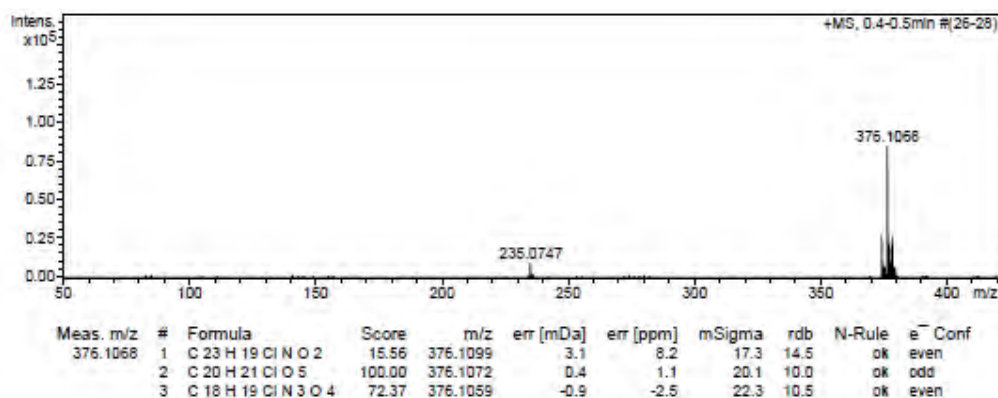
N-[2-(3-chlorophenyl)ethyl]-3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5e**)



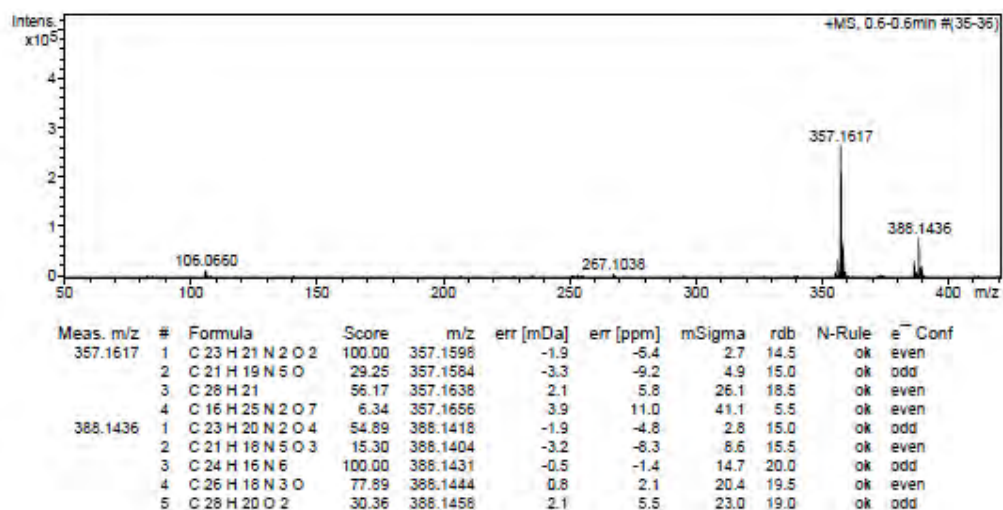
N-[(3-chlorophenyl)methyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5f**)



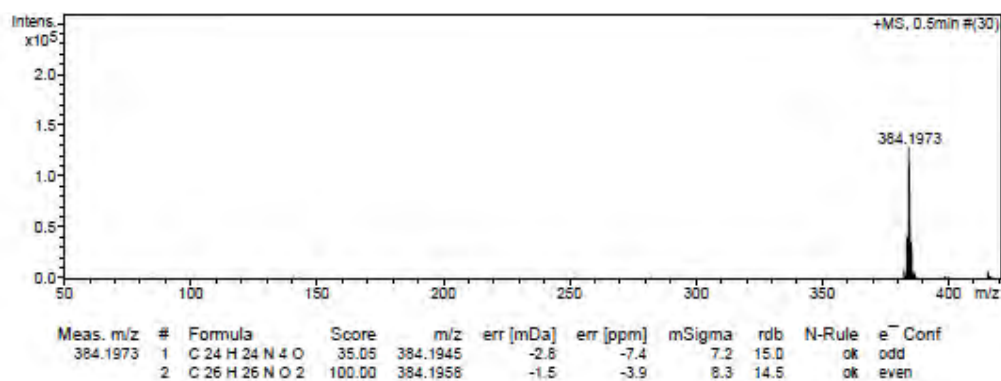
N-[(4-chlorophenyl)methyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5g**)



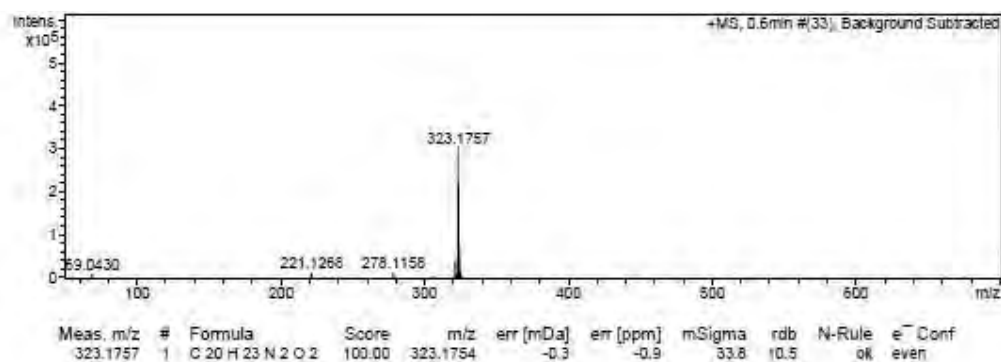
3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-[2-(pyridin-2-yl)ethyl]benzamide (**5h**)



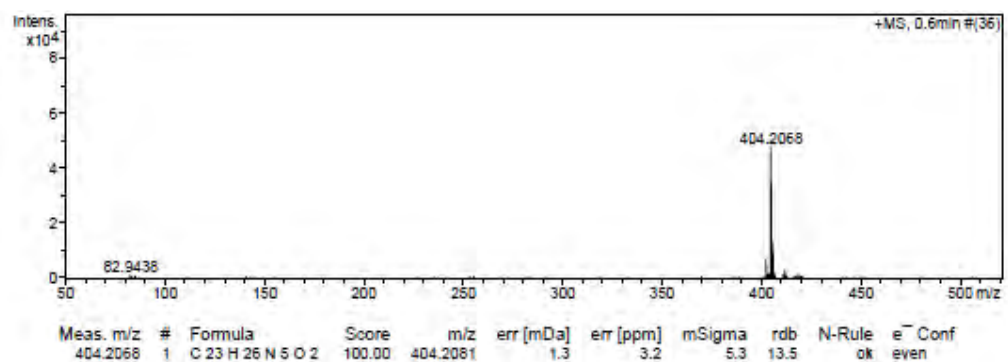
3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-*N*-(4-phenylbutyl)benzamide (**5i**)



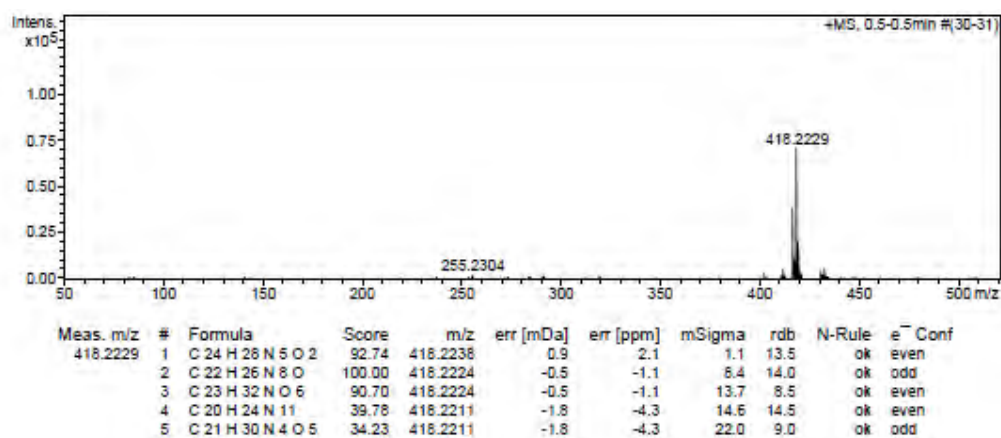
N-[2-(dimethylamino)ethyl]-3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]benzamide (**5j**)



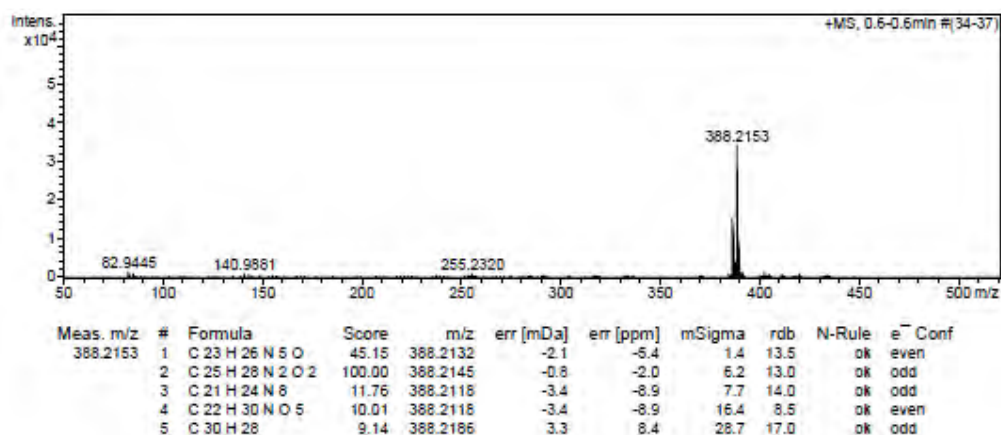
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(morpholin-4-yl)ethyl]benzamide (**6a**)



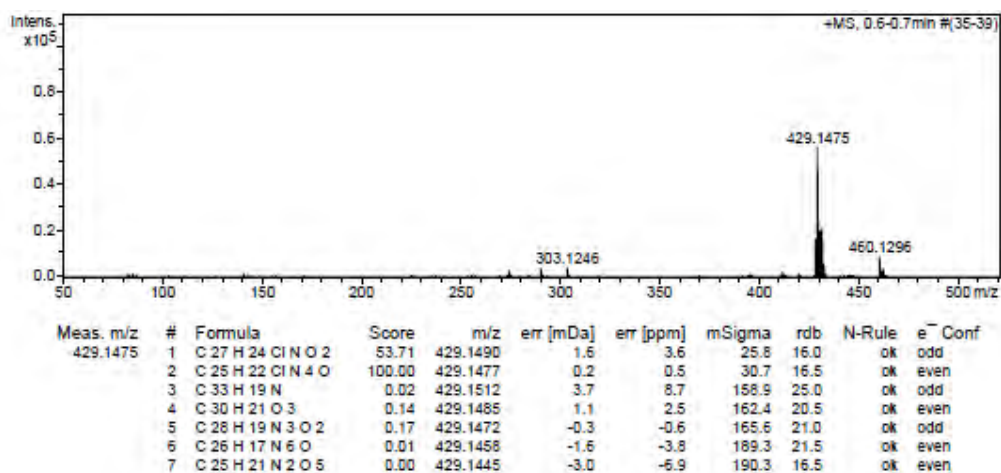
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[3-(morpholin-4-yl)propyl]benzamide (**6b**)



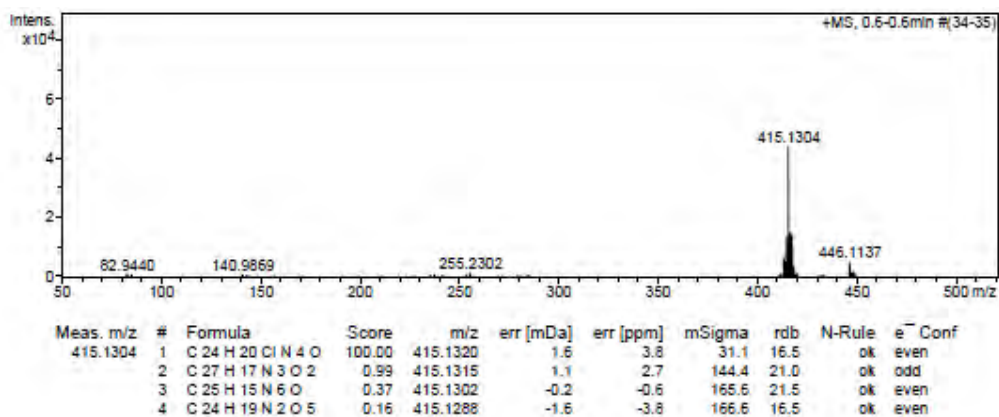
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(pyrrolidin-1-yl)ethyl]benzamide (**6d**)



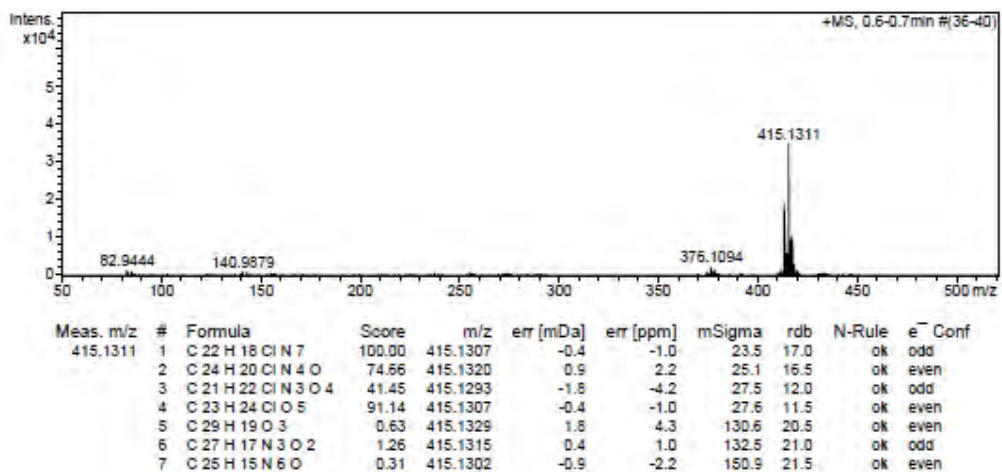
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(3-chlorophenyl)ethyl]benzamide (**6e**)



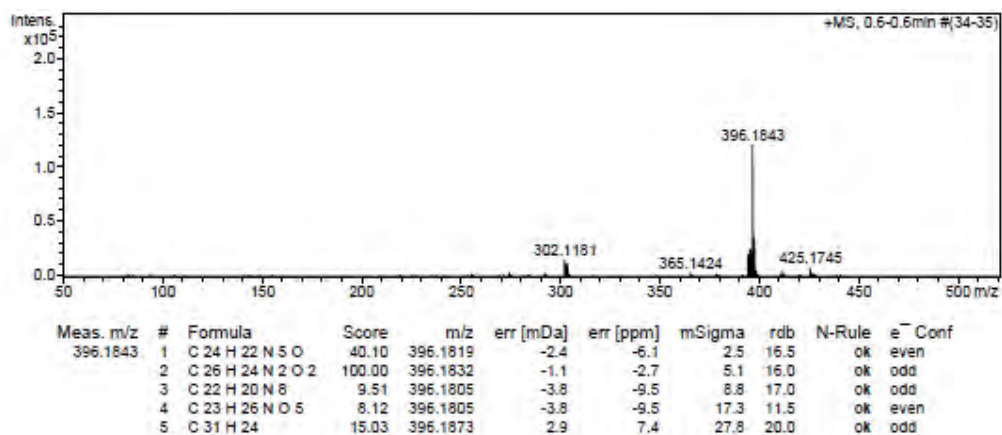
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[(3-chlorophenyl)methyl]benzamide (**6f**)



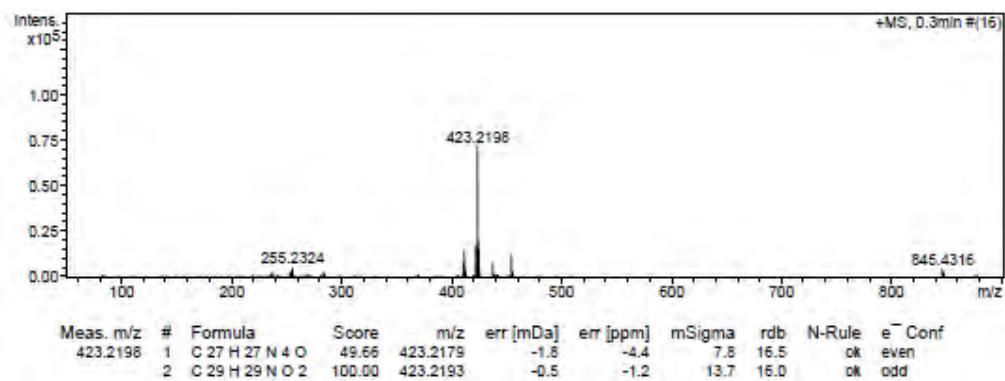
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[(4-chlorophenyl)methyl]benzamide (**6g**)



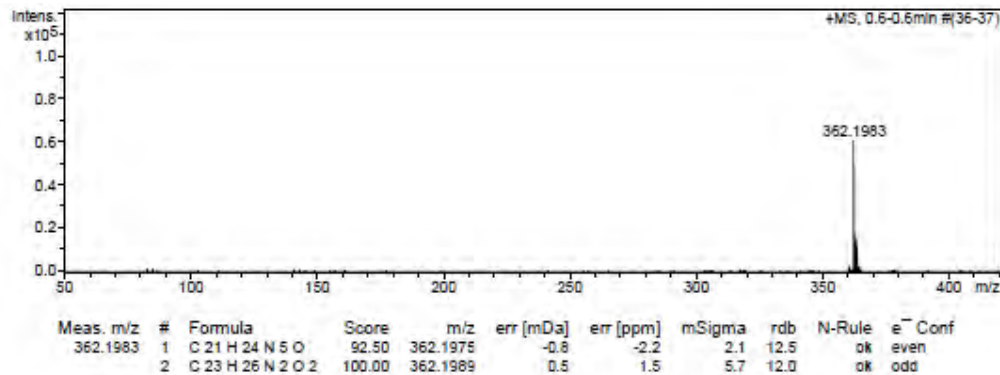
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(pyridin-2-yl)ethyl]benzamide (**6h**)



3-(2-amino-6-phenylpyrimidin-4-yl)-N-(4-phenylbutyl)benzamide (**6i**)

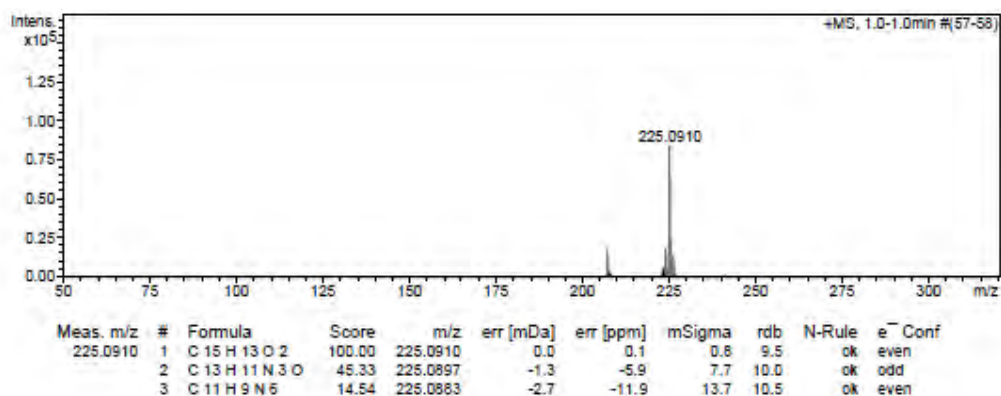


3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(dimethylamino)ethyl]benzamide (**6j**)

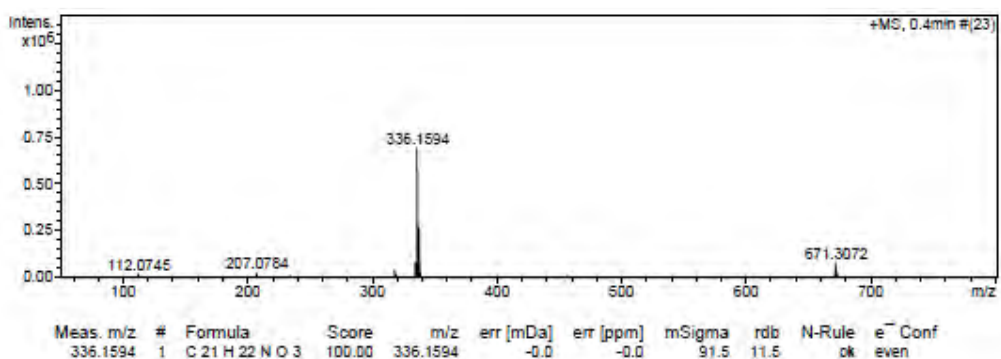


Mass spectra: Article 3

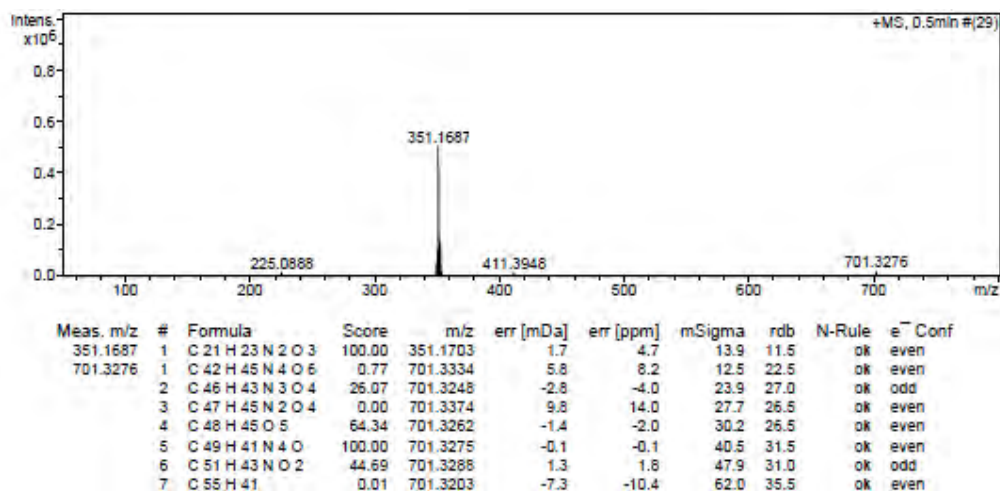
(2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**8**)



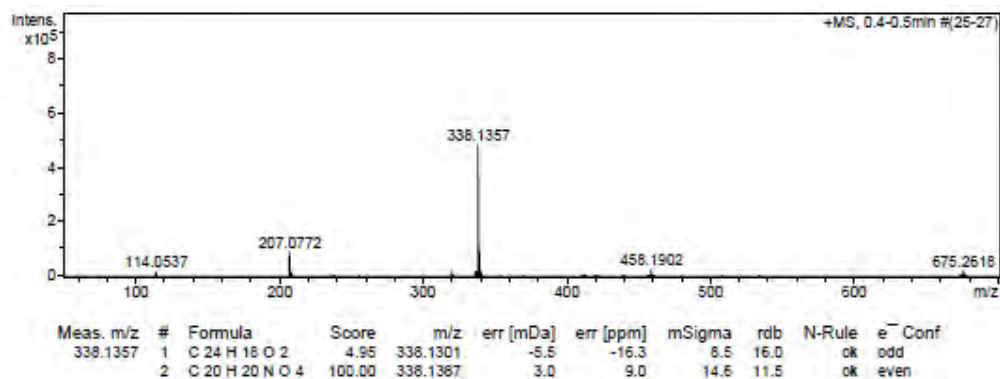
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl piperidine-1-carboxylate (**9a**)



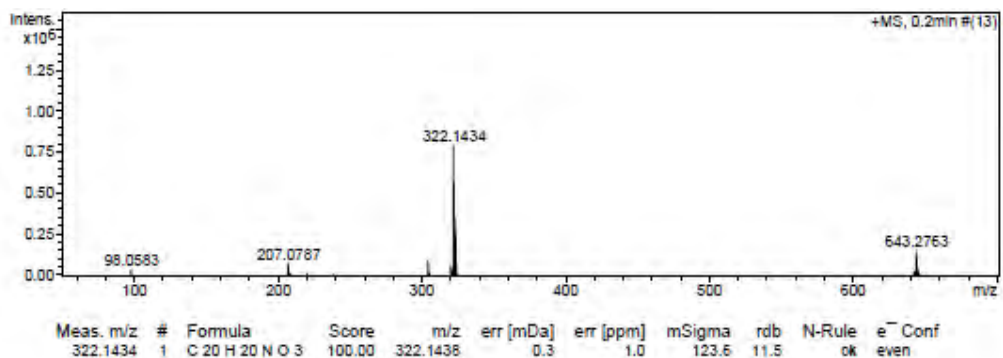
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl 4-methylpiperazine-1-carboxylate (**9b**)



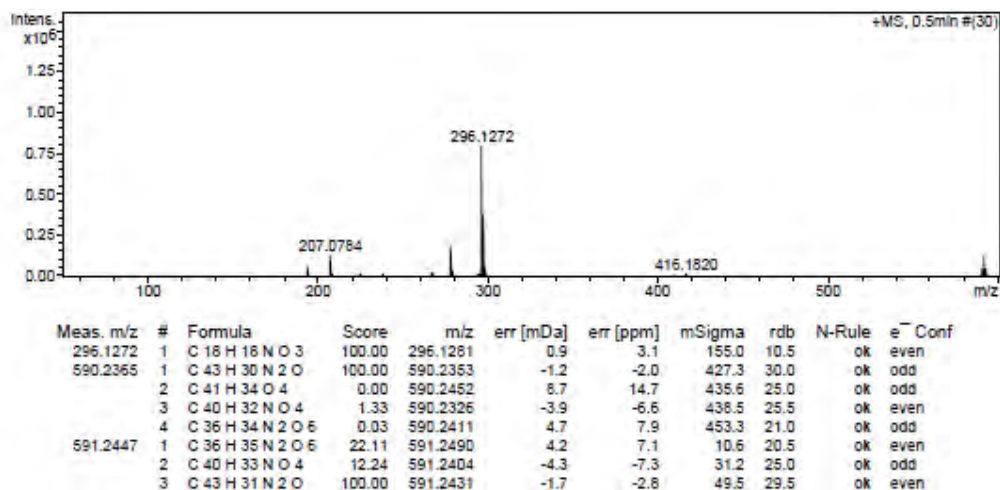
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl morpholine-4-carboxylate (**9c**)



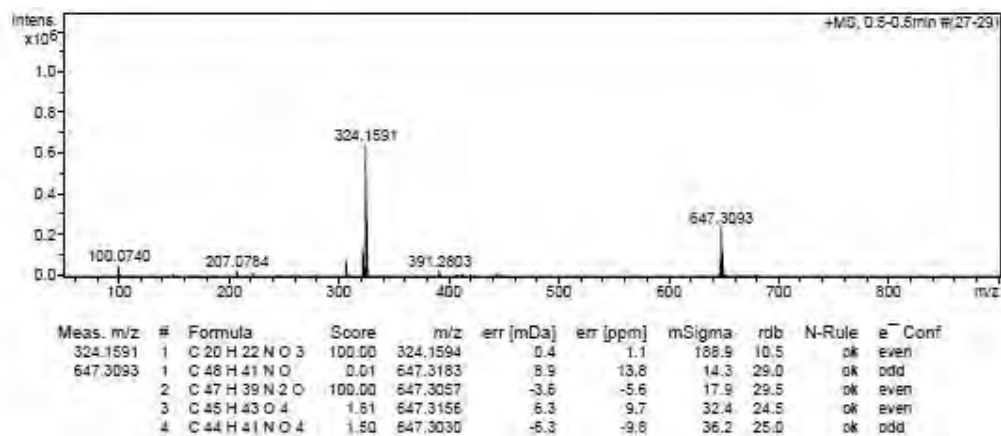
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl pyrrolidine-1-carboxylate (**9d**)



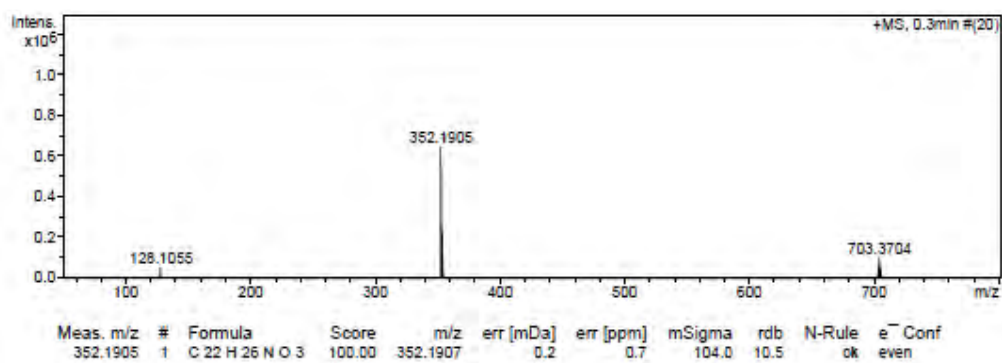
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-dimethylcarbamate (**9e**)



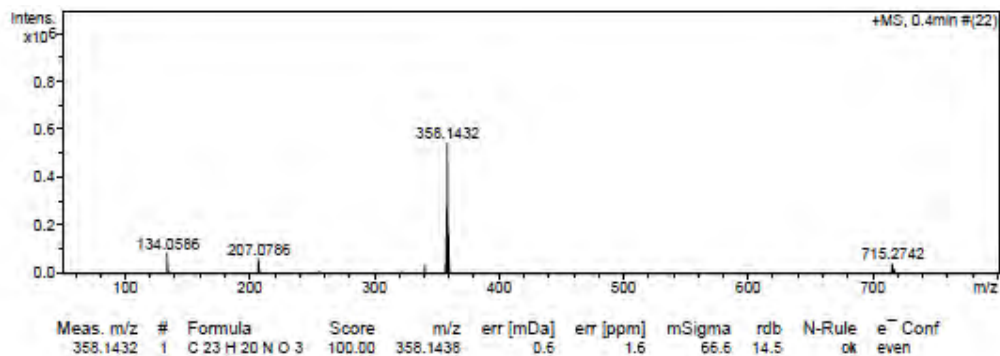
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-diethylcarbamate (**9f**)



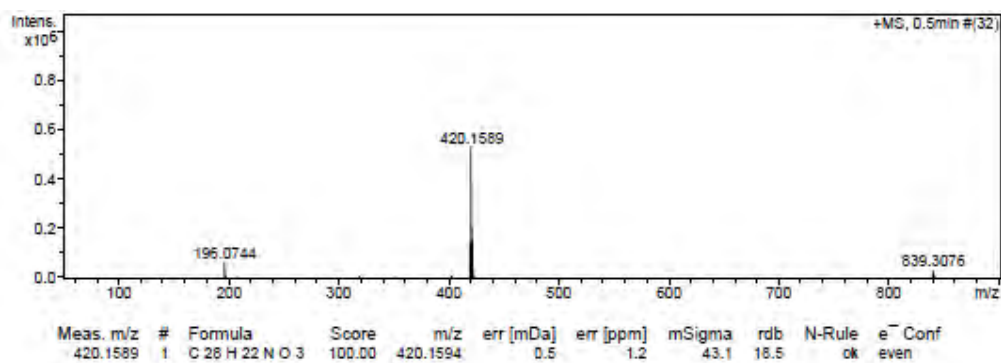
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-bis(propan-2-yl)carbamate (**9g**)



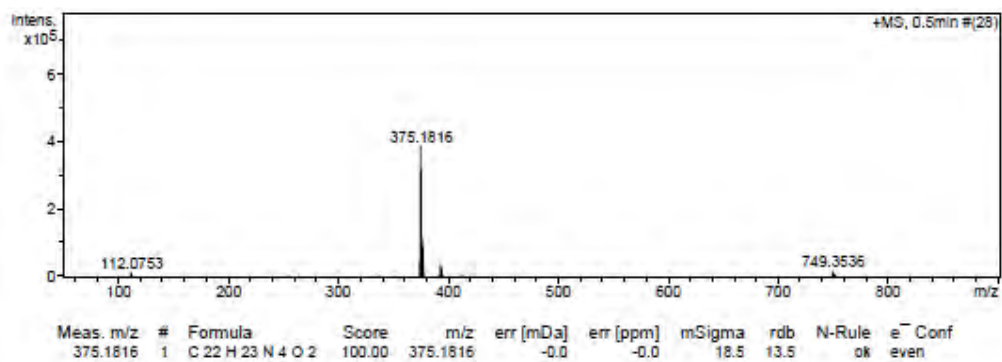
3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N*-methyl-*N*-phenylcarbamate (**9h**)



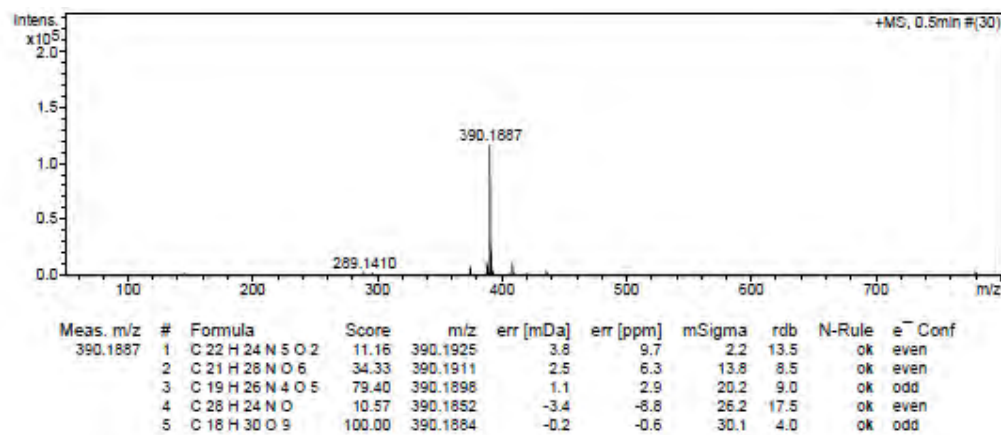
3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenyl *N,N*-diphenylcarbamate (**9i**)



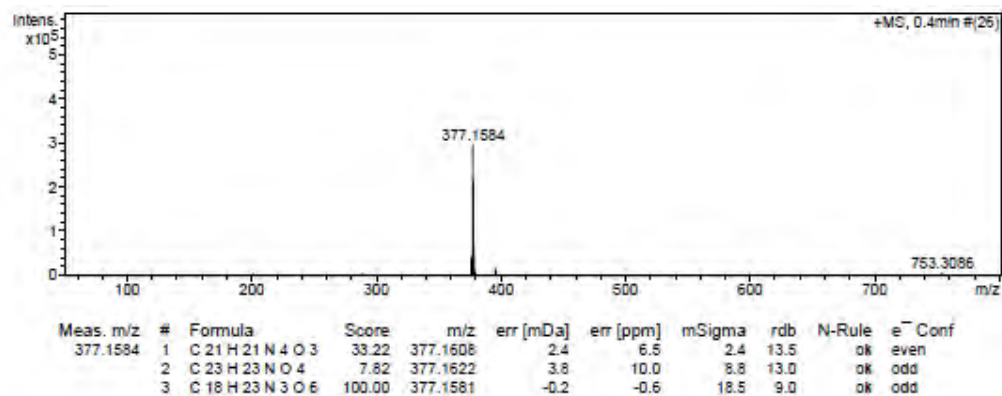
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl piperidine-1-carboxylate (**4a**)



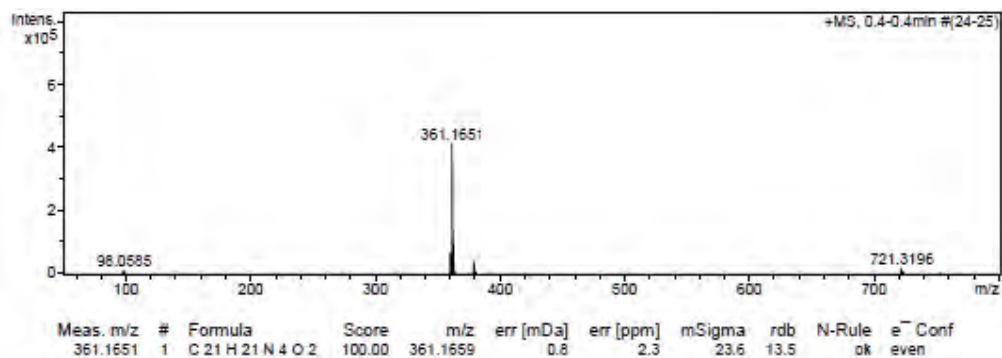
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl 4-methylpiperazine-1-carboxylate (**4b**)



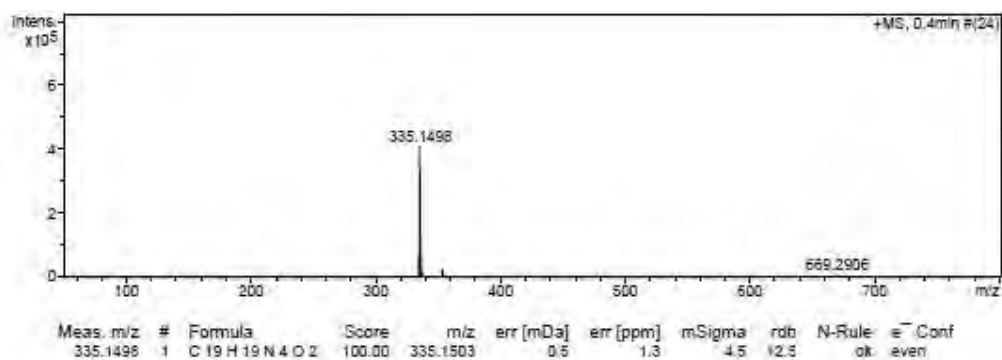
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate (**4c**)



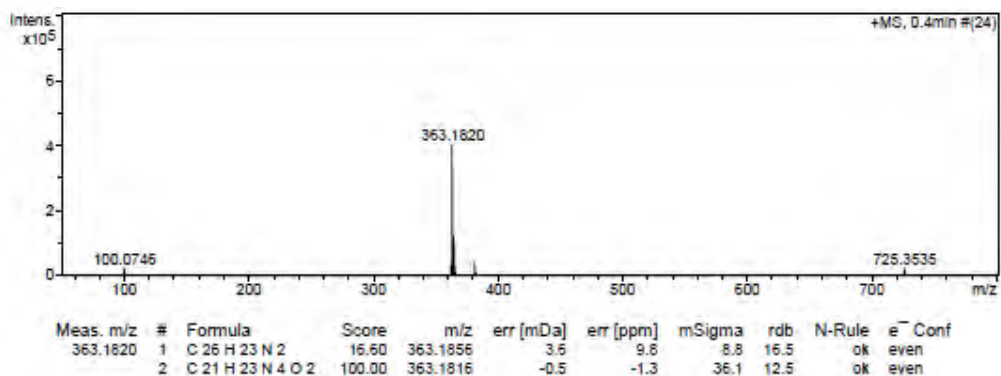
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl pyrrolidine-1-carboxylate (**4d**)



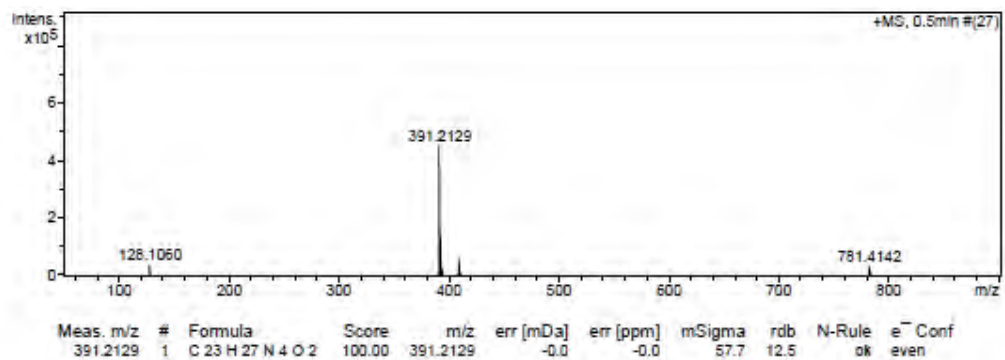
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-dimethylcarbamate (**4e**)



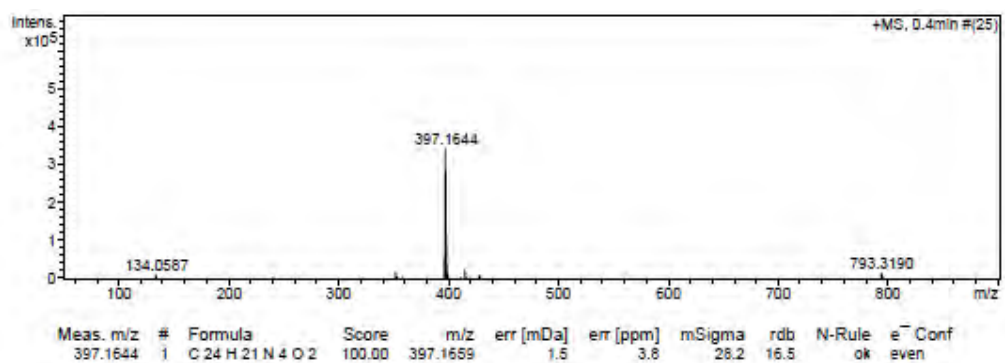
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-diethylcarbamate (**4f**)



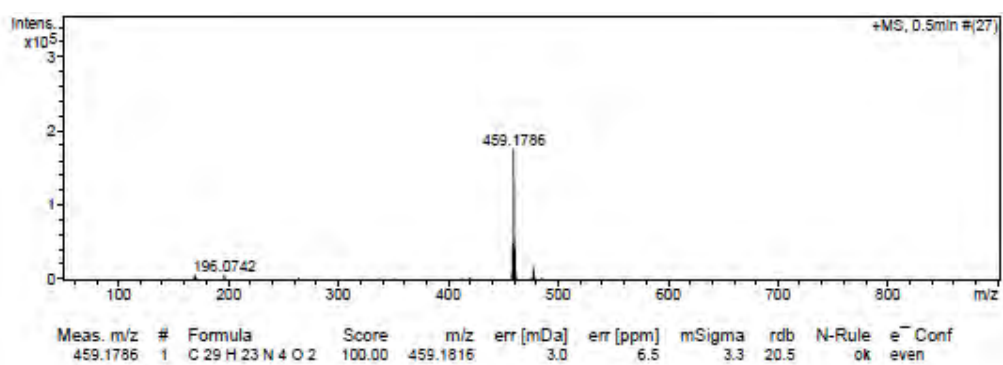
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-bis(propan-2-yl)carbamate (**4g**)



3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N*-methyl-*N*-phenylcarbamate (**4h**)

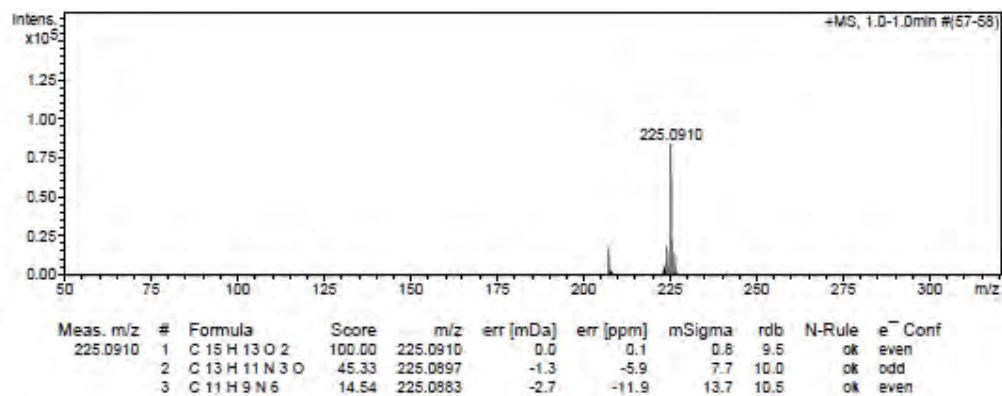


3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-diphenylcarbamate (**4i**)

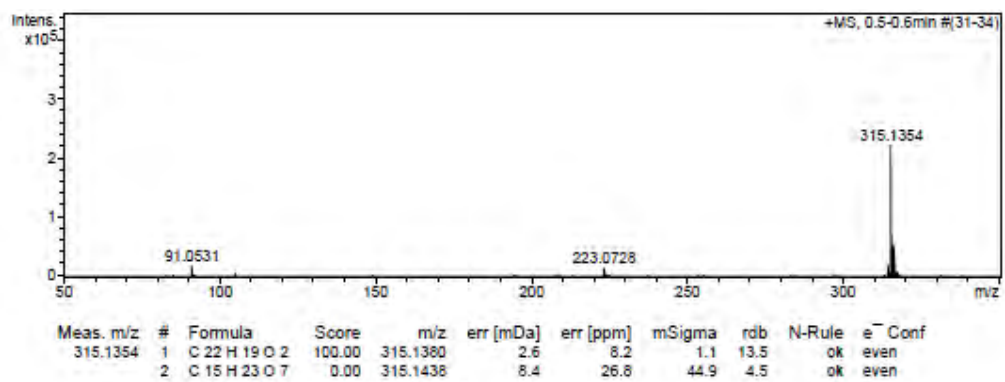


Mass spectra: Article 4

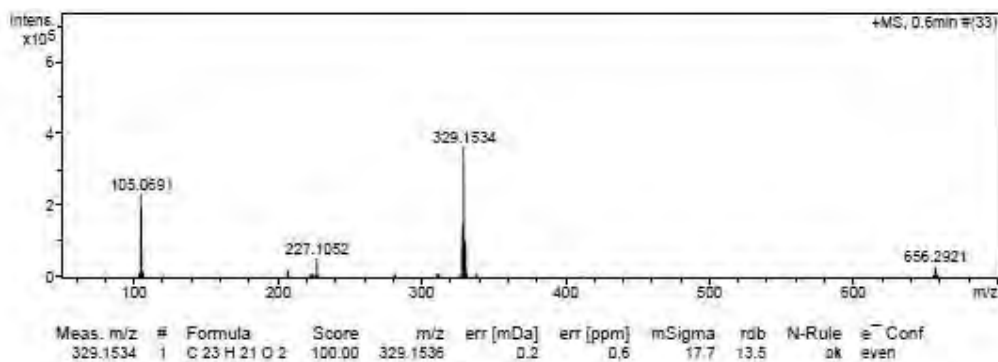
(2E)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**6**)



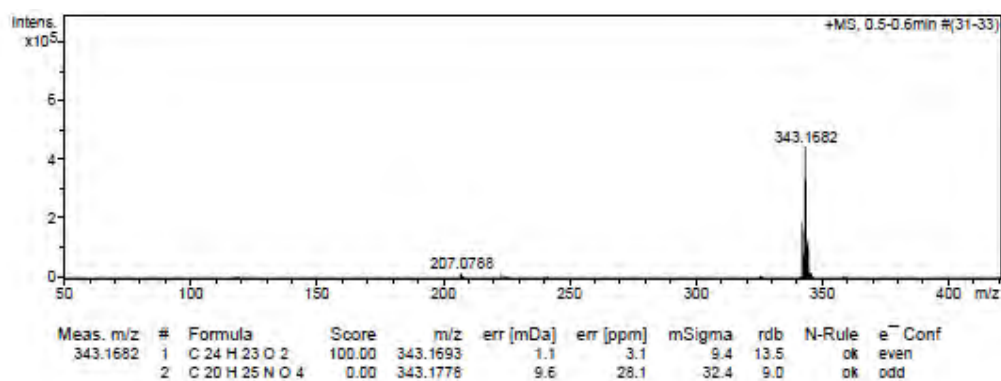
(2E)-3-[3-(benzyloxy)phenyl]-1-phenylprop-2-en-1-one (**7a**)



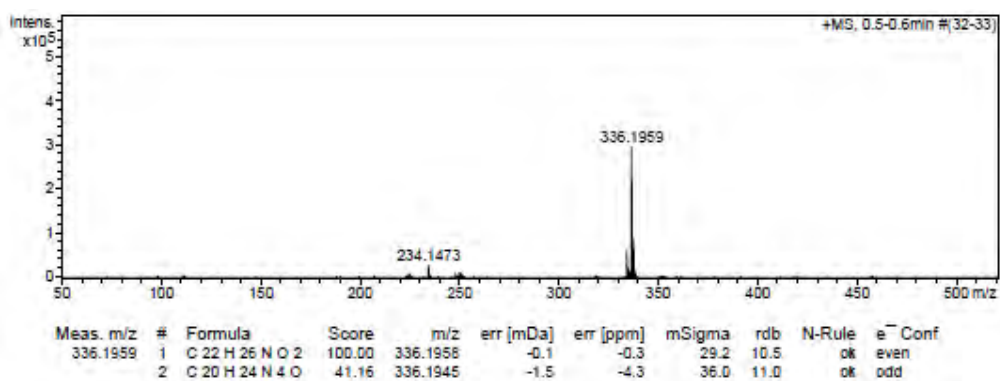
(2E)-1-phenyl-3-[3-(2-phenylethoxy)phenyl]prop-2-en-1-one (**7b**)



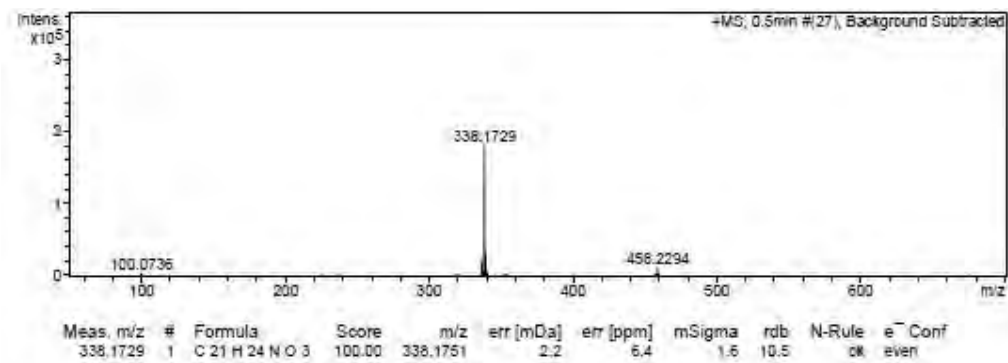
(2E)-1-phenyl-3-[3-(3-phenylpropoxy)phenyl]prop-2-en-1-one (7c)



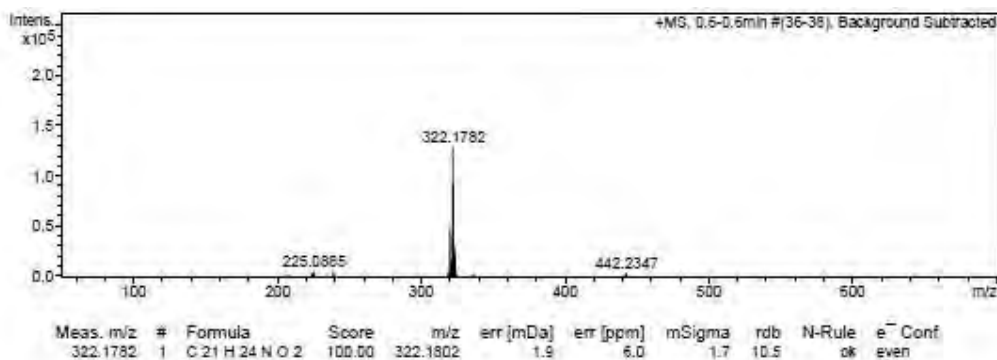
(2E)-1-phenyl-3-{3-[2-(piperidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (7d)



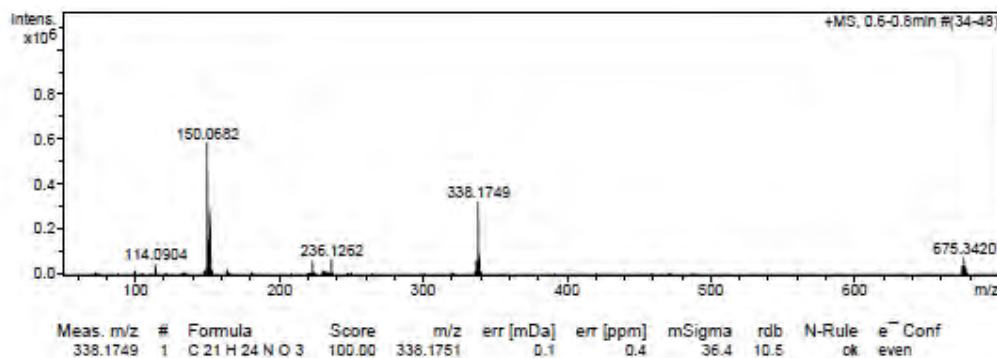
(2E)-3-{3-[2-(morpholin-4-yl)ethoxy]phenyl}-1-phenylprop-2-en-1-one (7e)



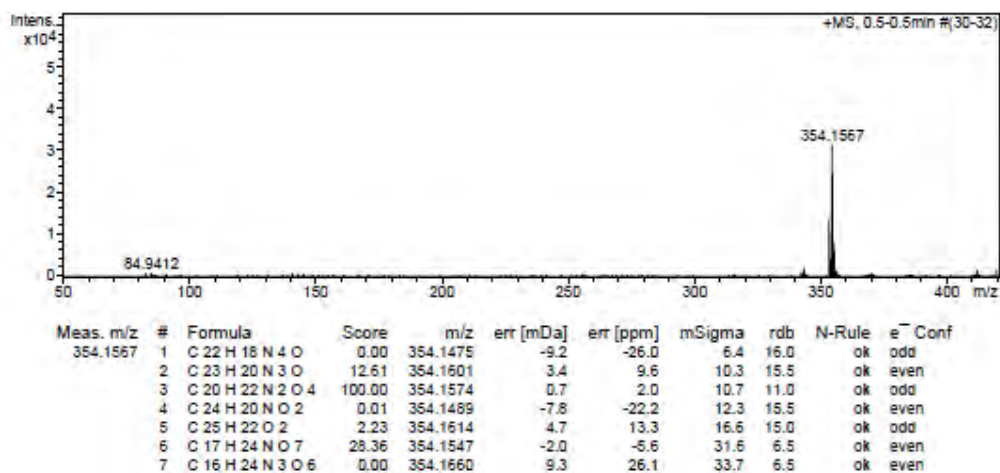
(2*E*)-1-phenyl-3-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}prop-2-en-1-one (**7f**)



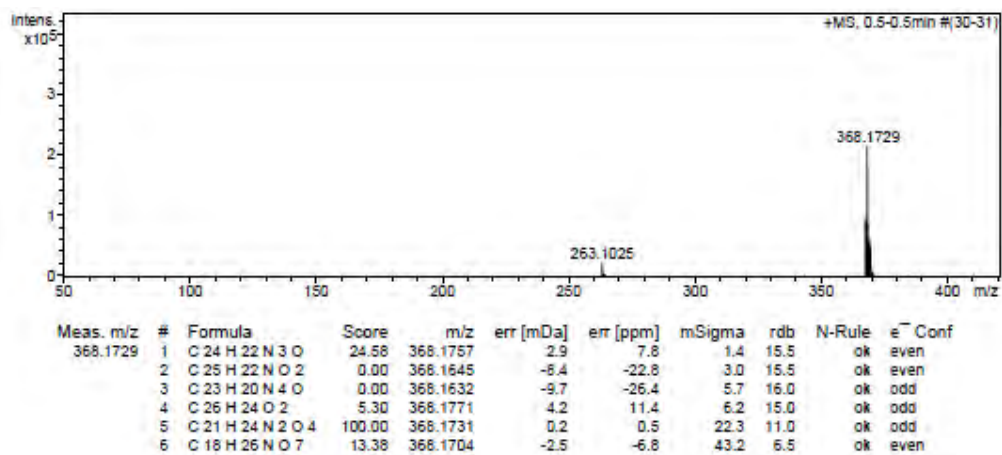
N,N-diethyl-2-{3-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]phenoxy}acetamide (**7g**)



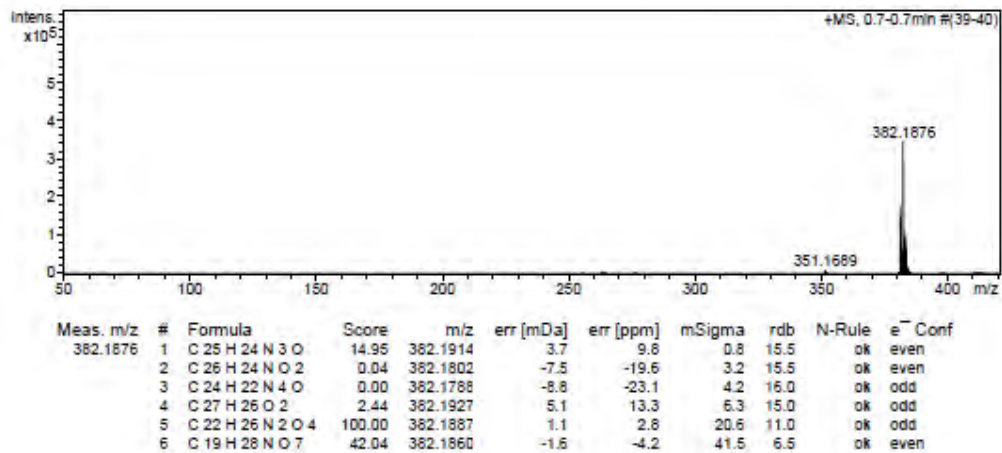
4-[3-(benzyloxy)phenyl]-6-phenylpyrimidin-2-amine (**3a**)



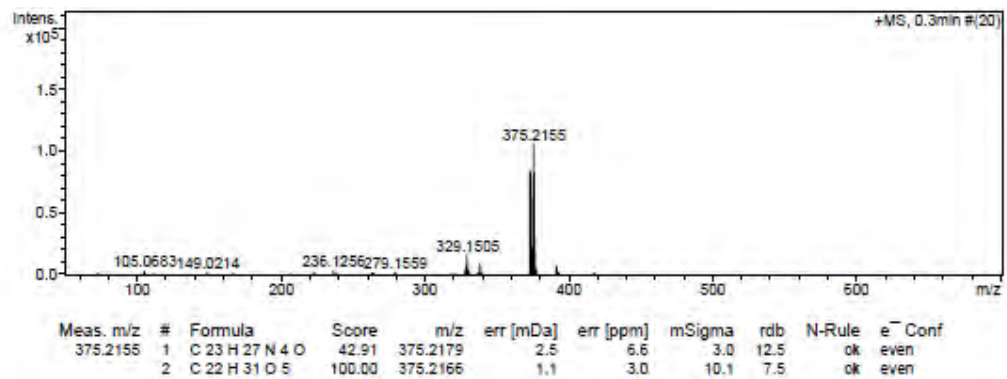
4-phenyl-6-[3-(2-phenylethoxy)phenyl]pyrimidin-2-amine (**3b**)



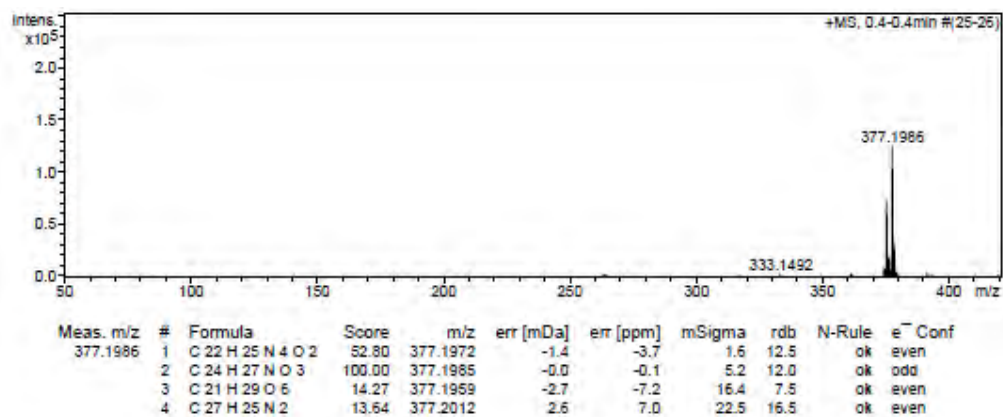
4-phenyl-6-[3-(3-phenylpropoxy)phenyl]pyrimidin-2-amine (**3c**)



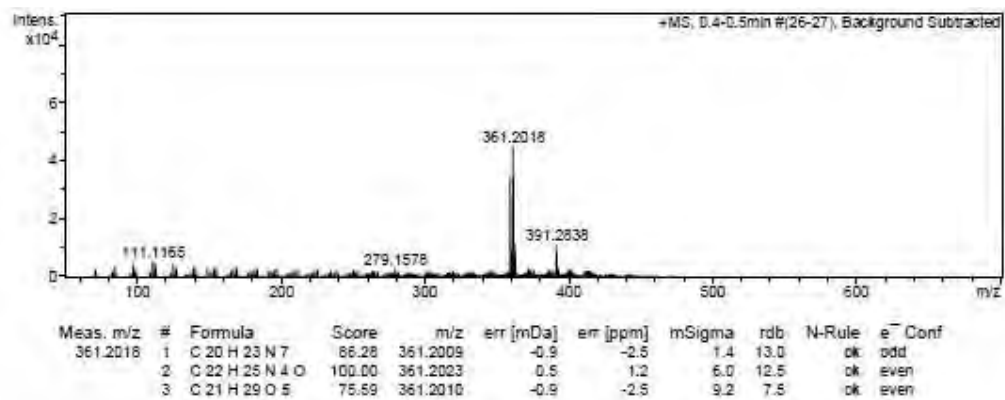
4-phenyl-6-[3-[2-(piperidin-1-yl)ethoxy]phenyl]pyrimidin-2-amine (**3d**)



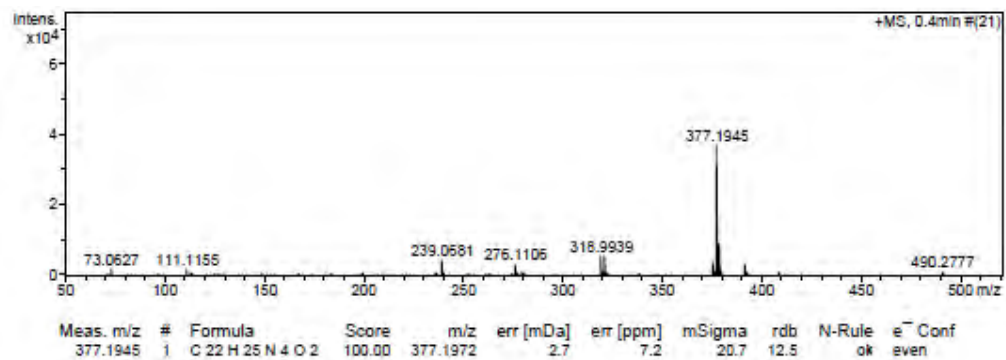
4-{3-[2-(morpholin-4-yl)ethoxy]phenyl}-6-phenylpyrimidin-2-amine (**3e**)



4-phenyl-6-{3-[2-(pyrrolidin-1-yl)ethoxy]phenyl}pyrimidin-2-amine (**3f**)

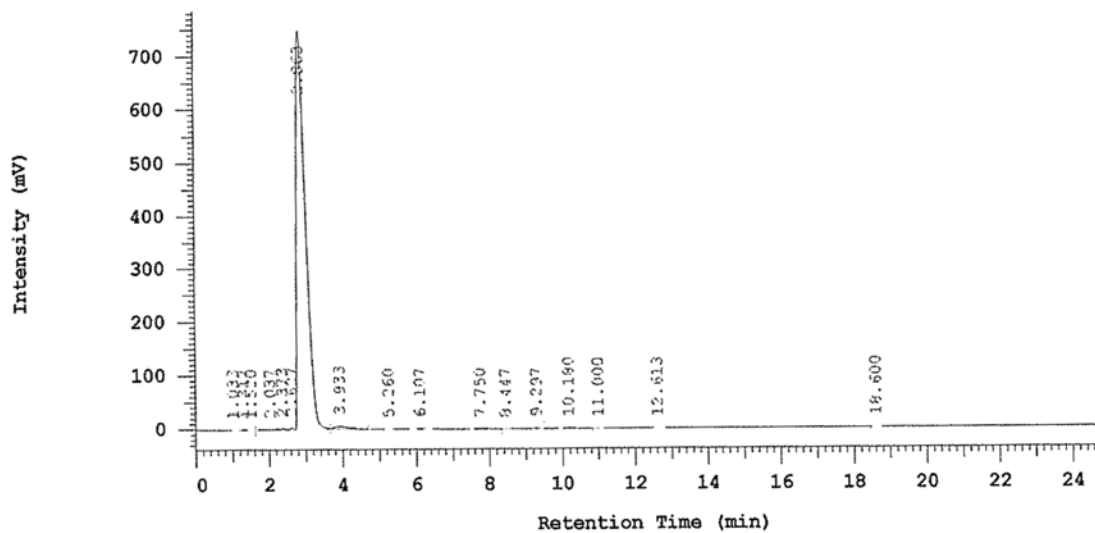


2-[3-(2-amino-6-phenylpyrimidin-4-yl)phenoxy]-*N,N*-diethylacetamide (**3g**)

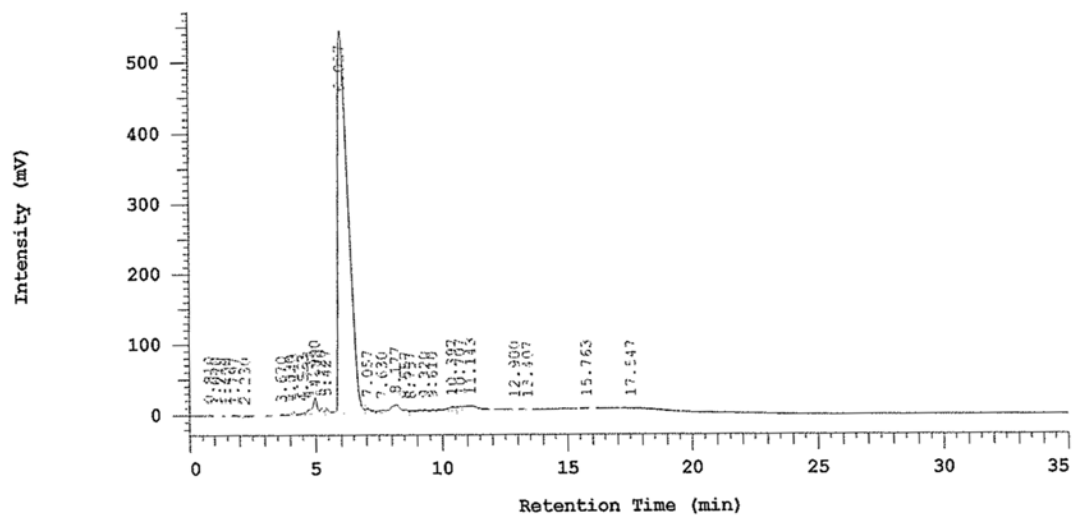


HPLC chromatograms: Article 2

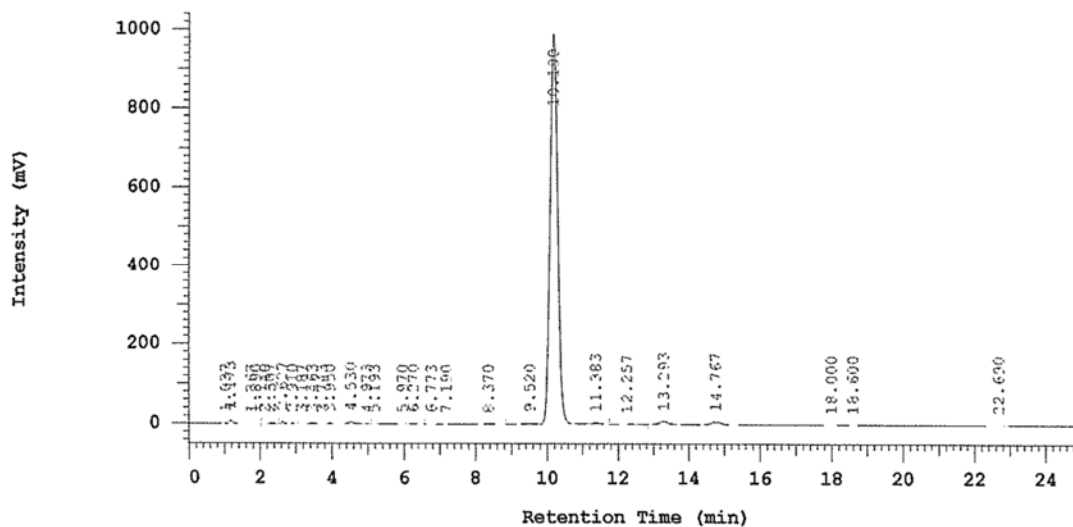
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(morpholin-4-yl)ethyl]benzamide (**6a**)



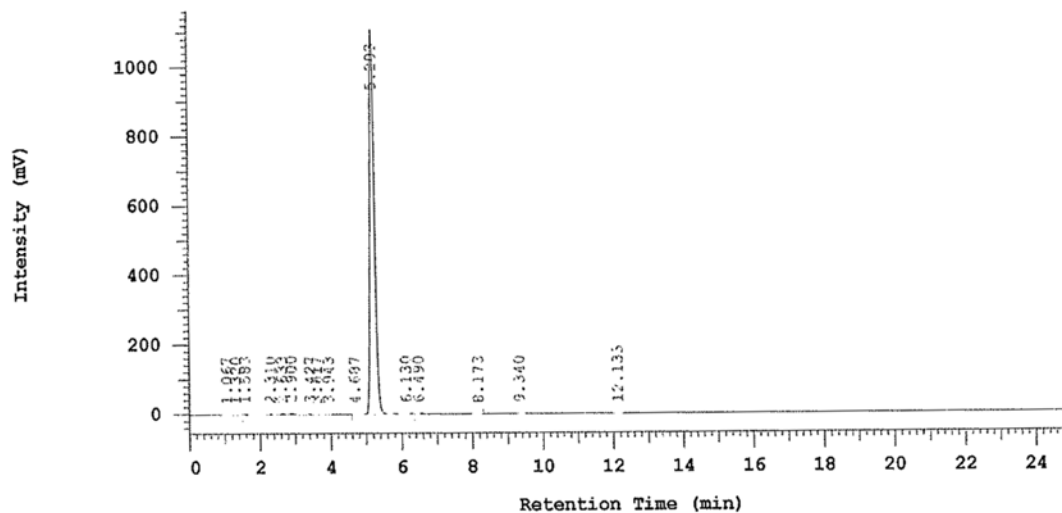
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[3-(morpholin-4-yl)propyl]benzamide (**6b**)



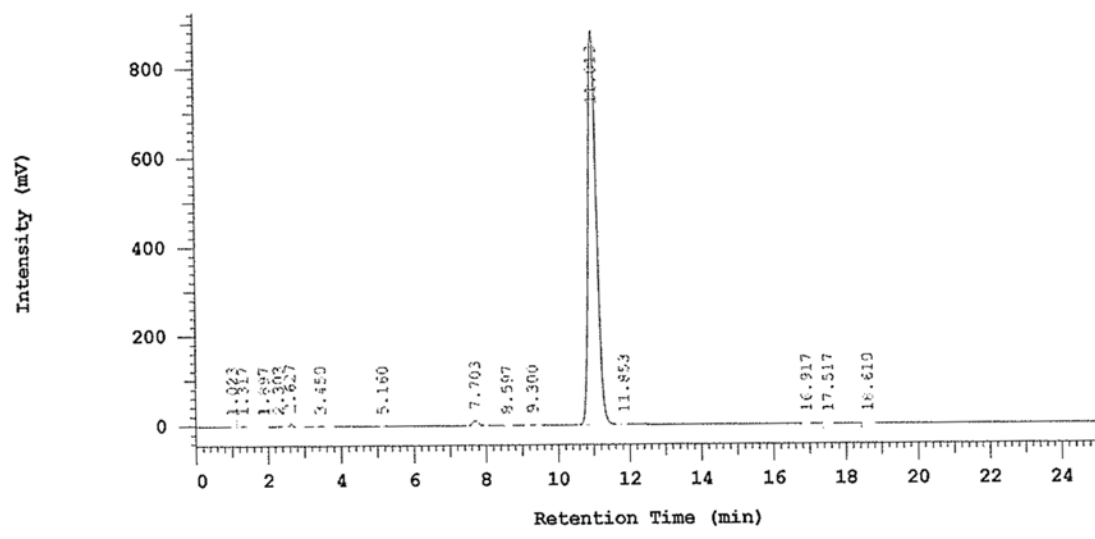
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(piperidin-1-yl)ethyl]benzamide (**6c**)



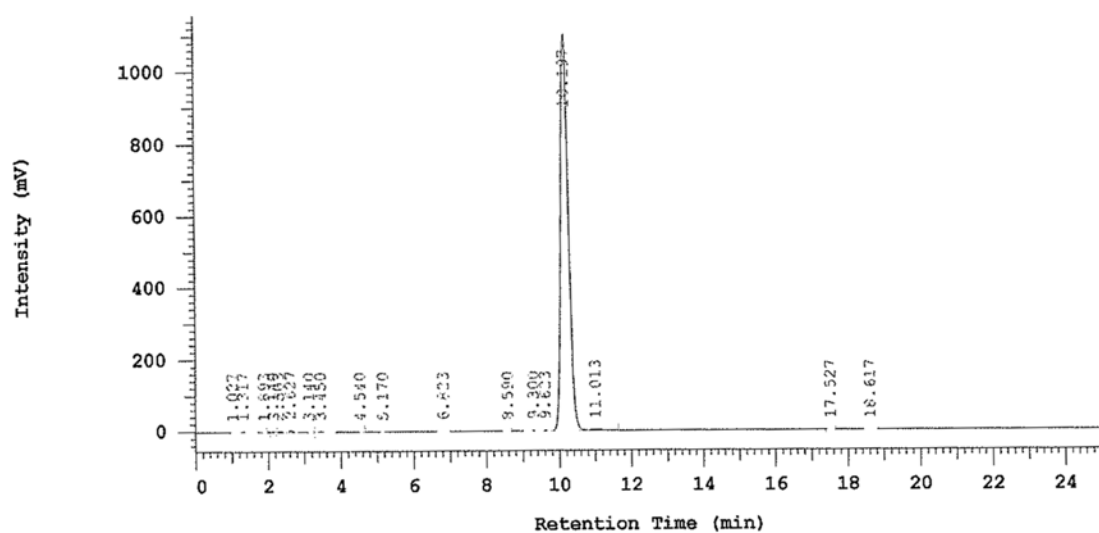
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(pyrrolidin-1-yl)ethyl]benzamide (**6d**)



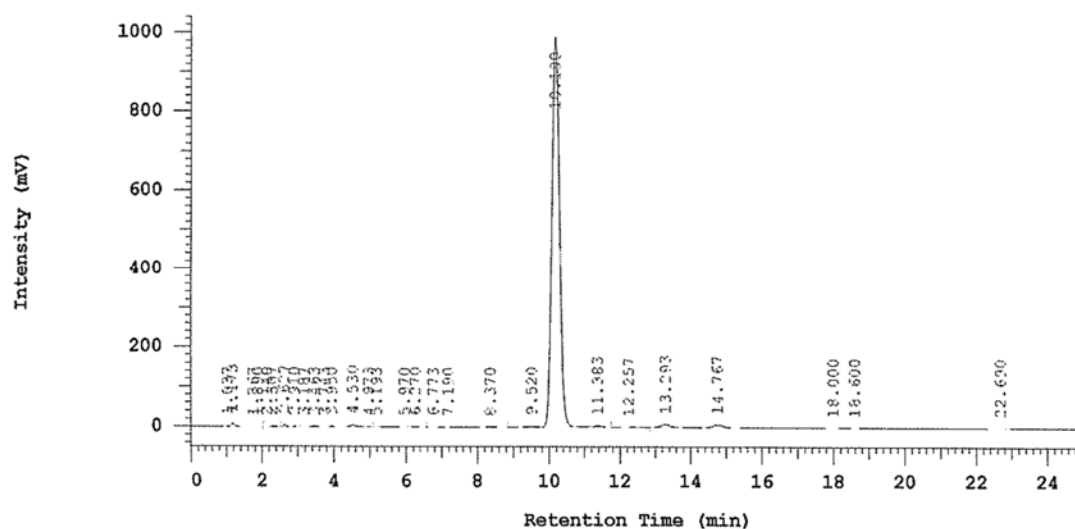
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[2-(3-chlorophenyl)ethyl]benzamide (6e)



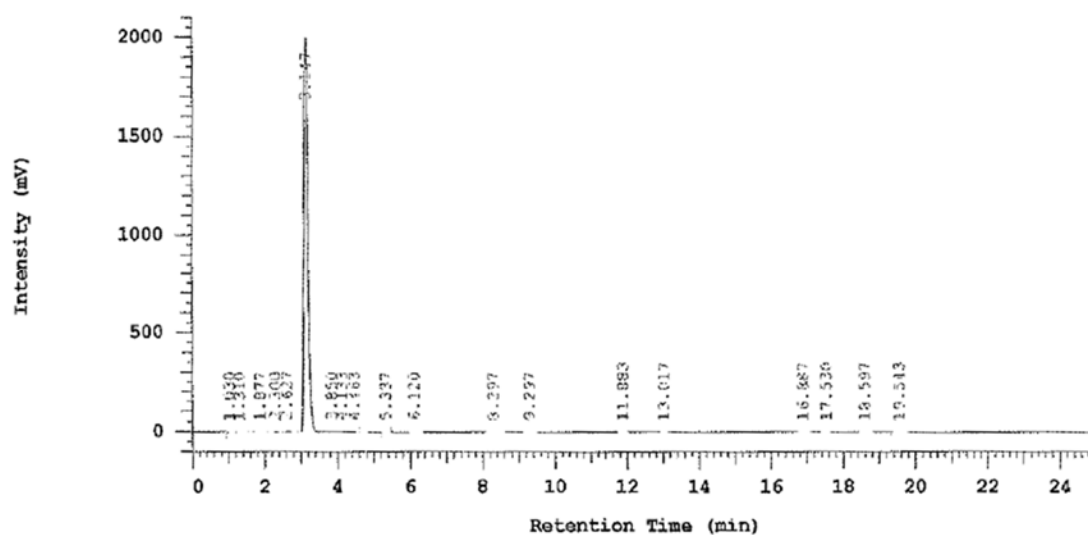
3-(2-amino-6-phenylpyrimidin-4-yl)-N-[(3-chlorophenyl)methyl]benzamide (6f)



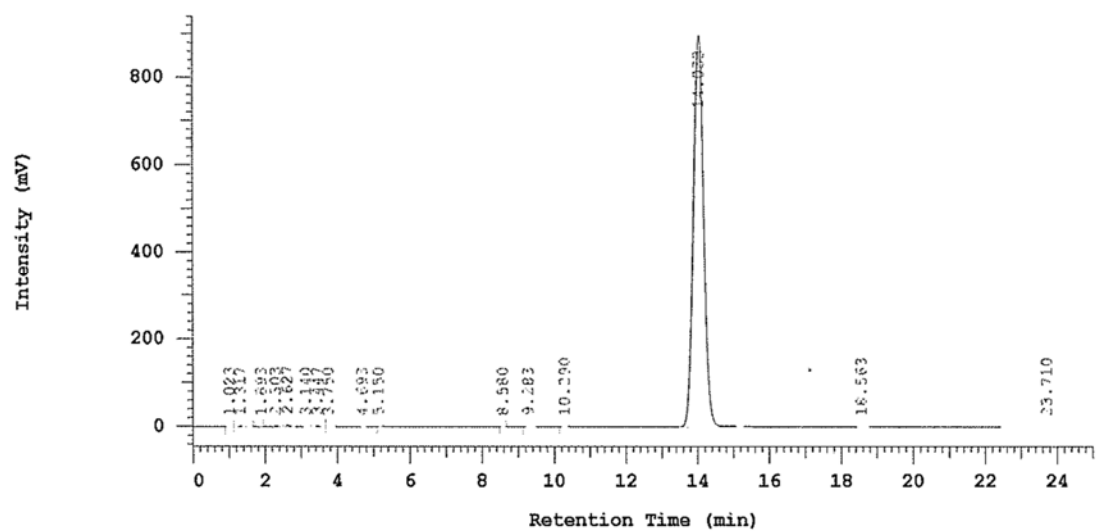
3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[(4-chlorophenyl)methyl]benzamide (**6g**)



3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-[2-(pyridin-2-yl)ethyl]benzamide (**6h**)

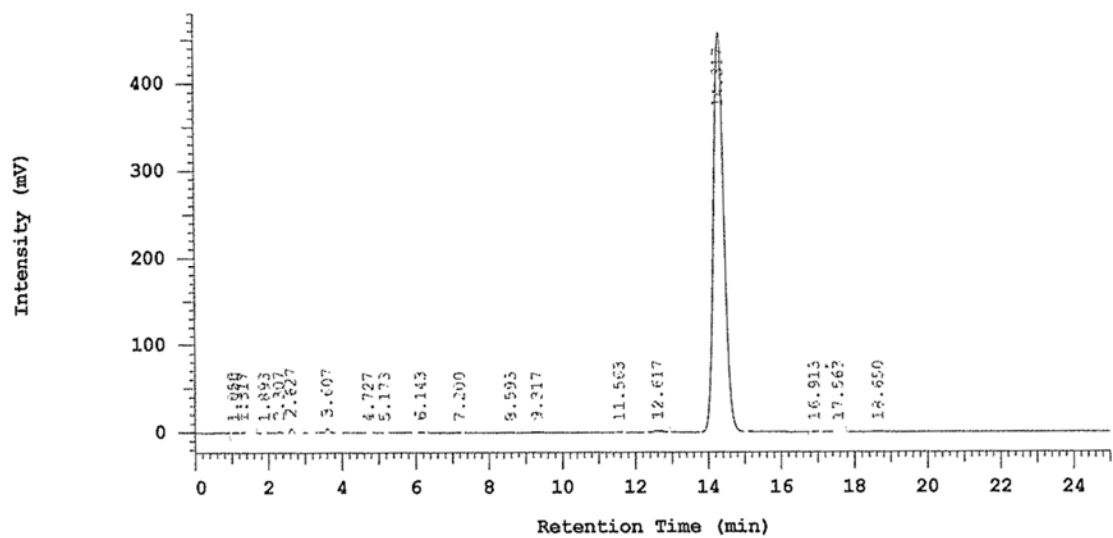


3-(2-amino-6-phenylpyrimidin-4-yl)-*N*-(4-phenylbutyl)benzamide (**6i**)

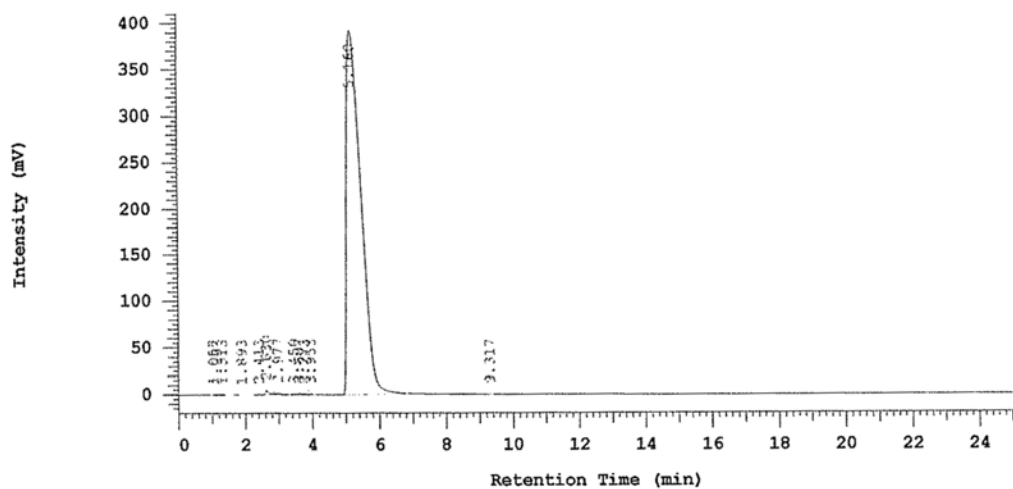


HPLC chromatograms: Article 3

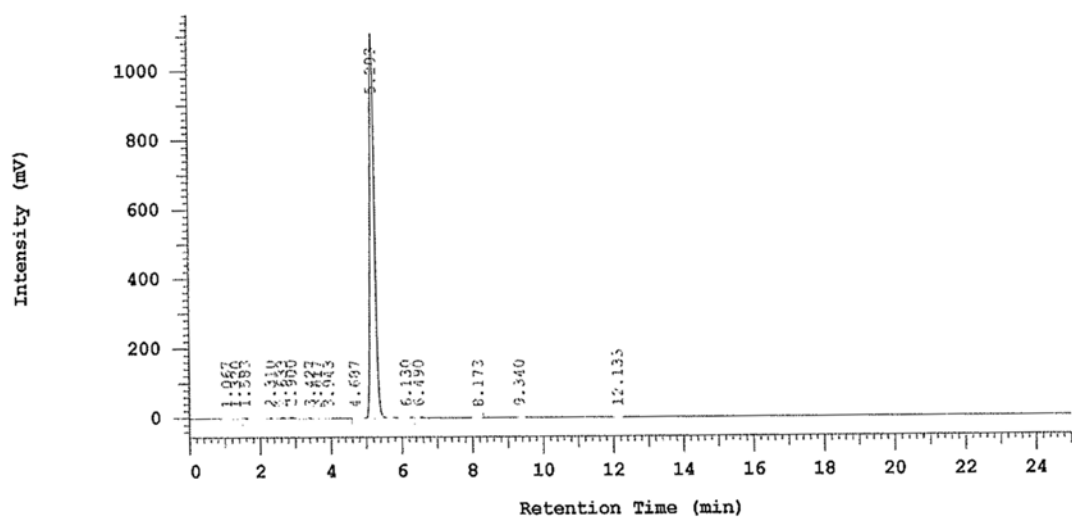
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl piperidine-1-carboxylate (**4a**)



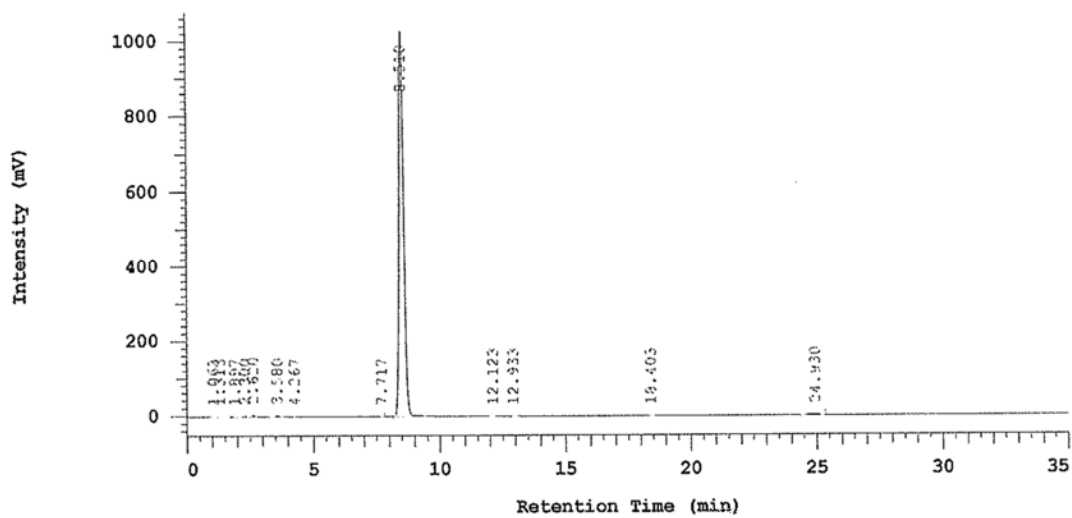
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl 4-methylpiperazine-1-carboxylate (**4b**)



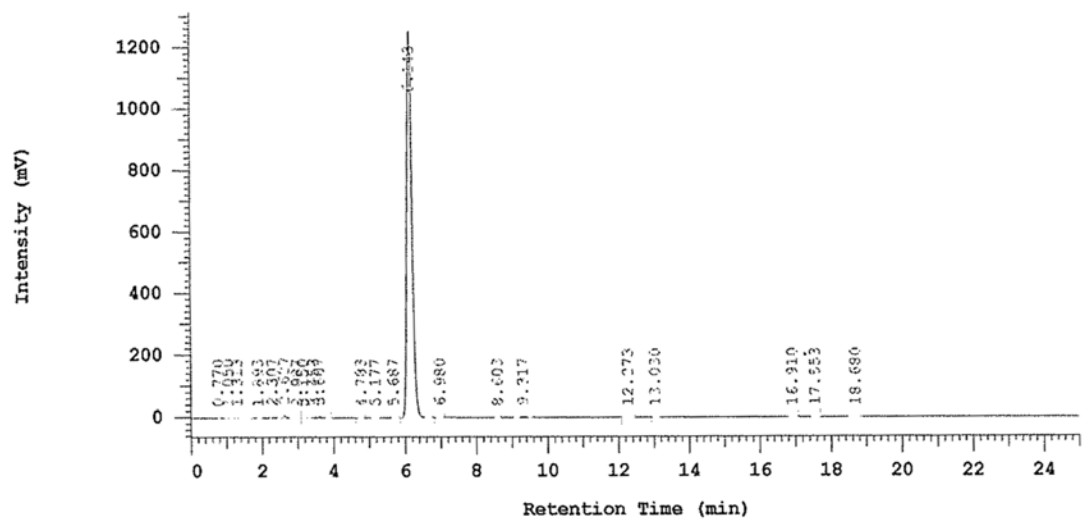
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate (**4c**)



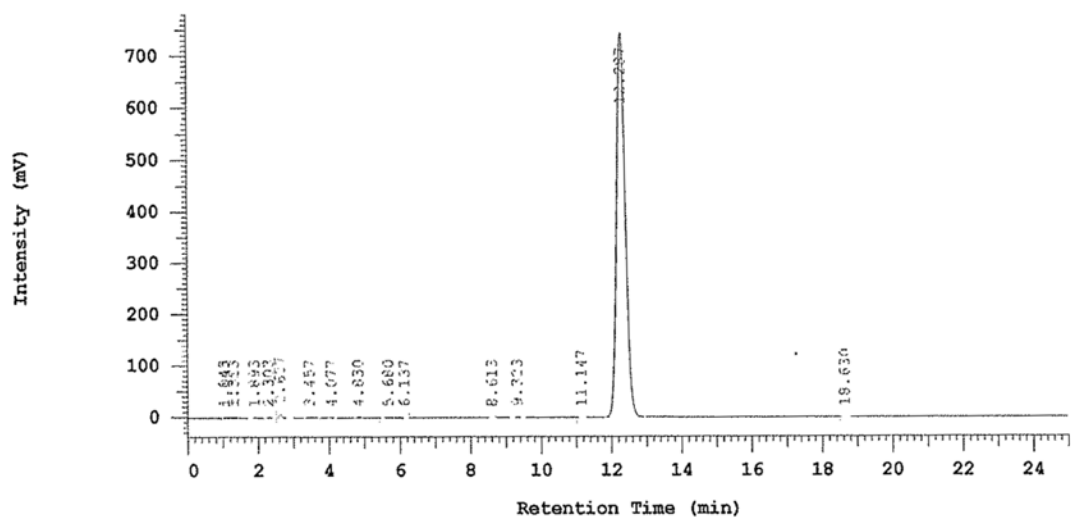
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl pyrrolidine-1-carboxylate (**4d**)



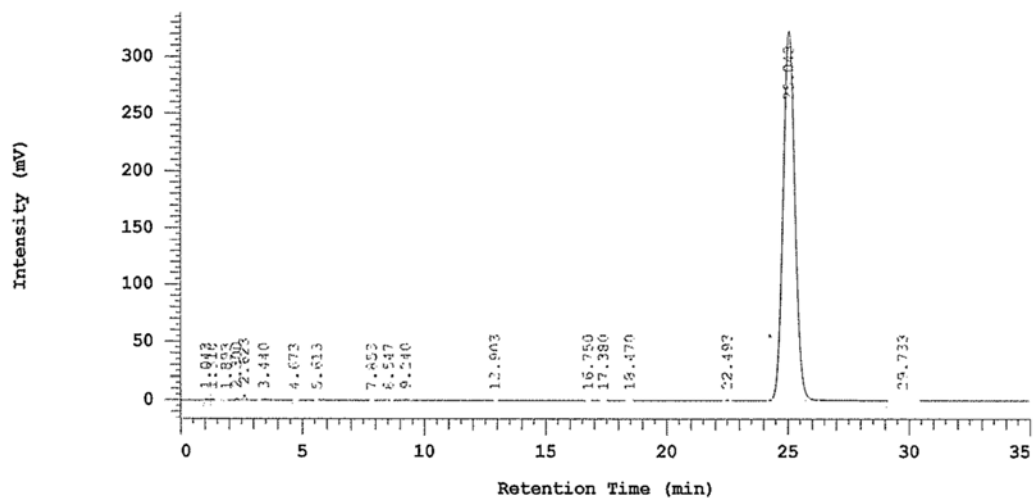
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-dimethylcarbamate (**4e**)



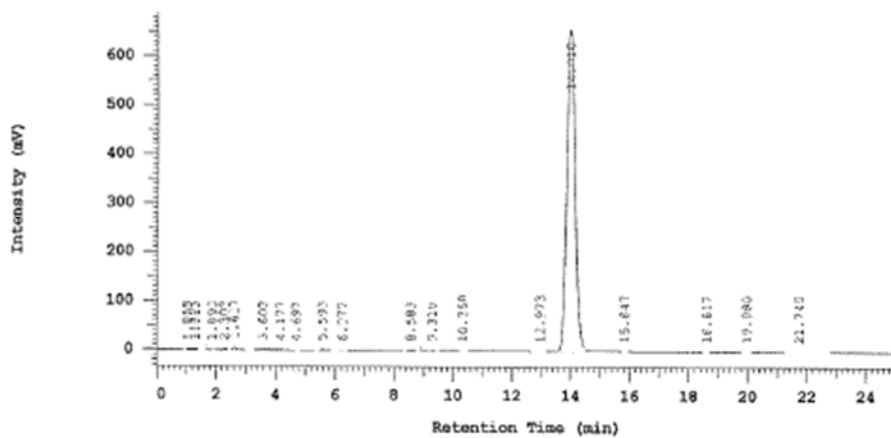
3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-diethylcarbamate (**4f**)



3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-bis(propan-2-yl)carbamate (**4g**)



3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N*-methyl-*N*-phenylcarbamate (**4h**)



3-(2-amino-6-phenylpyrimidin-4-yl)phenyl *N,N*-diphenylcarbamate (**4i**)

