5-Hydroxy-1-tetralone analogues as dual A_1/A_{2A} receptor antagonists for the potential treatment of neurological conditions

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Dissertation submitted in fulfilment of the requirements for the degree *Magister Scientiae* in Pharmaceutical Chemistry at the North-West University

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Graduation May 2018

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The financial assistance of the South African Medical Research Council (MRC) and National Research Foundation (NRF) (Grant specific unique reference numbers (UID) 96135) towards this study is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the MRC or NRF.

PREFACE

This dissertation is submitted in article format, comprising of a research article, in accordance with the General Academic Rules (A.13.7.33) of the North-West University (NWU). The article was published in *Bioorganic Chemistry* and the said journal granted the author permission to include the published article in this dissertation (**Annexure K**). All scientific research for this dissertation was conducted by Miss H.D. Janse van Rensburg at the NWU.

Letter of agreement from co-authors of the research article as well as a table of contributions and contributors is also included.

LETTER OF AGREEMENT

November 2017

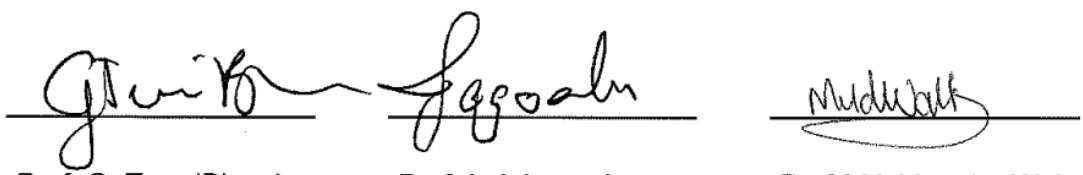
To whom it may concern,

CO-AUTHORSHIP ON RESEARCH ARTICLE

The undersigned are co-authors of the research article listed and, hereby, give permission to Miss H.D. Janse van Rensburg to submit this article as part of the degree *Magister Scientiae* in Pharmaceutical Chemistry at the North-West University (NWU).

- 5-Substituted 2-benzylidene-1-tetralone derivatives as A_1 and/or A_{2A} antagonists for the potential treatment of neurological conditions

Yours faithfully,



Prof. G. Terre'Blanche | Prof. L.J. Legoabe | Dr. M.M. Van der Walt

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c) HPLC	Prof. J. Du Preez
d) melting points	H.D. Janse van Rensburg
e) radioligand binding assays and GTP shift assays	H.D. Janse van Rensburg and Dr. M.M. Van der Walt
Contributed new reagents and/or analytic tools for:	
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b) radioligand binding assays and GTP shift assays	Prof. G. Terre'Blanche
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b) HPLC	H.D. Janse van Rensburg – with critical feedback from Prof. L.J. Legoabe
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ABSTRACT

Parkinson's disease (PD), a classic movement disorder, is the second most common neurological condition after Alzheimer's disease, with higher incidence and prevalence in advanced age — consequently, PD patients' quality of life is reduced and, in addition, the disease has a high socio-economic cost. The pharmacological treatment of PD is based on the dopaminergic system and only addresses the motor symptoms of PD and not the non-motor symptoms (such as cognitive deficits and depression) or neurodegeneration. Additionally, L-3,4-dihydroxyphenylalanine (Levo-dopa/L-dopa) is associated with adverse effects such as motor and non-motor fluctuations, dyskinesias and drug-induced psychosis. Therefore, non-dopaminergic treatment that addresses motor and non-motor symptoms, as well as neurodegeneration, is in demand. The manipulation of adenosine receptors (AR's) may be the solution to the PD-conundrum, as an epidemiological study has established an association between the consumption of coffee or caffeine and a reduced risk of developing PD — caffeine is a xanthine derivative and non-selective A_1 and A_{2A} AR antagonist.

The present study investigates novel, potent and selective A_1 and A_{2A} AR antagonists for the pharmacological treatment of PD. Most A_1 and A_{2A} AR antagonists are xanthine and non-xanthine derivatives. The xanthine core forms the basis of numerous potent and selective A_1 and A_{2A} AR antagonists, however, these compounds display low water solubility — limiting their *in vivo* application. This encouraged the design, synthesis and evaluation of non-xanthine derivatives, generally amino-substituted heterocyclic compounds. Additionally, the less explored N-free heterocyclic ring systems, such as flavonoids (exhibiting wide-ranging biological activity) — specifically aurones, may be a novel approach to non-xanthine A_1 and A_{2A} AR blockade. Structurally related to aurones are benzylidene tetralones, which also possess relatively good A_1 and/or A_{2A} AR antagonistic activity and selectivity.

Therefore, the current study aimed to gain insight into the importance of structural modifications to ring A and B of the benzylidene tetralone scaffold necessary for A_1 and/or A_{2A} AR affinity in order to identify potential drug candidates for PD treatment.

Acid catalysed aldol condensation reactions were used to synthesise novel benzylidene tetralones. The synthesised compounds were characterised via nuclear magnetic resonance (NMR) spectrometry, mass spectrometry (MS) and melting points. Furthermore, the purities of these compounds were determined by high performance liquid chromatography (HPLC). The A_1 and/or A_{2A} AR affinity of all synthesised compounds were ascertained by means of radioligand binding assays, while GTP shift assays determined selected compounds' functionality as A_1 AR agonists or antagonists.

It was found that C5-OH substitution on ring A of the benzylidene tetralones in combination with *meta* (C3')- and/or *para* (C4')-OH substitution on phenyl ring B of these scaffolds are ideal for A_1 and/or A_{2A} AR affinity. Furthermore, substitution of phenyl ring B of the benzylidene tetralones with a 2-aminopyrimidine ring resulting in moderate to high A_{2A} AR affinity. In general, conversion from fused 6- and 5-membered rings (aurones) to fused 6- and 6-membered rings (2-benzylidene-1-tetralones) in combination with ring B substitutions improved A_1 and A_{2A} AR affinity.

In conclusion, the current study involved the synthesis, characterisation and evaluation of novel 5-substituted 2-benzylidene-1-tetralone analogues to understand the importance of structural modifications to ring A and B of the aurone and 2-benzylidene-1-tetralone scaffold in gaining or even losing A_1 and/or A_{2A} AR affinity. The evaluated compounds are promising novel potent and selective A_1 and/or A_{2A} AR antagonists and, thus, possible lead compounds for the non-dopaminergic treatment of PD.

Key terms:

Parkinson's disease, adenosine receptors, A_1 adenosine receptor antagonists, A_{2A} adenosine receptor antagonists, 2-benzylidene-1-tetralones

OPSOMMING

Parkinson se siekte (PD), ‘n klassieke bewegingsteurnis, is die tweede mees algemene neurologiese toestand na Alzheimer se siekte, met ‘n hoër voorkoms in gevorderde ouderdom — gevolglik, het patiënte met PD ‘n verlaagde lewenskwaliteit en lei dié siekte tot hoë sosio-ekonomiese koste. Die farmakologiese behandeling van PD is op die dopamienergiese stelsel gemik en spreek slegs die motoriese simptome van PD aan en nie die nie-motoriese simptome (soos kognitiewe probleme en depressie) of neurodegenerasie nie. Daarbenewens, word L-3,4-dihidroksiefenielalanien (Levo-dopa/L-dopa) met newe-effekte soos motoriese en nie-motoriese fluktuasies, diskinesie and geneesmiddel-geïnduseerde psigose geassosiëer. Om hierdié redes, word nie-dopamienergiese behandeling — wat beide motoriese en nie-motoriese simptome asook neurodegenerasie aanspreek — benodig. Die manipulasie van adenosienreseptore (AR’s) mag die antwoord op die PD-vraagstuk wees, aangesien ‘n epidemiologiese studie ‘n verwantskap tussen die drink van koffie of kaffeïen en ‘n verlaagde kans op PD gevind het. Kaffeïen is ‘n metielxantien en nie-spesifieke A_1 en A_{2A} AR antagonis.

Die huidige studie ondersoek nuwe, potente en selektiewe A_1 en A_{2A} AR antagoniste vir die behandeling van PD. Die meeste A_1 en A_{2A} AR antagoniste is óf xantien derivate óf nie-xantien derivate (byvoorbeeld amino-gesubstitueerde heterosikliese verbindings). Die xantien kern vorm die hoeksteen van verskeie potente en selektiewe A_1 en A_{2A} AR antagoniste. Ongelukkig is hierdié verbindings swak wateroplosbaar — wat hul *in vivo* gebruik belemmer. Dit het die ontwikkeling van nie-xantien derivate aangemoedig, byvoorbeeld N-vrye heterosikliese ring sisteme, soos flavonoiëde (met ‘n wye reeks biologiese aktiwiteite) — spesifiek aurone, wat ‘n nuwe aanslag op nie-xantien A_1 en A_{2A} AR antagonisme mag wees. Bensielidien tetraloon verbindings is struktureel verwant aan aurone en besit ook relatiewe goeie A_1 en/of A_{2A} AR aktiwiteit en selektiwiteit.

Hierdié studie het dus gepoog om insig ten opsigte van die belangrikheid van strukturele veranderinge aan ring A en ring B van die bensielidien tetraloon verbindings te verkry noodsaaklik vir ‘n wins of verlies aan A_1 en/of A_{2A} AR affiniteit.

Suur gekataliseerde aldol kondensasie reaksies is gebruik om die bensielidien tetraloon verbindings te sintetiseer. Die gesintetiseerde verbindings is deur middel van kern magnetiese resonans (KMR) spektrofotometrie, massaspektrofotometrie (MS) en smeltpunte gekarakteriseer. Verder, is die suiwerheid van hierdié verbindings met behulp van hoë druk vloeistof chromatografie (HPLC) bepaal. Die A_1 en/of A_{2A} AR affiniteit van al die gesintetiseerde verbindings is deur middel van radioligand bindingstudies bepaal, terwyl sekere verbindings se

funksionaliteit as óf ‘n agonis óf ‘n antagonis met behulp van ‘n GTP verskuiwingstudie bepaal is.

Dié studie het bevind dat C5-OH substitusie op ring A van die bensielidien tetraloon verbindings in kombinasie met *meta* (C3’)- en/of *para* (C4’)-OH substitusie op ring B met goeie A_1 en/of A_{2a} affiniteit gepaard gaan. Substitusie van feniel ring B van die bensielidien tetraloon verbindings met ‘n 2-aminopirimidien ring lei tot relatiewe hoë A_{2A} AR affiniteit. Oor die algemeen, het verandering van die saamgevoegde 6- en 5-lid ringe (aurone) na saamgevoegde 6- en 6-lid ringe (2-bensielidien-1-tetralone) in kombinasie met ring B substitusies verbeterde A_1 en A_{2A} AR affiniteit tot gevolg gehad.

Ter samevatting, het die huidige studie die sintese, karakterisering en evaluering van nuwe 5-gesubstitueerde 2-bensielidien-1-tetraloon verbindings behels om sodoende die belangrikheid van strukturele veranderinge aan ring A en ring B van die aurone en 2-benzylidene-1-tetraloon verbindings se invloed op A_1 en/of A_{2A} AR affiniteit te verstaan. Die geëvalueerde verbindings is belowende nuwe, potente en selektiewe A_1 en/of A_{2A} AR antagoniste en, dus, moontlike leierverbindings vir nie-dpamienergiese behandeling van PD.

Sleutelterme:

Parkinson se siekte, adenosienreseptore, A_1 adenosienreseptorantagoniste, A_{2A} adenosienreseptorantagoniste, 2-bensielidien-1-tetraloon

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

A	
AC	adenyl cyclase
AR(‘s)	adenosine receptor(s)/adenosienreseptore
APCI	atmospheric pressure chemical ionisation
C	
cAMP	cyclic adenosine monophosphate
CF3	trifluoromethyl/trifluorometiel
Cl	chlorine/chloor
COMT	catechol-O-methyltransferase
CPA	N6-cyclopentyladenosine
D	
d	doublet
dd	doublet of doublets
DMSO-d6	deuterated dimethylsulfoxide
DOPAC	dihydroxyphenylacetic acid
E	
EDS	excessive daytime somnolence
F	
F	fluorine/fluoor
G	
GABA	γ-aminobutyric acid
GPe	external segment of globus pallidus
GPi	internal segment of globus pallidus
GTP	guanosine triphosphate/guanosientrifosfaat
H	
HPLC	high performance liquid chromatography/hoë druk vloeistof chromatografie

K

K_i	dissociation constant
KMR	kern magnetiese resonans

L

L-dopa	Levo-dopa/L-3,4-dihydroxyphenylalanine
LP	Lewy pathology

M

m	multiplet
MAO	monoamine oxidase
MAO-A	monoamine oxidase type A
MAO-B	monoamine oxidase type B
MCI	mild cognitive impairment
MPP^+	1-methyl-4-phenylpyridium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	mass spectrometry/massaspektrofotometrie

N

N	nitrogen/stikstof
NMDA	N-methyl-D-aspartate
NMR	nuclear magnetic resonance
NSAID's	nonsteroidal anti-inflammatory drugs

O

OCH_3	methoxy/metoksie
OH	hydroxy/hidroksie

P

PD	Parkinson's disease/Parkinson se siekte

R

REM	rapid eye movement

S

s	singlet
SEM	standard error of mean
SI	selectivity index
$Si(CH_3)_4$	tetramethylsilane
SNpc	substantia nigra *pars compacta*
SNr	substantia nigra *pars reticulata*
STN	subthalmic nucleus

T

t	triplet
td	triplet of doublets
TLC	thin layer chromatography

Q

q	quartet
δ	parts per million
[^{3}H]DPCPX	[^{3}H]-8-cyclopentyl-1,3-dipropylxanthine
[^{3}H]NECA	5'-N-[^{3}H]-ethylcarboxamideadenosine

CHAPTER 1

INTRODUCTION

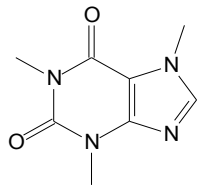
1.1 Background

The neurodegenerative disorder Parkinson's disease (PD) — characterised pathologically by neuronal loss in the nigrostriatal pathway and clinically by motor and non-motor symptoms — is the second-most common neurological condition and affects 2–3% of the population over 65 years of age (Poewe *et al.*, 2017).

Existing treatment for PD is controversial; on the one hand the gold standard of PD treatment, namely L-3,4-dihydroxyphenylalanine (Levo-dopa/L-dopa) — dopamine's precursor — effectively relieves motor symptoms, yet, on the other hand, its adverse effects include motor and non-motor fluctuations, dyskinesias and drug-induced psychosis (Schwarzchild *et al.*, 2006). Additionally, it only elevates the concentrations and effects of dopamine in the brain and, in so doing, does not address non-motor symptoms or neurodegeneration (Kalia & Lang, 2015). Other drugs used for the treatment of PD motor symptoms, also associated with adverse effects, are dopamine agonists, monoamine oxidase B inhibitors, catechol-*O*-methyltransferase inhibitors, anticholinergic drugs and amantadine (Abdel-Salam, 2015).

Justly, a novel drug that addresses all said problems is needed, seeing that non-dopaminergic treatment may possibly improve PD patients' quality of life and lighten the socio-economic burden associated with the disease (Butler, 2010).

The adenosine receptor (AR) antagonists may be the solution to the PD-conundrum; as an epidemiological study has established an association between the consumption of coffee or caffeine and a reduced risk of developing PD (Ross *et al.*, 2000) — caffeine is a xanthine derivative and acts as a non-selective A_1 and A_{2A} AR antagonist (Van der Walt & Terre'Blanche, 2015).



Caffeine
A_1K_i = 43.9 µM; $A_{2A}K_i$ = 47.2 µM

Adenosine has widespread effects in the human body. In the brain, specifically, it is a neuromodulator in charge of various neurotransmitters, receptors and signalling pathways

(Chen *et al.*, 2014). It acts through inhibitory (A_1 and A_3) or stimulatory (A_{2A} and A_{2B}) G-protein coupled receptors (Palmer & Stiles, 1995). Also, co-expression of AR's with each other (e.g. A_1/A_{2A}) or neurotransmitter receptors (e.g. A_{2A}/D_2) occur (Stockwell *et al.*, 2017). A_1 AR's are greatly expressed in the cortex and hippocampus and A_{2A} AR's in the basal ganglia, whereas A_{2B} and A_3 AR's show low brain expression (Stehle *et al.*, 1992). Consequently, the A_1 and A_{2A} AR's are associated with normal and abnormal brain function (Wei *et al.*, 2011), ranging from brain processes such as cognition (A_1), locomotion (A_{2A}), behaviour (A_{2A}) and neurodegeneration (A_{2A}) to PD (A_1 & A_{2A}) (Chen *et al.*, 2014).

As a result, A_1 and A_{2A} AR's are targets for the non-dopaminergic pharmacological treatment of PD and a dual A_1/A_{2A} AR antagonist may well attend to motor symptoms and non-motor symptoms (e.g. cognitive deficits and depression) of PD, as well as neurodegeneration.

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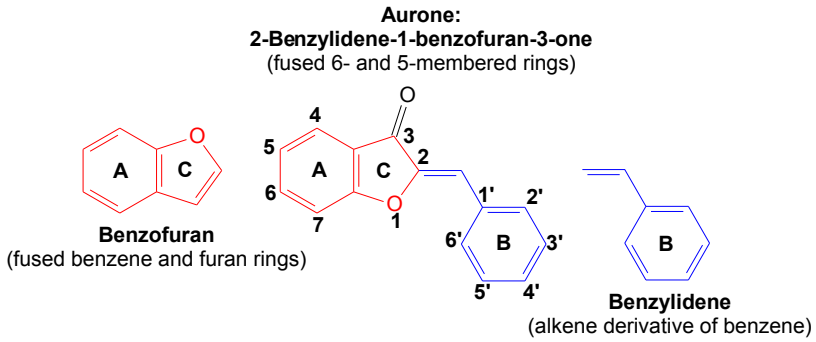
The present Pharmaceutical Chemistry study investigates novel, potent and selective A_1 and A_{2A} AR antagonists for the potential pharmacological treatment of PD. Firstly, this chapter provides the background, rationale, hypothesis and aims and objectives of the current research. Secondly, Chapter 2 and Chapter 3 appropriately contain a brief literature review of PD and AR's. The literature review of PD describes the epidemiology, clinical features, pathological features, etiology, pathogenesis and current pharmacological treatment of the disease, while the literature review of AR's describes the role A_1 and A_{2A} AR's, as well as their antagonists, play in PD. Thirdly, the findings are presented as a research article, provided in Chapter 4. The goals of the research article are to synthesise and evaluate 5-substituted 2-benzylidene-1-tetralones as A_1 and/or A_{2A} AR antagonists for the treatment of neurological conditions, such as PD. Lastly, Chapter 6 summarises the present study and suggests future research.

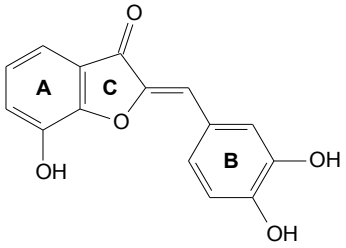
1.2 Rationale

Most A_1 and A_{2A} AR antagonists may be divided into xanthine and non-xanthine derivatives. The xanthine core forms the basis of numerous potent and selective A_1 and A_{2A} AR antagonists (Yuzlenko & Kieć-Kononowicz, 2006), however, these compounds display low water solubility — limiting their *in vivo* application (Müller *et al.*, 2002). This encouraged the design, synthesis and evaluation of non-xanthine derivatives, generally amino-substituted heterocyclic compounds (Yuzlenko & Kieć-Kononowicz, 2006). Additionally, the less explored N-free heterocyclic ring systems, such as flavonoids (exhibiting wide-ranging biological activity) — specifically aurones, may be a novel approach to non-xanthine A_1 and A_{2A} AR blockade (Jacobson *et al.*, 2002; Zwergel *et al.*, 2011).

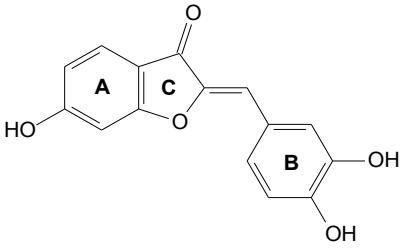
Aurones — a flavonoid subclass (Zwergel *et al.*, 2011) — are heterocyclic compounds containing fused 6- and 5-membered rings, possessing either *(E)*- or *(Z)*-configuration (Jacobson *et al.*, 2002). These compounds comprise of a benzofuran-like backbone and a benzylidene side-chain.



Hispidol is an aurone derivative that exists in the (*E*)-configuration and was found to be a selective A_1 AR antagonist with a dissociation constant (K_i) value of 0.352 μM in a radioligand binding assay of rat AR's (Jacobson *et al.*, 2002). Maritimetin, another aurone derivative, possess affinity for both the A_1 (A_1K_i = 3.47 μM) and A_{2A} ($A_{2A}K_i$ = 9.35 μM)) AR's (Jacobson *et al.*, 2002).



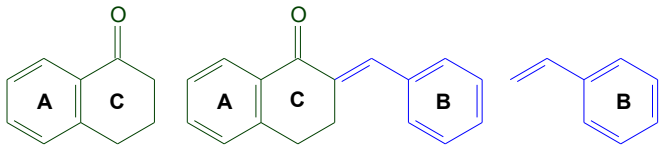
Hispidol
A_1K_i = 0.352 μM



Maritimetin
A_1K_i = 3.47 μM; $A_{2A}K_i$ = 9.35 μM

Benzylidene tetralones, structurally related to aurones, also possess A_1 and A_{2A} AR affinity. Demonstrating the aforementioned is (*E*)-2-benzylidene-5-hydroxy-1-tetralone which exhibits affinity for the A_1 and A_{2A} AR's (A_1K_i = 6 μM; $A_{2A}K_i$ = 3 μM), with a selectivity index of 2 towards the A_{2A} AR (Legoabe *et al.*, 2017). This compound has a basic benzylidene tetralone backbone (fused 6- and 6-membered rings, namely ring A and ring C), where ring C bears a C2-phenyl substituted side-chain (ring B).

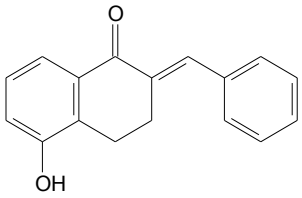
Benzylidene tetralone:
2-Benzylidene-1-tetralone
(fused 6- and 6-membered rings)



1-Tetralone
(ketone derivative of tetralin)

Benzylidene
(alkene derivative of benzene)

It was found that C5-OH substitution on ring A is ideal for A_1 and A_{2A} AR affinity, whereas C6- or C7-OH substitution on ring A favours only A_1 AR binding. Interestingly, *para* (4')-OH substitution on ring B in combination with C6- or C7-OH substitution lead to compounds with both A_1 and A_{2A} AR affinity. Modifications to ring A also showed that C6- or C7-OH substitution is preferred over C6- and C7-OCH_3 substitution for A_1 AR affinity, moreover, C6- and C7-OCH_3 substitution diminished A_{2A} AR affinity.



(*E*)-2-Benzylidene-5-hydroxy-1-tetralone
A_1K_i = 5.93 μM; $A_{2A}K_i$ = 2.90 μM

Based on the above, the aurone derivatives hispidol and maritimetin and the benzylidene tetralone (*E*)-2-benzylidene-5-hydroxy-1-tetralone may be used to design a scaffold for novel and potent A_1 and A_{2A} AR antagonists, such as 5-substituted 2-benzylidene-1-tetralones. The 2-benzylidene-1-tetralone parent scaffold will be structurally modified to include changes to ring A and B. Firstly, C5-OH or -OCH_3 substitution on ring A will be made. Secondly, substitution at *meta* (3') and/or *para* (4') position(s) of ring B with polar and non-polar groups, such as halogens (Cl and F), CF_3-, OH- and OCH_3-groups will be investigated. Additionally, phenyl ring B will be replaced with a pyridine ring where the N is at position 3 or 4 and a 2–amino–pyrimidine ring — seeing that the 2–aminopyrimidine moiety is associated with compounds possessing good AR activity (Shook *et al.*, 2012). Accordingly, the 5-substituted 2-benzylidene-1-tetralone analogues will be evaluated to identify structural features essential for dual A_1/A_{2A} AR affinity. (**Figure 1-1**)

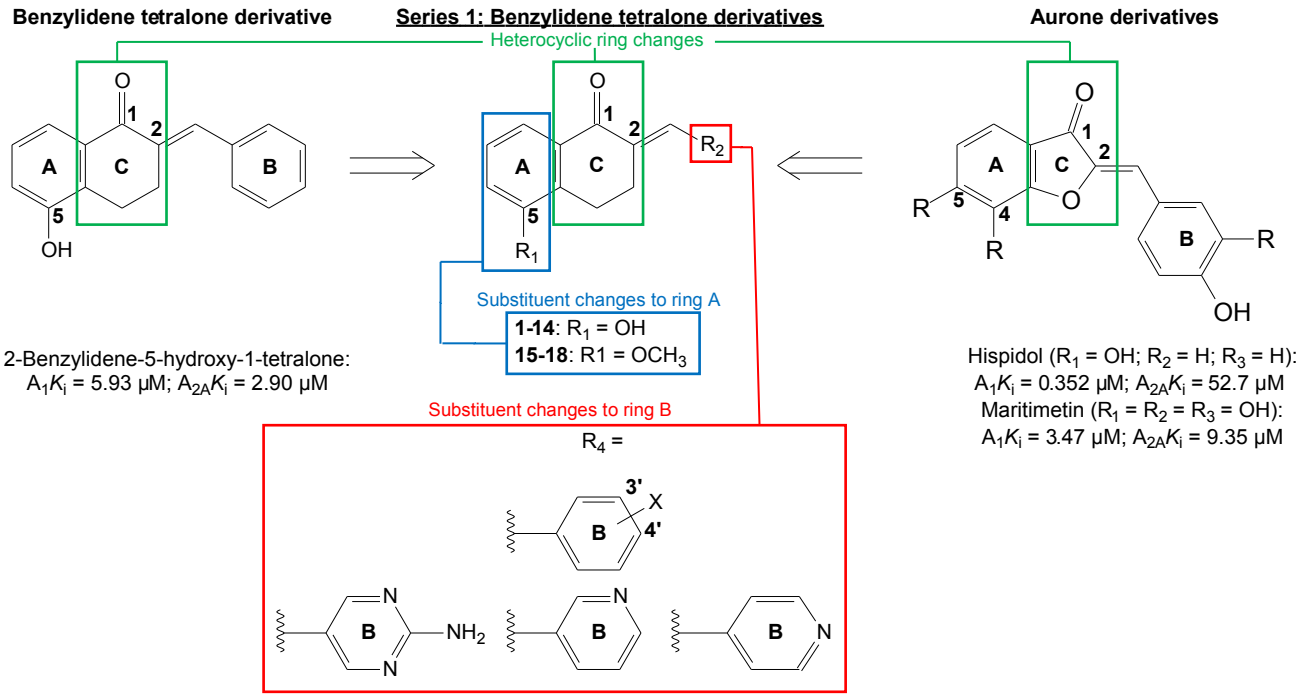


Figure 1-1: Structural and heterocyclic ring changes to hispidol, maritimetin and (*E*)-2-benzylidene-5-hydroxy-1-tetralone to determine features essential for dual A_1/A_{2A} AR antagonistic activity.

1.3 Hypothesis

Since the aurones hispidol and maritimetin and the benzylidene tetralone (*E*)-2-benzylidene-5-hydroxy-1-tetralone exhibit relatively good A_1 and/or A_{2A} AR affinity, it is hypothesised that substituent changes to ring A and B and heterocyclic changes to ring C of these compounds, yielding various benzylidene tetralones might increase A_1 and A_{2A} AR affinity of these compounds and reveal structure activity relationships that govern AR activity.

1.4 Aim and objectives

The focus of this dissertation is the design, synthesis, characterisation and evaluation of novel, potent and selective A_1 and/or A_{2A} AR antagonists for the potential treatment of neurological conditions, such as PD. Accordingly, the aim of this study is to gain insight into the importance of structural modifications to ring A and B of the benzylidene tetralone scaffold necessary for A_1 and/or A_{2A} AR affinity in order to identify potential drug candidates for PD treatment.

In short, the objectives of this study are:

- The design of novel benzylidene tetralones as A_1 and/or A_{2A} AR antagonists, drawing from the aurones hispidol and maritimetin and the benzylidene tetralone (*E*)-2-benzylidene-5-hydroxy-1-tetralone.

- The synthesis of proposed benzylidene tetralones via acid catalysed aldol condensation reactions.
- The characterisation of the synthesised benzylidene tetralones with proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectrometry, mass spectrometry (MS) and melting points.
- Purity determination of the synthesised benzylidene tetralones by high performance liquid chromatography (HPLC).
- The *in vitro* evaluation, by means of radioligand binding assays, of the synthesised benzylidene tetralones A_1 and/or A_{2A} AR antagonists.
- Functional characterisation of selected benzylidene tetralones as A_1 AR agonists or antagonists via a GTP shift assay.
- To ascertain structure activity relationships of benzylidene tetralone based derivatives essential for A_1 and A_{2A} AR affinity.
- Publication of study as a research article in an academic journal.

CHAPTER 2

PARKINSON'S DISEASE

2.1 Introduction

James Parkinson wrote "An Essay on the Shaking Palsy" in 1817; in this pioneering monograph Parkinson described the "tedious and most distressing malady" that would later bear his name. Two centuries later our comprehension of PD continues to change. Sensibly Parkinson wrote; "Until we are better informed respecting the nature of this disease, the employment of internal medicine is scarcely warrantable..." (Parkinson, 2002).

PD is a common, but complex, neurodegenerative disorder characterised by motor features associated with deterioration of the nigrostriatal pathway and Lewy pathology (Kalia & Lang, 2015). Bradykinesia, rigidity, resting tremor and postural instability are the four cardinal signs of PD (Gibb & Lees, 1988). Although motor symptoms generally define PD, non-motor symptoms should not be dismissed. Non-motor symptoms range from dribbling saliva, constipation, depression, sleep disorders, apathy, hallucinations and dementia (Chaudhuri *et al.*, 2005). Therefore symptomatology and pathology of PD are diverse. The etiology of PD is unknown, but age or aging (Pringsheim *et al.*, 2014), genetics and/or environmental factors (Noyce *et al.*, 2012) might be part of the cause. Pathogenic mechanisms are interactive, as no one mechanism of neurodegeneration is critical for the development of PD (Olanow, 2007). Current treatment of PD is symptomatic and consists of drugs that restore dopamine concentrations and/or effects (Kalia & Lang, 2015).

Parkinson hoped that "some remedial process may ere long be discovered, by which, at least, the progress of the disease may be stopped" (Parkinson, 2002). Unfortunately, an ideal drug that treats motor symptoms, non-motor symptoms and is neuroprotective has not been discovered. Nevertheless PD "ought not to be considered as one against which there exists no countervailing remedy" (Parkinson, 2002).

2.2 Epidemiology

It is estimated that between 4.1 and 4.6 million individuals over age 50 had PD in 2005 and between 8.7 and 9.3 million individuals will have PD by 2030 (Dorsey *et al.*, 2007), making PD the most common neurological disorder after Alzheimer's disease (Alzheimer's Association, 2015). Prevalence of PD appears higher in Europe (Von Campenhausen *et al.*, 2005), North America (Strickland & Bertoni, 2004) and South America (Bauso *et a.l*, 2012) compared to Africa (Okubadejo *et al.*, 2006), Asia (Muangpaisan *et al.*, 2009) and Arabic countries (Benamer

et al., 2008). Incidence varies by ethnicity (Hispanic > non-Hispanic Whites > Asians > Blacks), gender (men > women) and age (> 60 years) (Van Den Eeden *et al.*, 2003).

2.3 Clinical features

PD is a neurological disorder with both motor and non-motor features (Kalia & Lang, 2015). The classical motor symptoms of PD are bradykinesia (slowness of movement), rigidity (resistance to movement), resting tremor (rhythmic back and forth motion of thumb and forefinger at three beats per second, "pill rolling") and postural instability (impaired balance and coordination) (Gibb & Lees, 1988), which often present in an asymmetric fashion (Münchau & Bhatia, 2000) (**Figure 2-1**.). In contrast to motor symptoms, non-motor symptoms are under-recognized and undertreated (Shulman *et* al., 2002). Non-motor features include neuropsychiatric symptoms (depression, mild cognitive impairment (MCI) and dementia), sleep disorders (Rapid Eye Movement (REM) sleep behaviour disorder and excessive daytime somnolence (EDS)), autonomic symptoms (urinary symptoms, orthostatic hypotension and constipation), sensory symptoms (pain and hyposmia), and other symptoms such as fatigue, diplopia, blurred vision, seborrhoea and weight loss (Chaudhuri *et al.*, 2005) (**Figure 2-1**). A decline in health-related quality of life is associated with non-motor symptoms (Duncan *et al.*, 2014).

The UK Parkinson's Disease Society Brain Bank criteria are used to diagnose PD (Gibb & Lees, 1988). Diagnosis follows the onset of motor symptoms (bradykinesia plus rigidity and resting tremor, postural instability is characteristic of more advanced PD) (**Figure 2-1: Motor, Time 0 years to 20 years**). Yet the motor features may be preceded by a pre-motor or prodromal phase characterised by non-motor features (Postuma *et al.*, 2012) (**Figure 2-1: Non-motor, Time -20 years to 0 years**). As PD progresses additional non-motor features develop, causing disability (Hely *et al.*, 2005; Hely *et al.*, 2008) (**Figure 2-1: Non-motor, Time 0 years to 20 years**). Adverse effects of dopaminergic treatment such as motor fluctuations ("wearing-off phenomenon" and "on-off phenomenon"), dyskinesia and drug-induced psychosis also contribute to disability (Hely *et al.*, 2005) (**Figure 2-1: Complications, Time 5 years to 20 years**).

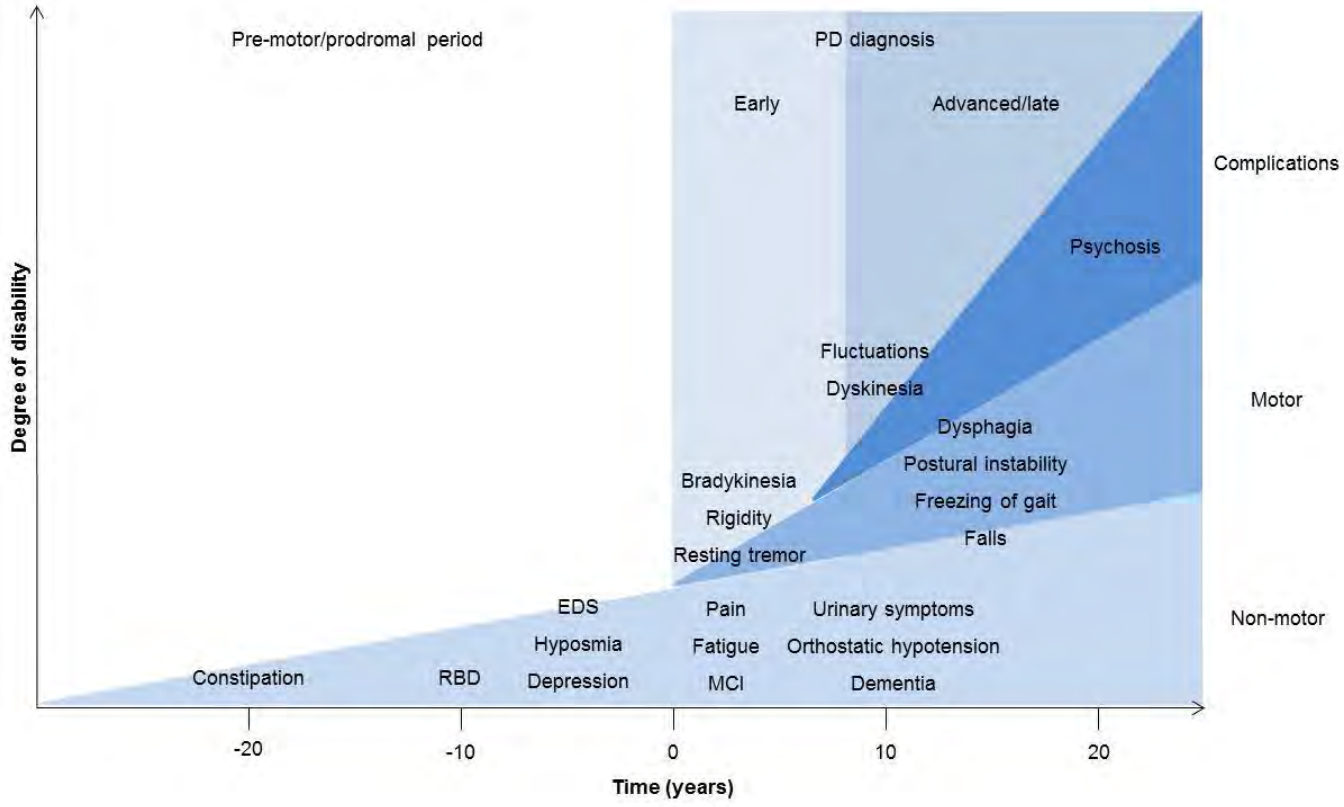


Figure 2-1: The clinical features, complications of dopaminergic treatment, and time course of PD. Adapted from Kalia & Lang (2015) and reproduced with permission from Elsevier.

2.4 Pathological features

The central pathological feature of PD is neuronal loss in the nigrostriatal pathway (Ehringer & Hornykiewicz, 1998). In the nigrostriatal pathway, dopaminergic neurons project from the substantia nigra *pars compacta* (SNpc) to the basal ganglia and synapse in the caudate and putamen of the striatum to generate purposeful movement (Knierim, 1997.). The loss of pigmented dopaminergic neurons within the SNpc and the subsequent decrease of dopamine in the striatum (putamen > caudate) are responsible for the motor features of PD (Dexter & Jenner, 2013). At the onset of motor symptoms approximately 60% of dopaminergic neurons within the SNpc are lost and dopamine in the putamen is depleted by about 80% (Dauer & Przedborski, 2003). Depigmentation of the SNpc follows the loss of pigmented dopaminergic neurons (Dickson *et al.*, 2009) (**Figure 2-2**). Neuronal loss is not restricted to the SNpc and affects other non-dopaminergic nuclei (Dickson, 2012). Non-dopaminergic degeneration may be the cause of non-motor features of PD (for example, MCI and/or autonomic symptoms (Schapira, 2008).

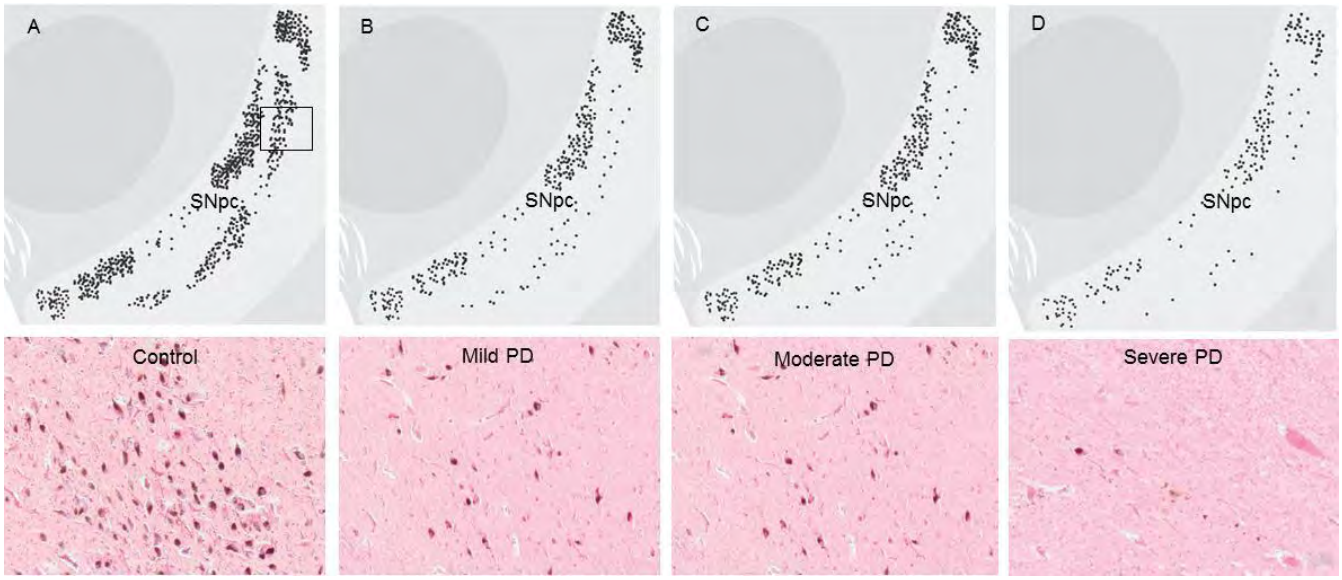


Figure 2-2: **The density of pigmented dopaminergic neurons within the SNpc. Top images show the distribution of pigmented neurons in healthy controls (A) and in patients with PD with mild (B), moderate (C) or severe (D) loss of pigmented dopaminergic neurons. The severity of depigmentation in PD is not homogenous and should be primarily assessed in the ventral and lateral regions of the SNpc (boxed area in A), to correlate with the severity of motor symptoms. Bottom images show the density of pigmented neurons in this region from actual cases. Adapted from Dickson and co-workers (2009) and reproduced with permission from Elsevier.**

Another pathological feature of PD is Lewy pathology (LP) (Kalia & Lang, 2015). Aggregates of insoluble misfolded proteins (for example, α-synuclein, parkin, ubiquitin and/or neurofilaments) form intracellular inclusions in cell bodies (Lewy bodies) and processes (Lewy neurites) of neurons in the brain (Spillantini *et al.,* 1997) (**Figure 2-3**). LP can also be found in the spinal cord and peripheral nervous system (Iwanaga *et al.*, 1999; Fumimura *et al.*, 2007; Beach *et al.*, 2010; Del Tredici *et al.*, 2010). Several non-motor features may be attributed to LP (Samii *et al.*, 2004), for example, hyposmia is associated with the presence of LP in the olfactory bulb and brain centers such as the amygdala and perirhinal nucleus (Witt *et al.*, 2009). The role LP plays in neurodegeneration is controversial (Dauer & Przedborski, 2003).

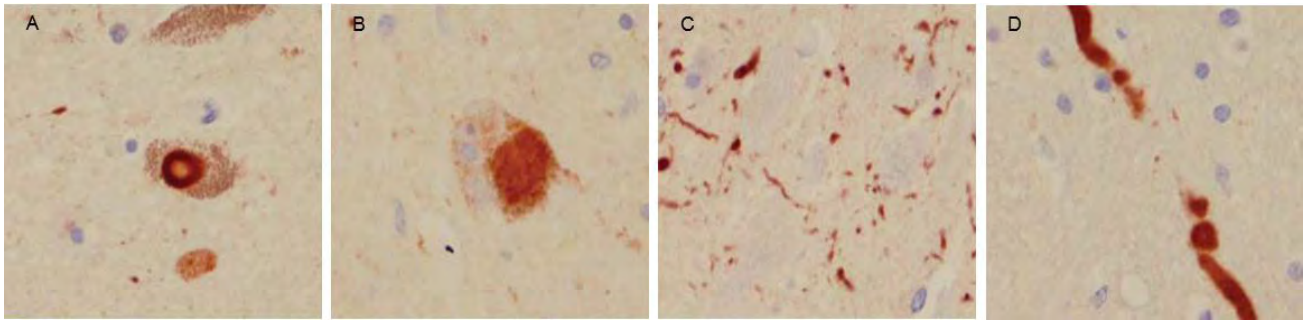


Figure 2-3: Microscopic findings in PD with α-synuclein immunohistochemistry. A typical brainstem type Lewy body (A), a pale staining "cortical type" Lewy body (B), Lewy neurites in CA2 sector of hippocampus (C) and intraneuritic Lewy bodies in medulla. Adapted from Dickson (2012) and reproduced with permission from Cold Spring Harbor Laboratory Press.

Neuroinflammation is an additional pathological feature of PD (Kalia & Lang, 2015). An active inflammatory response mediated by astrocytes and microglia in the brain is present in PD (Tansey & Goldberg, 2010). Reactive gliosis and microgliosis (from activated astrocytes and microglia, respectively) are associated with areas of neurodegeneration in PD (Phani *et al.*, 2012). Whether neuroinflammation promotes or protects against neurodegeneration is unknown (Kalia & Lang, 2015).

2.5 Etiology

The biochemical deficiency in PD is well known, however, the cause of cell death is unknown (Wu en Frucht, 2005). Chronological age or the aging process is a risk factor for the development of PD as prevalence and incidence of the disease increase with age (Pringsheim *et al.*, 2014) and peaks after 80 years of age (Driver *et al.*, 2009). Gene mutations of PARK1 (α-synuclein), PARK2 (parkin) and PARK8 (leucine-rich repeat kinase 2) are associated with inherited PD (Bezard & Przedborski, 2011); implicating genetics in the etiology of PD. Environmental factors may increase the possibility of developing PD (pesticide exposure > prior head injury > rural living > β-blocker use > agricultural occupation > well water drinking) (Noyce *et al.*, 2012). Interestingly, the following factors are speculated to decrease the development of PD: tobacco smoking > caffeine > nonsteroidal anti-inflammatory drugs > calcium channel blockers > alcohol (Noyce *et al.*, 2012). The risk of developing PD is clearly multifactorial, but the intricate interaction between age or aging, genetics and environmental factors are just beginning to be deciphered (Kalia & Lang, 2015).

2.6 Pathogenesis and/or mechanism of neurodegeneration

While the exact pathogenesis of PD is unknown, mechanisms of neurodegeneration may be attributed to oxidative stress, mitochondrial dysfunction, altered proteolysis, inflammation, excitotoxicity and apoptosis (Dexter & Jenner, 2013). The aforementioned mechanisms are interactive (**Figure 2-4**); no one pathogenic mechanism is critical for the development of PD and the pattern of cell death could differ from patient to patient (Olanow, 2007). Indeed, agents that cause oxidative stress can damage mitochondria and proteasomes and, in turn, mitochondrial dysfunction can lead to oxidative stress and altered proteolysis (Okada *et al.*, 1999; Ding & Keller, 2001; Jha *et al.*, 2002; Hoglinger *et al.*, 2003; Shamoto-Nagai *et al.*, 2003). Similarly, inhibition of proteasomal and lysosomal function can cause oxidative stress (Kikuchi *et al.*, 2003), mitochondrial dysfunction (Okada *et al.*, 1999; Kikuchi *et al.*, 2003; Sullivan *et al.*, 2004), inflammation (Rockwell *et al.*, 2000) and apoptosis (Jesenberger & Jentsch, 2002). Furthermore, oxidative stress and altered proteolysis can act synergistically to promote protein misfolding (Okada *et al.*, 1999; Mytilineou *et al.*, 2004).

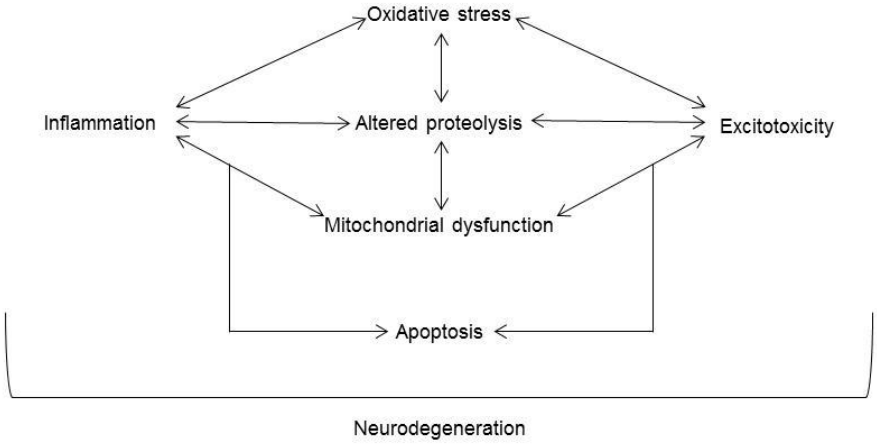


Figure 2-4: Key pathogenic mechanisms that could contribute to neurodegeneration in PD. Adapted from Olanow (2007) and reproduced with permission from John Wiley and Sons.

2.7 Pharmacological treatment

Existing treatment for PD focuses on the dopaminergic system since the motor features of PD are caused by a loss of dopaminergic neurons in the nigrostriatal pathway (AlDakheel *et al.*, 2014). Symptomatic treatment is effective in early PD, however, it is associated with motor and non-motor fluctuations, dyskinesias and drug-induced psychosis (Hely *et al.*, 2005; AlDakheel *et al.*, 2014). Moreover, non-dopaminergic features develop and dominate advanced PD, resulting in treatment-resistant disability (AlDakheel *et al.*, 2014). Non-dopaminergic treatment for motor and non-motor symptoms is in demand and neuroprotective treatment will prevent the

debilitating complications of advanced PD, while alleviation of motor and non-motor symptoms by non-dopaminergic treatment will increase health-related quality of life (Kalia & Lang, 2015).

2.7.1 Drugs for neuroprotection

Neuroprotection is multifaceted (Schapira, 2008); it entails interventions that influence PD pathogenesis and/or mechanisms of neurodegeneration (See **Figure 2-5**) and, in so doing, prevents neuronal loss in the nigrostriatal pathway, ending or decreasing disease progression (AlDakheel *et al.*, 2014). As of yet, no drug convincingly stops or, at least, slows neuronal loss in the nigrostriatal pathway of PD patients (Münchau & Bhatia, 2000).

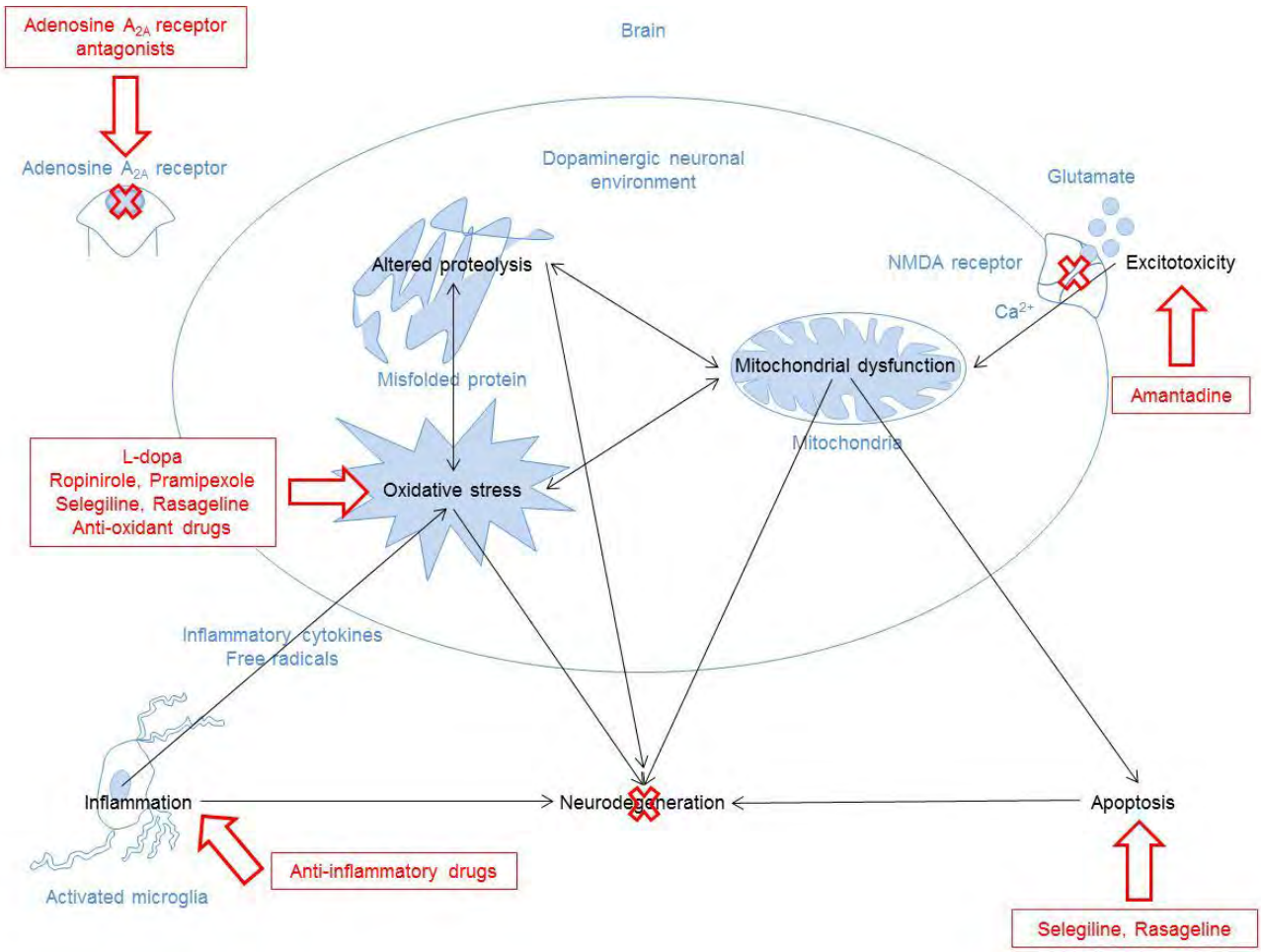


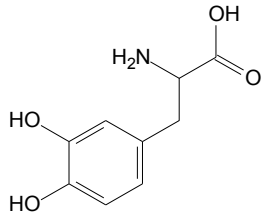
Figure 2-5: Possible neuroprotective therapy for PD that influence PD pathogenesis and/or mechanisms of neurodegeneration.

2.7.1.1 Dopaminergic drugs

Although developed for the symptomatic treatment of PD, drugs with dopaminergic properties may well be neuroprotective (LeWitt & Taylor, 2008).

2.7.1.1.1 L-3,4-dihydroxyphenylalanine

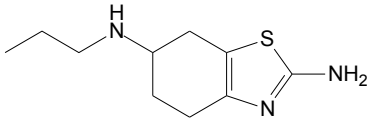
The effect of L-dopa on dopaminergic neurons in the nigrostriatal pathway of PD patients is debatable (AlDakheel *et al.*, 2014). On the one hand L-dopa may advance neurodegeneration through oxidative metabolites after dopamine metabolism (Fahn, 1996) and on the other hand, animal models have demonstrated the neuroprotective effects of L-dopa (Murer *et al.*, 1998; Datla *et al.*, 2001). L-dopa can act as a pro-oxidant or an anti-oxidant depending on concentration; low concentrations improves production of protective molecules and high concentrations cause toxicity in culture models (Schapira, 2010).



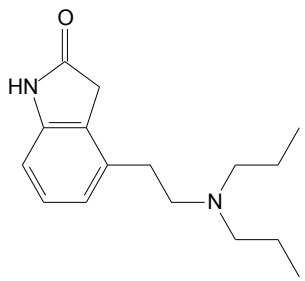
L-dopa

2.7.1.1.2 Dopamine agonists

Dopamine agonists ropinirole and pramipexole may well protect dopaminergic neurons from degeneration (Olanow *et al.*, 1998). Studies show that dopamine agonists protect dopaminergic neuronal function in toxin model systems; pramipexole reduces toxicity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridium (MPP^+), rotenone, and 6-hydroxydopamine, and both pramipexole and ropinirole delay the rate of cell loss (Schapira, 2002). In theory, dopamine agonists may be neuroprotective by means of a L-dopa sparing effect, stimulation of dopamine autoreceptors resulting in decreased dopamine synthesis, release and metabolism, direct anti-oxidant effects and restoration of dopaminergic tone to the dopamine-denervated brain to restore inhibition to the subthalamic nucleus and thereby diminish subthalamic nucleus-mediated excitotoxicity (Olanow *et al.*, 1998).



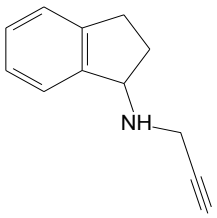
Pramipexole



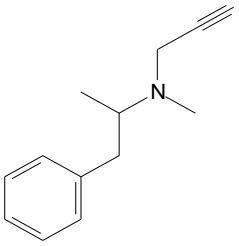
Ropinirole

2.7.1.2 Monoamine oxidase B inhibitors

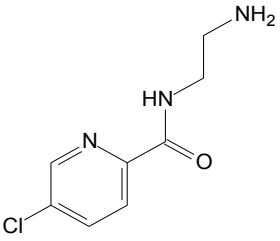
The oxidative deamination of monoamine neurotransmitters, neuromodulators and exogenous bioactive monoamines is catalysed by monoamine oxidase (MAO) (Mandel *et al.*, 2003). Two types of MAO exist; MAO type A (MAO-A) and MAO type B (MAO-B) (Mandel *et al.*, 2003). MAO-B is predominant in the striatum and metabolises dopamine in the brain (Goldenberg, 2008). The irreversible MAO-B inhibitors selegiline and rasagiline inhibit MAO-B. Selegiline and rasagiline are prescribed as monotherapy in early PD and as adjunctive therapy in late PD (Lew *et al.*, 2010; Mizuno *et al.*, 2010; Reichman & Jost, 2010), additionally, these drugs may be neuroprotective (AlDakheel *et al.*, 2014). In all probability neuroprotection by selegiline is multifold; firstly it may offer protection against free radicals (Chiueh *et al.*, 1992), neurotoxins (Chiba *et al.*, 1984) and apoptosis (Maruyama & Naoi, 1999) and secondly, it may affect neurotrophic factors (Mizuta *et al.*, 2000). Rasagiline, a compound similar in structure to selegiline, is a more potent MAO-B inhibitor (Youdim *et al.*, 2001) and conceivably neuroprotective due to its propargyl moiety, and not its MAO-B inhibition properties (Youdim & Weinstock, 2002; Maruyama *et al.*, 2002). The aminoindan metabolite of rasagiline could confer additional neuroprotection (Bar-*Am et al.*, 2010). Lazabemide is a reversible highly selective MAO-B inhibitor which might be neuroprotective, however, it causes severe liver toxicity (Teo & Ho, 2013).



Rasagiline



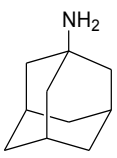
Selegiline



Lazabemide

2.7.1.3 Amantadine

Neuroprotection may be facilitated by amantadine - an antiviral drug and N-methyl-D-aspartate glutamate (NMDA) receptor antagonist - by reducing the release of pro-inflammatory factors from activated microglia, and/or an increase in expression of neurotrophic factors (Kim *et al.*, 2012).



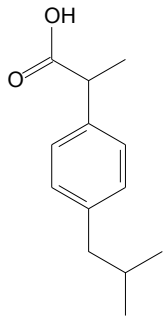
Amantadine

2.7.1.4 Anti-oxidant drugs

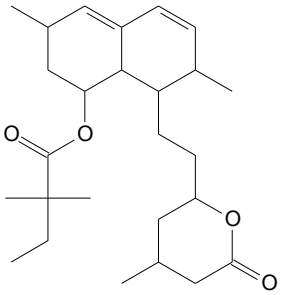
Oxidative stress - the result of increased reactive free radicals that occur either because of an overproduction of these free radicals or a failure of mechanisms that limit their accumulation - plays an evident role in the pathogenesis and/or mechanism of neurodegeneration in PD (AlDakheel *et al.*, 2014). Reduction of oxidative stress with anti-oxidant drugs such as selegiline, rasagiline, and vitamin E (α-tocopherol) has not convincingly demonstrated neuroprotection (Athauda & Foltynie, 2015). Several clinical trials have been or are currently being conducted using anti-oxidants, such as N-acetylcysteine, glutathione, inosine, mitoquinone, zonisamide, co-enzyme Q-10 and green tea polyphenol (AlDakheel *et al.*, 2014).

2.7.1.5 Anti-inflammatory drugs

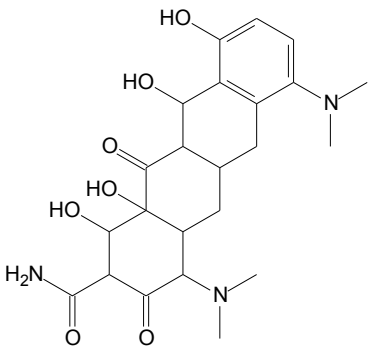
Neuroprotection by nonsteroidal anti-inflammatory drugs (NSAID's) such as aspirin, ibuprofen, meclofenamic acid, sulindac sulfide and ketoprofen is controversial; contradicting results were obtained from studies regarding neuroprotection and NSAID's (AlDakheel *et al.*, 2014). An epidemiological study found that NSAID's reduce the risk of developing PD by 45% (Chen *et al.*, 2003), yet the same researchers later found that only ibuprofen had this effect (Chen *et al.*, 2005). Another line of attack may be via statins, for example simvastatin. Besides lowering cholesterol, statins also possess anti-inflammatory properties (Selley, 2004). The tetracycline derivative minocycline may be neuroprotective by anti-inflammatory and anti-apoptotic mechanisms of action (NINDS NET-PD Investigators, 2006). Theoretically, pioglitazone – a proliferator activated receptor-γ agonist – diminish pro-inflammatory cytokines by destructive activated microglia and spare favourable activated microglia (Simuni *et al.*, 2015).



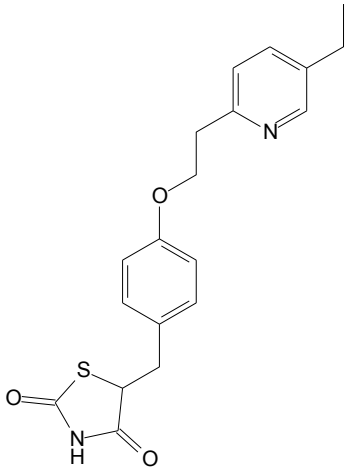
Ibuprofen



Simvastatin



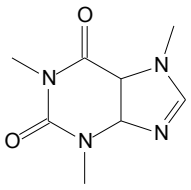
Minocycline



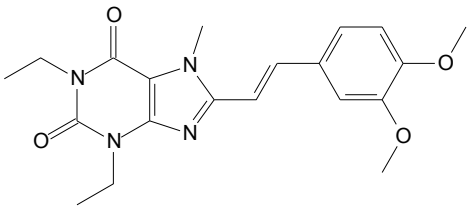
Pioglitazone

2.7.1.6 A_{2A} adenosine receptor antagonists

A_{2A} AR's have been identified as a drug target for neuroprotection in PD (Richardson *et al.*, 1997; Mihara *et al.*, 2007). An epidemiological study has established an association between the consumption of coffee or caffeine and a reduced risk of developing PD — caffeine is a xanthine derivative and non-selective A_1 and A_{2A} AR antagonist (Ross *et al.*, 2000). Furthermore, caffeine has demonstrated protection against neurotoxicity and deterioration of dopaminergic neurons in a mouse MPTP neurotoxin model of PD (Chen *et al.*, 2001). Thus, a selective A_{2A} AR antagonist like KW-6002 (istradefylline) might protect dopaminergic neurons from deterioration and exhibit neuroprotective properties (Chen *et al.*, 2001). A detailed discussion of AR's and their antagonists follows in Chapter 3.



Caffeine



KW-6002

2.7.2 Drugs for symptomatic treatment

Current treatment of PD is symptomatic and consists of drugs that restore dopamine concentrations and/or effects (Kalia & Lang, 2015) (**Figure 2-6**). Symptomatic treatment of PD improves function and quality of life of PD patients (Connolly & Lang, 2014).

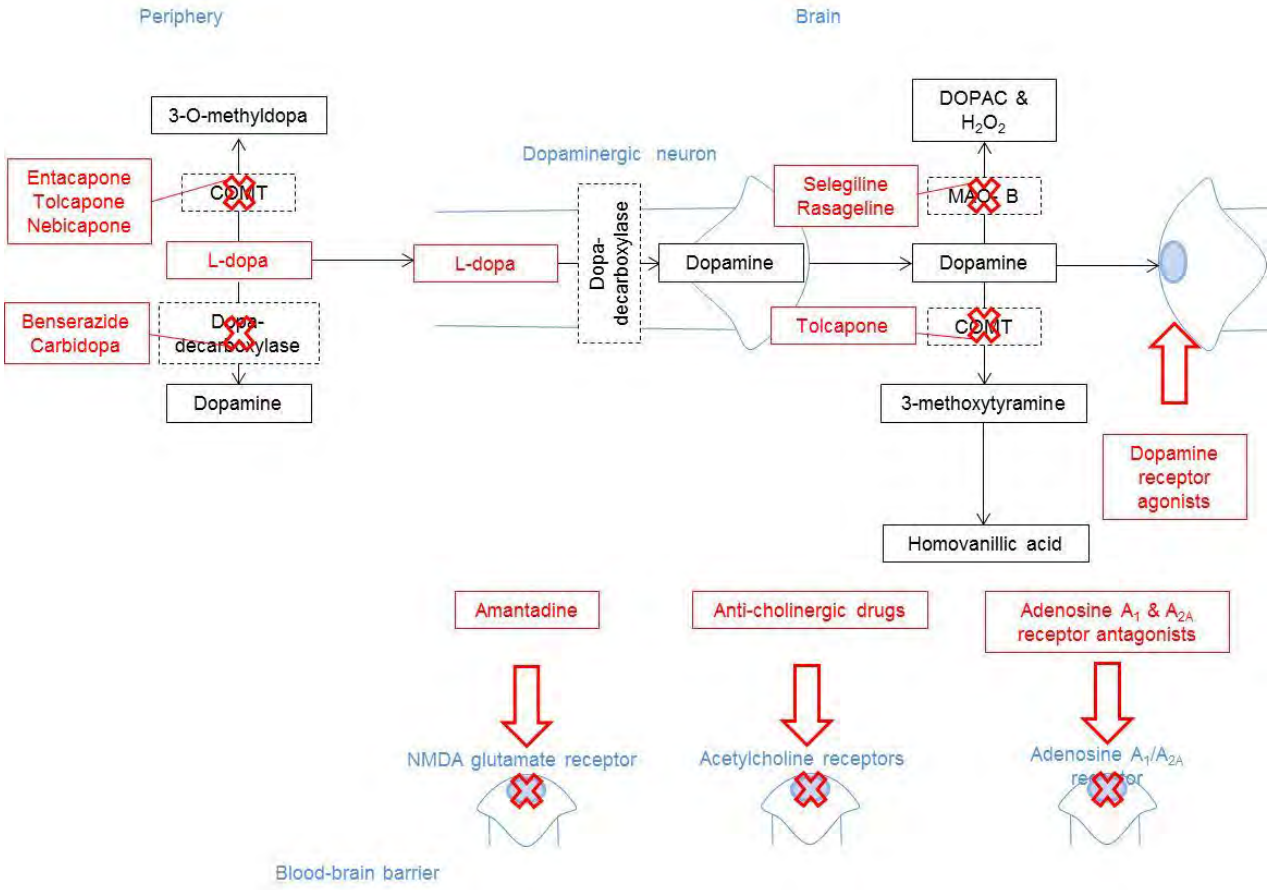


Figure 2-6: Symptomatic treatment of PD.

2.7.2.1 Motor symptoms

2.7.2.1.1 L-3,4-dihydroxyphenylalanine (in combination with benserazide or carbidopa)

L-dopa, dopamine's immediate precursor, is considered the most effective drug for the treatment of the motor symptoms of PD (Calne, 1993). Though L-dopa provides the greatest symptomatic relief, its adverse effects include motor complications ("wearing-off phenomenon" and "on-off phenomenon"), non-motor complications, dyskinesia and drug-induced psychosis (Cotzias *et al.*, 1969). L-dopa is generally combined with benserazide or carbidopa; aromatic amino acid decarboxylases inhibitors which do not cross the blood-brain barrier but prevent the conversion of L-dopa to dopamine peripherally (Münchau & Bhatia, 2000). Consequently, adverse effects are minimised, central delivery improved and the dosage of L-dopa can be reduced (Soares-da-Silva *et al.*, 1997). Other than L-dopa's adverse effects, a concern that L-dopa is neurotoxic exist - as L-dopa is metabolised to toxic metabolites and free radicals, both possible mechanisms of neurodegeneration in PD (Graham, 1978; Basma *et al.*, 1995).

2.7.2.1.2 Dopamine agonists

Drugs in this class act directly on dopamine receptors; imitating the endogenous neurotransmitter, dopamine (Münchau & Bhatia, 2000; Abdel-Salam, 2015). Dopamine agonists are categorized as ergot-derivatives (bromocriptine, cabergoline, lisuride and pergolide) and non-ergolines (apomorphine, pramipexole and ropinirole) (Münchau & Bhatia, 2000; Abdel-Salam, 2015). In practice, dopamine agonists are prescribed as monotherapy in younger patients (Abdel-Salam, 2015) and adjunctive therapy to L-dopa in patients with motor complications (Pezzoli *et al.*, 1995; Nohria & Partiot, 1997). Dopamine agonists, compared to L-dopa, have various advantages. Firstly, dopamine agonists delay or diminish dyskinesias and motor complications due to L-dopa (Stowe *et al.*, 2008), secondly dopamine agonists are not metabolised to toxic metabolites or free radicals (Jamrozik & Janik, 1997; Brooks, 2000) and thirdly dopamine agonists may be neuroprotective (Marek *et al.*, 2002).

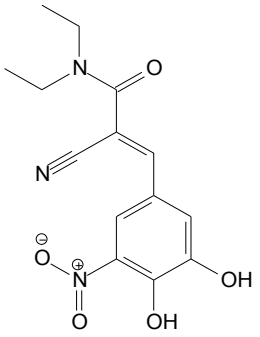
2.7.2.1.3 Monoamine oxidase B inhibitors

MAO-B inhibitors selegiline and rasagiline irreversibly inhibit metabolism of dopamine via MAO-B to dihydroxyphenylacetic acid (DOPAC) and hydrogen peroxide (Münchau & Bhatia, 2000). Selegiline undergoes first pass metabolism to L-methamphetamine, L-amphetamine and desmethyldeprenyl - which cause sleep disorders (Abdel-Salam, 2015). Rasagiline's main metabolite is aminoindan, which has no amphetamine-like properties (Knudsen, 2011). Selegiline and rasagiline are prescribed as monotherapy in early PD and as adjunctive therapy in late PD (Lew *et al.*, 2010; Mizuno *et al.*, 2010; Reichman & Jost, 2010). These drugs provide mild symptomatic benefits, compared with L-dopa and dopamine agonists (Abdel-Salam, 2015).

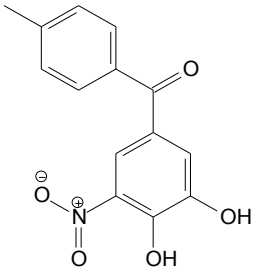
2.7.2.1.4 Catechol-*O*-methyltransferase inhibitors

Besides metabolism of L-dopa to dopamine by aromatic amino acid decarboxylase, substantial metabolism of L-dopa is also facilitated by catechol-*O*-methyltransferase (COMT) which catalysis the *O*-methylation of L-dopa to 3-*O*-methyldopa (Münchau & Bhatia, 2000). Entacapone and tolcapone reversibly inhibit COMT, nebicapone is a new COMT inhibitor (Abdel-Salam, 2015). Tolcapone is a longer acting and more potent COMT inhibitor than nebicapone and entacapone (Kaakkola, 2010). These drugs decrease metabolism of L-dopa, extend its half-life and increase bioavailability (Kaakkola *et al.*, 1994). COMT inhibition translates to a decrease in “off” time and an increase in “on” time in patients with fluctuating L-dopa concentrations (Münchau & Bhatia, 2000; Abdel-Salam, 2015). The COMT inhibitors are used adjunctively to L-dopa for the symptomatic treatment of PD with motor fluctuations (Abdel-Salam, 2015). Similarly, by stabilizing L-dopa concentrations, tolcapone and entacapone permit an uninterrupted stimulation of dopamine receptors which, ideally, would lessen motor

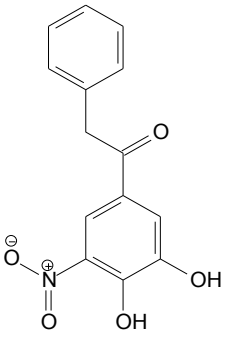
complications (Abdel-Salam, 2015). Dyskinesia is the most common adverse effect of entacapone and tolcapone (Mizuno *et al.*, 2007; Abdel-Salam, 2015), additional adverse effects include nausea, diarrhoea, orange discoloration of urine, and sleep disturbances (Münchau & Bhatia, 2000). Tolcapone is associated with an elevation of liver enzymes and is used cautiously in PD patients with decreased liver function (Münchau & Bhatia, 2000).



Entacapone



Tolcapone



Nebicapone

2.7.2.1.5 Amantadine

Amantadine, an antiviral drug, exert antiparkinsonian effects; such as improvement of bradykinesia, rigidity, and resting tremor (Schwab *et al.*, 1969), and is primarily used as adjunctive treatment for L-dopa-induced dyskinesia in late PD (Abdel-Salam, 2015). Amantadine is an NMDA receptor antagonist (Abdel-Salam, 2015), however, several mechanisms of action for amantadine have been suggested (Münchau & Bhatia, 2000). Adverse effects include blurred vision, visual hallucinations, peripheral edema (Malkani *et al.*, 2012), reversible corneal edema after long-term use (Chang *et al.*, 2008), auditory hallucinations (Gondim *et al.*, 2010), myoclonus, hallucination, delirium (Nishikawa *et al.*, 2009), cardiac arrest, ventricular tachycardia and prolonged QTc interval (Manini *et al.*, 2007; Schwartz *et al.*, 2008).

2.7.2.1.6 Anticholinergic drugs

The first pharmacological treatment of PD was with anticholinergic drugs (Brocks, 1999). However, these drugs are rarely used today and limited to early PD and younger patients with bothersome resting tremor (Olanow *et al.*, 2009) as anticholinergic drugs are burdened by cognitive, neuropsychiatric and autonomic adverse effects and best prescribed cautiously in the elderly (Brocks, 1999; Münchau & Bhatia, 2000; Olanow *et al., 2009*). Anticholinergic drugs are of little value in the treatment of bradykinesia, rigidity and postural instability (Olanow *et al.*, 2009). Examples are benztropine, biperiden, diphenhydramine, ethopropazine, orphenadrine, procyclidine and trihexyphenidyl (Brocks, 1999).

2.7.2.1.7 A_{2A} adenosine receptor antagonists

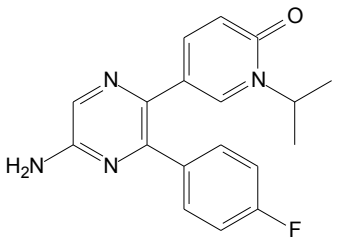
Adenosine plays a role opposite to dopamine in the brain (Ferré *et al.*, 2001). Agonists and antagonists of AR's produce behavioural effects similar to antagonists and agonists of dopamine receptors, respectively (Ferré *et al.*, 1992). Therefore, the antagonism of A_{2A} AR's in striatopallidal neurons reduce postsynaptic effects of dopamine depletion and sequentially reduce motor symptoms of PD (Schwarzschild *et al.*, 2006). Also, the combination of A_{2A} AR antagonists and L-dopa could reduce the risk of developing dyskinesia associated with long term L-dopa treatment (Kanda *et al.*, 2000). A detailed discussion of AR's and their antagonists follows in Chapter 3.

2.7.2.2 Non-motor symptoms

Non-motor symptoms in PD are a major source of disability, and ought to be treated promptly to improve the quality of life of PD patients (Ranawaya & Suchowersky, 2010). Numerous treatments are available and, for a number of patients, these treatments control or improve debility from non-motor symptoms like depression, sleep disorders, and autonomic symptoms (urinary symptoms, orthostatic hypotension, and constipation) (Kalia & Lang, 2015). Novel treatment for these non-motor symptoms may include A_1 and A_{2A} AR antagonists.

A_{2A} AR antagonists may potentially address non-motor symptoms of PD by acting as an antidepressant (El Yacoubi *et al*, 2001). For example, the A_{2A} AR antagonist KW-6002 alone or in combination with currently available antidepressants might treat depression; as demonstrated by a decrease in immobility time during the forced swim test and the tail suspension test in rodents (El Yacoubi *et al*., 2001; Yamada *et al.*, 2013).

Cognitive impairment associated with PD may well improve through A_1 AR antagonism (Mihara *et al.*, 2007). For example, the cognitive effects of caffeine may be due to the antagonism of A_1 AR's in the hippocampus and cortex - brain areas associated with learning and memory (Fredholm *et al.*, 1999). Additional evidence for the improvement of cognitive impairment associated with PD through the antagonism of the A_1 AR demonstrated by a study using a mixed A_1 and A_{2A} AR antagonist, ASP-5854 (Mihara *et al.*, 2007). This drug reversed scopolamine-induced memory deficits in rats, whereas a specific A_{2A} AR antagonist, KW-6002, did not (Mihara *et al.*, 2007). A detailed discussion of AR's and their antagonists follows in Chapter 3.



ASP-5854

2.8 Conclusion

Headway has been made in PD since James Parkinson's "An Essay on the Shaking Palsy", written 200 years ago. No more is PD "generally regarded by the sufferers in this point of view, so discouraging to the employment of remedial means" (Parkinson, 2002). Existing treatment relieves patients of the four cardinal symptoms of PD; bradykinesia, rigidity, resting tremor and postural instability, as well as some adverse effects associated with chronic L-dopa treatment (Lang & Obeso, 2004). Regrettably, no drug convincingly stops or, at least, slows neuronal loss in the nigrostriatal pathway of PD patients and treats both motor and non-motor symptoms (Münchau & Bhatia, 2000). Dual A_1/A_{2A} AR antagonists may address the aforesaid problems (Ferré *et al.*, 1992) and, perhaps, PD will no longer be considered "an evil, from the domination of which one has no prospect of escape" (Parkinson, 2002).

CHAPTER 3

ADENOSINE RECEPTORS

3.1 Introduction

The endogenous purine nucleoside adenosine has widespread effects in the human body (Chen *et al.*, 2014). In the brain, specifically, it is a neuromodulator in charge of various neurotransmitters, receptors and signalling pathways (Chen *et al.*, 2014). It acts through inhibitory (A_1 and A_3) and stimulatory (A_{2A} and A_{2B}) metabotropic G–protein coupled receptors, where it, respectively, increases and decreases cyclic adenosine monophosphate (cAMP) (Palmer & Stiles, 1995). The A_1 and A_{2A} AR's possess high adenosine affinity, while the A_{2B} and A_3 AR's do not (Chen *et al.*, 2014). Also, co-expression of AR's AR's with each other (e.g. A_1/A_{2A}) and neurotransmitter receptors (e.g. A_{2A}/D_2) occur (Stockwell *et al.*, 2017). A_1 AR's are greatly expressed in the prefrontal cortex and hippocampus and A_{2A} AR's in the basal ganglia and olfactory bulb, while A_{2B} and A_3 AR's show low brain expression (Stehle *et al.*, 1992). As a result, A_1 and A_{2A} AR's are associated with physiological and pathological processes in the central nervous system; ranging from the sleep/wake cycle, learning and memory, movement and neurodegeneration to the neurological condition PD (Chen *et al.*, 2014). A_1 and A_{2A} AR's are, therefore, rational drug targets for the treatment of PD and may possibly address motor symptoms and non-motor symptoms (cognitive deficits and depression), as well as neurodegeneration. (**Figure 3-1**)

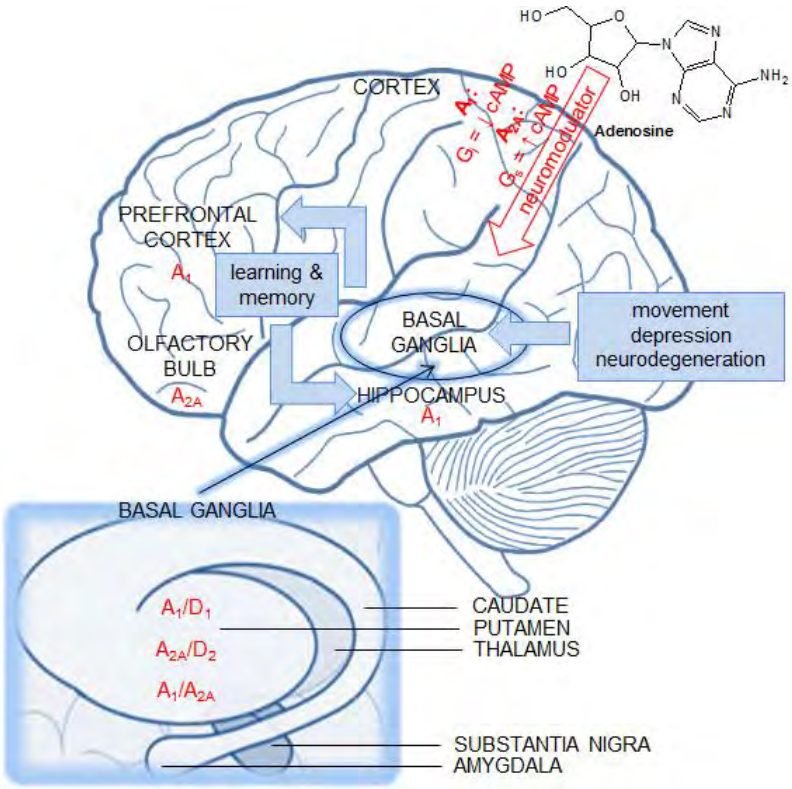


Figure 3-1: The distribution, expression and function of A_1 and A_{2A} AR's in the brain, related to PD.

3.2 Adenosine receptors and Parkinson's disease

The significance of AR's in PD is based on the xanthine derivative caffeine and its ability to act as a non-selective A_1 and A_{2A} AR antagonist and, in so doing, affect brain function (e.g. sleep/wake cycle, cognition, locomotion etc.) as well as neurological conditions leading to brain dysfunction (e.g. PD) (Ribeiro & Sebastião, 2010).

Epidemiological studies established an association between the consumption of caffeine — present in coffee and tea — and a reduced risk of developing PD (Ross *et al.*, 2000). Moreover, caffeine also decreased freezing of gait in PD (Kitagawa *et al.*, 2007) and improved pharmacokinetic properties of L-3,4-dihydroxyphenylalanine (Levo-dopa/L-dopa) in PD patients (Deleu *et al.*, 2006). Another study found that elderly women who drank relatively large quantities of coffee over their lifetimes perform better in cognitive function tests than their counterparts who drank no coffee (Johnson-Kozlow *et al.*, 2002). Caffeine consumption is also linked to a decreased risk of depression (Wang *et al.*, 2016). A pharmacological study conferred neuroprotection by caffeine in animal models of PD (Chen *et al.*, 2001).

The structure of caffeine, elucidated by Hermann Fischer in the nineteenth century, is similar to that of adenosine (Ribeiro & Sebastião, 2010). Caffeine and other A_1 and A_{2A} AR antagonists

exert effects contrary to endogenous adenosine (Prediger, 2010) and, in so doing, affects various neurotransmitters, receptors and signalling pathways (Chen *et al.*, 2014).

Therefore, caffeine and other A_1 and A_{2A} AR antagonists may be non-dopaminergic drugs for the symptomatic treatment of both PD motor symptoms (for example bradykinesia, rigidity, resting tremor and postural instability) and PD non-motor symptoms (for example cognitive deficits such as executive dysfunction with secondary visuospatial and mnemonic disturbances and depression) as well as exhibit neuroprotective properties (Prediger, 2010).

3.2.1 Motor symptoms

The nigrostriatal pathway is one of the major dopaminergic pathways in the brain and is involved in movement via the indirect and direct pathway, otherwise known as the striatopallidal pathway and the striatonigral pathway, respectively (Mori, 2014). The striatopallidal (indirect) pathway — which inhibits undesired movement — projects to the external segment of the globus pallidus (GPe), then to the subthalmic nucleus (STN) and lastly to the internal segment of the globus pallidus (GPi) and the substantia nigra *pars reticulata* (SNr), i.e. the GPi/SNr complex (Mori, 2014). While the striatonigral (direct) pathway projects straight to the GPi/SNr complex and enables movement (Mori, 2014). By means of A_{2A} AR's and dopamine D_2 receptors the striatopallidal (indirect) pathway facilitates inhibition, and the striatonigral (direct) pathway facilitates excitation through A_1 AR's and dopamine D_1 receptors (Mori, 2014) Thus, projection from the SNpc to the striatum offers contrasting influences on these γ-aminobutyric acid (GABA) output pathways. (**Figure 3-2 A**)

Once dopaminergic neurons in the SNpc are lost – as is the case in PD – the inhibitory and excitatory regulation of the striatum through dopamine receptors are compromised; ensuing both increased excitation of the striatopallidal (indirect) pathway and decreased activity of the striatonigral (direct) pathway (Mori, 2014). Disproportionate inhibition of the thalamocortical pathway causes hypokinetic movement as, firstly, the GPe reduces GABAergic inhibition of the STN and, secondly, the STN increases glutamatergic stimulation of the GPi/SNr complex (Mori, 2014). (**Figure 3-2 B**)

In a normal state – that is at physiological conditions – balance exists between the A_{2A} AR mediated excitatory and dopamine D_2 receptor mediated inhibitory modulation of the indirect pathway (Mori, 2014) (See **Figure 3-2 A**). In PD, however, the dopamine D_2 receptor system is damaged (due to a loss of dopaminergic neurons in the SNpc) and A_{2A} ARmediated excitatory modulation is relatively dominant in the indirect pathway, resulting in increased excitation (Mori, 2014). The aforementioned induces a disturbance of basalganglia-thalamocortical circuit and causes hypokinetic movement (Mori, 2014). (**Figure 3-2 B**)

Motor symptoms in PD are associated with the indirect pathway, rather than the direct pathway (Obeso *et al.*, 2014); given that an increased density of A_{2A} AR's and decreased density of dopamine D_2 receptors are seen in PD patients (Mori, 2014).

A_{2A} AR antagonists block A_{2A} AR mediated dual excitatory modulation in both the striatum and GPe on the indirect pathway: the excessive excitation of the pathway is reduced, resulting in the entire basal ganglia-thalamocortical balance to shift toward normalization — even with the loss of dopamine (Mori, 2014) (**Figure 3-2 C**).

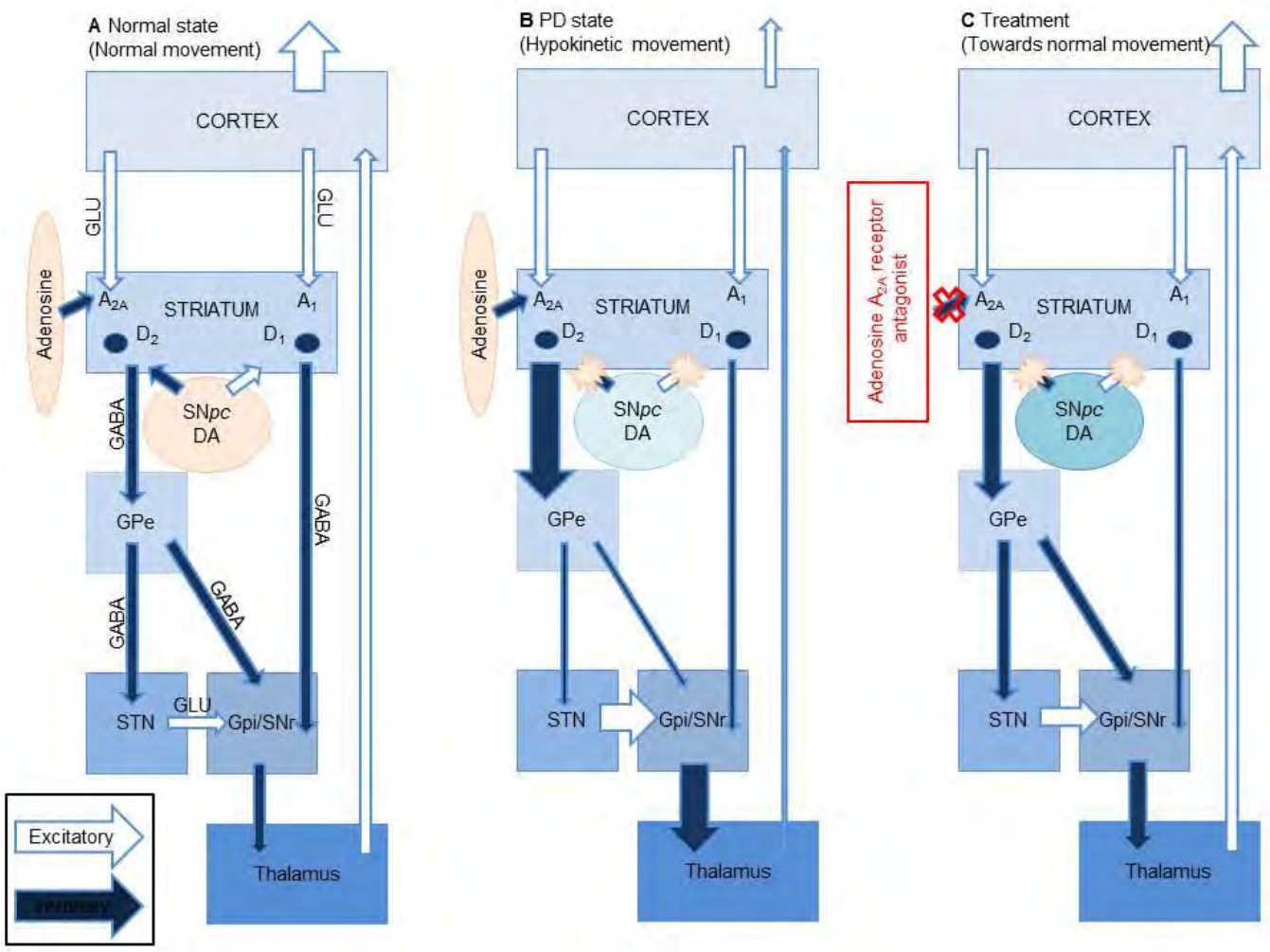


Figure 3-2: **Schematic diagram of basal ganglia-thalamocortical circuit for (A) normal state, (B) PD state and (C) treatment of PD state with an A_{2A} AR antagonist. Activity indicated by thickness of arrows. Adapted from Mori (2014) and reproduced with permission from Elsevier.**

Pharmacological studies support the above by means of the selective A_{2A} AR antagonist KW-6002 (istradefylline) which is active in animal models of PD (El Yacoubi *et al.*, 2001). This activity may be due to close anatomical and functional relationship between A_{2A} AR's and dopamine D_2 receptors on the indirect striatopallidal GABAergic pathway (Ferré *et al.*, 2001).

Additionally, concomitant administration of an A_{2A} AR antagonist to L-dopa reduces adverse effects of L–dopa, such as dyskinesias (Kanda *et al*, 2000).

Interestingly, blockade of both A_1 and A_{2A} AR's synergistically improve motor control by increasing presynaptic dopamine release via A_1 AR inhibition and postsynaptic dopamine response via A_{2A} AR inhibition (Shook & Jackson, 2011).

3.2.2 Non-motor symptoms

3.2.2.1 Cognitive deficits

Synaptic plasticity; the ability of synapses to strengthen or weaken in response to increases or decreases in their activity, is the basis for learning and memory (Takahashi *et al.*, 2008). Brain areas associated with cognition are the prefrontal cortex and hippocampus (Takahashi *et al.*, 2008). Endogenous adenosine via A_1 AR's — which are abundantly expressed in the prefrontal cortex and hippocampus — modulates synaptic plasticity phenomena long-term depression and long-term potentiation and, in so doing, inhibits learning and memory (Ribeiro *et al.*, 2003).

A_1 AR antagonists block A_1 AR mediated inhibitory modulation of synaptic plasticity in the prefrontal cortex and hippocampus: neurotransmitter release and synaptic transmission is increased, facilitating synaptic plasticity and, thus, learning and memory (Takahashi *et al.*, 2008).

Pharmacological studies support the above, by means of the selective A_1 AR antagonists BIIP20 and FR194921 which are active in animal models of cognitive deficits (Pitsikas & Borsini, 1997; Maemoto *et al.*, 2004).

3.2.2.2 Depression

Endogenous adenosine, via its receptors — specifically the A_{2A} AR, mediates behaviour (El Yacoubi *et al.*, 2001). For example, adenosine produces a "depressant"-like state in animal models relevant to neuropsychiatric disorders such as depression (El Yacoubi *et al.*, 2001), additionally, an increase in adenosine or stimulation of AR's induce a state of "learned helplessness" in animal models — similar to that observed in animal models of depression (Minor *et al.*, 1994; Woodsen *et al*, 1998). Consistently, A_{2A} AR knockout mice are less sensitive to "depressant" challenges than their wild counterparts (El Yacoubi *et al.*, 2001). The aforementioned effects of adenosine may be explained by the antagonistic interaction between A_{2A} AR's and dopamine D_2 receptors in the striatum; stimulation of A_{2A} AR's lead to effects resembling inhibition of dopamine D_2 receptors and *vice versa* (Ferré *et al.*, 1992).

Pharmacological studies support the above, as the selective A_{2A} AR antagonist SCH58261's antidepressant activity was prevented by the dopamine D_2 receptor antagonist haloperidol in animal models of depression (El Yacoubi *et al.*, 2001). Consistently, A_{2A} AR antagonists (SCH58261 and KW-6002) reduced total immobility time in the tail suspension test and forced swim test, both animal models of depression (El Yacoubi *et al.*, 2001).

3.2.3 Neurodegeneration

Endogenous adenosine released during brain insults, for example hypoxia, diminishes consequent neuronal damage via A_1 AR activation, moreover, A_{2A} AR agonism may possibly cause neurotoxicity (Simola *et al.*, 2014). However, caffeine and other A_{2A} AR antagonists, such as KW-6002, are neuroprotective and reduce glial cell activation in neurotoxin animal models of PD (Chen *et al.*, 2001).

Several animal studies of PD demonstrated that A_{2A} AR antagonists counteract both the loss of dopaminergic neurons within the SNpc and subsequent decrease of dopamine in the striatum (Schwarzschild *et al.*, 2006; Morelli *et al.*, 2010). Additionally, A_{2A} AR antagonists are also effective in paradigms of Alzheimer's and Huntington's disease-like neurotoxicity (Stone *et al.*, 2009). This suggests that A_{2A} AR antagonists act by mechanisms which are not selective towards dopaminergic neurons; mechanisms of action may be modulation of glutamate-induced excitotoxicity and neuroinflammation (Simola *et al.*, 2014).

Glutamate-induced excitotoxicity is important to neurodegeneration in PD; on the one hand, dopaminergic neurons are vulnerable to fluctuations in extracellular glutamate concentrations and, on the other hand, dysregulation of glutamate transmission is present in PD (Lancelot & Beal, 1998; Greenamyre, 2001). The stimulation of A_{2A} AR's increase extracellular concentrations of glutamate, while inhibition of A_{2A} AR's decrease extracellular concentrations which, in turn, relieves excitotoxicity and affords neuroprotection to dopaminergic neurons (Popoli *et al.*, 1995). As stated, A_{2A} AR antagonists block A_{2A} AR mediated dual excitatory modulation in both the striatum and GPe on the striatopallidal (indirect) pathway, resulting in the entire basal ganglia-thalamocortical balance to shift toward normalization — even with the loss of dopamine (Mori, 2014). Furthermore, studies showed that both lesion of STN and blockade of NMDA receptors in the STN reduce the loss of dopaminergic neurons in neurotoxin animal models of PD (Piallat *et al.*, 1996; Blandini *et al.*, 2001; Carvalho & Nikkhah, 2001). Therefore, glutamate release from the STN may be a mechanism of neurodegeneration in PD and it is plausible that A_{2A} AR antagonists, by regulating the striatopallidal (indirect) pathway, might attenuate degeneration of dopaminergic neurons through modulation of STN firing (Simola *et al.*, 2014). (**Figure 3-3**)

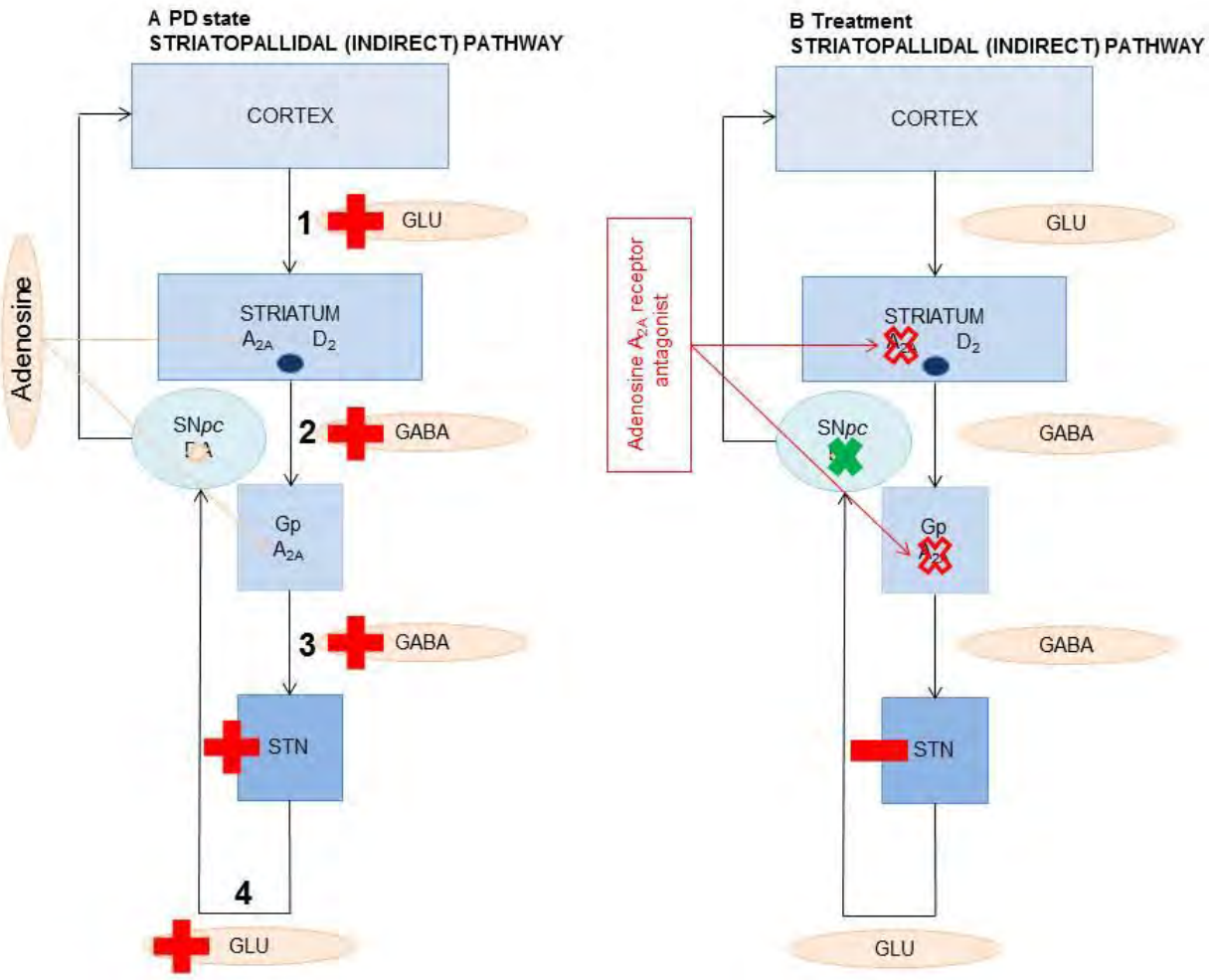


Figure 3-3: **Proposed actions of A_{2A} AR antagonists on striatopallidal (indirect) pathway. (A) PD state: (1) Degeneration of dopaminergic neurons within SNpc increases glutamatergic input from the cortex to the striatum and (2) increases GABAergic indirect output from the striatum to the GPe, (3) leading to increased STN activity. (4) In turn, increased STN activity contributes to excitotoxic degeneration of dopaminergic neurons within SNpc. (B) Treatment with an A_{2A} AR antagonist: A_{2A} AR's control excitability of striatopallidal (indirect) pathway and, thus, A_{2A} AR antagonism neutralises degeneration of dopaminergic neurons within SNpc.**

Another mechanism of neurodegeneration in PD may be neuroinflammation; it leads to the activation of glial cells and the successive production of inflammatory mediators (Simola *et al.*, 2014). Glial cells possess A_{2A} AR's and blockade of these receptors inhibit the complex cascade that is neuroinflammation (Armentero *et al.*, 2011).

Pharmacological studies support the above, by means of the selective A_{2A} AR antagonists SCH58261, DMPX and KW-6002 which attenuated striatal dopamine depletion in the MPTP animal model of PD (Chen *et al.*, 2001). Also, genetic studies demonstrated that degeneration of dopaminergic neurons by the neurotoxin MPTP is reduced in A_{2A} AR knockout mice (Chen *et al.*, 2001).

3.3 Adenosine receptor antagonists

A_1 and A_{2A} AR antagonists are divided into xanthine derivatives and non-xanthine derivatives (generally amino-substituted heterocyclic compounds) (Yuzlenko & Kieć-Kononowicz, 2006). Among xanthine based compounds some common features may be pointed out (**Figure 3-4: Xanthine derivatives**): A_1 AR blockers that exhibit biological activity contain long alkyl chains at positions 1 and 3 as well as bulky substituents at position 8, whereas A_{2A} AR blockers with high affinity contain an alkyl or alkynyl moiety at position 3 and a styryl function or heterocyclic ring at position 8. Non-xanthine blockers include non-fused monocyclic ring systems in addition to fused bi- and tricyclic ring systems with various heteroatoms. Most often non-xanthine A_1 AR blockers are adenine-based (**Figure 3-4: Non-xanthine derivatives**) with a bulky substituent at the amino group and variable N-atoms in different positions, as well as a short alkyl or phenyl moiety at position 8 , while A_{2A} AR blockers contain a furanyl ring — vital to AR binding — in the fused bi- and tricyclic ring systems. Additionally, biologically active A_1 and A_{2A} AR antagonists are often N-containing tricyclic fused ring systems with an alkylphenyl moiety (Yuzlenko & Kieć-Kononowicz, 2006).

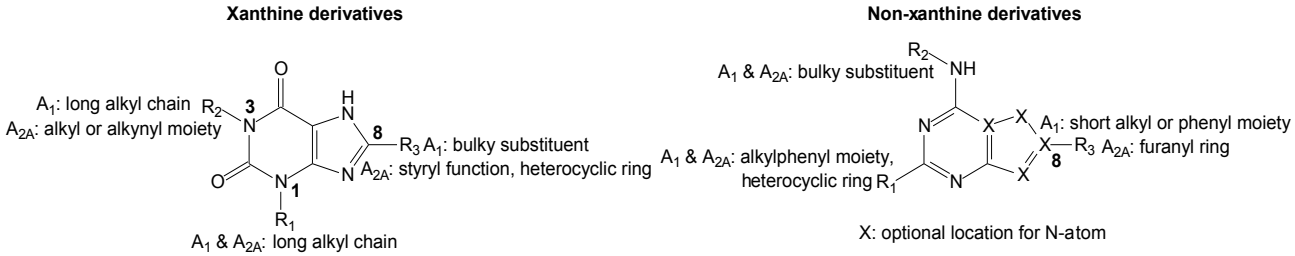
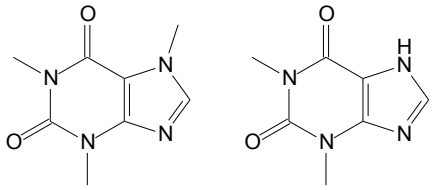


Figure 3-4: Summary of general features of xanthine and non-xanthine derivatives essential to A_1 and A_{2A} AR antagonists. Adapted from Yuzlenko & Kieć-Kononowicz (2006) and reproduced with permision from Bentham Science.

3.3.1 Adenosine A_1 receptor antagonists

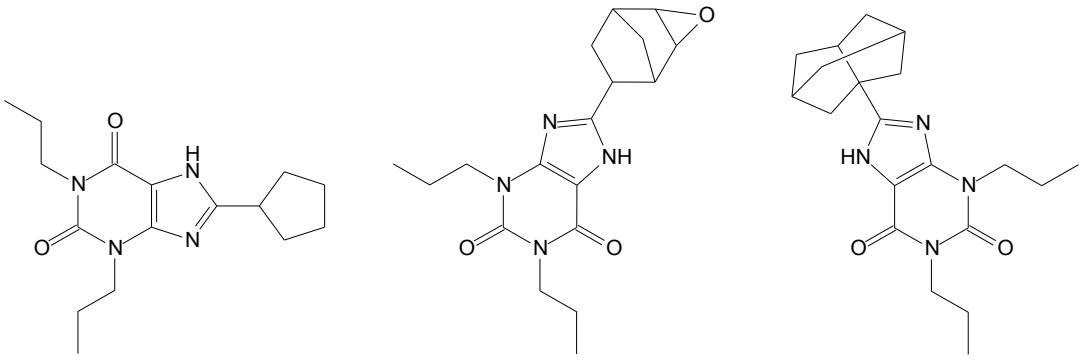
3.3.1.1 Xanthine derivatives

The classical A_1 AR antagonists are the xanthines caffeine and theophylline; however, natural xanthines are non-selective AR antagonists and have low affinity for the A_1 AR (Yuzlenko & Kieć-Kononowicz, 2006).



Caffeine **Theophylline**

Several potent and selective xanthine derivatives as A_1 AR antagonists have been identified and among these the 8-cycloalkyl xanthine derivatives are effective A_1 AR antagonists (Yuzlenko & Kieć-Kononowicz, 2006). For example, DPCPX exhibit high selectivity for A_1 AR's in animal studies with a K_i value of 0.45 nM in rat fat cells (Lohse *et al.*, 1987). Other selective A_1 AR antagonists worth mentioning are BG-9719 and KW-3902 (Yuzlenko & Kieć-Kononowicz, 2006). BG-9719 has roughly a 100 fold higher affinity for the A_1 AR than the A_{2A} AR; with an A_1K_i (rat) value of 5.12 nM *versus* an $A_{2A}K_i$ (rat) value of 551 nM (Auchampach *et al.*, 2004). The A_1K_i (rat) value of KW-3902 is 12.6 nM, with an $A_{2A}K_i$ (rat) value of 510 nM (Auchampach *et al.*, 2004).



DPCPX **BG-9719** **KW-3902**

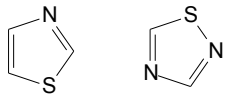
It is speculated that xanthine derivatives with increased chain length at position N1 and N3, such as the dipropyl groups of DPCPX, BG-9719 and KW-3902, as well as bulky C8-substituents (e.g. cyclopentyl (DPCPX), epoxynorbornyl (BG-9719) and 3-noradamantyl (KW-3902)) could possess enhanced A_1 AR activity (Müller, 2001).

3.3.1.2 Non–xanthine derivatives

Although non-xanthine derivatives, comprising of mono-, bi- and tricyclic (fused) heteroatomic ring systems differ by means of their chemical structures, similarities may be found between these compounds (Chang *et al.*, 2004). Generally, these compounds are adenine-based; with a substituted amino-group and contain various numbers of N-atoms within the heteroatomic rings systems (Yuzlenko & Kieć-Kononowicz, 2006).

3.3.1.2.1 Monocyclic heteroatomic ring systems

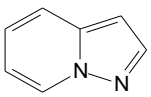
Non-fused rings are rarely A_1 AR antagonists and have generally low affinity for the A_1 AR, except for the five-membered heterocycles, namely thiazoles and thiadiazoles (Yuzlenko & Kieć-Kononowicz, 2006). The thiazole N-(4-phenylthiazol-2-yl)-4-chlorobenzamide show relatively good affinity and selectivity for the A_1 AR (A_1K_i (rat) = 18 nM), whereas the thiadiazole VUF-5472 (N-(3-phenyl-1,2,4-thiadiazol-5-yl)-*trans*-4-hydroxycyclohexanamide) has an A_1K_i (rat) value of 20 nM. The most potent five-membered heterocyclic is LUF-5437 (N-(3-phenyl-1,2,4-thiadiazol-5-yl)-4-hydroxybenzamide) with an A_1K_i (rat) value of 7 nM.



Thiazole and 1,2,4-thiadiazole basic structures

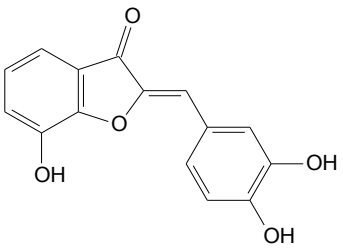
3.3.1.2.2 Bicyclic fused heteroatomic ring systems

The bicyclic heteroatomic ring systems consist of 6:5 fused, 6:6 fused and 5:7 fused rings, containing either two, three, four or five N-atoms, yet, natural heteroatomic ring systems (for example benzofurans and flavonoids) contain no N-atoms (Chang *et al.*, 2004). The 6:5 fused heteroatomic compounds entail the largest group of non-xanthine based antagonists and pyrazolo[1,5-*α*]pyridines, containing two N-atoms, are the most investigated class of compounds in this group (Chang *et al.*, 2004; Yuzlenko & Kieć-Kononowicz, 2006).

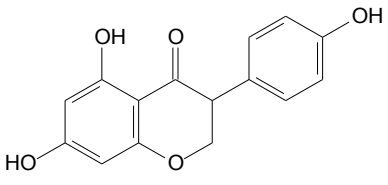


Pyrazolo[1,5-*a*]pyridine basic structure

Aurones, a type of flavonoid, also contain a 6:5 fused heteroatomic ring system and show promise as novel non-xanthine A_1 AR antagonists, as seen with the selective A_1 AR antagonist Hispidol (A_1K_i (rat) = 0.352 μM) (Jacobson *et al.*, 2002). Additionally, isoflavones (heterocyclic fused 6- and 6-membered rings) such as Genistein (A_1K_i (rat) = 12.6 μM) too posess affinity for the A_1 AR (Jacobson *et al.*, 2002).

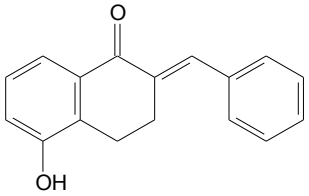


Hispidol



Genistein

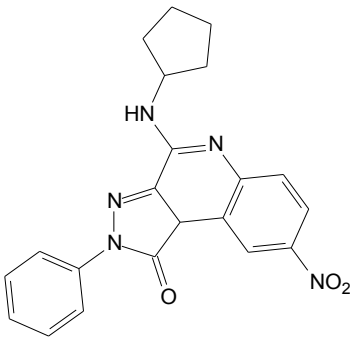
Recently, Legoabe and co-workers (2017) evaluated 2-benzylidene-1-tetralone derivatives as A_1 AR antagonists and found that these 6:6 fused ring systems – which are structurally related to aurones and isoflavones – readily bind to the A_1 AR, for example (*E*)-2-benzylidene-5-hydroxy-1-tetralone with a A_1K_i (rat) value of 5.93 μM.



(*E*)-2-Benzylidene-5-hydroxy-1-tetralone

3.3.1.2.3 Tricyclic fused heteroatomic ring systems

The tricyclic heteroatomic ring systems consist of 6:6:5 fused, 6:5:6 fused and 5:6:5 fused rings, containing various N-atoms (Chang *et al.*, 2004). The 6:6:5 fused N-heteroatomic compounds form the largest group of the tricyclic antagonists (Chang *et al.*, 2004). These compounds are similar to the bicyclic fused heteroatomic ring systems and some are just further developments of the bicyclic antagonists (Chang *et al.*, 2004). For example, the 6:6:5 fused compound 4-(cyclopentylamino)-8-nitro-2-phenyl-1H,2H,9bH-pyrazolo[3,4-c]quinolin-1-one with an A_1K_i (bovine) value of 0.35 nM (Yuzlenko & Kieć-Kononowicz, 2006).



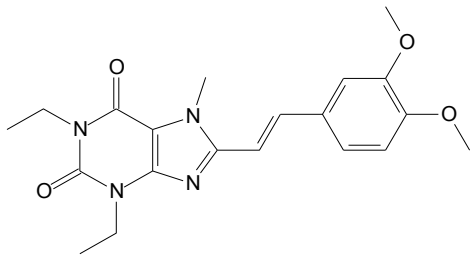
4-(Cyclopentylamino)-8-nitro-2-phenyl-1H,2H,9bH-pyrazolo[3,4-c]quinolin-1-one

3.3.2 Adenosine A_{2A} receptor antagonists

3.3.2.1 Xanthine derivatives

The methylxanthines caffeine and theophylline are, as mentioned, non-selective AR antagonists with weak affinity, not only for the A_1 AR, but also for the A_{2A} AR (Yuzlenko & Kieć-Kononowicz, 2006).

The first xanthine based A_{2A} AR antagonist was DMPX, unfortunately it was a non-selective and weak A_{2A} AR antagonist. Various selective 8-styrylxanthines followed; including CSC, MSX-2 and KW-6002 (istradefylline) (Yuzlenko & Kieć-Kononowicz, 2006). Gold was struck with the discovery of the potent and selective KW-6002; it has an $A_{2A}K_i$ (rat) value of 2 nM and is approved as an antiparkinsonian drug in Japan (Pinna, 2014). Regrettably, a problem of 8-styrylxanthines is low water solubility (Yuzlenko & Kieć-Kononowicz, 2006). Other xanthine derivatives are the tricyclic imidazo[2,1-*i*]purin-5-ones (exhibit increased water solubility), with (*S*)-1,4-dimethyl-8-ethyl-2-styryl-imidazo[2,1-*i*]purinone being the most potent compound of these derivatives ($A_{2A}K_i$ (rat) = 0.42 μM) (Yuzlenko & Kieć-Kononowicz, 2006). Constrained bioisosteric derivatives of the 8-styrylxanthines proved to be relatively good A_{2A} AR antagonists, i.e. oxygen- and nitrogen-containing theophylline analogues, such as oxazolo-, oxazino- and oxazepinopurindiones, as well as imidazo-, pyrimido- and diazepinopurindiones (Yuzlenko & Kieć-Kononowicz, 2006).



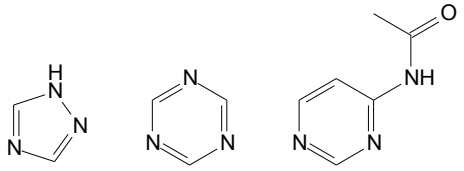
KW-6002

3.3.2.2 Non-xanthine derivatives

Due to the xanthine derivatives' low selectivity for the A_{2A} AR and low water solubility, non-xanthine based A_{2A} AR antagonists consisting of three main classes, namely mono-, bi- and tricyclic derivatives were explored (Azam *et al.*, 2009). Non-xanthine antagonists of the A_{2A} AR often comprise of nitrogen-containing monocyclic and fused bicyclic or tricyclic ring systems without the ribose moiety of adenosine (confers agonism) and include hydrophobic substituents (convey selectivity) (Azam *et al.*, 2009).

3.3.2.2.1 Monocyclic fused heteroatomic ring systems

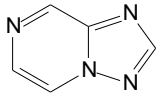
A number of monocyclic prototypes have been explored, including 1,2,4-triazole, thiazole, thiadiazole and triazine and pyrimidine acetamide derivatives exhibit high selectivity and affinity for the A_{2A} AR (Azam *et al.*, 2009). For example, the potent and selective *N*-Pyrimidinyl-2-phenoxyacetamides with $A_{2A}K_i$ (human) values ranging from 0.2 nM to 3.7 nM (Azam *et al.*, 2009).



1,2,4-Triazole, 1,3,5-triazine and pyrimidine acetamide basic structures

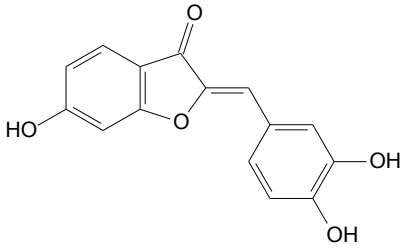
3.3.2.2.2 Bicyclic fused heteroatomic ring systems

The bicyclic moiety is linked to less molecular flexibility (Veber et al., 2002), which correlates with good oral bioavailability and CNS penetration (Crivori *et al.*, 2000). Structurally diverse compounds serve as non-xanthine A_{2A} AR antagonists; including [1,2,4]triazol[1,5-*a*][1,3,5]triazine, [1,2,4]triazolo[1,5-*c*]pyrimidine, [1,2,4]triazolo[1,5-*a*]pyridine, [1,2,3]triazolo[4,5-*d*]pyrimidine and [1,2,4]triazolo[1,5-*a*]pyrazine derivatives (Azam *et al.*, 2009). For example, the [1,2,4]Triazolo[1,5-*a*]pyrazine derivatives which are highly selective toward A_{2A} AR's and possess $A_{2A}K_i$ (human) values of 1 nM (Azam *et al.*, 2009).



[1,2,4]Triazolo[1,5-a]pyrazine basic structure

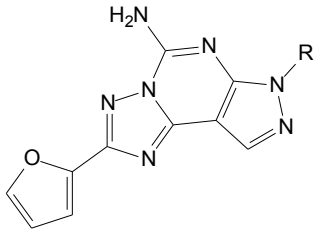
Additionaly, the less investigated aurones – a type of flavonoid – posess A_{2A} AR affinity, for example the 6:5 fused heteroatomic compound Maritimetin ($A_{2A}K_i$ (rat) = 9.35 μM) (Jacobson *et al.*, 2002). The structurally related 2-benzylidene-1-tetralone derivatives also show promise as A_{2A} AR antagonists, as demonstrated by (*E*)-2-benzylidene-5-hydroxy-1-tetralone, with a $A_{2A}K_i$ (rat) value of 2.90 μM (Legoabe *et al.*, 2017).



Maritimetin

3.3.2.2.3 Tricyclic fused heteroatomic ring systems

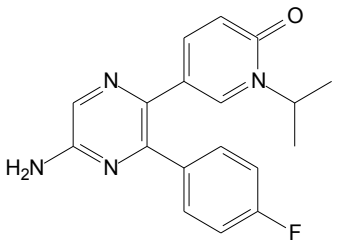
Of the non-xanthine heterocycles containing a tricyclic nucleus, several of these compounds are A_{2A} AR antagonists (Azam *et al.*, 2009). Initially, triazolo[1,5-*c*]quinazolines, triazolo[4,3-*a*]quinoxalines, triazolo[4,3-*a*]quinoxalin-1-ones, triazolo[1,5-*a*]quinoxalines, 1,2,4-triazolo[5,1-*i*]purine, pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines, pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines, and indeno[1,2-*d*]pyrimidines showed interesting properties, however, these compounds also interacted with other AR's (Azam *et al.*, 2009). Current developments with the tricyclic moiety generally include pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines (Azam *et al.*, 2009).



Pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine

3.4 Dual adenosine A_1 and A_{2A} receptor antagonists

The original dual A_1 and A_{2A} AR antagonist is the xanthine caffeine with low affinity for both A_1 and A_{2A} AR's (Jacobson *et al*, 1993). However, the non-xanthine derivative and pyrazolopyrimidine derivative, namely ASP-5854, is a potent and selective dual A_1/A_{2A} AR antagonist (Mihara *et al.*, 2007). The K_i values of ASP-5854 for the A_1 and A_{2A} AR's are 9.03 nM and 1.76 nM, respectively (Mihara *et al.*, 2007). What is more, Mihara and co-workers (2007) demonstrated that the orally active ASP-5854 improves motor symptoms and is neuroprotective via A_{2A} AR antagonism, additionally, it also enhances cognition through A_1 AR antagonism in animal models of PD.



ASP-5854

3.5 Conclusion

The significance of AR's in PD is based on the xanthine derivative caffeine and its ability to act as a non-selective A_1 and A_{2A} AR antagonist and, in so doing, affect brain function as well as

neurological conditions leading to brain dysfunction, for example the neurodegenerative disorder PD – burdened with motor dysfunction, cognitive deficits and depression. A_1 and A_{2A} AR's are, therefore, rational drug targets for the treatment of PD and a dual A_1/A_{2A} AR antagonist may possibly address motor symptoms and non-motor symptoms (cognitive deficits and depression), as well as neurodegeneration.

CHAPTER 4

PUBLISHED ARTICLE

Bioorganic Chemistry 74 (2017) 251–259

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

5-Substituted 2-benzylidene-1-tetralone analogues as A_1 and/or A_{2A} antagonists for the potential treatment of neurological conditions

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ARTICLE INFO

Article history:
Received 27 June 2017
Revised 15 August 2017
Accepted 22 August 2017
Available online 30 August 2017

Keywords:
Parkinson's disease
Alzheimer's disease
5-Hydroxy-2-benzylidene-1-tetralones
A_1 adenosine receptor antagonists
A_{2A} adenosine receptor antagonists

ABSTRACT

Adenosine A_1 and A_{2A} receptors are attracting great interest as drug targets for their role in cognitive and motor deficits, respectively. Antagonism of both these adenosine receptors may offer therapeutic benefits in complex neurological diseases, such as Alzheimer's and Parkinson's disease. The aim of this study was to explore the affinity and selectivity of 2-benzylidene-1-tetralone derivatives as adenosine A_1 and A_{2A} receptor antagonists. Several 5-hydroxy substituted 2-benzylidene-1-tetralone analogues with substituents on ring B were synthesized and assessed as antagonists of the adenosine A_1 and A_{2A} receptors via radioligand binding assays. The results indicated that hydroxy substitution in the *meta* and *para* position of phenyl ring B, displayed the highest selectivity and affinity for the adenosine A_1 receptor with K_i values in the low micromolar range. Replacement of ring B with a 2-amino-pyrimidine moiety led to compound **12** with an increase of affinity and selectivity for the adenosine A_{2A} receptor. These substitution patterns led to enhanced adenosine A_1 and A_{2A} receptor binding affinity. The para-substituted 5-hydroxy analogue **3** behaved as an adenosine A_1 receptor antagonists in a GTP shift assay performed with rat whole brain membranes expressing adenosine A_1 receptors. In conclusion, compounds **3** and **12**, showed the best adenosine A_1 and A_{2A} receptor affinity respectively, and therefore represent novel adenosine receptor antagonists that may have potential with further structural modifications as drug candidates for neurological disorders.

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1. Introduction

Parkinson's disease (PD) is a common but complex neurodegenerative disease characterized by a loss of dopaminergic neurons in the substantia nigra *pars compacta* [1] and abnormal aggregates of α-synuclein protein called Lewy bodies and Lewy neurites [2]. The decrease of dopamine in the basal ganglia leads to classical motor symptoms associated with PD, which include bradykinesia, rigidity, resting tremor, and postural instability [3]. Even though the four cardinal signs generally define PD, it is important not to dismiss the non-motor symptoms associated with PD [4]. Non-motor symptoms range from dribbling saliva, constipation, depression, sleep disorders, apathy, hallucinations, and dementia [5].

It is estimated that between 4.1 and 4.6 million individuals over age 50 had PD in 2005 and that between 8.7 and 9.3 million individuals will have PD by 2030, making PD the most common neurological condition after Alzheimer's disease [6]. Consequently PD has a high socioeconomic cost [7].

Current treatment of PD is symptomatic and consists of drugs that restore dopamine concentrations and/or effects [8]. L-3,4-dihydroxyphenylalanine (Levo-dopa/L-dopa), dopamine's immediate precursor, is considered the most effective drug for treating PD-associated motor symptoms [9]. Though L-dopa provides the greatest symptomatic relief, its adverse effects include motor fluctuations ("wearing-off phenomenon" and "on-off phenomenon"), non-motor fluctuations, dyskinesia, and drug-induced psychosis [10]. Other drugs used for the treatment of PD are dopamine agonists, catechol-O-methyltransferase inhibitors, monoamine oxidation inhibitors, amantadine, and anti-cholinergic drugs [11]. These drugs are focussed on either replacing the concentrations and/or effects of dopamine in the brain and only address motor symptoms and not non-motor symptoms [12]. Therefore an ideal drug would address motor symptoms and non-motor symptoms, be disease modifying, and neuroprotective. Adenosine receptor (AR) antagonists may address the aforementioned problems [13].

Adenosine is a neuro- and homeostatic modulator with wide ranging effects throughout the human body [14,15]. Currently four AR subtypes, namely A_1, A_{2A}, A_{2B} and A_3, exist [16]. Adenosine acts through G-protein coupled receptors [17]. Activation of A_1 and A_3

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http://dx.doi.org/10.1016/j.bioorg.2017.08.013
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ARs inhibit adenyl cyclase (AC) [18,19], whereas A_{2A} and A_{2B} ARs stimulate AC [20]. A_1 ARs are highly expressed throughout the brain, while A_{2A} AR expression is limited to the striatum, nucleus accumbens and the olfactory tubercle [21]. A_{2B} ARs are distributed diffusely throughout the brain [20] though at a considerably lower density than A_1 and A_{2A} ARs [22]. A_3 ARs show low brain expression [23]. Therefore the impact of adenosine on brain function generally depends on the actions of A_1 and A_{2A} ARs [22]. A_1 and A_{2A} ARs have been identified as drug targets for the treatment of PD and other neurological conditions [24,25]. For example an epidemiological study has established an association between the consumption of coffee or caffeine (Fig. 1) and a reduced risk of developing PD [26]. Furthermore caffeine (Fig. 1) has been demonstrated to protect against neurotoxicity and deterioration of dopaminergic neurons in a mouse 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine (MPTP) neurotoxin model of PD [27]. The effects of the xanthine derivative, caffeine, on the brain are mediated by the non-selective antagonism of A_1 and A_{2A} ARs (Fig. 1 caffeine) [28].

Adenosine plays a role opposite to dopamine in the brain [29]. Agonists and antagonists of ARs produce behavioural effects similar to antagonists and agonists of dopamine receptors, respectively [13]. Therefore the antagonism of A_{2A} ARs in striatopallidal neurons reduce postsynaptic effects of dopamine depletion, and sequentially reduce motor symptoms of PD [30]. Furthermore A_{2A} ARs antagonists might protect dopaminergic neurons from deterioration and thus be neuroprotective [27]. The combination of A_{2A} AR antagonists and L-dopa could reduce the risk of developing dyskinesia associated with long term L-dopa treatment [31]. A_{2A} AR antagonists may also address non-motor symptoms of PD by acting as an antidepressant [32]. For example the A_{2A} AR antagonist KW-6002 (Fig. 1) alone or in combination with currently available antidepressants, has demonstrated to decrease immobility time during the forced swim test and the tail suspension test in rodents [32,33]. Furthermore the cognitive impairment associated with PD may be improved through A_1 AR antagonism [25], the cognitive effects of caffeine may be due to the antagonism of A_1 ARs in the hippocampus and cortex – the brain areas mostly involved in cognition [28]. Additional evidence for the improvement of cognitive impairment associated with PD through the antagonism of the A_1 AR came from a study using a mixed A_1/A_{2A} receptor antagonist, ASP-5854 (Fig. 1) [25]. ASP-5854 reversed scopolamine-induced memory deficits in rats, whereas a specific A_{2A} AR antagonist, KW-6002, did not [25].

It is plausible that A_1 and/or A_{2A} AR antagonist may have significance in the treatment of PD as it reduces motor symptoms and is neuroprotective through the antagonism of A_{2A} ARs, and improves cognitive impairment through the antagonism of A_1 ARs [25]. A dual-target A_1/A_{2A} AR antagonist may have a synergistic motor activating effect; since antagonism of the A_1 AR facilitates presynaptic dopamine release and antagonism of the A_{2A} AR facilitates postsynaptic dopamine release [34]. This synergistic motor effect was observed with the simultaneous administration of a selective A_1 antagonist and a selective A_{2A} antagonist on the locomotor behaviour in rodents [35]. Thus, selective or dual-targeted A_1/A_{2A} AR antagonists are considered as potential drug candidates in the treatment of PD.

Affinity for both the A_1 and A_{2A} ARs are exhibited by aurone derivatives [38]. An aurone (2-benzylidene-1-benzofuran-3-one) is a heterocyclic chemical compound (fused 6- and 5-membered rings) and a type of flavonoid [39]. The latter compound may exist as either an (*E*)- or a (*Z*)-configuration [40]. For example hispidol is an aurone derivative with (*E*)-configuration, with a single hydroxy substituent on ring A, and was found to be a selective A_1 AR antagonist with a K_i value of 0.352 μM for A_1 AR and a K_i value of 52.7 μM for A_{2A} AR [38]. Maritimetin, another aurone derivative with a dihydroxy (position 6 and 7) on ring A, was found to possess affinity and selectivity for both the A_1 and A_{2A} ARs with a K_i value of 3.47 μM and 9.35 μM, respectively [38]. Aureusidin with a dihydroxy on position 4 and 6 of ring A also displayed A_1 AR affinity, but A_{2A} AR affinity was diminished (Fig. 2) [38]. Benzylidene tetralones (two fused 6-membered rings) are structurally related to the aurones. The basic tetralone back bone represents an interesting scaffold for designing novel AR antagonists.

Based on the aforementioned findings that aurone derivatives possess A_1 and A_{2A} AR affinity, hydroxy and methoxy substitution at C5 of ring A and secondly substitution at the *para* (4′) or *meta* (3′) position of ring B with polar and non-polar groups (F, Cl, Br, OH, OCH_3) will be investigated. Additionally phenyl ring B will be replaced with a pyridine ring where the N is at position 3′ or 4′ (Fig. 2). Furthermore, 2-amino-pyrimidine will also be explored at ring B due to a previous observation that 2-amino-pyrimidine has been associated with compounds possessing good A_1 and A_{2A} AR antagonist activity [41]. Therefore, in a continuing effort to identify potential compounds with antagonism for the AR, the C5 substituted 2-benzylidene-1-tetralone derivatives with a range of structural diversity will be evaluated in order to find the structural features essential for A_1/A_{2A} AR affinity.

2. Results and discussion

2.1. Chemistry

The tetralone analogues **1** and **15** were commercially available from Sigma Aldrich. In turn, the 2-benzylidene-1-tetralone

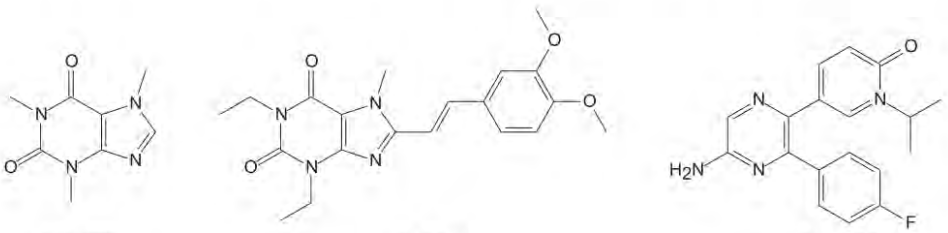


Fig. 1. The structures and AR affinities of caffeine [36], KW-6002 [37], and ASP-5854 [25].

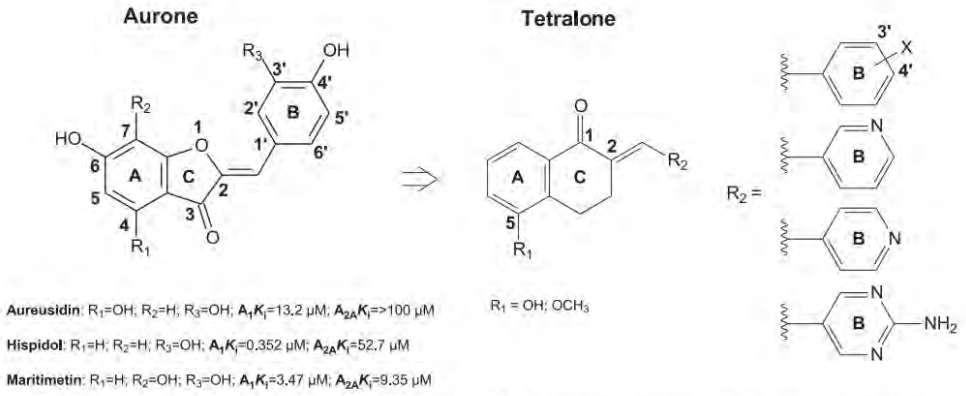


Fig. 2. Structural changes to 5-substituted-2-benzylidene-1-tetralones to find the structural features essential for A_1/A_{2A} antagonistic activity.

analogues (**2–14, 16–18**) were synthesized via acid catalyzed aldol condensation, as outlined in Scheme 1. 5-Hydroxy-1-tetralone (**2–14**) or 5-methoxy-1-tetralone (**16–18**) was reacted with a commercially available heteroatomic aldehyde to attain the desired 2-benzylidene-1-tetralone derivatives. The synthesized compounds were obtained in fair yields (16–82%) after purification via recrystallization. The reaction conditions and compound characterization are described in Sections 4.1 and 4.2. Structures of the novel compounds (**2–14, 16–18**) were verified by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry and the purities were evaluated by HPLC analysis, as cited in the Supplementary material.

2.2. Radioligand binding assays

The affinities of the 2-benzylidene-1-tetralones (**1–18**) at rat A_1 and A_{2A} AR subtypes were determined with radioligand competition experiments as described previously [36,42]. Two reference compounds (CPA and DPCPX) were included in the study and the results of the radioligand binding experiments for the reference and test compounds (**1–18**) are summarized in Table 1. Furthermore, the experimental results of CPA and DPCPX were found in accordance with literature values (Table 1).

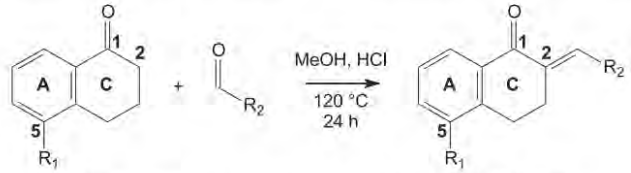
The impact of C5-hydroxy and -methoxy substitution on ring A with combined C3′/C4′ substitution on ring B were evaluated by comparing the dissociation constant (K_i) values of the test compounds (**3–14, 16–18**) to the unsubstitued compound **1**, **2** and **15**.

Structural modifications on ring A: The replacement of the hydroxy at C5 with a methoxy group resulted in a diminished affinity for both the A_1 and A_{2A} AR (compound **2** *vs* compound **16**; compound **3** *vs* compound **17**). Overall the investigated compounds favoured hydroxy substitution of ring A over substitution with a methoxy group.

Structural modification on ring B: Noteworthy, compound **1** without the 2-benzylidene side chain (ring B) showed no affinity for either the A_1 AR or the A_{2A} AR subtypes ($A_1K_i = A_{2A}K_i = >100$ μM) when compared to the lead compound **2** ($A_1K_i = 5.93$ μM; $A_{2A}K_i = 2.90$ μM) which possess an unsubstituted 2-benzylidene side chain (ring B). With the introduction of a 3′-hydroxy group to the 2-benzylidene ring of compound **2** ($A_1K_i = 5.93$ μM; $A_{2A}K_i = 2.90$ μM), compound **3** was obtained with a 3.6-fold increase in A_1 AR affinity, a 1.8-fold decrease in A_{2A} AR affinity ($A_1K_i = 1.62$ μM; $A_{2A}K_i = 5.46$ μM) and an increase in A_1 AR selectivity (SI = 3.4). Shifting the 3′-hydroxy group to the 4′-position to obtain compound **4**, showed the same pattern with a 3.6-fold increase in A_1 AR affinity ($A_1K_i = 1.64$ μM), a further 3.4-fold decrease in A_{2A} AR affinity ($A_{2A}K_i = 9.92$ μM) and higher selectivity for the A_1 AR (SI = 6). However, substitution with a 4′-methoxy led to a non-selective compound **5** (SI = 1.3) with a decrease in both A_1 and A_{2A} AR affinity ($A_1K_i = 12.9$ μM; $A_{2A}K_i = 16.8$ μM).

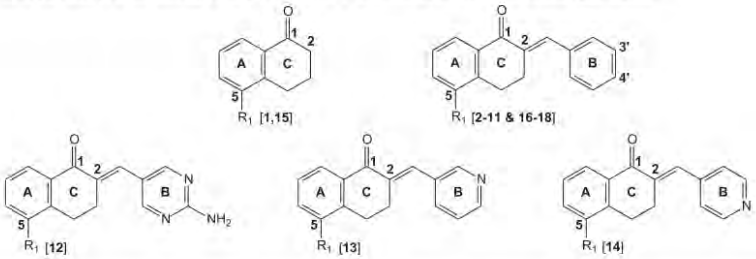
As part of the investigative study, halogen substitutions on ring B (retaining the C5-OH ring A), was additionally explored, to gain further insight into structural requirements. In general, the test compounds (**7–11**) possessing halogen substitution on ring B exhibited no affinity towards the A_1 and A_{2A} AR, with the only exception being compound **7** (3′-chloro) and compound **9** (3′,4′-dichloro) that exerted a more favourable effect on the A_1 AR with K_i values of 9.38 and 23.29 respectively. Overall, both A_1 and A_{2A} AR binding affinity are favoured by ring B bearing a C3′ or C4′-hydroxy group (**3** and **4**), whereas other substituents on phenyl ring B led to a decreased or loss in A_1 and A_{2A} AR affinity.

Further we explored other ring systems. The pyridine ring was included with the N at position 3′ or 4′ (compounds **13** and **14**). In addition the 2-amino-pyrimidine (**12**) was also included due to previous observations that the 2-amino-pyrimidine moiety has been associated with compounds possessing good A_1 and A_{2A} AR affinity [41]. Compounds **13** and **14** containing an unsubstituted pyridine ring (ring B) was devoid of A_1 and A_{2A} AR affinity. As expected compound **12** containing the 2-amino-pyrimidine ring showed the best A_{2A} AR affinity ($A_{2A}K_i = 1.61$ μM) with a 2-fold increase and selectivity in A_{2A} AR when compared to the



Scheme 1. Synthetic pathway to 2-benzylidene-1-tetralones (**2–14, 16–18**).

Table 1
The dissociation constant values (K_i values) for the binding of the 2-benzylidene-1-tetralones at rat adenosine A_1 and A_{2A} receptors.



#	Ring A (R_1) 5	Ring B (R_2) 3′	Ring B (R_2) 4′	$K_i \pm$ SEM (μM)[a] (% displacement)[b] A_1[c] vs [^{3}H]DPCPX	$K_i \pm$ SEM (μM)[a] (% displacement)[b] A_{2A}[c] vs [^{3}H]NECA	SI[d] (A_{2A}/A_1)	$K_i \pm$ SEM (μM)[a] A_1 + GTP[e] vs [^{3}H]DPCPX	GTP Shift[f]
1	OH	–	–	>100 (60%)[b]	>100 (86%)[b]	–	–	–
2	OH	H	H	5.93 ± 0.45[a]	2.90 ± 0.66[a]	0.5[d]	–	–
3	OH	OH	H	1.62 ± 0.36[a]	5.46 ± 0.49[a]	3.4[d]	1.64 ± 0.10[a]	1.0
4	OH	H	OH	1.64 ± 0.16[a]	9.92 ± 1.10[a]	6.0[d]	–	–
5	OH	H	OCH_3	12.9 ± 0.4[a]	16.8 ± 0.4[a]	1.3[d]	–	–
6	OH	H	$N(CH_3)_2$	>100 (38%)[b]	>100 (61%)[b]	–	–	–
7	OH	Cl	H	9.38 ± 0.14[a]	>100 (31%)[b]	–	–	–
8	OH	H	Cl	>100 (46%)[b]	>100 (53%)[b]	–	–	–
9	OH	Cl	Cl	23.29 ± 0.74[a]	>100 (79%)[b]	–	–	–
10	OH	H	F	>100 (46%)[b]	>100 (38%)[b]	–	–	–
11	OH	H	CF_3	>100 (37%)[b]	>70 (24%)[b]	–	–	–
12	OH	–	–	11.4 ± 0.5[a]	1.61 ± 0.23[a]	0.14[d]	–	–
13	OH	–	–	>100 (41%)[b]	>100 (28%)[b]	–	–	–
14	OH	–	–	>100 (48%)[b]	>100 (43%)[b]	–	–	–
15	OCH_3	–	–	>100 (53%)[b]	>100 (40%)[b]	–	–	–
16	OCH_3	H	H	>100 (32%)[b]	>100 (64%)[b]	–	–	–
17	OCH_3	OH	H	>100 (32%)[b]	>100 (30%)[b]	–	–	–
18	OCH_3	OH	OH	>100 (39%)[b]	>100 (73%)[b]	–	–	–
CPA (A_1 agonist)				0.0068 ± 0.0001[a] (0.0079)[g];(0.015)[h]	0.163 ± 0.001[a] (0.331)[i]	24[d] (22)[g]	0.099 ± 0.015[a] (0.099)[h]	15 (14)[h]
DPCPX (A_1 antagonist)				0.0004 ± 0.0002[a] (0.0005)[i]; (0.0003)[i]	0.545 ± 0.204[a] (0.530)[i]; (0.340)[i]	1363[d] (958)[g]; (1130)[i]	0.0004 ± 0.0002[a] (0.0004)[h]	1.0

[a] All K_i values determined in triplicate and expressed as mean ± SEM.
[b] Percentage displacement of the radioligand at the indicated concentration.
[c] Rat receptors were used (A_1: rat whole brain membranes; A_{2A}: rat striatal membranes).
[d] Selectivity index (SI) for the A_1 AR isoform calculated as the ratio of K_i (A_{2A})/K_i (A_1).
[e] GTP shift assay, where the 100 μM GTP was added to the A_1 AR radioligand binding assay.
[f] GTP shifts calculated by dividing the K_i in the presence of GTP by the K_i in the absence of GTP.
[g] Literature value obtained from Ref.[43].
[h] Literature value obtained from Ref.[42].
[i] Literature value obtained from Ref.[44].

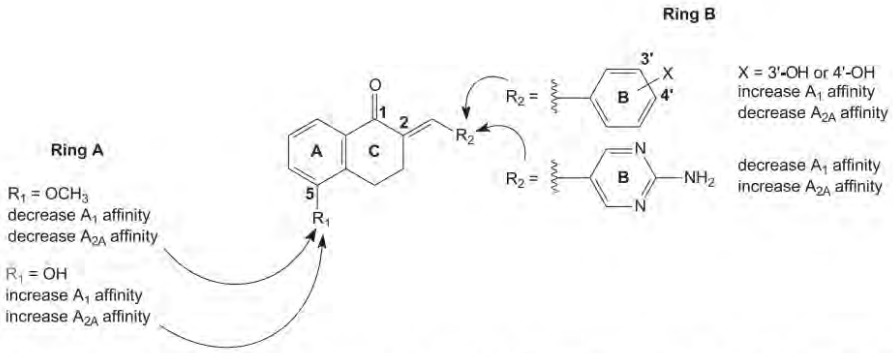


Fig. 3. A general depiction showing the influence of substitution on ring A and B at the 2-benzylidene-1-tetralone core on the A_1 and A_{2A} AR affinity.

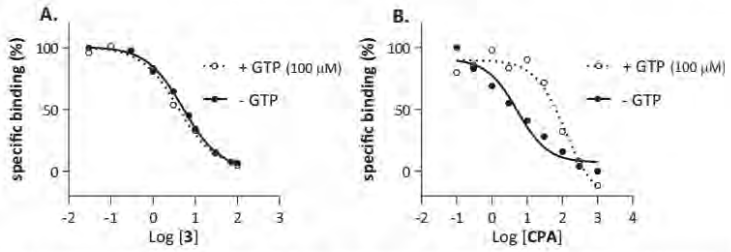


Fig. 4. The binding curves of CPA (reference compound) and compound **3**, indicating their A_1 AR agonist/antagonistic action as determined via GTP shift assays (with and without 100 μM GTP) in rat whole brain membranes expressing A_1 ARs with [^{3}H]DPCPX as radioligand. (A) GTP shift of 1 calculated for the A_1 AR antagonist compound **3**, and (B) GTP shift of 15 calculated for the A_1 AR agonist CPA.

unsubstituted compound **2** ($A_{2A}K_i$ = 2.90 μM). Contrary to the expected compound **12** retained A_1 affinity but a 2-fold decrease in A_1 AR affinity was observed (compound **2** A_1K_i = 5.93 μM vs compound **12** A_1K_i = 11.4 μM).

It may be concluded among the 5-hydroxy substituted 2-benzylidene-1-tetralones investigated, that replacing the hydroxy with a methoxy on ring A is detrimental to A_1 and A_{2A} AR affinity. Furthermore, 3′ and 4′ hydroxy substitution on ring B enhances A_1 AR affinity and selectivity, and by replacing the phenyl ring B with a 2-amino-pyridine ring an increase in affinity and selectivity for the A_{2A} AR subtype is obtained (Fig. 3).

2.3. Functional characterization

In order to determine if the test compounds that exhibit A_1 AR affinity, act as agonists or antagonists, GTP shift experiments were carried out. For this purpose compound **3** was chosen as it exhibited the highest A_1 AR binding affinity among the investigated compounds. The affinities of the reference (CPA and DPCPX) and test compound **3** were determined in the absence and presence of 100 μM GTP and are reported with the calculated GTP shifts in Table 1. The calculated GTP shift results for CPA and DPCPX (Table 1) was found to correspond with literature values, where CPA act as an agonist (Fig. 4) and DPCPX as an antagonist. Generally, a rightward shift of the binding curve in the presence of GTP (due to an uncoupling of the A_1 AR from its G_i protein) is expected for an A_1 AR agonist [42,45]. In the case of an A_1 AR antagonist no significant shift is anticipated in the presence of GTP [42,45]. The results suggest that compound **3** act as an A_1 AR antagonist, since no significant rightward shift of the binding curve was observed in the presence of GTP (Fig. 4).

3. Conclusion

The influence of a C5 hydroxy and methoxy substitution on ring A in combination with a 3′ and 4′ hydroxy substitution pattern (ring B) on A_1 and A_{2A} AR affinity has not been explored, yet. Accordingly, the aim of the study was to gain insight to the importance of structural modifications at both rings A and B of the 2-benzylidene-1-tetralone scaffold for attaining and even losing A_1 and/or A_{2A} AR affinity. On examination it was found that methoxy substitution on ring A is detrimental to A_1 and A_{2A} AR affinity and that 3′ and 4′ hydroxy substitution on phenyl ring B enhances A_1 AR affinity and selectivity, thereby affording compounds **3** and **4** with K_i values below 2 μM. Furthermore, replacing the phenyl ring B with a 2-amino-pyridine ring led to an increase in affinity and selectivity for the A_{2A} AR, with compound **12** possessing the highest A_{2A} AR affinity ($A_{2A}K_i$ = 1.62 μM) and a SI of 7 towards the A_{2A} AR. Compound **2** was found to display a SI of 2 towards the A_{2A} AR subtype and possessed the second highest A_{2A} AR binding (A_1-K_i = 5.93 μM; $A_{2A}K_i$ = 2.90 μM) among the test compounds. Considering the above, compounds **2**, **3**, **4** and **12** are ideal drug candidates for future optimization of the 2-benzylidene-1-tetralone scaffold to afford potent, selective antagonists for the A_1 and A_{2A} AR for the treatment of neurodegenerative disorders.

4. Experimental section

4.1. Chemistry

Starting materials were procured from Sigma-Aldrich and used without further purification – unless otherwise noted. Both, proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 151 MHz, respectively, in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts are reported in parts per million (δ) in relation to the signal of tetramethylsaline (Si(Ch_3)$_4$). Spin multiplicities are indicated as follow: s (singlet), d (doublet), dd (doublet of doublets), td (triplet of doublets), t (triplet), q (quartet) and m (multiplet). High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric pressure chemical ionisation (APCI) mode. High performance liquid chromatography (HPLC) analyses were determined on an Agilent 1100 HPLC system. Melting points (mp) were measured with a Buchi B545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was done using silica gel 60 (Merck) with UV254 fluorescent indicator.

4.2. Synthetic preparation

Compounds **2**–**14** and **16**–**18** were synthesized via acid catalyzed aldol condensation. In short, the ketone becomes a nucleophilic donor (enolate ion) and reacts with the aldehyde (electrophilic acceptor) to form a β-hydroxyl carbonyl compound, followed by dehydration to give an α,β-unsaturated carbonyl compound [46].

5-hydroxy-1-tetralone (**2**–**14**) or 5-methoxy-1-tetralone (**16**–**18**) (1.233 mmol) and the appropriate benzaldehyde (1.233 mmol) were suspended in MeOH (4 mL) and HCl (32%; 6 mL) and mechanically stirred at 120 °C under reflux for 24 h. The reaction mixture was then cooled to room temperature, subsequently ice (20 g) was added and the precipitate was filtered, dried and recrystallized from a suitable solvent.

The synthesized compounds possess *E*-configuration. In theory, these compounds may be either *E*- or *Z*-isomers [47]. However, the *E*-isomer is favourable – as it is thermodynamically stable [47]. The *Z*-isomer is undesirable due to steric interaction between the aryl and carbonyl groups [48].

*4.2.1. (E)-2-benzylidene-5-methoxy-3,4-dihydronaphthalen-1(2H)-one (**2**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one and benzaldehyde, yielded 88% product; brown powder; mp: 174.2–175.3 °C (EtOH). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 9.85 (s, 1H), 7.65 (s, 1H), 7.51 (d, J = 7.4 Hz, 2H), 7.45 (t, J = 7.3 Hz, 3H), 7.38 (t, J = 7.3 Hz, 1H), 7.20 (t, J = 7.9 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 3.03 (t, J = 5.8 Hz, 2H), 2.81 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 187.07, 154.35, 135.61, 135.30, 135.19, 134.02, 130.11, 129.88, 128.68, 128.58, 127.06, 119.27, 117.97, 26.10, 21.13; APCI-HRMS *m*/*z*: calculated for $C_{17}H_{14}O_2$ (MH^{+1}): 250.29186, found: 251.1049. Purity (HPLC): 98%.

*4.2.2. (E)-5-hydroxy-2-(3-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one (**3**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 3-hydroxybenzaldehyde, yielded 27% product; brown powder; mp: 177.1–207.4 °C (EtOH). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.61 (s, 1H), 7.56 (s, 1H), 7.45 (dd, J = 7.8, 1.0 Hz, 1H), 7.23 (dt, J = 32.5, 7.8 Hz, 2H), 7.07 (dd, J = 8.0, 1.1 Hz, 1H), 6.92 (dd, J = 21.7, 4.8 Hz, 2H), 6.85–6.77 (m, 1H), 3.03 (td, J = 6.7, 1.6 Hz, 2H), 2.82 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 186.96, 154.47, 139.56, 137.79, 133.83, 133.33, 130.47, 130.26, 128.52 (q, J = 31.8 Hz), 127.18, 125.38 (q, J = 3.4 Hz), 125.07, 123.26, 119.48, 118.02, 26.09, 21.16. APCI-HRMS *m*/*z* calculated for $C_{17}H_{15}O_3$ (MH^+): 267.1016, found: 267.0990. Purity (HPLC): 91%.

*4.2.3. (E)-5-hydroxy-2-(4-hydroxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one (**4**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 4-hydroxybenzaldehyde, yielded 82% product; brown crystals; mp: 267.0–272.1 °C (DCM). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.94 (s, 1H), 9.82 (s, 1H), 7.61 (s, 1H), 7.42 (dd, J = 11.3, 8.1 Hz, 3H), 7.18 (t, J = 7.8 Hz, 1H), 7.06 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 3.04 (t, J = 6.3 Hz, 2H), 2.81 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.96, 158.36, 154.28, 135.94, 134.33, 132.52, 132.12, 129.86, 126.97, 126.21, 119.07, 117.95, 115.56, 26.16, 20.97. APCI-HRMS *m*/*z* calculated for $C_{17}H_{15}O_3$ (MH^+): 267.1016, found: 267.1004. Purity (HPLC): 96.7%.

*4.2.4. (E)-5-hydroxy-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one (**5**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 4-methoxybenzaldehyde, yielded 29% product; brown crystals; mp: 310.1–310.2 °C (MeOH). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.84 (s, 1H), 7.64 (s, 1H), 7.51 (d, J = 8.7 Hz, 2H), 7.44 (dd, J = 7.8, 1.0 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 7.09–6.99 (m, 3H), 3.81 (s, 3H), 3.04 (td, J = 6.7, 1.6 Hz, 2H), 2.82 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.95, 159.70, 154.29, 135.34, 134.21, 133.48, 131.82, 129.89, 127.73, 126.98, 119.12, 117.93, 114.12, 55.28, 26.12, 20.99. APCI-HRMS *m*/*z* calculated for $C_{18}H_{17}O_3$ (MH^+): 281.1172, found: 281.1181. Purity (HPLC): 98.7%.

*4.2.5. (E)-2-(4-(dimethylamino)benzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(2H)-one (**6**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 4-(dimethylamino)benzaldehyde, yielded 62% product; light brown powder; mp: 211.2–216.3 °C (DCM). ^{1}H NMR (600 MHz, DMSO) δ 7.59 (s, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 7.7 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 7.13–7.03 (m, 3H), 3.08–2.95 (m, 8H), 2.78 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 187.44, 154.54, 148.28, 136.11, 134.62, 133.11, 132.21, 130.24, 127.70, 127.42, 119.56, 118.38, 115.33, 42.11, 26.52, 21.30. APCI-HRMS *m*/*z* calculated for $C_{19}H_{20}NO_2$ (MH^+): 294.1489, found: 294.1489. Purity (HPLC): 98.8%.

*4.2.6. (E)-2-(3-chlorobenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(2H)-one (**7**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 3-chlorobenzaldehyde, yielded 88% product; light beige powder; mp: 180.9–197.7 °C (DCM). ^{1}H NMR (600 MHz, DMSO) δ 9.89 (d, J = 3.1 Hz, 1H), 7.61 (s, 1H), 7.57 (s, 1H), 7.52–7.41 (m, 4H), 7.21 (t, J = 7.8 Hz, 1H), 7.08 (dd, J = 7.9, 1.1 Hz, 1H), 3.01 (t, J = 6.1 Hz, 2H), 2.83 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 186.94, 154.44, 137.54, 136.98, 133.88, 133.54, 133.31, 130.41, 130.21, 129.34, 128.41, 127.13, 119.42, 117.99, 26.09, 21.11. APCI-HRMS *m*/*z* calculated for $C_{17}H_{14}ClO_2$ (MH^+): 285.0677, found: 285.0679. Purity (HPLC): 97.6%.

*4.2.7. (E)-2-(4-chlorobenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(2H)-one (**8**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 4-chlorobenzaldehyde, yielded 64% product; light yellow crystals; mp: 233.3–264.6 °C (EtOH). ^{1}H NMR (600 MHz, DMSO) δ 9.88 (s, 1H), 7.63 (s, 1H), 7.57–7.49 (m, 4H), 7.45 (dd, J = 7.8, 1.0 Hz, 1H), 7.20 (t, J = 7.9 Hz, 1H), 7.08 (dd, J = 7.9, 1.1 Hz, 1H), 3.01 (td, J = 6.6, 1.6 Hz, 2H), 2.82 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 186.94, 154.40, 136.29, 134.21, 133.94, 133.83, 133.27, 131.68, 130.13, 128.62, 127.11, 119.36, 117.98, 26.07, 21.07. APCI-HRMS *m*/*z* calculated for $C_{17}H_{14}ClO_2$ (MH^+): 285.0677, found: 285.0660. Purity (HPLC): 100%.

*4.2.8. (E)-2-(3,4-dichlorobenzylidene-5-hydroxy-3,4-dihydronaphthalen-1(2H)-one (**9**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 3,4-dichlorobenzaldehyde, yielded 69% product; dark yellow crystals; mp: 190.6–197.3 °C (EtOH). ^{1}H NMR (600 MHz, DMSO) δ 9.89 (s, 1H), 7.78 (s, 1H), 7.73–7.68 (m, 1H), 7.59 (s, 1H), 7.50 (dd, J = 8.3, 0.6 Hz, 1H), 7.45 (dd, J = 7.8, 1.0 Hz, 1H), 7.21 (t, J = 7.9 Hz, 1H), 7.08 (dd, J = 7.9, 1.0 Hz, 1H), 3.00 (dd, J = 9.1, 3.8 Hz, 2H), 2.83 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 186.81, 154.43, 137.43, 136.12, 133.80, 132.51, 131.54, 131.33, 131.08, 130.66, 130.19, 129.88, 127.14, 119.44, 117.98, 26.04, 21.03. APCI-HRMS *m*/*z* calculated for $C_{17}H_{13}Cl_2O_2$ (MH^+): 319.0287, found: 319.0283. Purity (HPLC): 100%.

*4.2.9. (E)-2-(4-fluorobenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(2H)-one (**10**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 4-fluorobenzaldehyde, yielded 48% product; dark yellow crystals; mp: 217.5–218.6 °C (EtOH). ^{1}H NMR (600 MHz, DMSO-d_6) δ ^{1}H NMR (600 MHz, DMSO) δ 9.88 (s, 1H), 7.65 (s, 1H), 7.63–7.54 (m, 2H), 7.45 (dd, J = 7.8, 1.1 Hz, 1H), 7.33–7.25 (m, 2H), 7.20 (dd, J = 9.8, 6.0 Hz, 1H), 7.08 (dd, J = 7.9, 1.2 Hz, 1H), 3.02 (td, J = 6.7, 1.6 Hz, 2H), 2.82 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 187.02, 162.87, 161.23, 154.40, 135.48 (d, J = 0.9 Hz), 134.15, 134.02, 132.22 (d, J = 8.4 Hz), 131.83 (d, J = 3.2 Hz), 130.10, 127.10, 119.33, 117.99, 115.68, 115.54, 26.03, 21.07. APCI-HRMS *m*/*z* calculated for $C_{17}H_{14}FO_2$ (MH^+): 269.0972, found: 269.0995. Purity (HPLC): 95.4%.

*4.2.10. (E)-5-hydroxy-2-(4-trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (**11**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaphthalen-1(2H)-one and 4-(trifluoromethyl)benzaldehyde, yielded 47% product; light yellow crystals; mp: 201.3–202.3 °C (EtOH). ^{1}H NMR (600 MHz, DMSO-d_6) δ ^{1}H NMR (600 MHz, DMSO) δ 9.91 (s, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.75–7.65 (m, 3H), 7.47 (d,

J = 7.3 Hz, 1H), 7.22 (t, J = 7.9 Hz, 1H), 7.09 (dd, J = 7.9, 0.7 Hz, 1H), 3.03 (t, J = 5.8 Hz, 2H), 2.84 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO) δ 186.96, 154.47, 139.56, 137.79, 133.83, 133.33, 130.47, 130.26, 128.52 (q, J = 31.8 Hz), 127.18, 125.38 (q, J = 3.4 Hz), 125.07, 123.26, 119.48, 118.02, 26.09, 21.16. APCI-HRMS m/z calculated for $C_{18}H_{14}F_3O_2$ (MH^+): 319.0940, found: 319.0940. Purity (HPLC): 100%.

*4.2.11. (E)-2-((2-aminopyrimidin-5-yl)methylene)-5-hydroxy-3,4-dihydronaphthalen-1(2H)-one (**12**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaph thalen-1(2H)-one and 2-aminopyrimidine-5-carbaldehyde, yielded 40%; brown powder; mp: 51.6–51.6 °C (MeOH). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.33 (s, 1H), 10.55 (s, 1H), 9.30 (s, 1H), 8.33 (d, J = 9.3 Hz, 1H), 7.95 (d, J = 7.7 Hz, 1H), 7.82 (dd, J = 9.3, 2.8 Hz, 1H), 7.53 (d, J = 2.8 Hz, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 3.24 (t, J = 7.4 Hz, 2H), 3.06 (t, J = 7.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ^{13}C NMR (151 MHz, DMSO) δ 170.10, 158.37, 155.09, 151.58, 131.24, 130.48, 129.08, 128.74, 126.84, 126.39, 120.64, 118.49, 110.30, 25.19, 19.25. APCI-HRMS m/z calculated for $C_{15}H_{14}N_3O_2$ (MH^+): 268.1081, found: 268.1067. Purity (HPLC): 100%.

*4.2.12. (E)-5-hydroxy-2-(pyridin-3-ylmethylene)-3,4-dihydronaphthalen-1(2H)-one (**13**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaph thalen-1(2H)-one and 3-pyridinecarboxaldehyde, yielded 45%; orange crystals; mp: 256.4–258.5 °C (EtAc). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.03 (d, J = 1.4 Hz, 1H), 8.84 (dd, J = 5.4, 0.9 Hz, 1H), 8.56 (d, J = 8.1 Hz, 1H), 8.02 (dd, J = 8.0, 5.6 Hz, 1H), 7.68 (s, 1H), 7.47 (dd, J = 7.7, 0.9 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.16 (dd, J = 8.0, 1.2 Hz, 1H), 3.03 (td, J = 6.6, 1.6 Hz, 2H), 2.86 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.61, 154.60, 144.28, 143.80, 142.38, 140.08, 134.12, 133.56, 130.35, 128.84, 127.31, 126.46, 119.80, 118.04, 25.99, 21.07. APCI-HRMS m/z calculated for $C_{16}H_{14}NO_2$ (MH^+): 252.1019, found: 252.1006. Purity (HPLC): 97.8%.

*4.2.13. (E)-5-hydroxy-2-(pyridin-4-ylmethylene)-3,4-dihydronaphthalen-1(2H)-one (**14**)*

The title compound, prepared from 5-hydroxy-3,4-dihydronaph thalen-1(2H)-one and 4-pyridinecarboxaldehyde, yielded 61%; green powder; mp: 205.4–261.1 °C (EtAc). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.15 (d, J = 9.2 Hz, 1H), 8.94 (d, J = 6.6 Hz, 2H), 8.10 (d, J = 6.5 Hz, 2H), 7.69 (s, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.26–7.17 (m, 2H), 3.05 (dd, J = 8.9, 3.7 Hz, 2H), 2.87 (t, J = 6.4 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ^{13}C NMR (151 MHz, DMSO) δ 186.48, 154.66, 151.86, 142.89, 142.21, 133.37, 130.50, 129.82, 127.37, 127.03, 119.96, 118.05, 26.24, 21.10. APCI-HRMS m/z calculated for $C_{16}H_{14}NO_2$ (MH^+): 252.1019, found: 252.1024. Purity (HPLC): 100%.

*4.2.14. (E)-2-benzylidene-5-methoxy-3,4-dihydronaphthalen-1(2H)-one (**16**)*

The title compound, prepared from 5-methoxy-3,4-dihydro naphthalen-1(2H)-one and benzaldehyde, yielded 16%; beige crystals; mp: 399.9–400.0 °C (EtOH). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.68 (s, 1H), 7.59 (d, J = 7.3 Hz, 1H), 7.52 (d, J = 7.4 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.43–7.34 (m, 2H), 7.25 (d, J = 7.7 Hz, 1H), 3.84 (s, 3H), 3.05 (td, J = 6.6, 1.6 Hz, 2H), 2.85 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.83, 156.07, 135.42, 135.29, 135.22, 133.72, 131.74, 129.90, 128.75, 128.59, 127.39, 119.01, 115.05, 55.79, 25.97, 20.96. APCI-HRMS m/z calculated for $C_{18}H_{17}O_2$ (MH^+): 265.1223, found: 265.1123. Purity (HPLC): 95.9%.

*4.2.15. (E)-2-(3-hydroxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-1(2H)-one (**17**)*

The title compound, prepared from 5-methoxy3,4-dihydronaph thalen-1(2H)-one and 3-hydroxybenzaldehyde, yielded 21%; dark beige powder; mp: 30.8–30.9 °C (EtOH & PE). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.62 (s, 1H), 7.61–7.55 (m, 2H), 7.37 (t, J = 8.0 Hz, 1H), 7.29–7.22 (m, 2H), 6.92 (dd, J = 19.4, 4.6 Hz, 2H), 6.80 (dd, J = 8.0, 2.0 Hz, 1H), 3.84 (s, 3H), 3.04 (td, J = 6.6, 1.6 Hz, 2H), 2.85 (t, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.86, 157.37, 156.07, 136.41, 135.62, 135.11, 133.73, 131.74, 129.63, 127.38, 120.84, 119.00, 116.33, 115.91, 115.02, 55.78, 26.03, 20.98. APCI-HRMS m/z calculated for $C_{18}H_{17}O_3$ (MH^+): 281.1172, found: 281.1172. Purity (HPLC): 98.3%.

*4.2.16. (E)-2-(3,4-dihydroxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-1(2H)-one (**18**)*

The title compound, prepared from 5-methoxy-3,4-dihydro naphthalen-1(2H)-one and 3,4-dihydroxybenzaldehyde, yielded 52%; green powder; mp: 30.8–30.9 °C (EtOH & PE). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.34 (dd, J = 169.0, 6.1 Hz, 2H), 7.57–7.53 (m, 2H), 7.35 (t, J = 8.0 Hz, 1H), 7.22 (dd, J = 8.2, 0.7 Hz, 1H), 6.98 (d, J = 2.0 Hz, 1H), 6.88 (dd, J = 8.3, 1.9 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 3.83 (d, J = 7.9 Hz, 3H), 3.09–3.01 (m, 2H), 2.84 (t, J = 6.6 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.66, 155.99, 146.88, 145.19, 136.53, 134.03, 131.94, 131.42, 127.27, 126.56, 122.92, 118.96, 117.32, 115.73, 114.77, 55.77, 26.01, 20.80. APCI-HRMS m/z calculated for $C_{18}H_{17}O_4$ (MH^+): 297.1121, found: 297.1107. Purity (HPLC): 100%.

4.3. In vitro evaluation

General information: All commercially available reagents were obtained from various manufacturers: radioligands [^{3}H]NECA (specific activity 27.1 Ci/mmol) procured from PerkinElmer and [^{3}H]DPCPX (specific activity 120 Ci/mmol) from Amersham Biosciences, filter-count from PerkinElmer and Whatman GF/B 25 mm diameter filters from Merck. Radio activity was calculated by a Packard Tri-CARB 2810 TR liquid scintillation counter.

4.3.1. Radioligand binding assays

The collection of tissue samples for the A_1 and A_{2A} AR binding studies was approved by the Research Ethics Committee of the North-West University (application number NWU-0035-10-A5). The rat whole brains (expressing A_1 AR) and rat striata (expressing A_{2A} AR) were prepared according to the protocol described in literature [36,42].

The competition experiments were carried out in the presence of the radioligands [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX; 0.1 nM; K_d = 0.36 nM) and 5′-N-[^{3}H]-ethylcarboxamideadenosine ([^{3}H]NECA; 4 nM; K_d = 15.3 nM) for the A_1 and A_{2A} AR radioligand binding assay, respectively [36,42]. In addition, the A_{2A} AR binding studies were determined in the presence of N^6-cyclopentyladenosine (CPA) to minimize the binding of [^{3}H]NECA to A_1 AR. The nonspecific binding was defined by the addition of 100 μM CPA. The sigmoidal-dose response curves, via Graphpad Software Inc. package, were obtained by plotting the specific binding versus the logarithm of the test compound's concentrations. Subsequently, the K_i values were obtained by using the IC_{50} values that were determined from sigmoidal-dose response curves. All incubations were carried out in triplicate and the K_i values (dissociation constants) are expressed as the mean ± standard error of mean (SEM). CPA and DPCPX (unlabelled) were used as reference compounds and its assay results confirmed validity of the radioligand binding assays (Table 1).

4.3.2. GTP shift assays

In addition, compound **3** was explored via a GTP shift assay to determine the agonistic or antagonistic functionality of the investigated 2-benzylidene-1-tetralones towards the A_1 AR. The GTP shift assay was performed as described previously with rat whole brain membranes and [^{3}H]DPCPX (0.1 nM; K_d = 0.36 nM) in the absence and presence of 100 μM GTP (Table 1; Fig. 4) [42]. The nonspecific binding was defined by the addition of 10 μM DPCPX (unlabeled). If a calculated GTP shift of approximately 1 is obtained, that compound is considered to function as an antagonist. On the other hand, the presence of GTP affects the competition curve of an agonist and shifts the curve to the right as is demonstrated by the A_1 AR agonist CPA [42]. The sigmoidal-dose response curves were obtained via the Graphpad Software Inc. package and the K_i values determined as described above (Section 4.3.1) The GTP shift was calculated by dividing the K_i value of a compound reported in the presence of GTP by the K_i value obtained in the absence of GTP [42].

Acknowledgements

We are grateful to Dr. J. Jordaan and Mr. A. Joubert of the SASOL Centre for Chemistry, North-West University, for recording the NMR and MS spectra, respectively. We are also thankful to Prof. J. du Preez for HPLC analysis. Financial support for this work was provided by the North-West University, the National Research Foundation (Grant No: 96135) and the Medical Research Council, South Africa.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2017.08.013.

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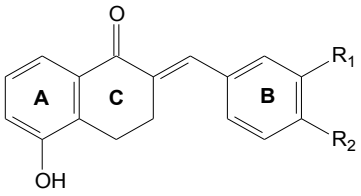
CHAPTER 5

CONCLUSION

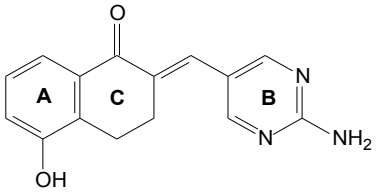
The neurodegenerative disorder PD is characterised pathologically by neuronal loss in the nigrostriatal pathway and clinically by motor and non-motor symptoms. The significance of AR's in PD is based on the xanthine derivative caffeine and its ability to act as a non-selective A_1 and A_{2A} AR antagonist and, in so doing, affect brain function (e.g. sleep/wake cycle, cognition, locomotion etc.) as well as neurological conditions leading to brain dysfunction (e.g. PD).

In this study, a series of A_1 and/or A_{2A} AR antagonists were synthesised, characterised and evaluated for the potential pharmacological treatment of neurological conditions, such as PD and presented as a research article published in an academic journal.

Based on a pilot study which explored 2-benzylidene-1-tetralones as AR antagonists, this study showed that 5-substituted 2-benzylidene-1-tetralone derivatives, specifically C5-OH substituted compounds, are potent and selective A_1 and/or A_{2A} AR antagonists. Substitution at C5 on ring A with OH is preferred to OCH_3 substitution; in fact, OCH_3 substitution is detrimental to both A_1 and A_{2A} AR affinity. The benzylidene side-chain is essential to AR affinity; seeing as the starting material 5-hydroxy-1-tetralone (**1**) has no A_1 or A_{2A} AR affinity, yet, the lead compound (**2**) — containing a benzylidene side-chain — retain both A_1 and A_{2A} AR affinity. A_1 AR affinity increases with *meta* (3')- or *para* (4')- OH substitution on phenyl ring B, affording compounds **3** and **4** K_i values in rat whole brains below 2 µM. Additionally, substitution of phenyl ring B with a 2-aminopyrimidine ring leads to potent and selective A_{2A} AR antagonism — providing compound **12** with the highest A_{2A} AR affinity ($A_{2A}K_i$ (rat) = 1.61 µM).



2: $R_1 = R_2 = OH$
3: $R_1 = OH$; $R_2 = H$
4: $R_1 = OH$; $R_2 = H$



12

Taking the above into account, compounds **3**, **4** and **12** are promising candidates for optimization of the 2-benzylidene-1-tetralone scaffold to yield effective dual A_1/A_{2A} AR antagonists. See **Figure 5-1** for a broad overview of ring A and B substitutions on 2-benzylidene-1-tetralone core's influence on A_1 and A_{2A} AR affinity.

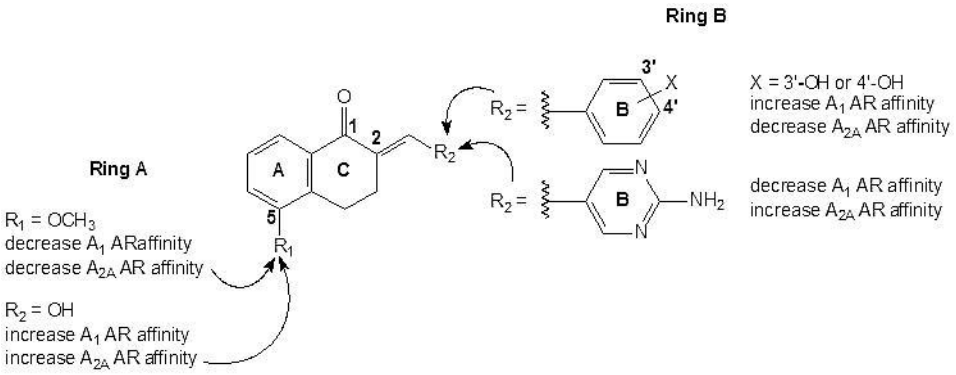


Figure 5-2: A broad overview of ring A and B substitutions on 2-benzylidene-1-tetralone core's influence on A_1 and A_{2A} AR affinity.

The aim of the study, as a whole, was to gain insight into the importance of structural modifications to ring A and B of the benzylidene tetralone scaffold necessary for A_1 and/or A_{2A} AR affinity in order to identify potential drug candidates for PD treatment. Through the objectives of the study, this goal was achieved and the hypothesis proved correct: substituent changes to ring A and B and heterocyclic changes to ring C of the aurones hispidol and maritimetin and the benzylidene tetralone (*E*)-2-benzylidene-5-hydroxy-1-tetralone yielded various novel benzylidene tetralones with potent and selective A_1 and/or A_{2A} AR affinity and structure activity relationships that govern AR activity were revealed.

In conclusion, 16 novel 2-benzylidene-1-tetralones were synthesised by means of acid catalysed aldol condensation reactions. The synthesised compounds were characterised via NMR spectrometry, MS and melting points. Furthermore, the purities of these compounds were determined by high performance liquid chromatography (HPLC). The A_1 and/or A_{2A} AR affinity of all synthesised compounds were ascertained by means of radioligand binding studies, while GTP shift assays determined selected compounds' functionality as either A_1 AR agonists or antagonists. The evaluated compounds are promising novel, potent and selective A_1 and/or A_{2A} AR antagonists and, thus, possible lead compounds for non-dopaminergic treatment of PD.

Future endeavours may well include the exploration of the structurally related indanone and benzofuran based cyclic chalcones as dual A_1/A_{2A} AR antagonists for the potential pharmacological treatment of neurological conditions, such as PD.

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ANNEXURE A: PUBLISHED ARTICLE

GRAPHICAL ABSTRACT

5-Substituted 2-benzylidene-1-tetralone analogues as A_1 and/or A_{2A} antagonists for the potential treatment of neurological conditions

HD Janse van Rensburg [a], G Terre'Blanche [a,b,], MM van der Walt [b] and LJ Legoabe [b*]

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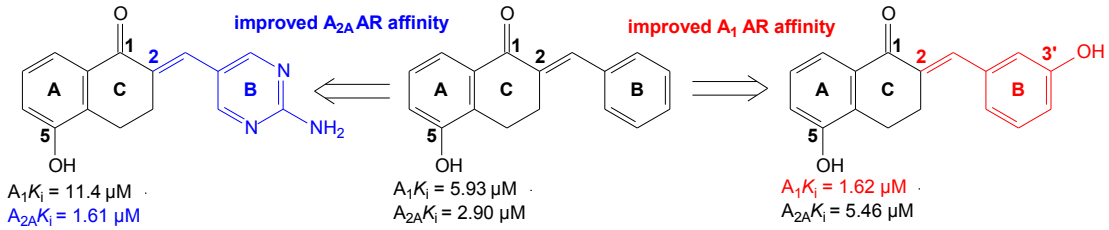

ANNEXURE B: PUBLISHED ARTICLE
SUPPLEMENTARY MATERIALS

5-Substituted 2-benzylidene-1-tetralone analogues as A_1 and/or A_{2A} antagonists for the potential treatment of neurological conditions

HD Janse van Rensburg [a], G Terre'Blanche [a,b,], MM van der Walt [b] and LJ Legoabe [b*]

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Chemicals and instrumentation

Starting materials were procured from Sigma-Aldrich and used without further purification — unless otherwise noted. Both, proton (1H) and carbon (13C) NMR spectra were recorded on a Bruker Avance III 600 spectrometer at frequencies of 600 MHz and 150 MHz, respectively, in deuterated dimethylsulfoxide (DMSO-d6). Chemical shifts are reported in parts per million (δ) in relation to the signal of tetramethylsaline (Si(CH3)4). Spin multiplicities are indicated as follows: s (singlet), d (doublet), dd (doublet of doublets), td (triplet of doublets), t (triplet), q (quartet) and m (multiplet). MS were recorded on a Bruker micrOTOF-Q II mass spectrometer in atmospheric pressure chemical ionisation (APCI) mode. HPLC analyses were determined on an Agilent 1100 HPLC system. Melting points were measured with a Buchi B545 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was done using silica gel 60 (Merck) with UV254 fluorescent indicator. Radioligand competition experiments were completed with commercially available components: radioligands [3H]NECA (specific activity 27.1 Ci/mmol) procured from PerkinElmer and [3H]DPCPX (specific activity 120 Ci/mmol) from Amersham Biosciences, filter-count from PerkinElmer and Whatman GF/B 25 mm diameter filters from Merck. Radio activity was calculated by a Packard Tri-CARB 2810 TR liquid scintillation counter.

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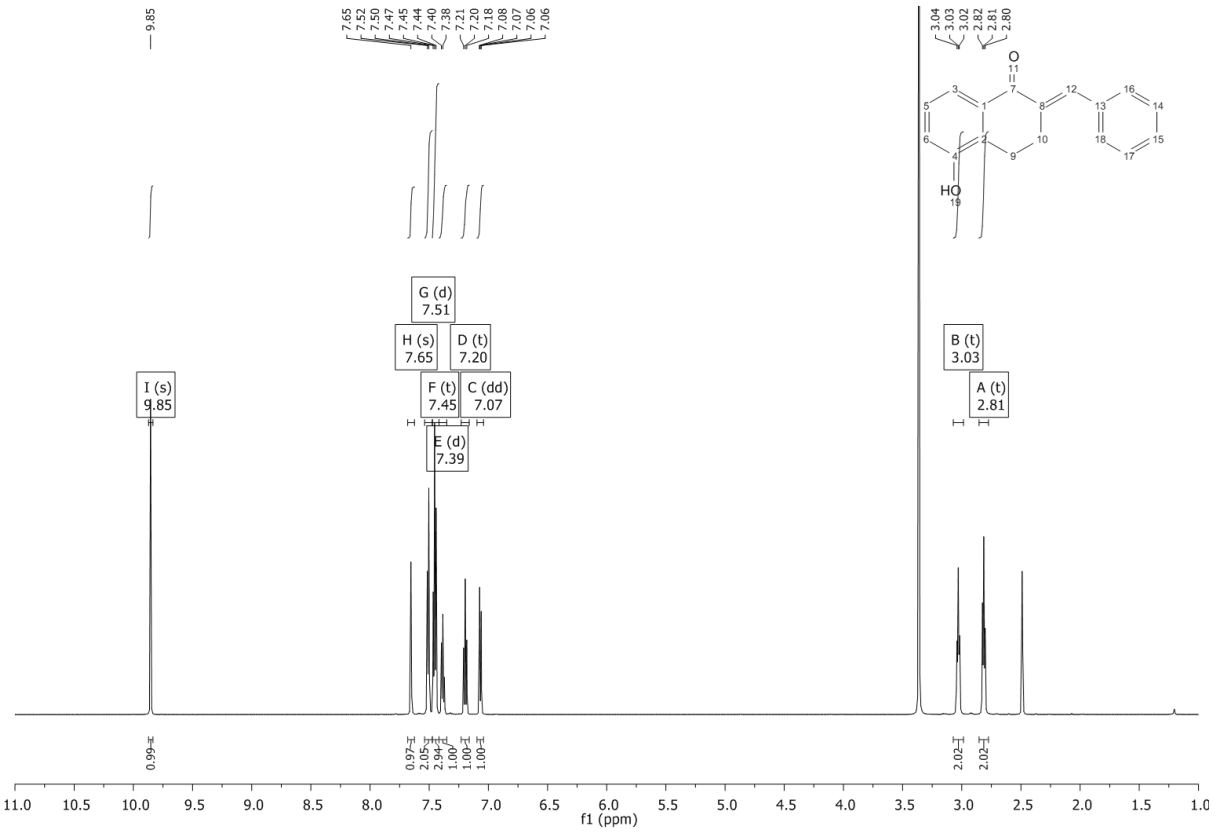

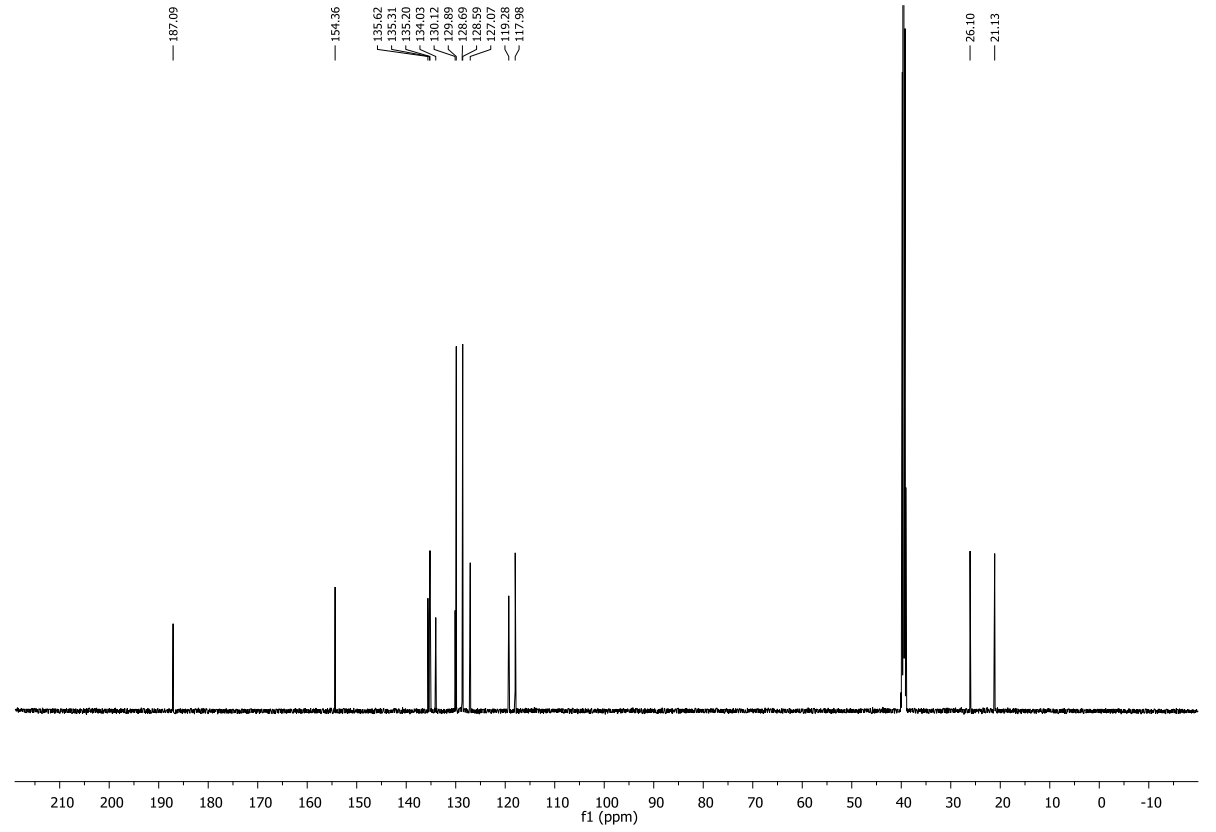

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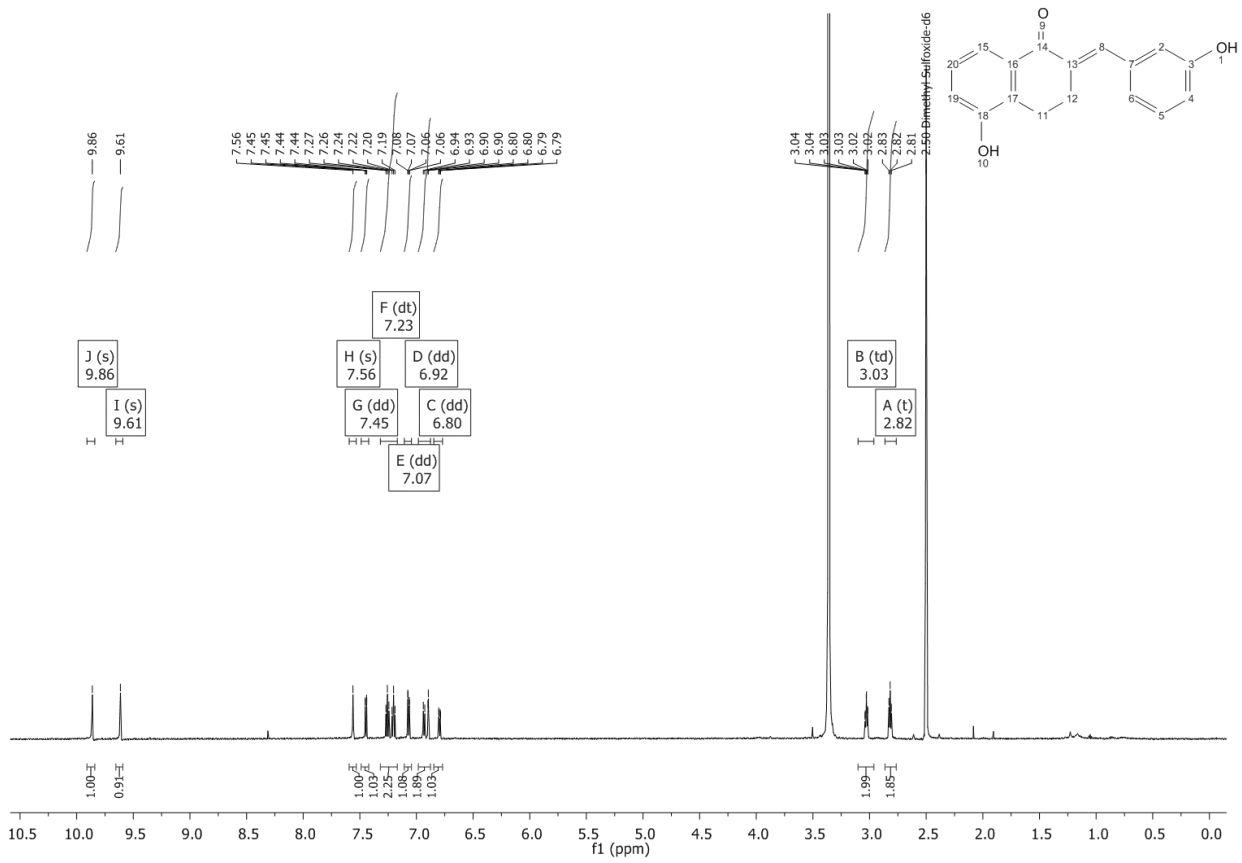

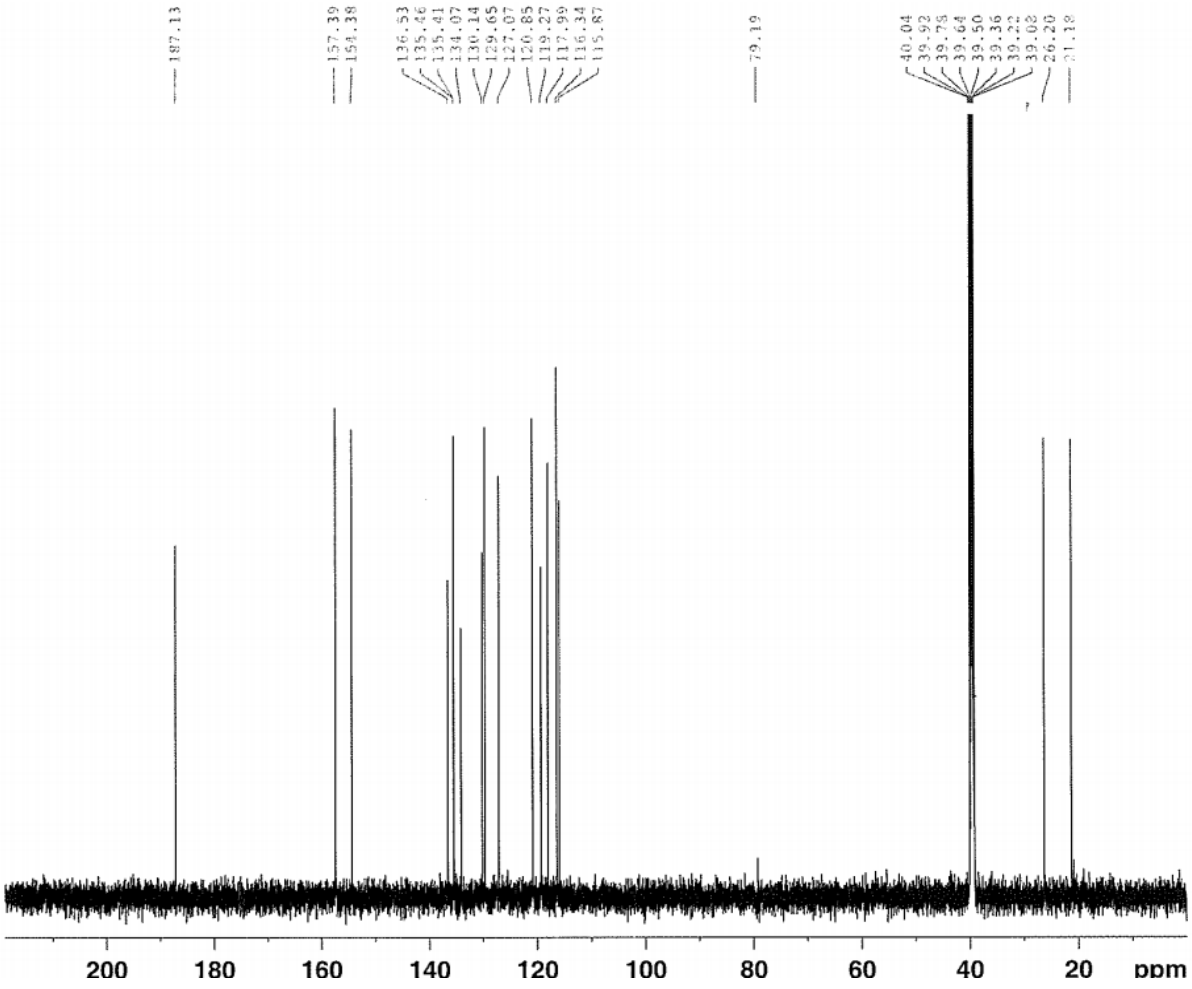

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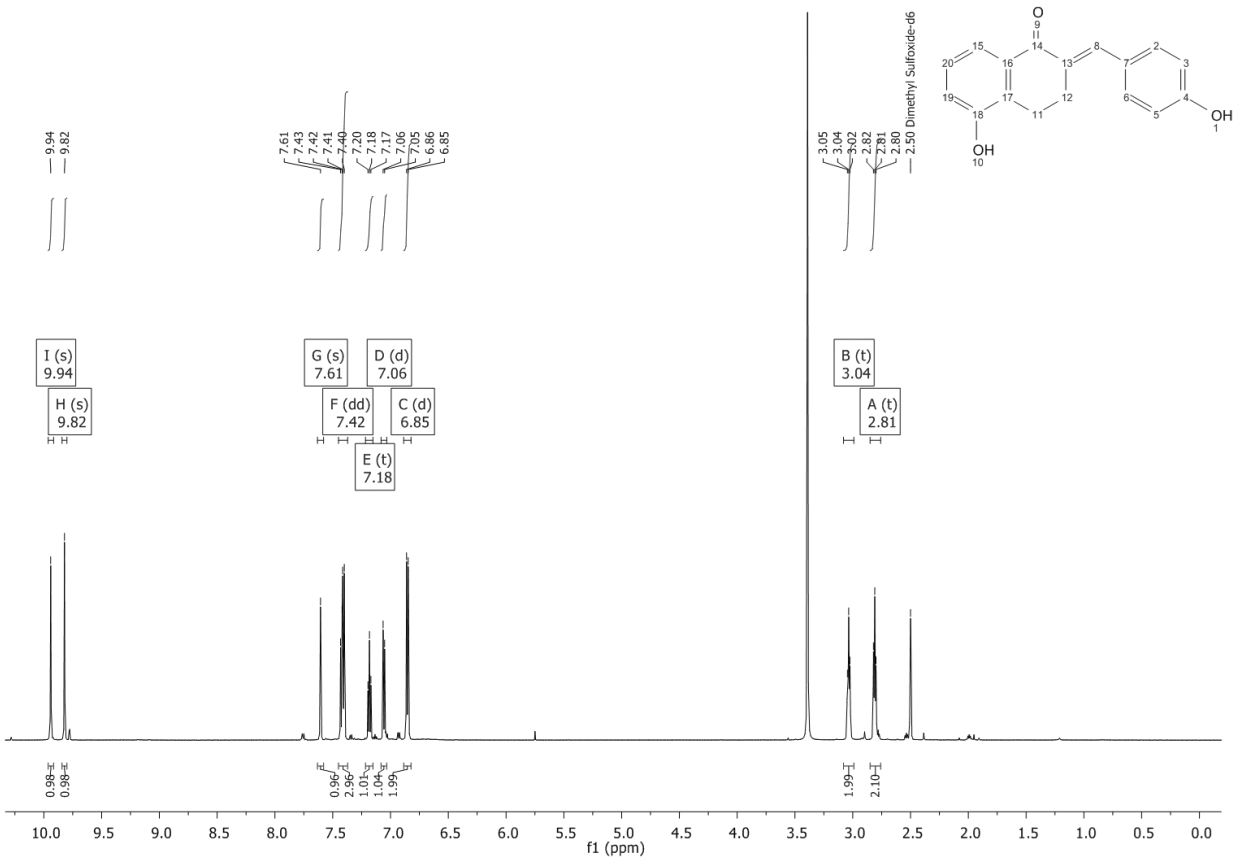

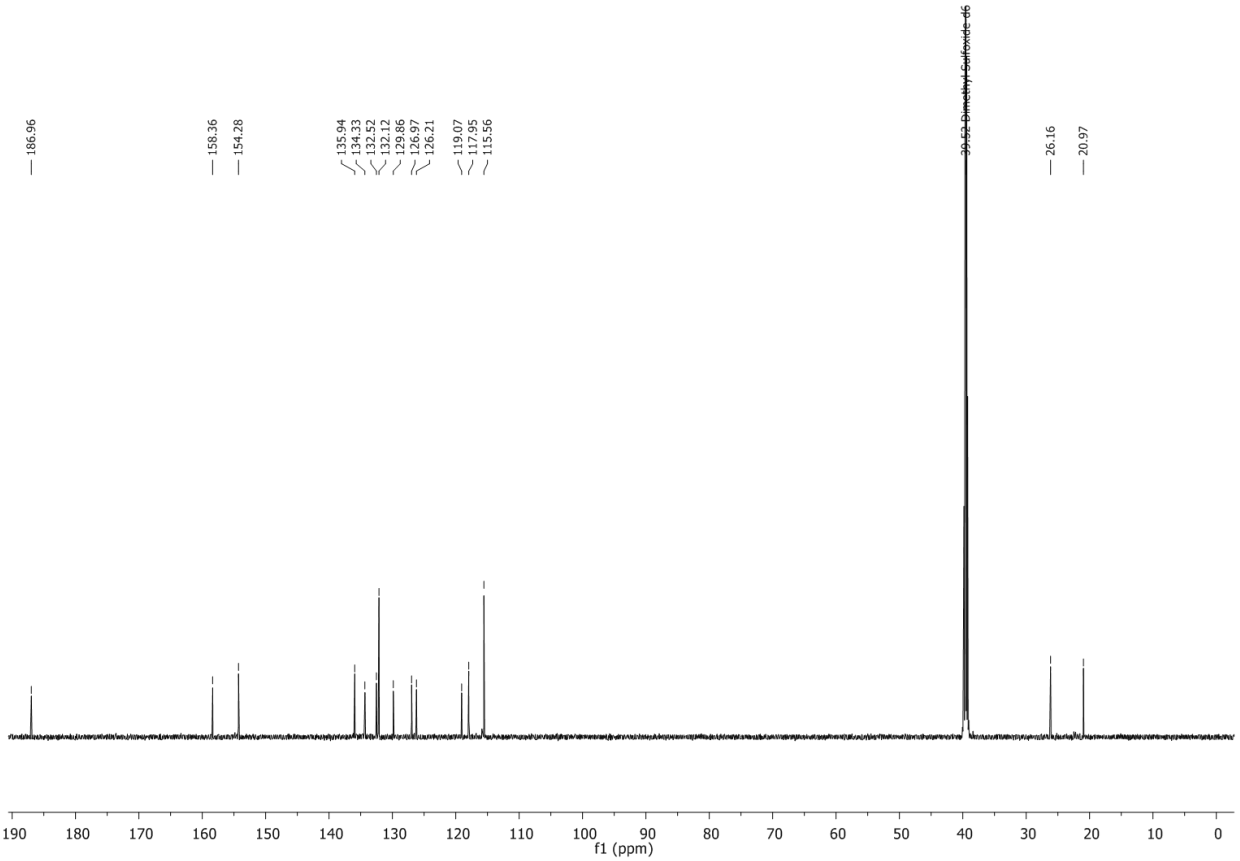

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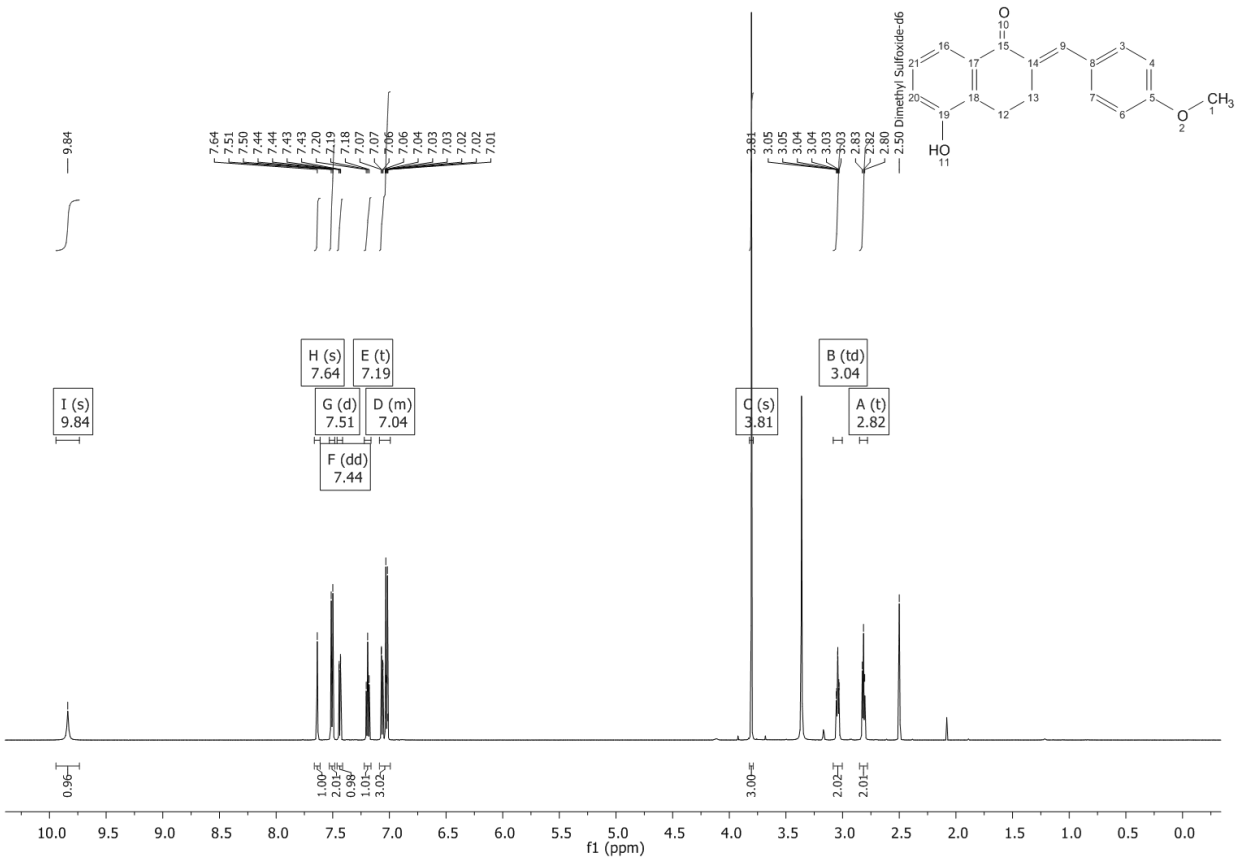

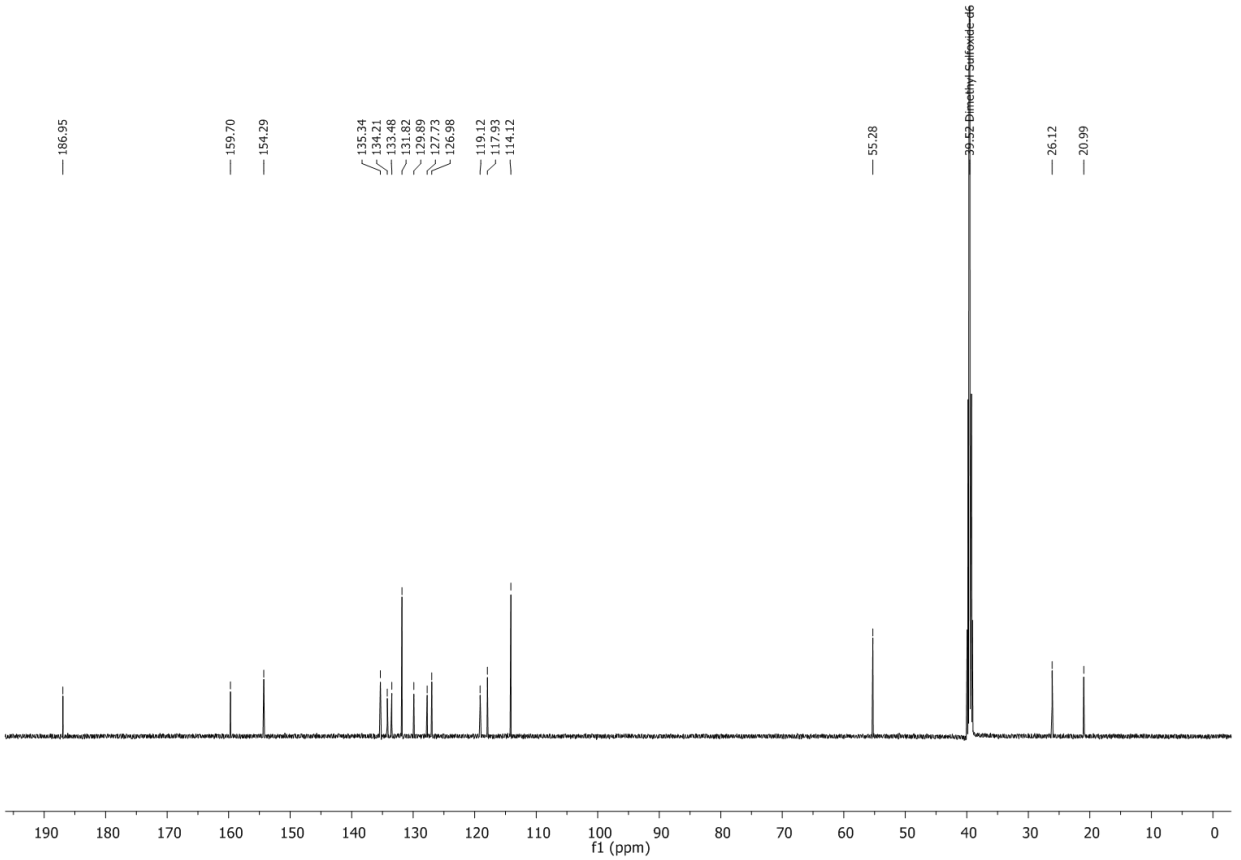

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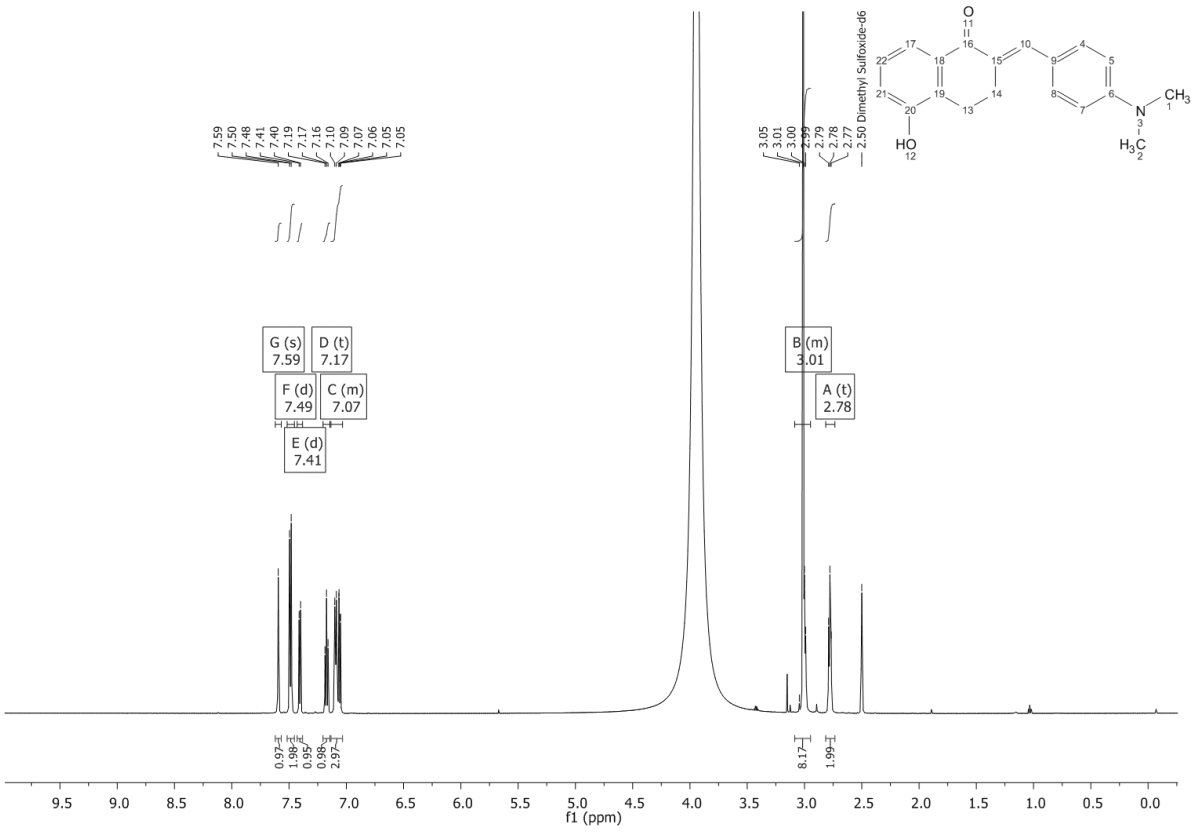

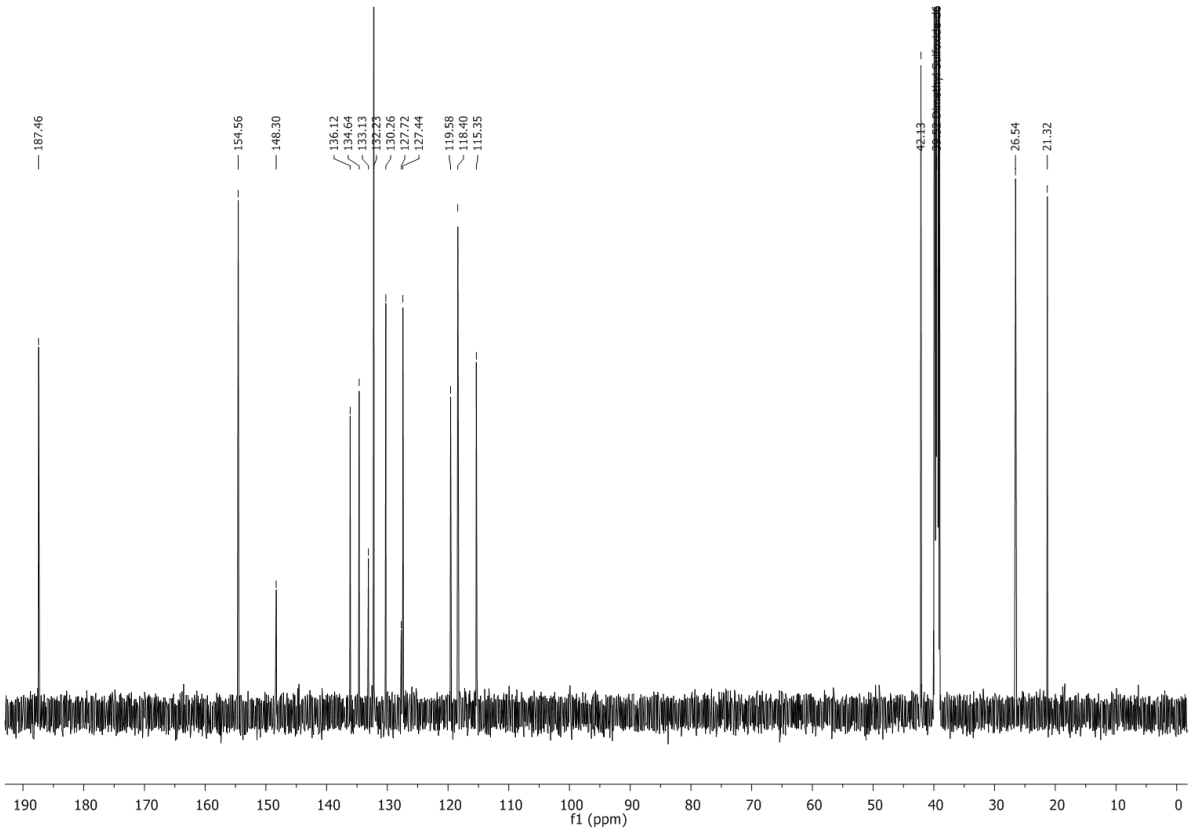

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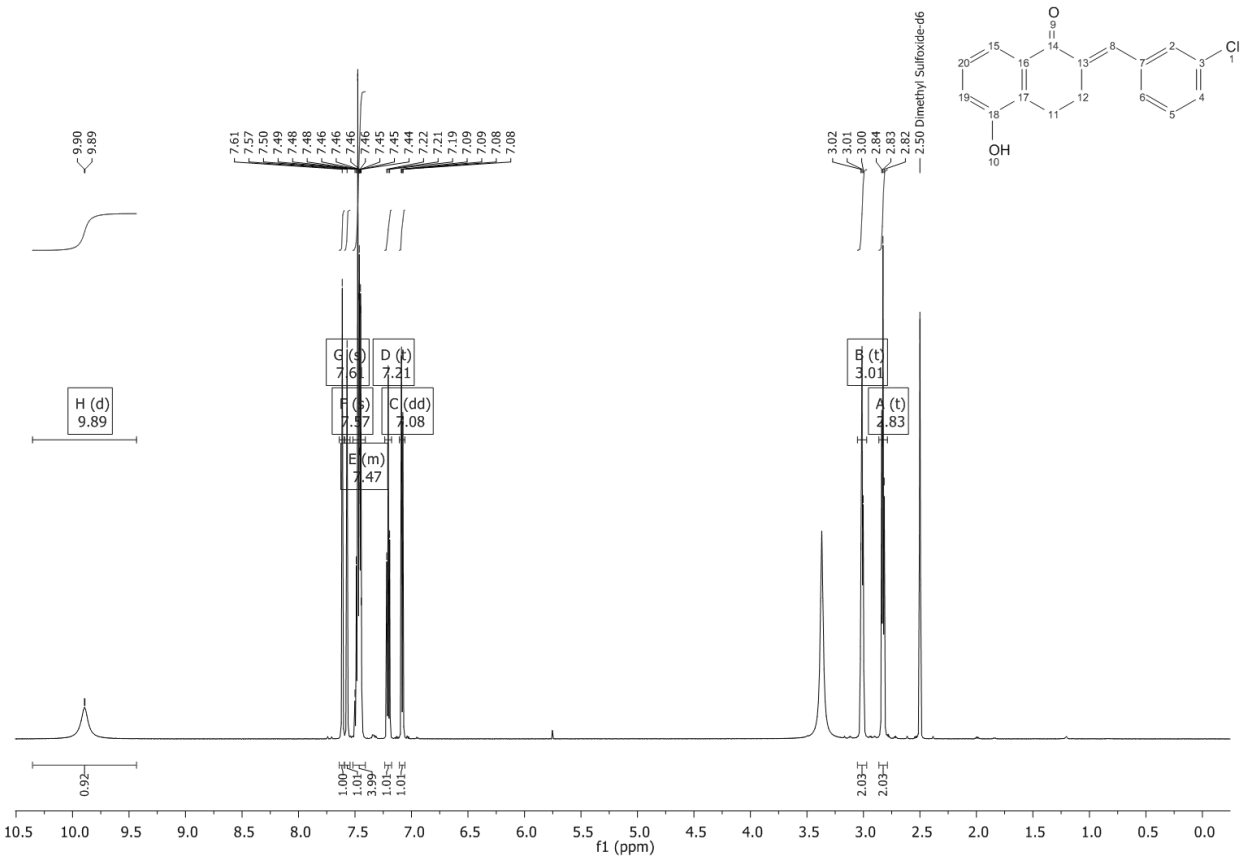

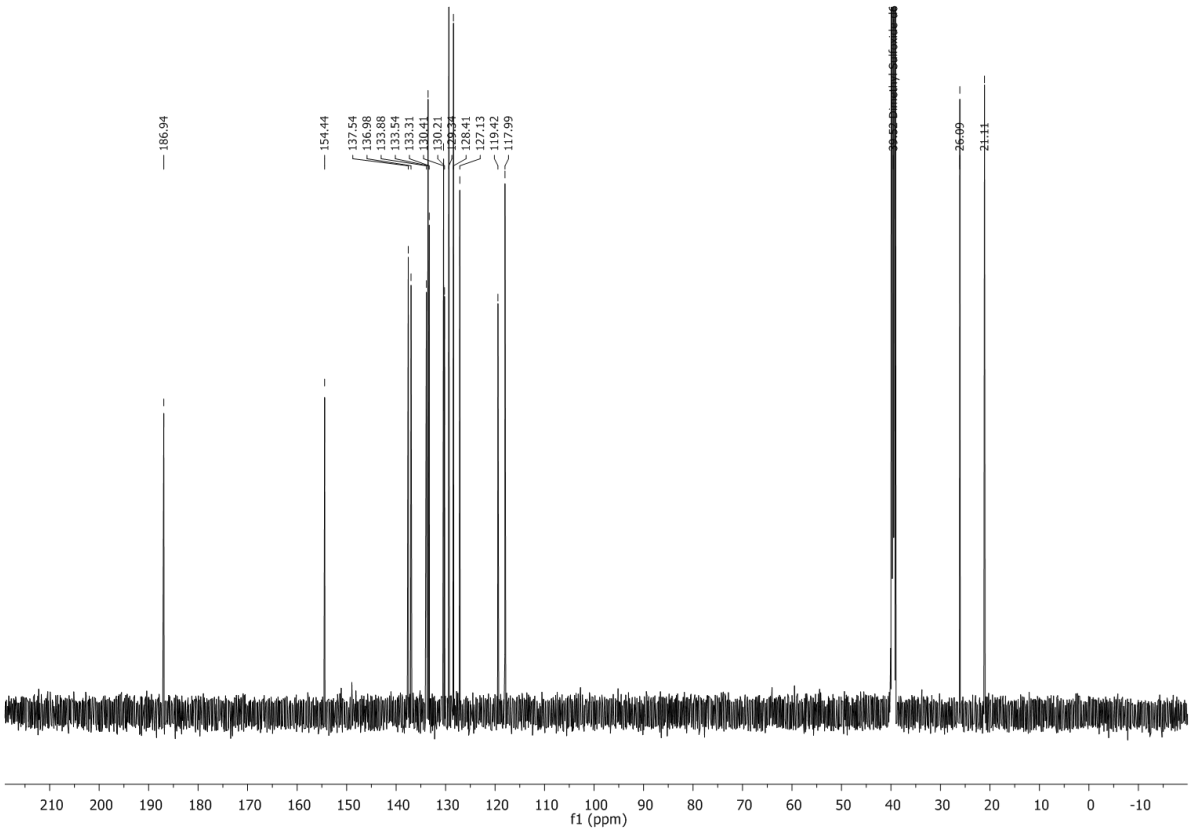

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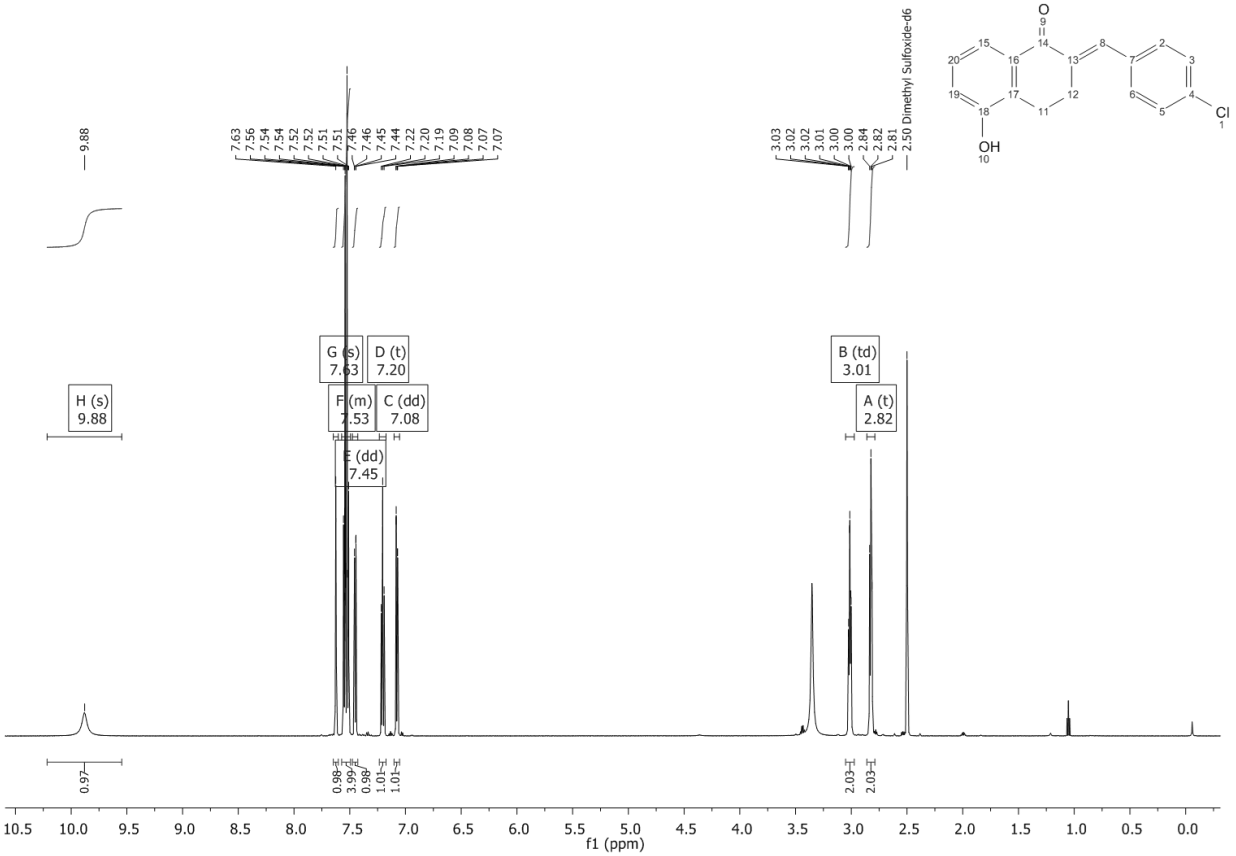

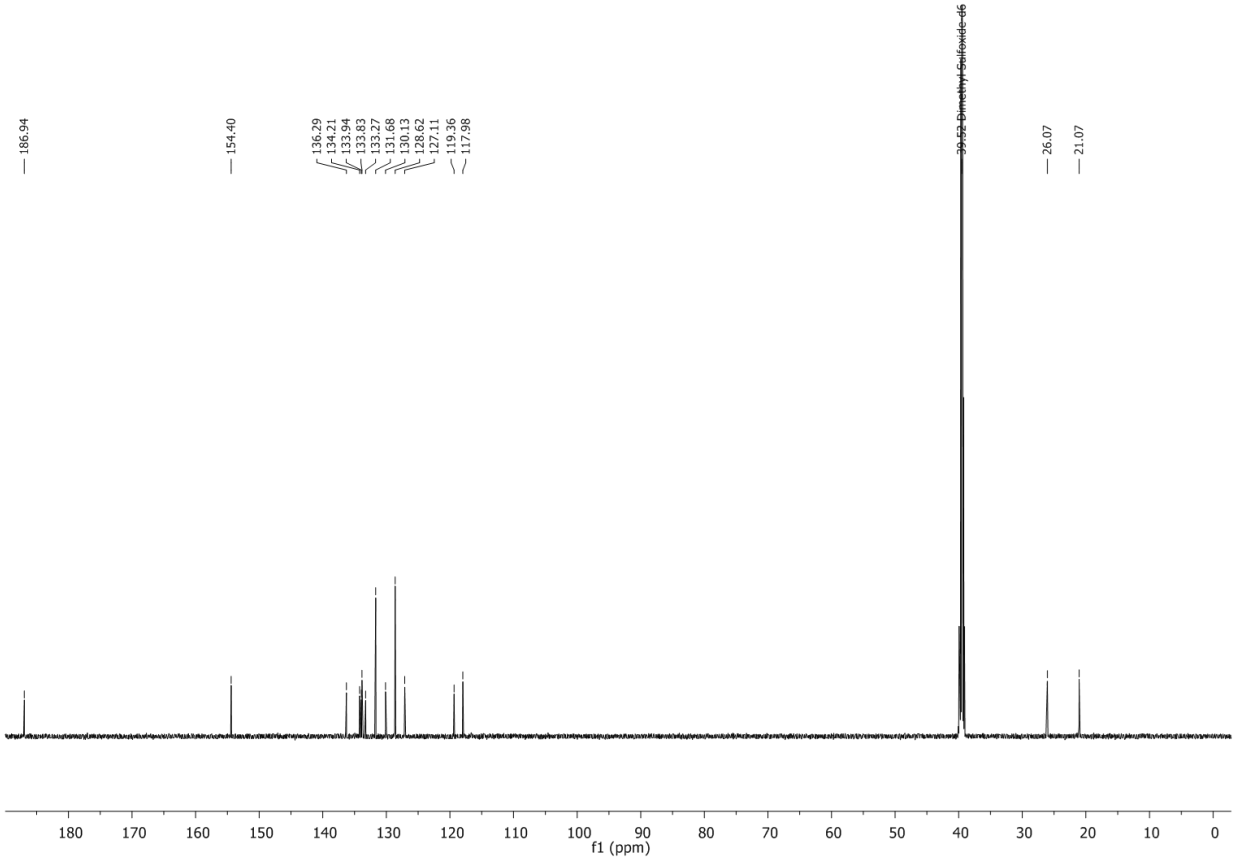

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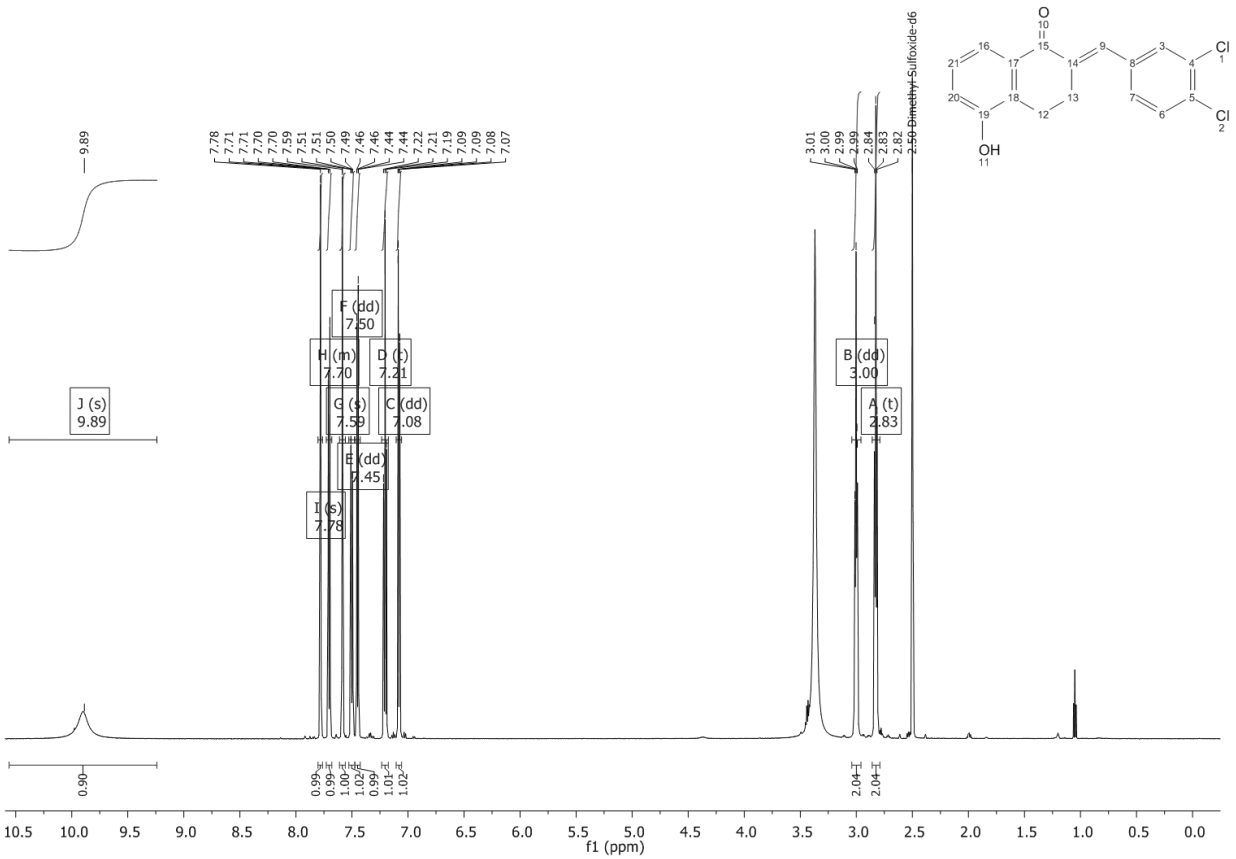

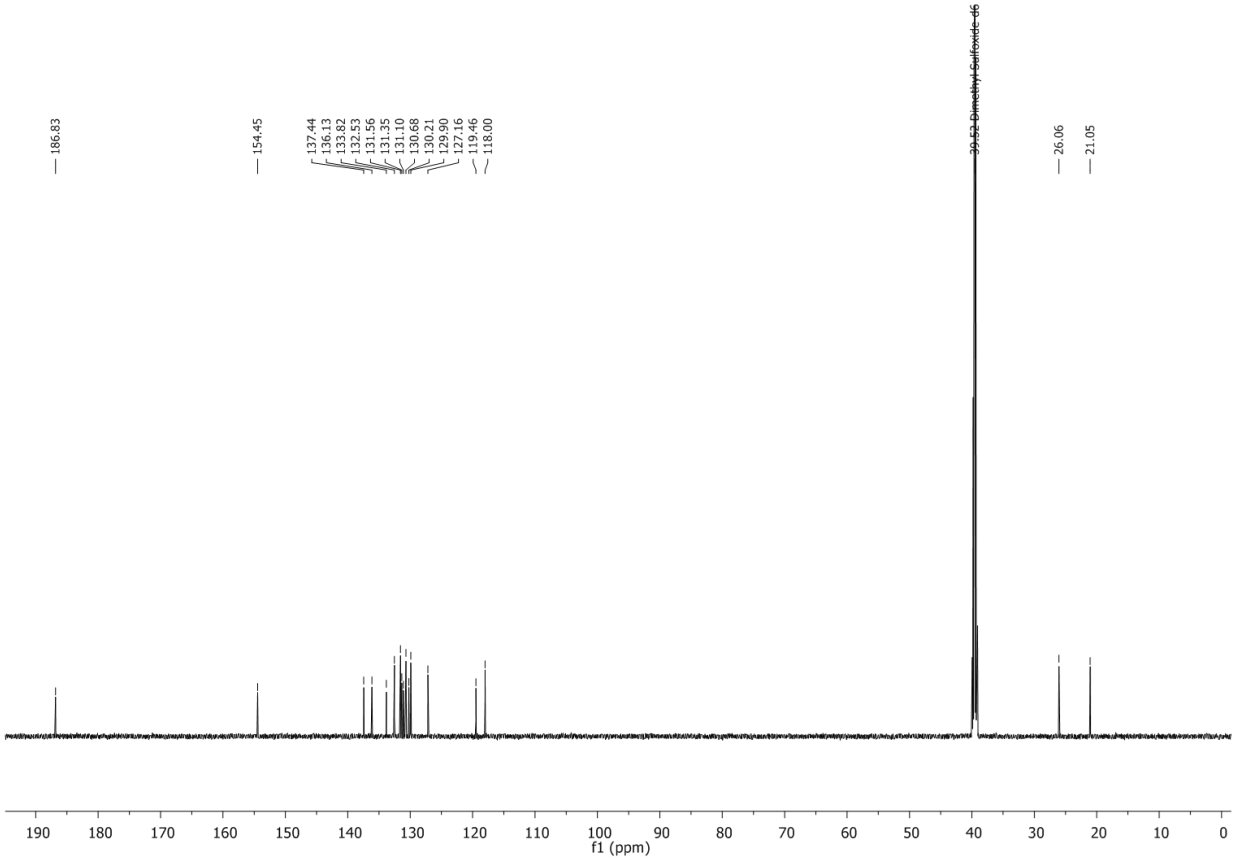

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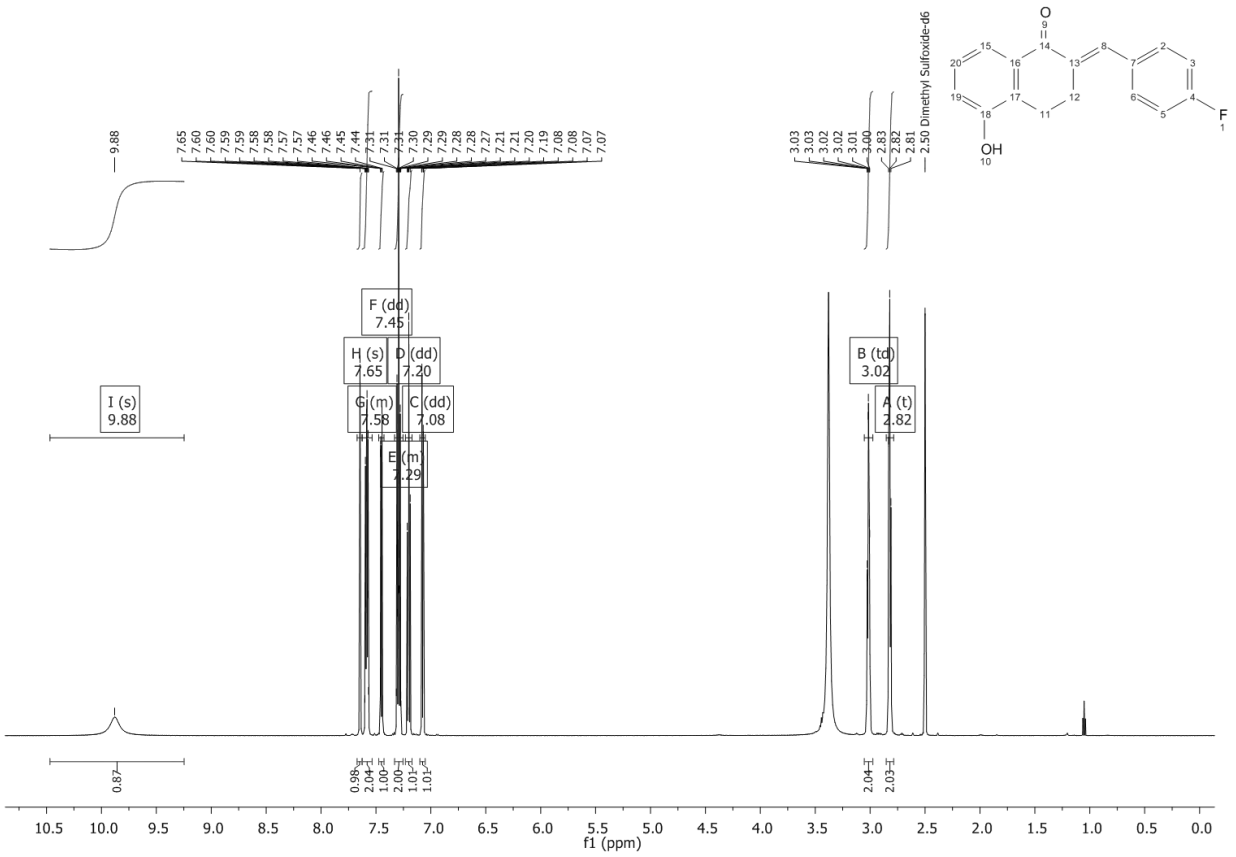

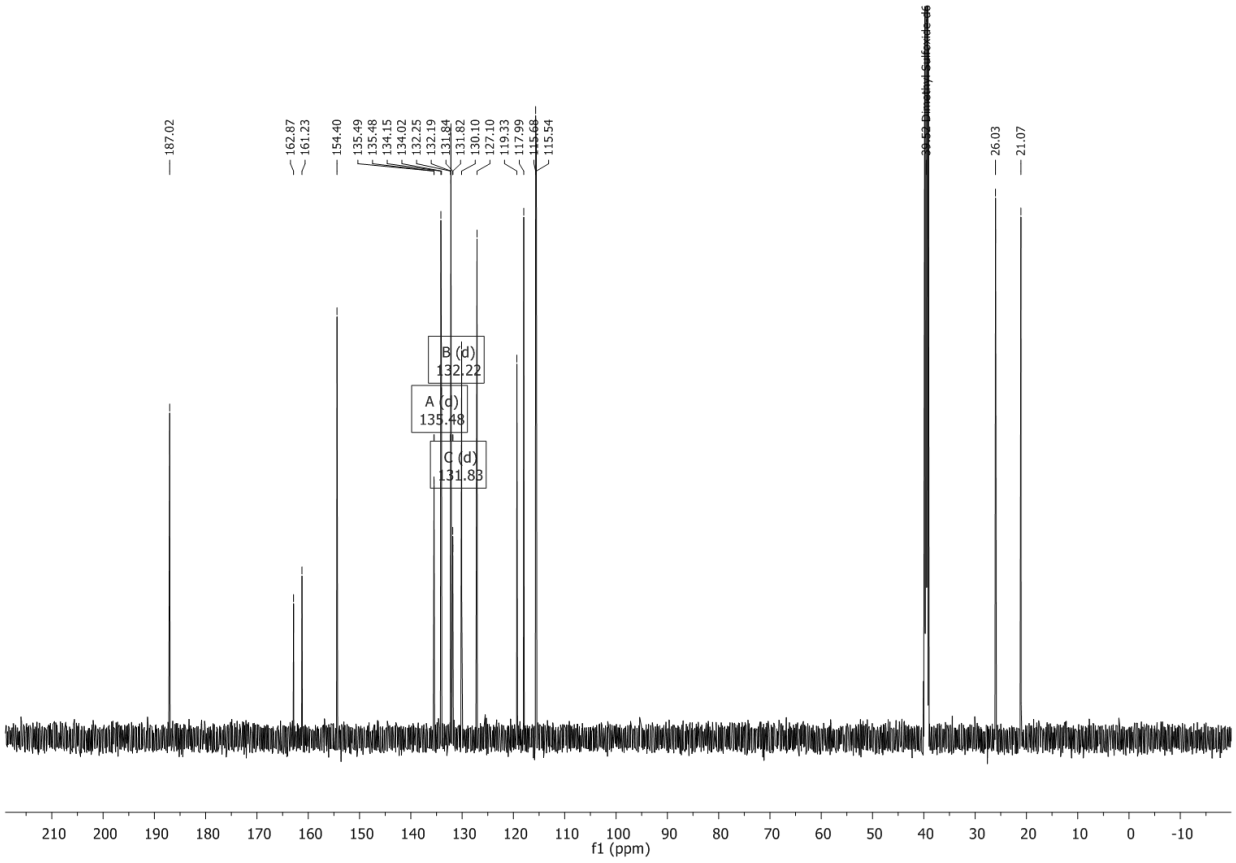

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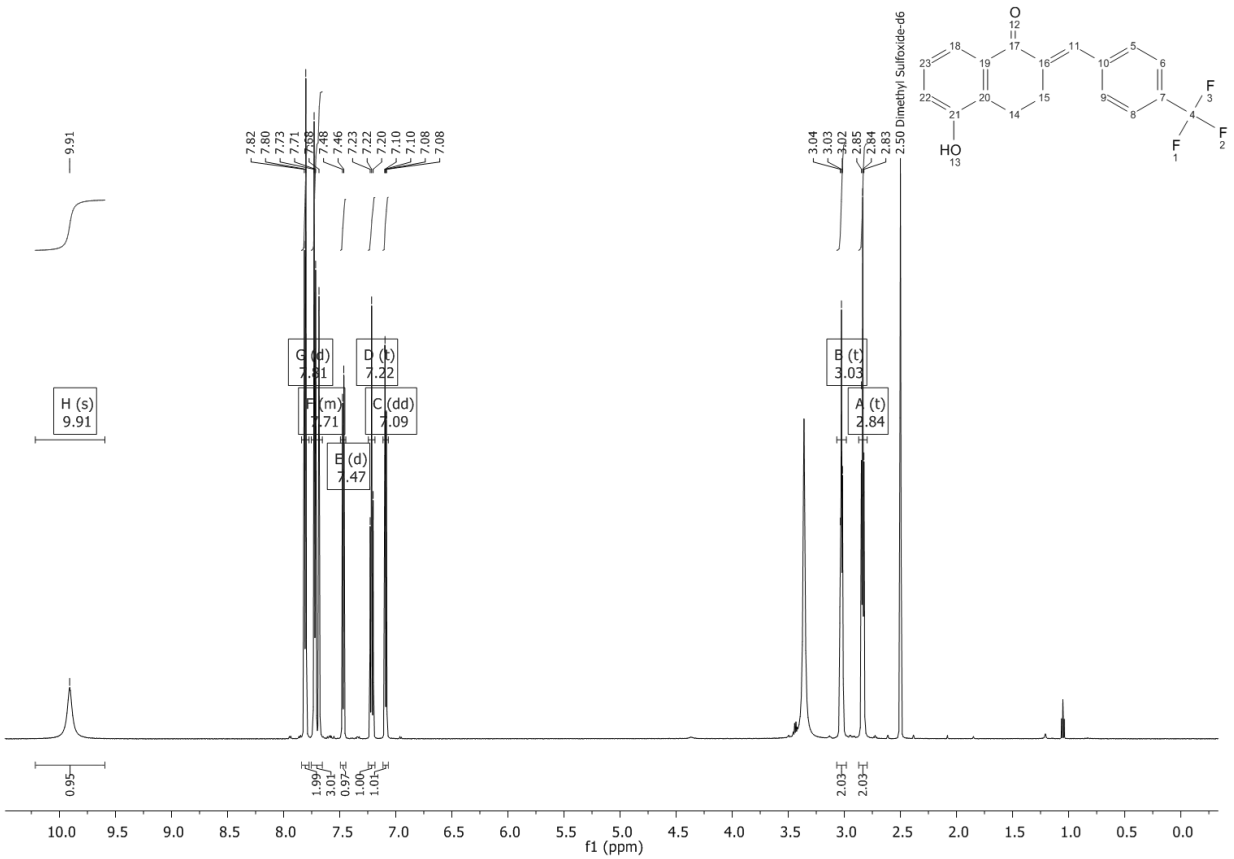

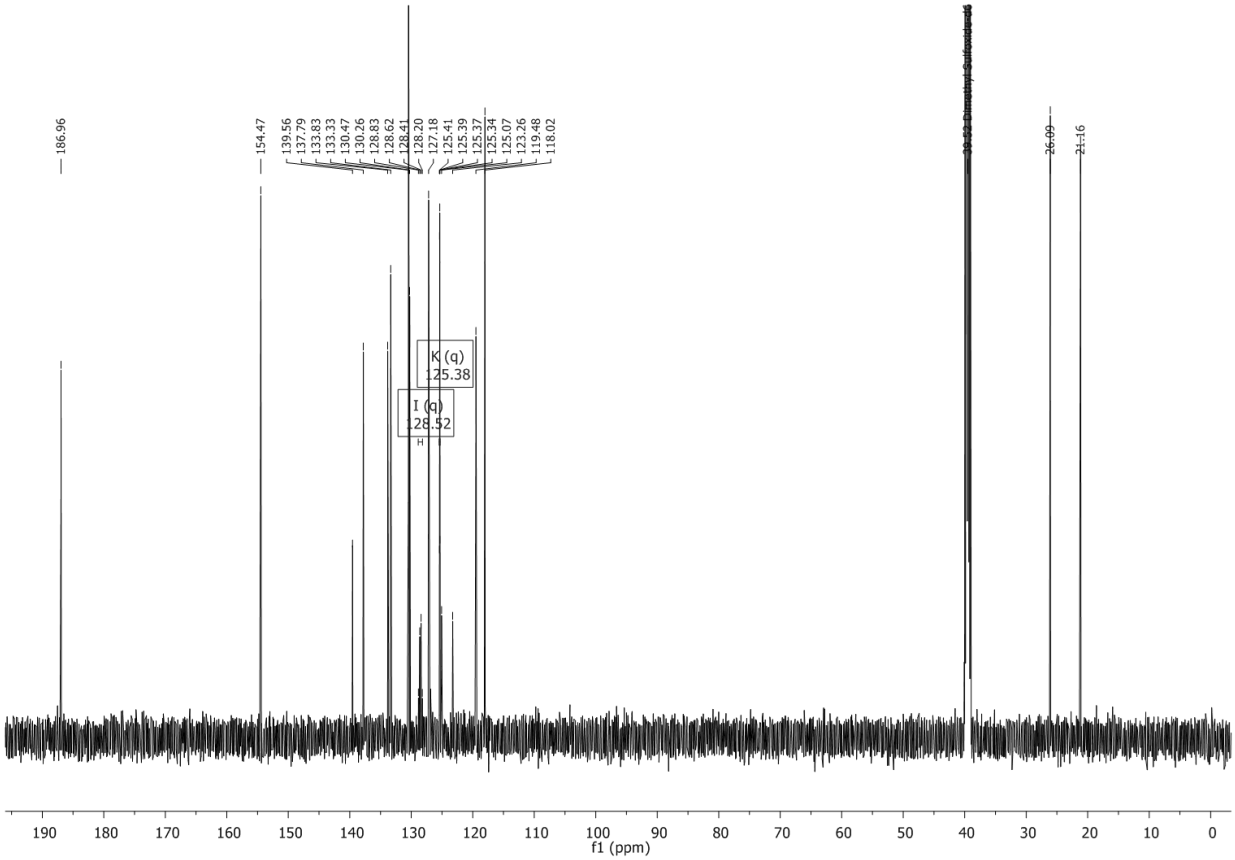

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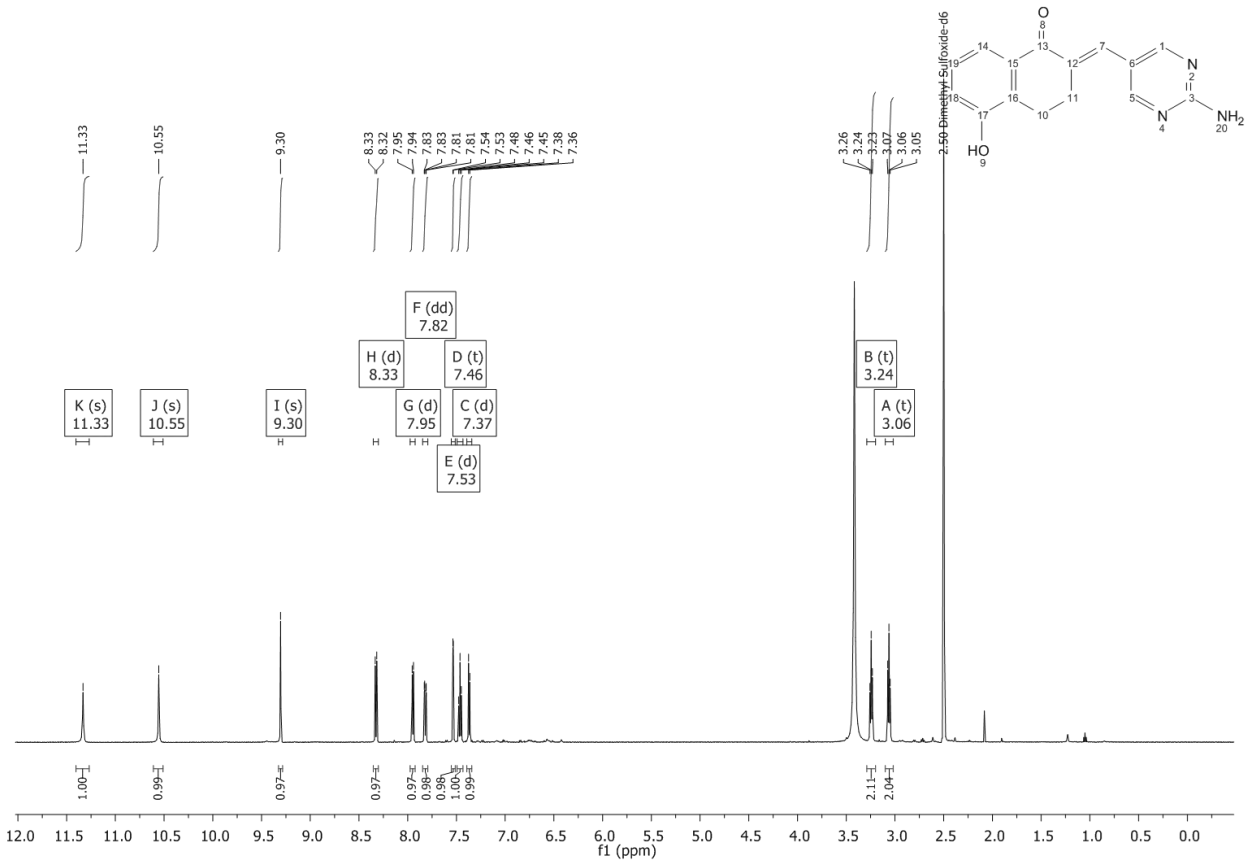

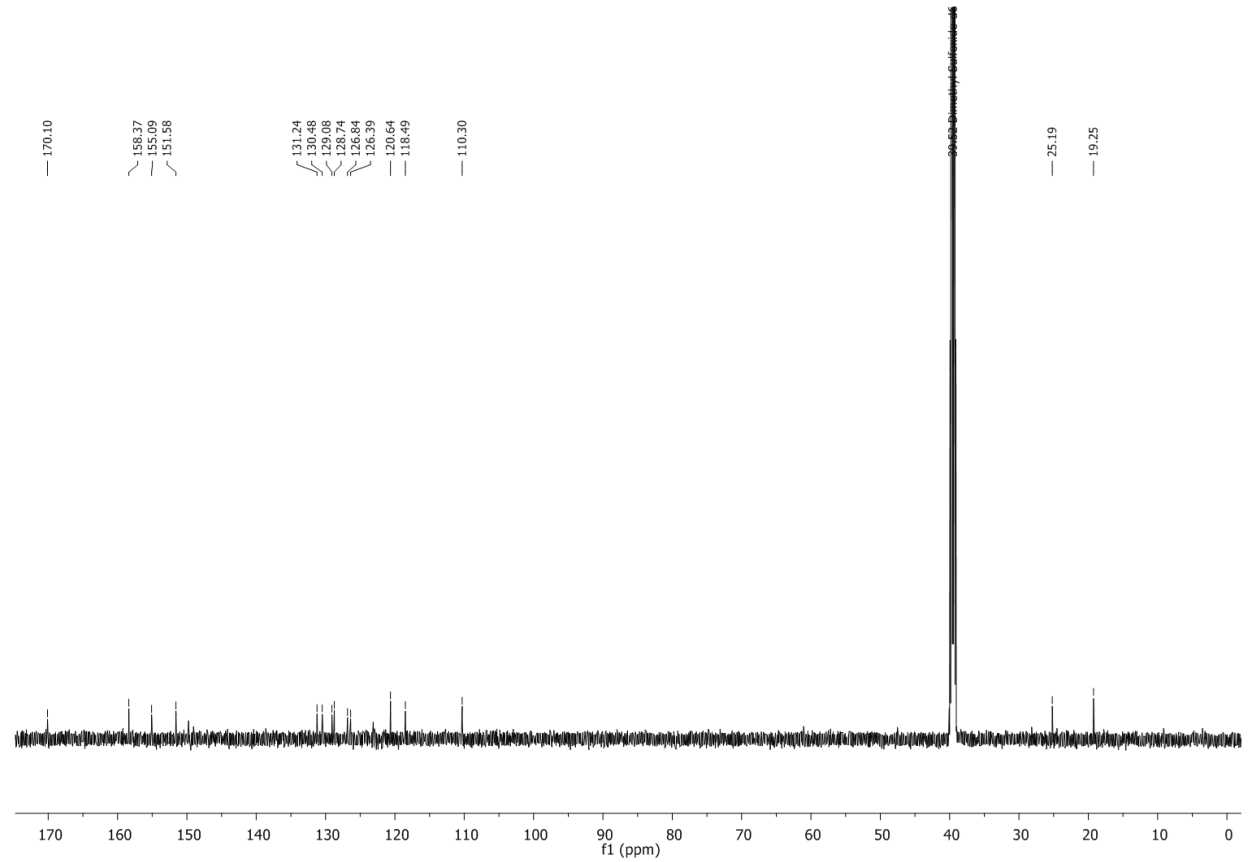

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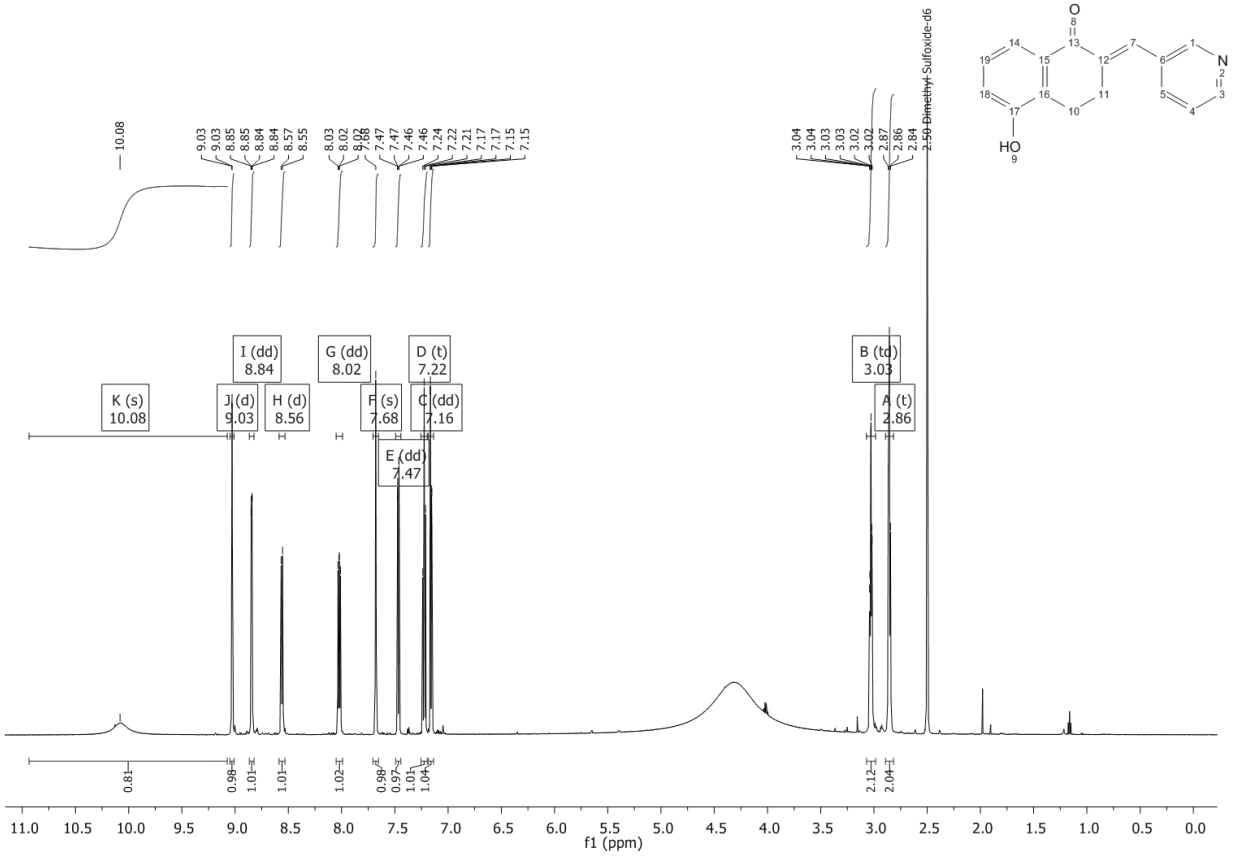
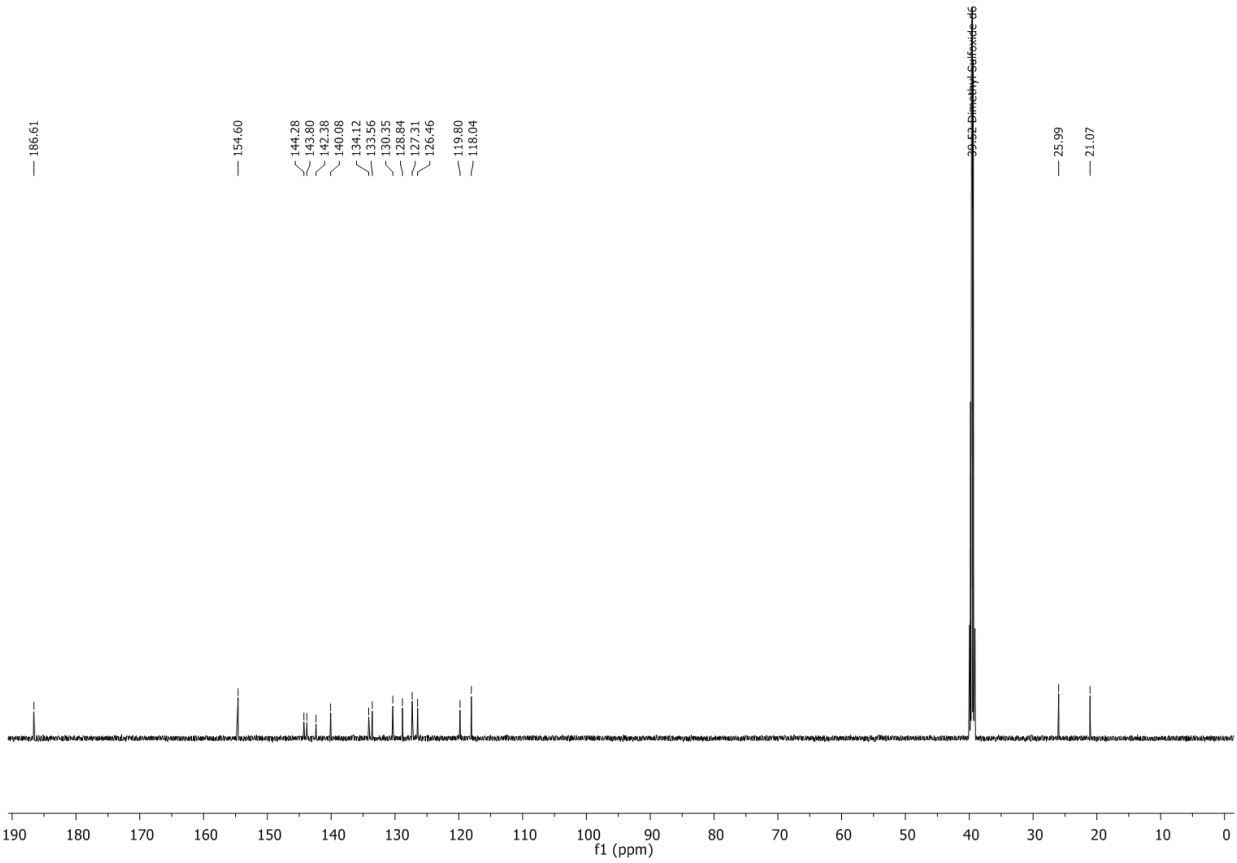
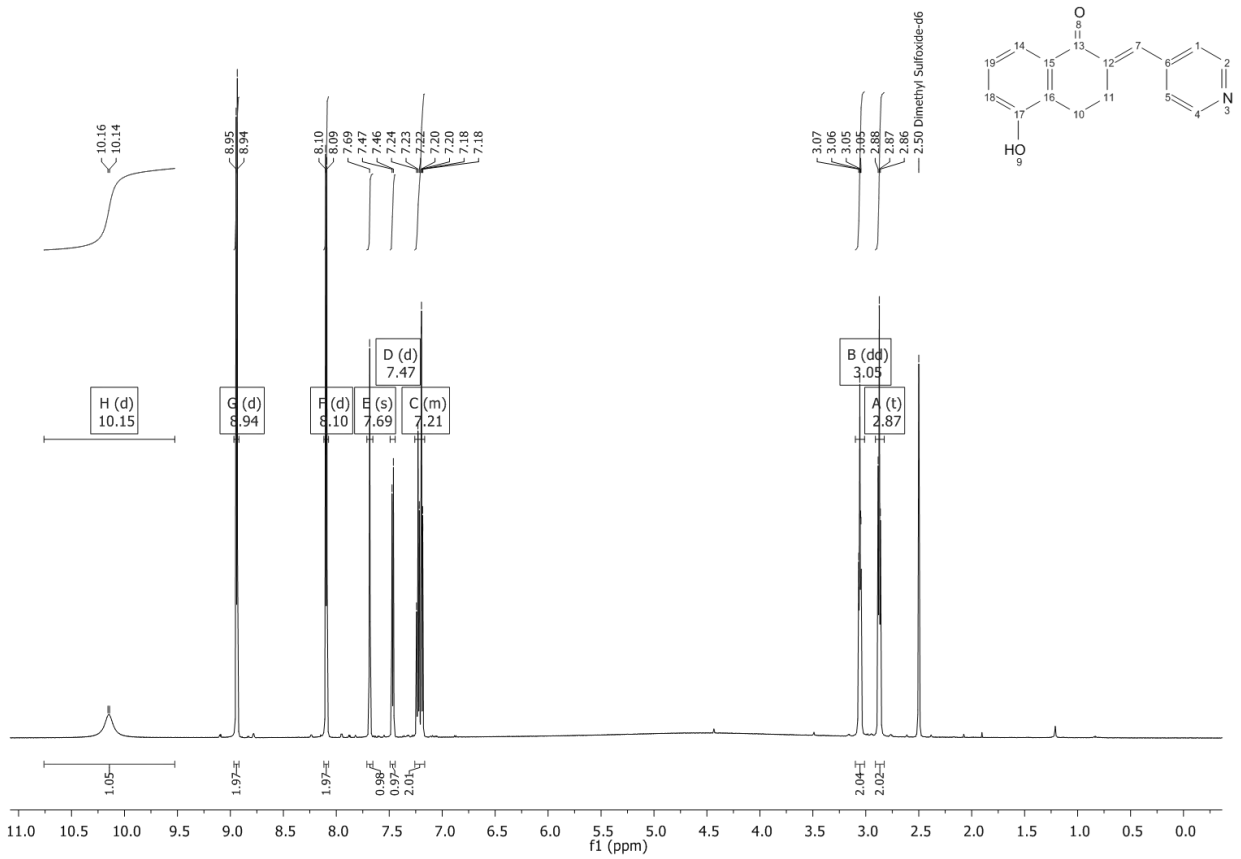
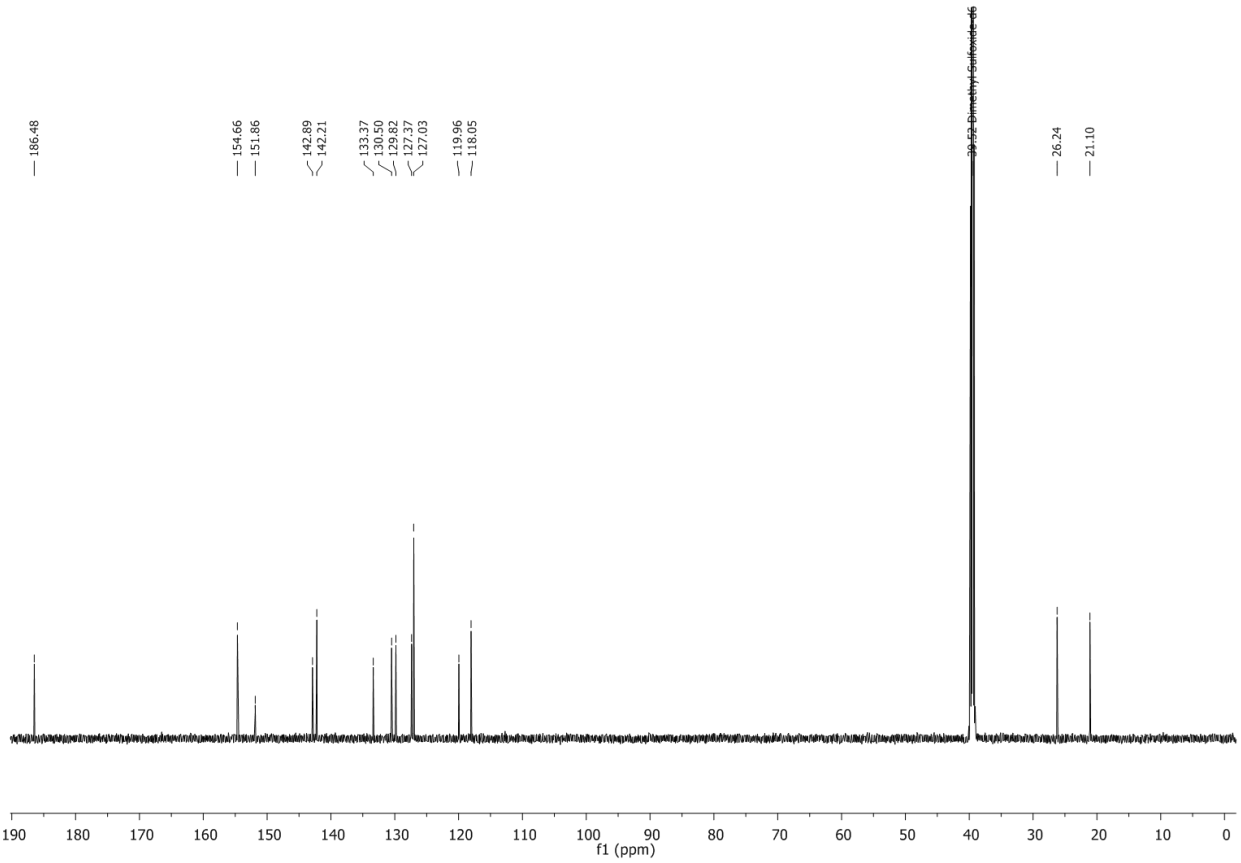


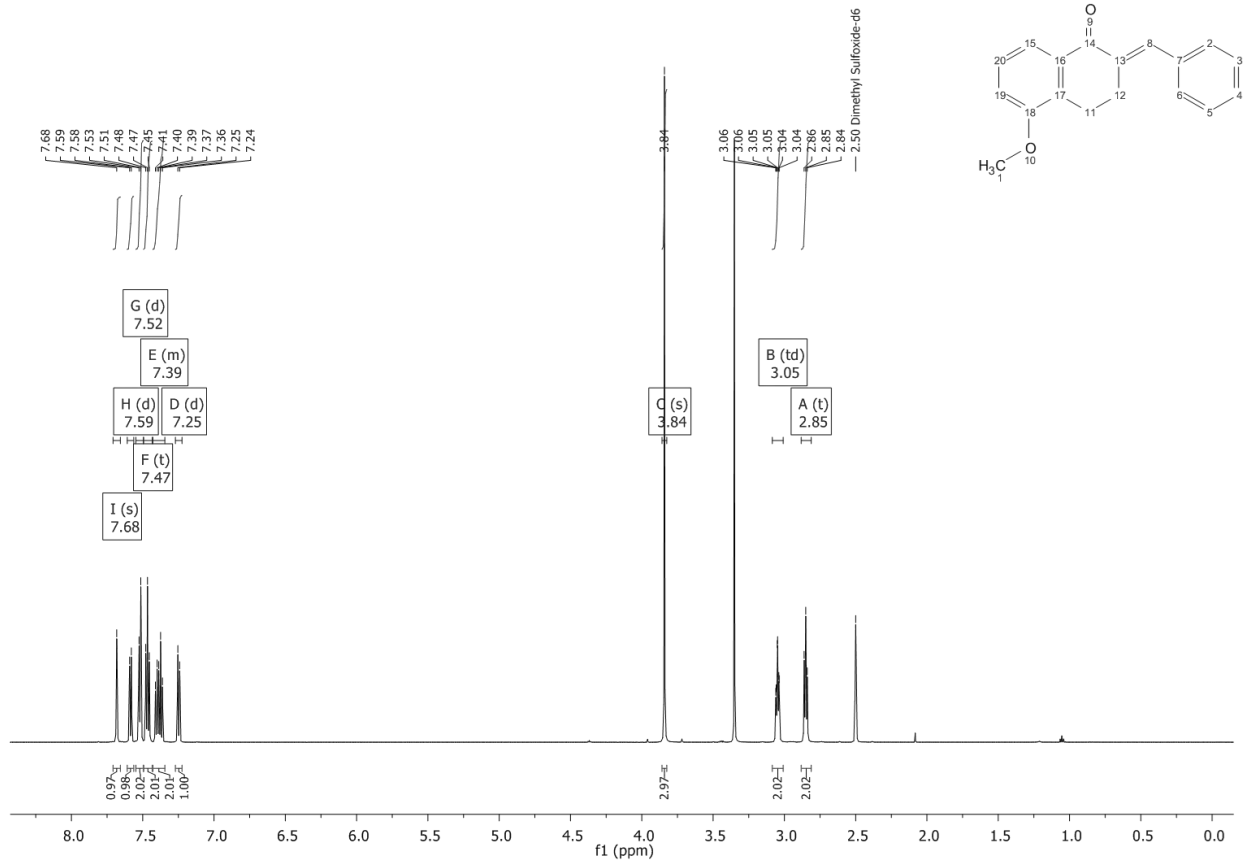


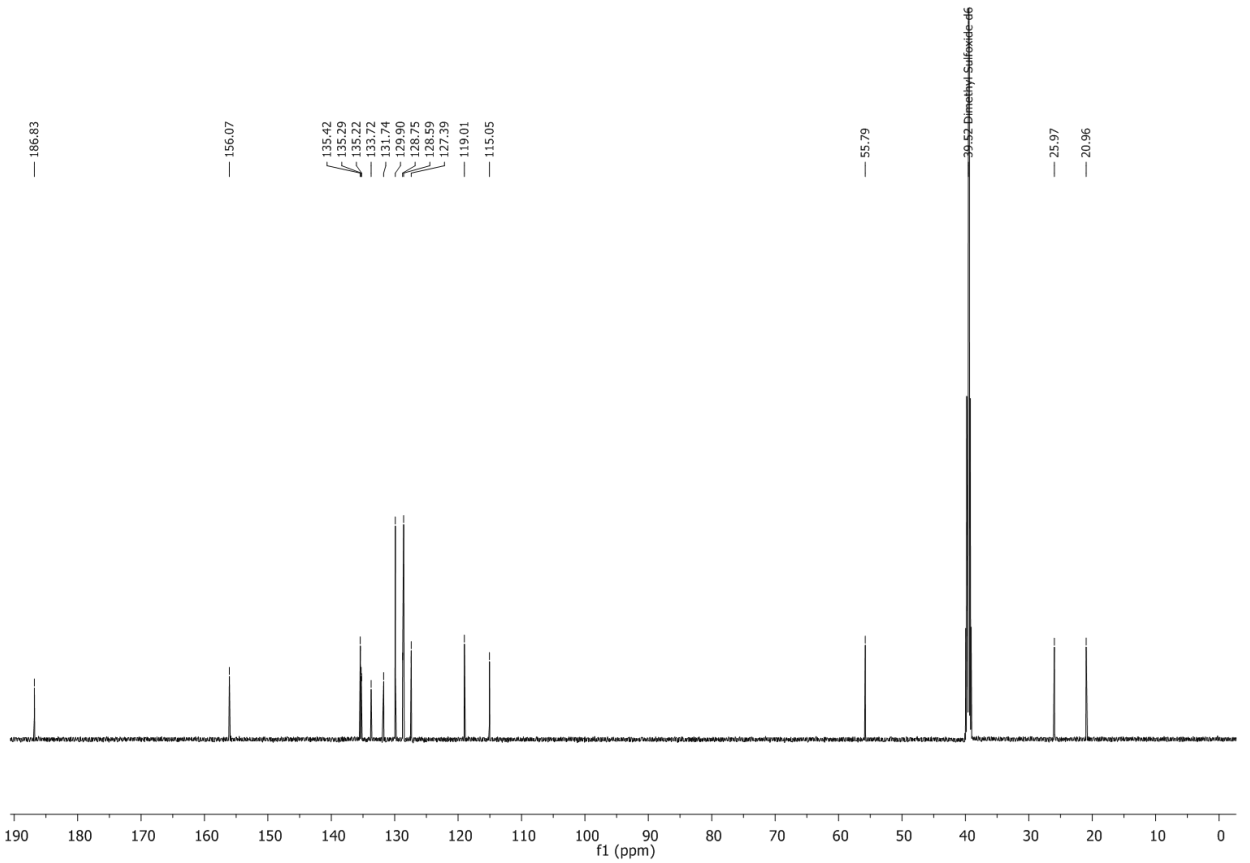
(*E*)-5-hydroxy-2-(pyridin-4-ylmethylene)-3,4-dihydronaphthalen-1(*2H*)-one (14)



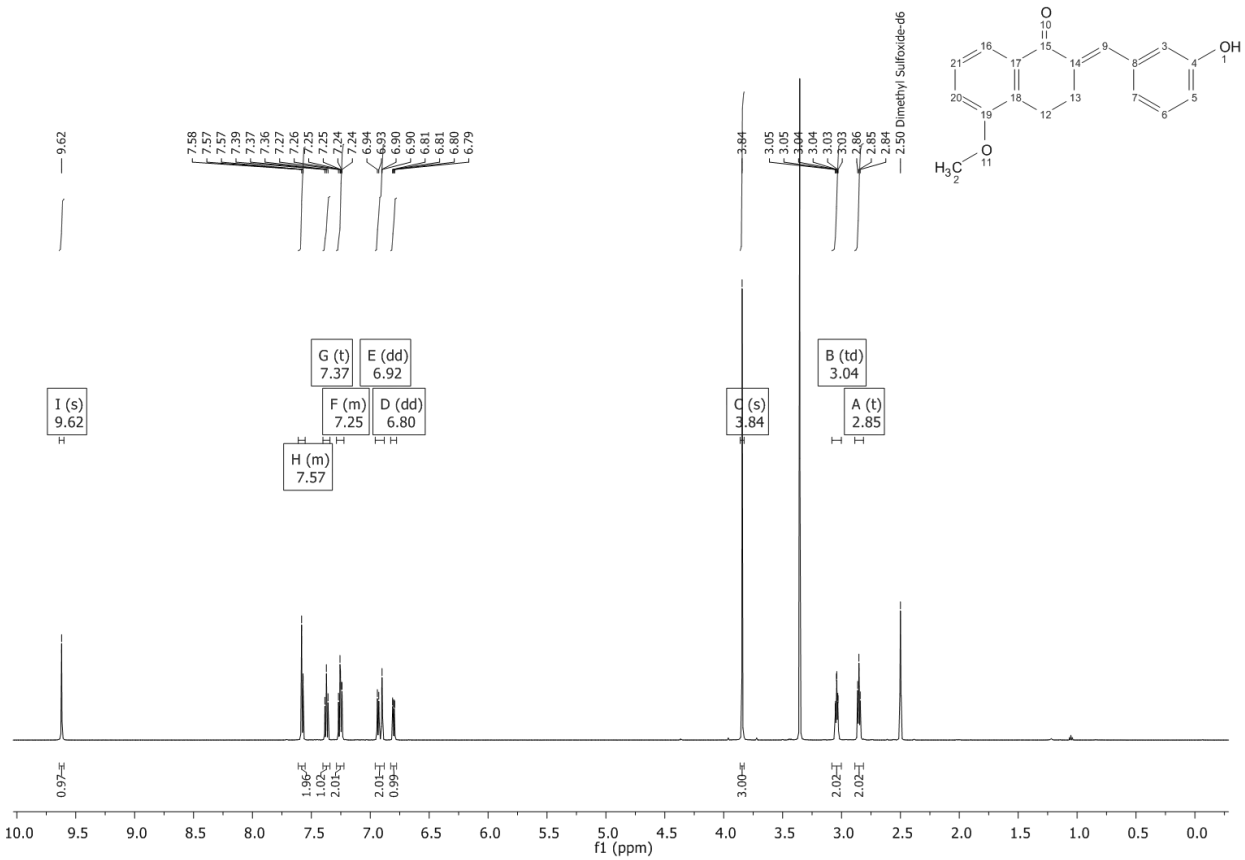


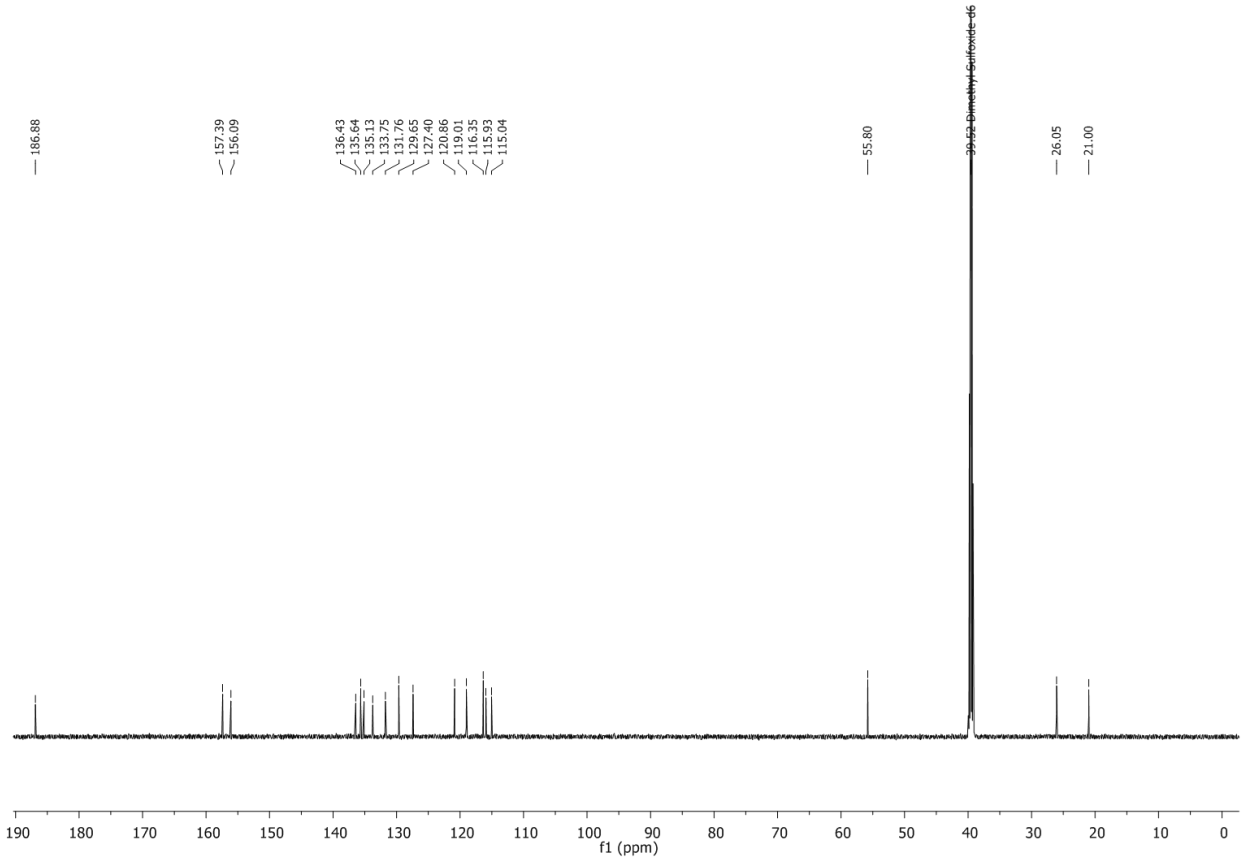
(*E*)-2-benzylidene-5-methoxy-3,4-dihydronaphthalen-1(*2H*)-one (16)



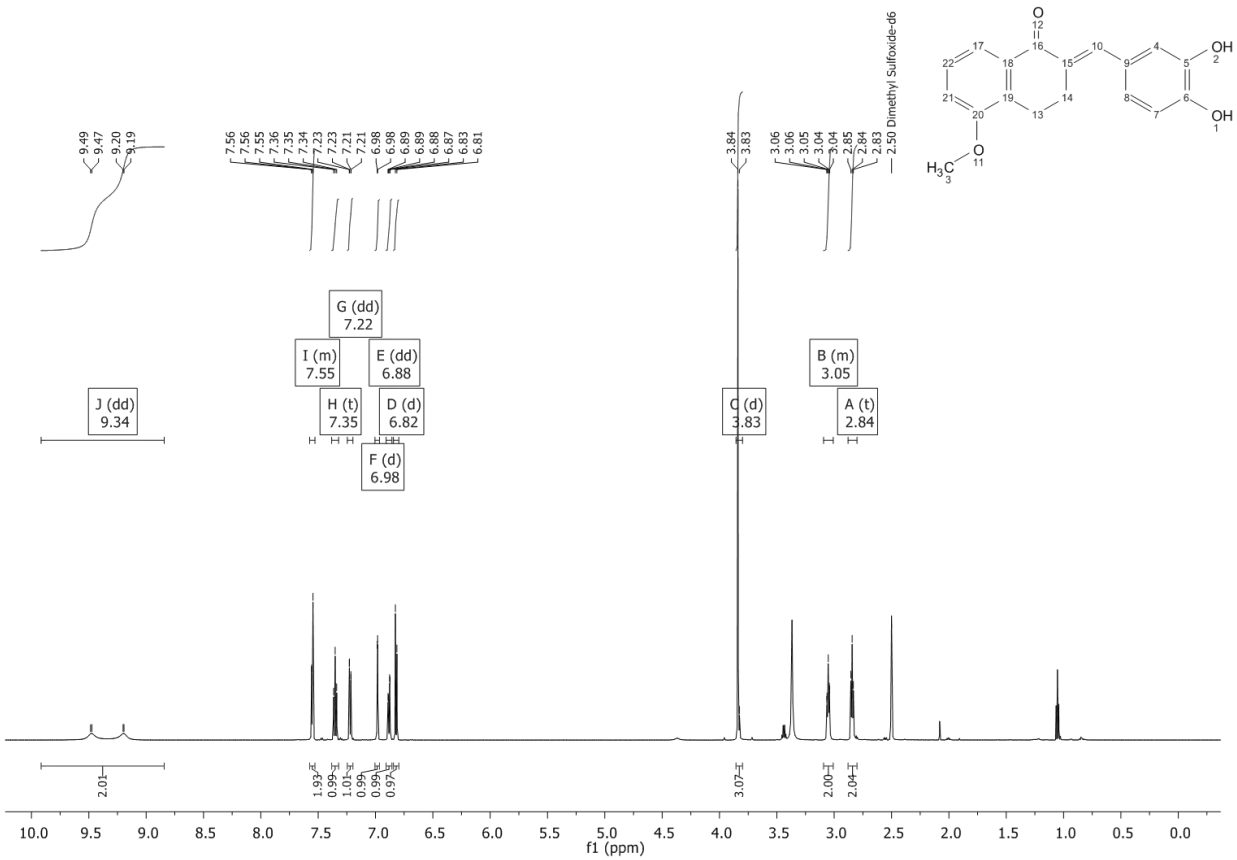


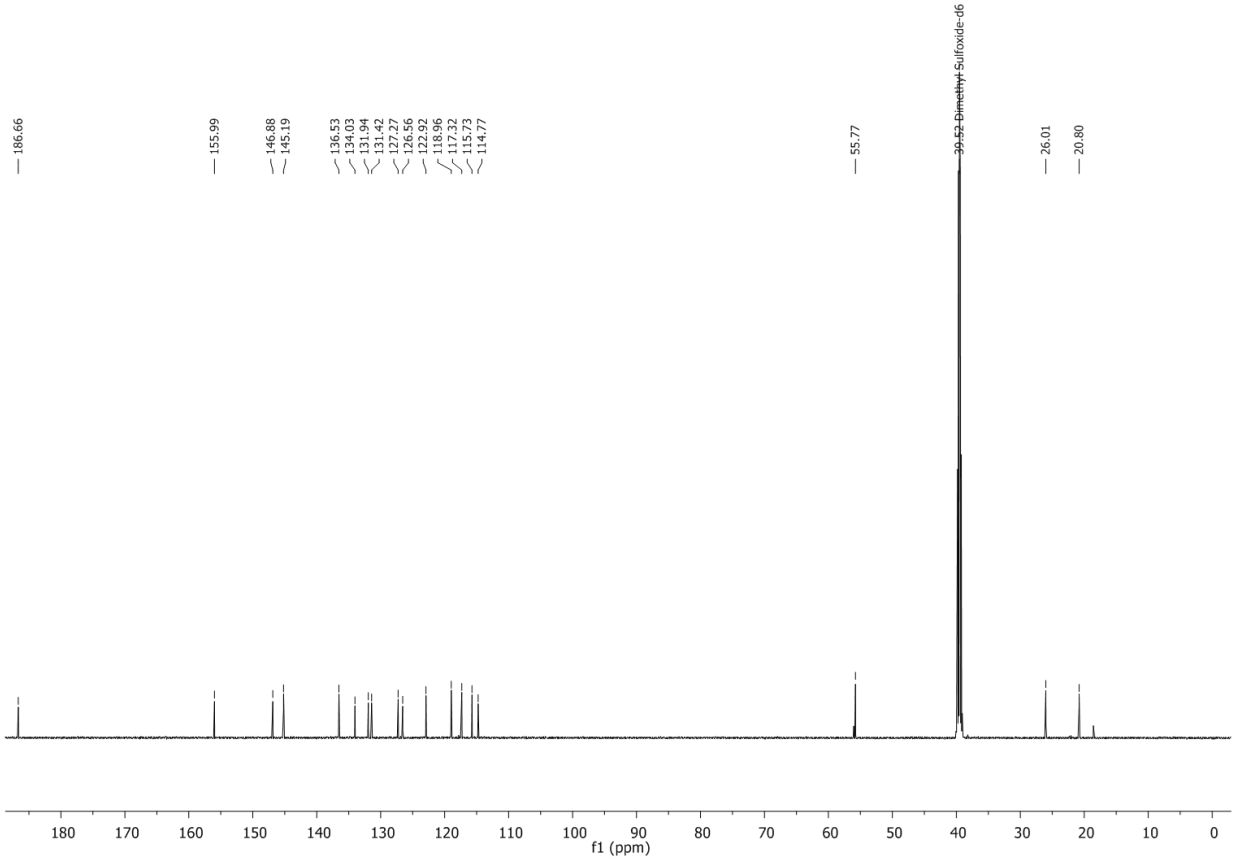
(*E*)-2-(3-hydroxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-1(*2H*)-one (17)





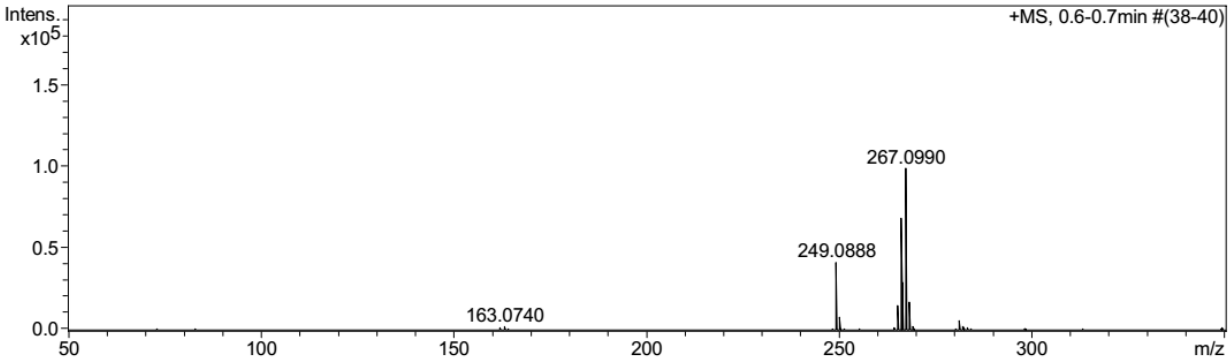
(*E*)-2-(3,4-dihydroxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-1(*2H*)-one (18)





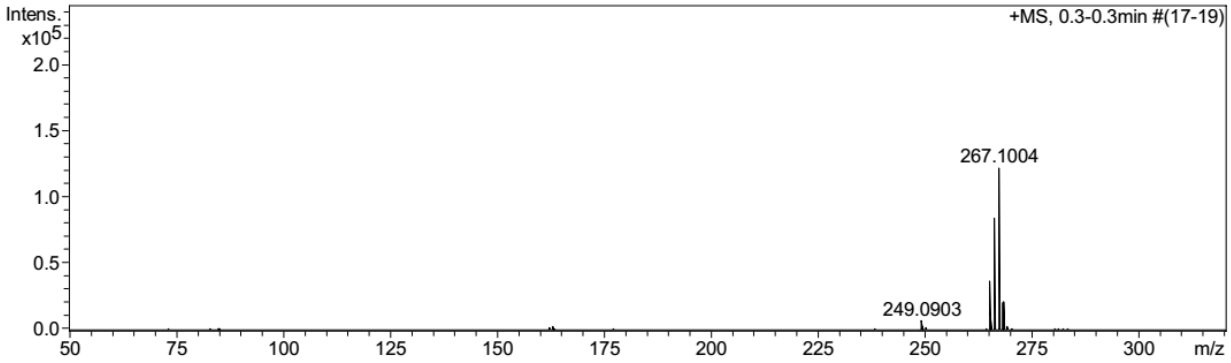
ANNEXURE C: PUBLISHED ARTICLE
MASS SPECTRA

(*E*)-5-hydroxy-2-(3-hydroxybenzylidene)-3,4-dihydronaphthalen-1(*2H*)-one (3)



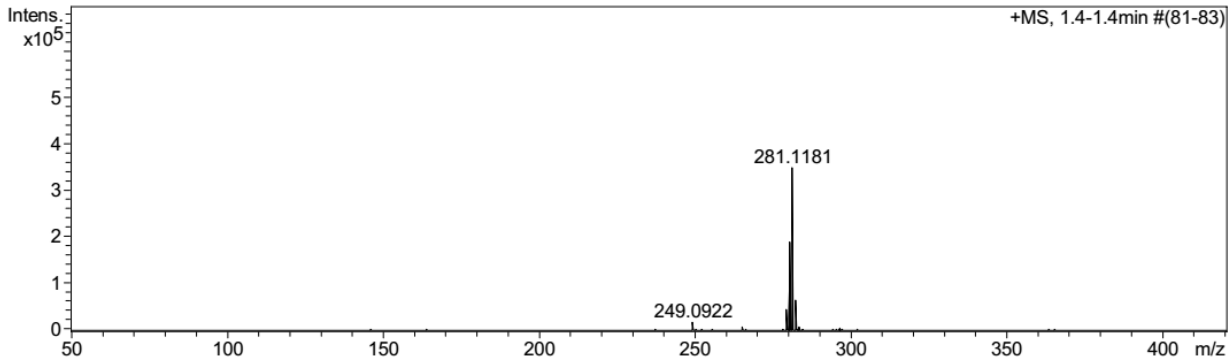
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
267.0990	1	C 17 H 15 O 3	100.00	267.1016	2.6	9.7	7.9	10.5	ok	even

(*E*)-5-hydroxy-2-(4-hydroxybenzylidene)-3,4-dihydronaphthalen-1(*2H*)-one (4)



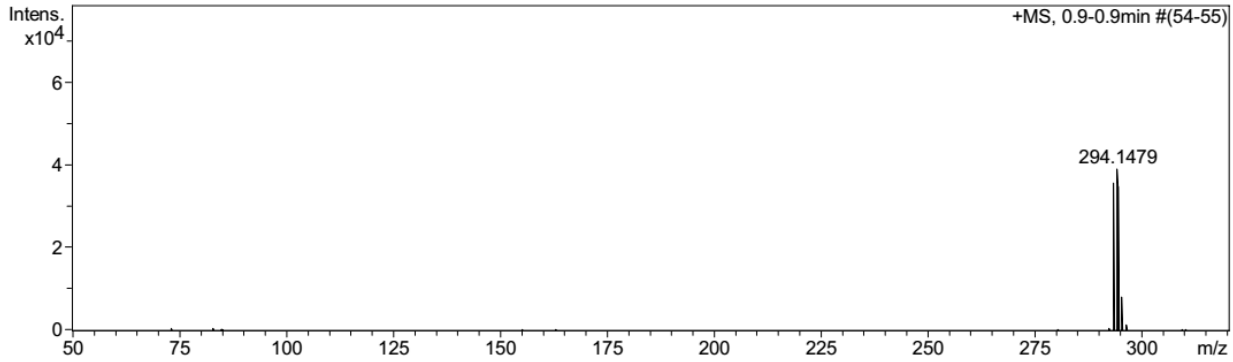
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
267.1004	1	C 17 H 15 O 3	100.00	267.1016	1.2	4.3	8.4	10.5	ok	even

(*E*)-5-hydroxy-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(*2H*)-one (5)



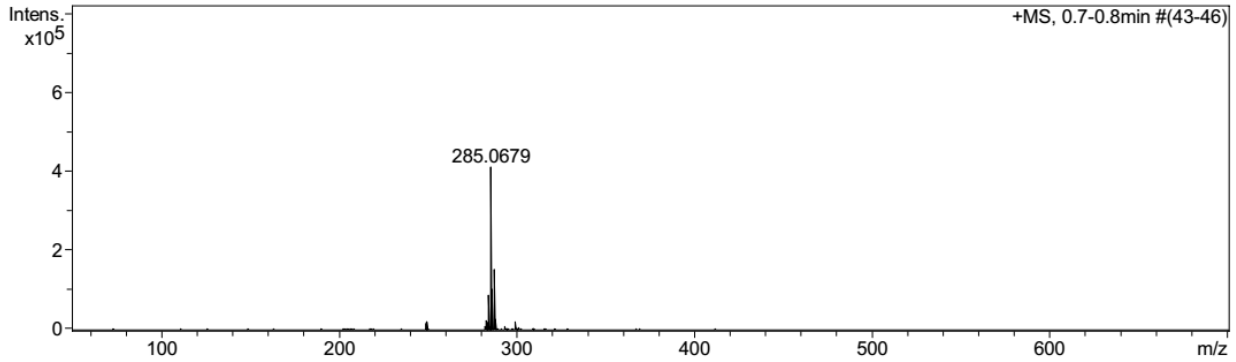
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
281.1181	1	C 18 H 17 O 3	100.00	281.1172	-0.9	-3.2	6.4	10.5	ok	even

(*E*)-2-(4-(dimethylamino)benzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one (6)



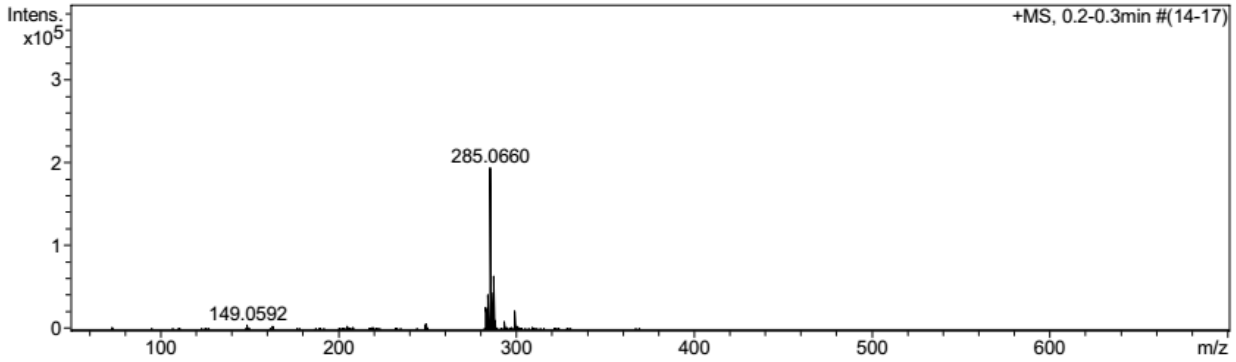
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
294.1479	1	C 19 H 20 N O 2	100.00	294.1489	1.0	3.2	9.6	10.5	ok	even

(*E*)-2-(3-chlorobenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one (7)



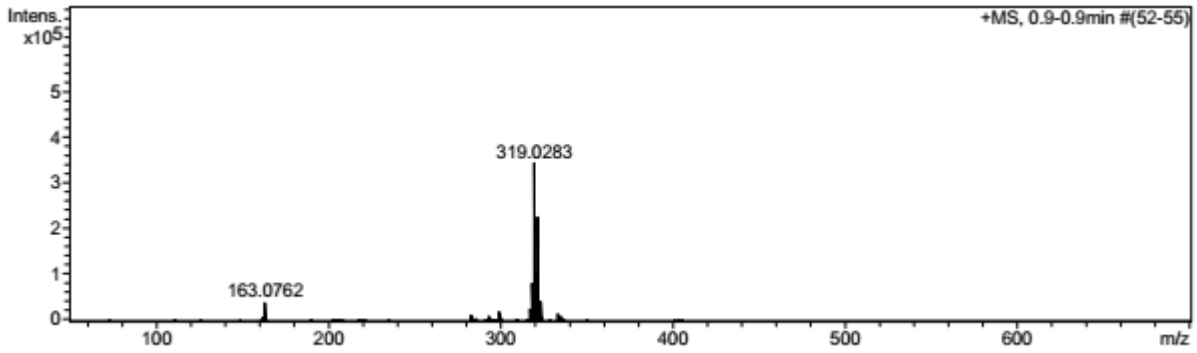
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
285.0679	1	C 17 H 14 Cl O 2	100.00	285.0677	-0.2	-0.7	32.8	10.5	ok	even
	2	C 23 H 9	0.01	285.0699	2.0	6.9	197.9	19.5	ok	even
	3	C 18 H 9 N 2 O 2	0.00	285.0659	-2.0	-7.2	203.3	15.5	ok	even
	4	C 16 H 7 N 5 O	0.00	285.0645	-3.4	-11.9	207.8	16.0	ok	odd

(*E*)-2-(4-chlorobenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one (8)



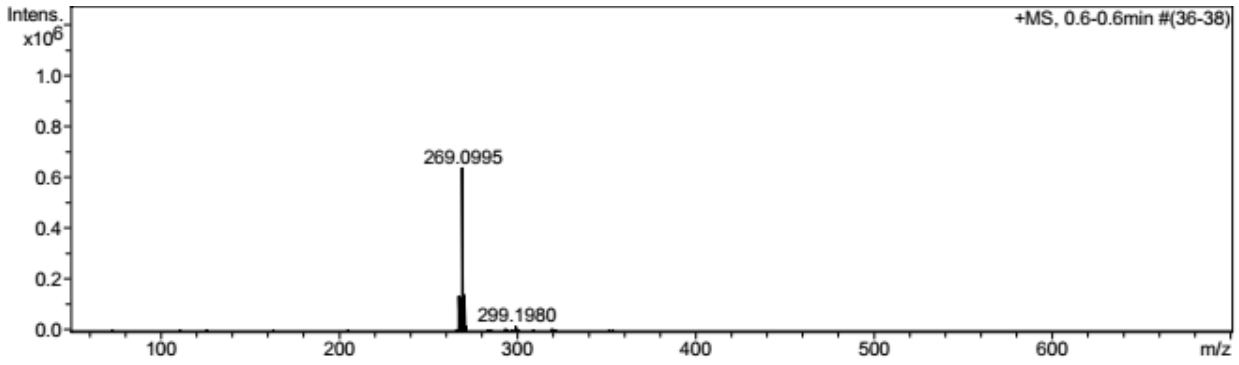
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
284.0583	1	C 17 H 13 Cl O 2	100.00	284.0599	1.6	5.6	524.7	11.0	ok	odd
	2	C 10 H 17 Cl O 7	0.00	284.0657	7.5	26.3	551.2	2.0	ok	odd
	3	C 13 H 13 Cl O 5	0.00	284.0446	-13.7	-48.1	623.7	7.0	ok	odd
	4	C 23 H 8	0.00	284.0621	3.8	13.3	638.9	20.0	ok	odd
	5	C 19 H 8 O 3	0.00	284.0468	-11.5	-40.4	656.4	16.0	ok	odd
	6	C 16 H 12 O 5	0.00	284.0679	9.7	34.0	669.8	11.0	ok	odd
	7	C 12 H 12 O 8	0.00	284.0527	-5.6	-19.7	687.9	7.0	ok	odd
285.0660	1	C 17 H 14 Cl O 2	50.88	285.0677	1.7	5.9	19.6	10.5	ok	even
	2	C 15 H 12 Cl N 3 O	100.00	285.0663	0.3	1.2	24.1	11.0	ok	odd
	3	C 23 H 9	0.00	285.0699	3.9	13.6	175.0	19.5	ok	even
	4	C 18 H 9 N 2 O 2	0.06	285.0659	-0.1	-0.5	178.7	15.5	ok	even
	5	C 16 H 7 N 5 O	0.02	285.0645	-1.5	-5.2	182.8	16.0	ok	odd
	6	C 14 H 5 N 8	0.01	285.0632	-2.8	-9.9	184.8	16.5	ok	even

(*E*)-2-(3,4-dichlorobenzylidene-5-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one (9)



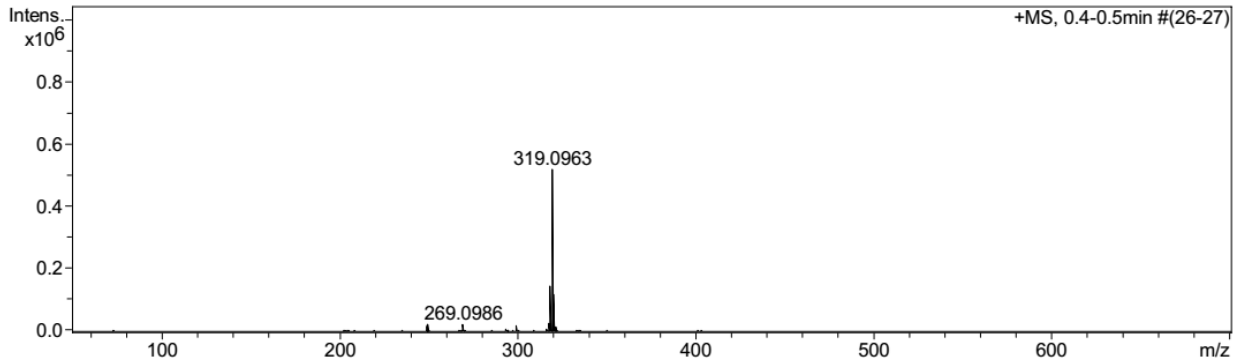
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
319.0283	1	C 17 H 13 Cl 2 O 2	100.00	319.0287	0.4	1.3	51.2	10.5	ok	even
	2	C 15 H 11 Cl 2 N 3 O	67.00	319.0274	-0.9	-2.9	56.0	11.0	ok	odd
	3	C 13 H 9 Cl 2 N 6	22.86	319.0260	-2.3	-7.1	60.8	11.5	ok	even
	4	C 7 H 9 Cl 2 N 10 O	0.48	319.0332	5.0	15.5	84.4	7.5	ok	even
	5	C 5 H 7 Cl 2 N 13	2.07	319.0319	3.6	11.3	89.3	8.0	ok	odd
	6	C 23 H 8 Cl	0.18	319.0309	2.6	8.2	151.6	19.5	ok	even
	7	C 18 H 8 Cl N 2 O 2	0.26	319.0269	-1.4	-4.4	160.4	15.5	ok	even
	8	C 16 H 6 Cl N 5 O	0.07	319.0255	-2.7	-8.6	164.9	16.0	ok	odd
	9	C 14 H 4 Cl N 8	0.01	319.0242	-4.1	-12.8	178.4	16.5	ok	even
	10	C 10 H 6 Cl N 9 O 2	0.00	319.0327	4.5	14.0	188.7	12.0	ok	odd
	11	C 8 H 4 Cl N 12 O	0.00	319.0314	3.1	9.8	195.5	12.5	ok	even
	12	C 6 H 2 Cl N 15	0.01	319.0301	1.8	5.6	198.4	13.0	ok	odd
	13	C H 2 Cl N 17 O 2	0.00	319.0260	-2.2	-7.0	211.8	9.0	ok	odd
	14	C 24 H 3 N 2	0.00	319.0291	0.8	2.5	361.7	24.5	ok	even
	15	C 19 H 3 N 4 O 2	0.00	319.0251	-3.2	-10.1	368.5	20.5	ok	even
	16	C 17 H N 7 O	0.00	319.0237	-4.6	-14.3	373.1	21.0	ok	odd
	17	C 11 H N 11 O 2	0.00	319.0309	2.6	8.3	381.6	17.0	ok	odd

(*E*)-2-(4-fluorobenzylidene)-5-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one (10)



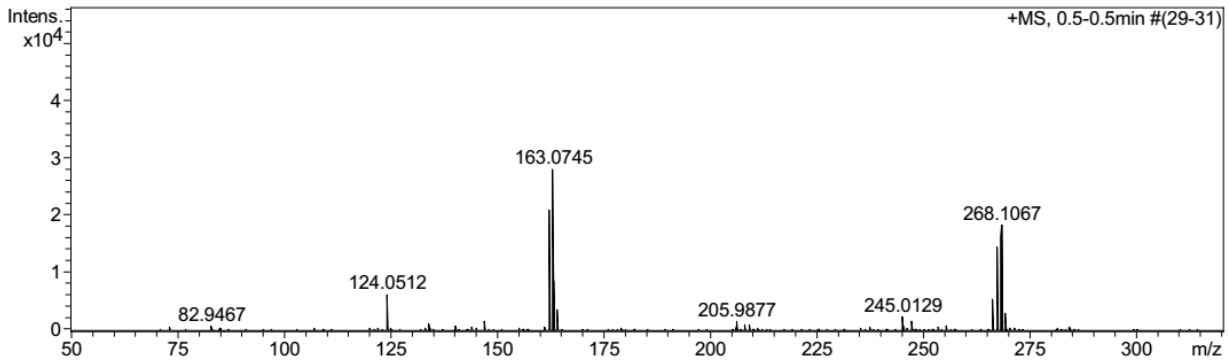
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
269.0995	1	C 20 H 13 O	49.22	269.0961	-3.4	-12.5	2.0	14.5	ok	even
	2	C 18 H 11 N 3	8.90	269.0947	-4.7	-17.5	8.1	15.0	ok	odd
	3	C 17 H 14 F O 2	100.00	269.0972	-2.2	-8.3	20.5	10.5	ok	even
	4	C 15 H 12 F N 3 O	24.15	269.0959	-3.6	-13.3	27.1	11.0	ok	odd
	5	C 14 H 13 N 4 O 2	16.18	269.1033	3.8	14.3	30.8	10.5	ok	even

(*E*)-5-hydroxy-2-(4-trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(*2H*)-one (11)



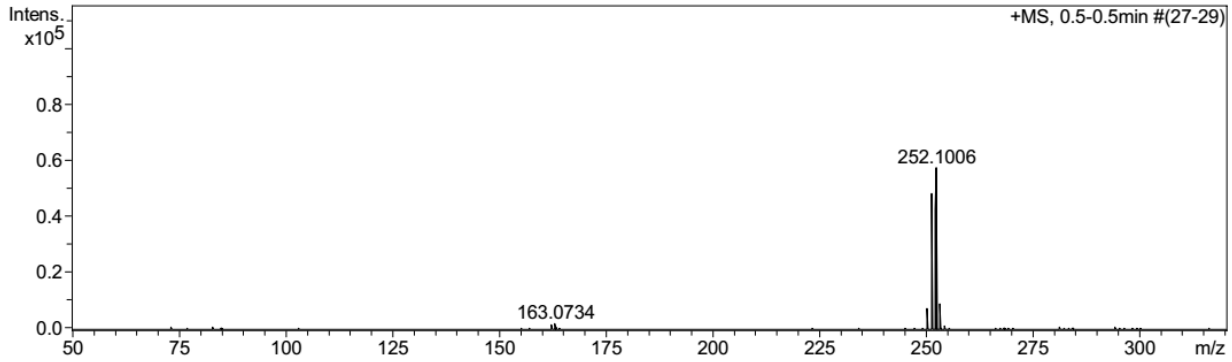
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e¯ Conf
319.0963	1	C 21 H 13 F 2 O	24.14	319.0929	-3.4	-10.5	1.3	14.5	ok	even
	2	C 20 H 14 F N O 2	10.32	319.1003	4.1	12.7	5.0	14.0	ok	odd
	3	C 19 H 11 F 2 N 3	4.39	319.0916	-4.7	-14.7	7.3	15.0	ok	odd
	4	C 21 H 11 N 4	100.00	319.0978	1.6	4.9	7.7	18.5	ok	even
	5	C 18 H 12 F N 4 O	37.69	319.0990	2.7	8.5	11.5	14.5	ok	even
	6	C 23 H 13 N O	29.87	319.0992	2.9	9.1	14.0	18.0	ok	odd
	7	C 16 H 10 F N 7	93.93	319.0976	1.4	4.3	17.8	15.0	ok	odd
	8	C 24 H 12 F	4.63	319.0918	-4.5	-14.1	18.2	18.5	ok	even
	9	C 16 H 11 N 6 O 2	40.46	319.0938	-2.5	-7.7	19.5	14.5	ok	even
	10	C 18 H 14 F 3 O 2	49.09	319.0940	-2.2	-6.9	19.8	10.5	ok	even
	11	C 16 H 12 F 3 N 3 O	11.91	319.0927	-3.6	-11.1	26.4	11.0	ok	odd
	12	C 15 H 13 F 2 N 4 O 2	7.76	319.1001	3.9	12.1	30.1	10.5	ok	even

(*E*)-2-((2-aminopyrimidin-5-yl)methylene)-5-hydroxy-3,4-dihydronaphthalen-1(*2H*)-one (12)



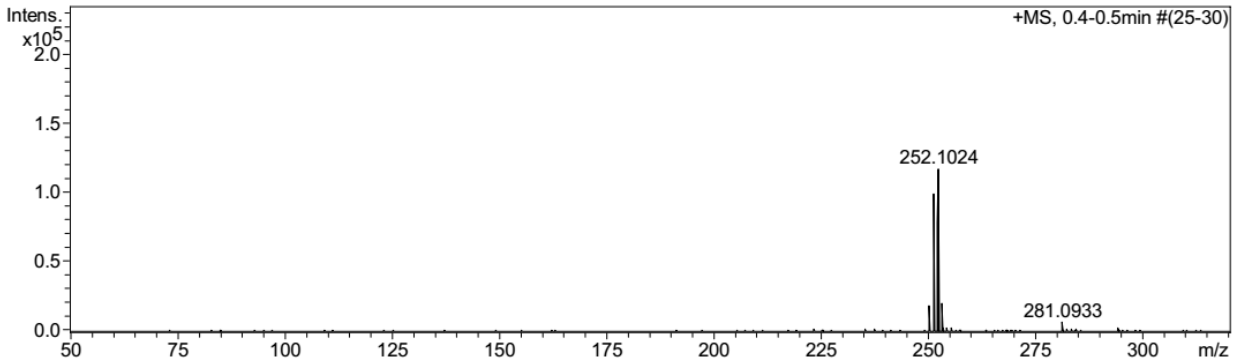
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e¯ Conf
163.0745	1	C 10 H 11 O 2	93.88	163.0754	0.9	5.3	13.1	5.5	ok	even
	2	C 8 H 9 N 3 O	100.00	163.0740	-0.5	-2.9	19.9	6.0	ok	odd
268.1067	1	C 15 H 14 N 3 O 2	100.00	268.1081	1.3	4.9	4.0	10.5	ok	even
	2	C 17 H 16 O 3	32.77	268.1094	2.6	9.9	9.5	10.0	ok	odd

(*E*)-5-hydroxy-2-(pyridin-3-ylmethylene)-3,4-dihydronaphthalen-1(*2H*)-one (13)



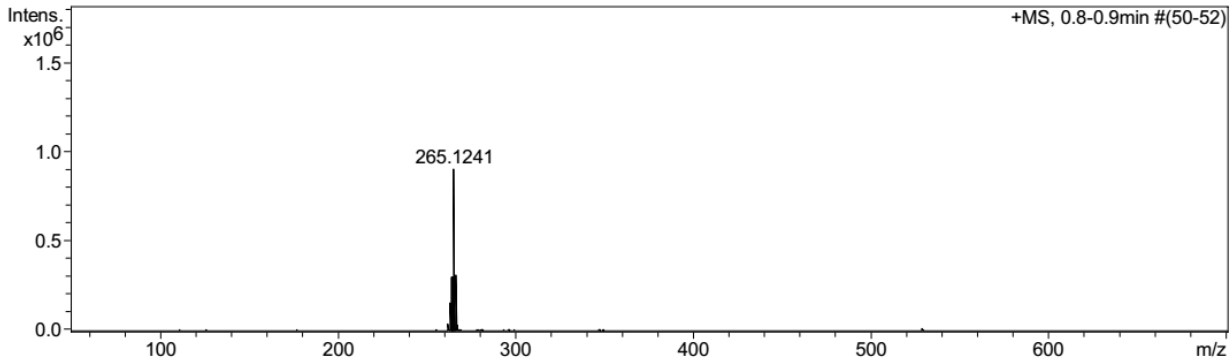
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e¯ Conf
251.0932	1	C 16 H 13 N O 2	100.00	251.0941	0.9	3.7	490.6	11.0	ok	odd
252.1006	1	C 16 H 14 N O 2	100.00	252.1019	1.3	5.2	9.4	10.5	ok	even

(*E*)-5-hydroxy-2-(pyridin-4-ylmethylene)-3,4-dihydronaphthalen-1(*2H*)-one (14)



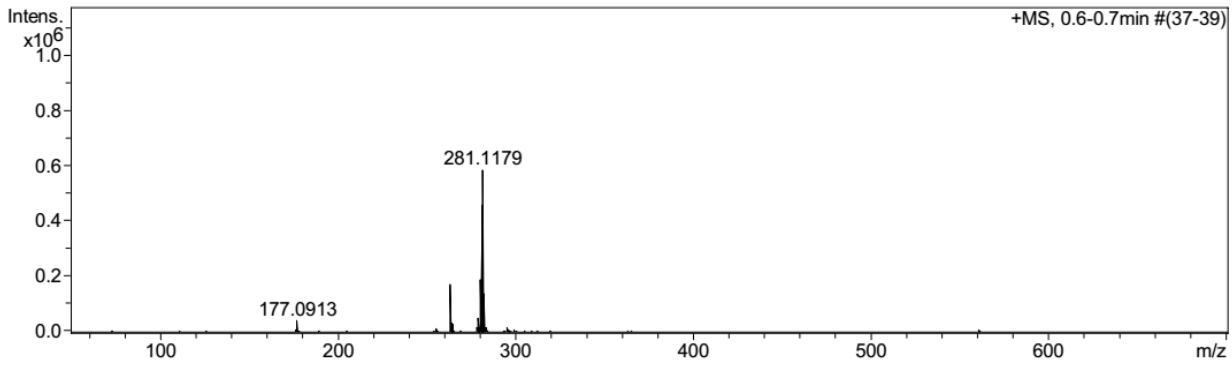
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e¯ Conf
251.0950	1	C 16 H 13 N O 2	100.00	251.0941	-0.9	-3.7	491.1	11.0	ok	odd
252.1024	1	C 16 H 14 N O 2	100.00	252.1019	-0.5	-2.0	3.5	10.5	ok	even

(*E*)-2-benzylidene-5-methoxy-3,4-dihydronaphthalen-1(*2H*)-one (16)



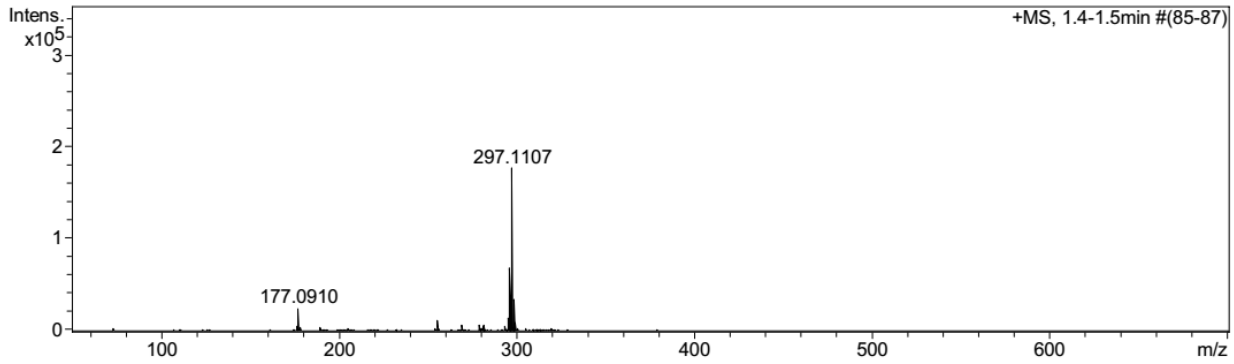
Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
265.1241	1	C 18 H 17 O 2	100.00	265.1223	-1.8	-6.7	84.6	10.5	ok	even
	2	C 16 H 15 N 3 O	24.04	265.1210	-3.1	-11.8	91.0	11.0	ok	odd
	3	C 14 H 13 N 6	3.93	265.1196	-4.5	-16.8	97.2	11.5	ok	even
	4	C 10 H 15 N 7 O 2	1.95	265.1282	4.1	15.4	120.0	7.0	ok	odd
	5	C 8 H 13 N 10 O	5.48	265.1268	2.8	10.4	127.1	7.5	ok	even
	6	C 6 H 11 N 13	10.99	265.1255	1.4	5.3	133.1	8.0	ok	odd

(*E*)-2-(3-hydroxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-1(*2H*)-one (17)



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
281.1179	1	C 21 H 15 N	63.63	281.1199	2.0	7.1	2.5	15.0	ok	odd
	2	C 18 H 17 O 3	100.00	281.1172	-0.7	-2.4	23.0	10.5	ok	even
	3	C 16 H 15 N 3 O 2	36.76	281.1159	-2.0	-7.2	29.3	11.0	ok	odd

(*E*)-2-(3,4-dihydroxybenzylidene)-5-methoxy-3,4-dihydronaphthalen-1(*2H*)-one (18)



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e¯ Conf
297.1107	1	C 18 H 17 O 4	54.75	297.1121	1.4	4.7	0.8	10.5	ok	even
	2	C 16 H 15 N 3 O 3	100.00	297.1108	0.1	0.2	6.8	11.0	ok	odd
	3	C 14 H 13 N 6 O 2	47.29	297.1095	-1.3	-4.3	13.3	11.5	ok	even
	4	C 19 H 13 N 4	14.96	297.1135	2.7	9.2	15.1	15.5	ok	even
	5	C 12 H 11 N 9 O	14.88	297.1081	-2.6	-8.8	20.3	12.0	ok	odd
	6	C 21 H 15 N O	3.20	297.1148	4.1	13.7	21.0	15.0	ok	odd

ANNEXURE D: ETHICS

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The Applicant / Primary Investigator

Faculty of Health Sciences
Ethics Office for Research, Training and Support
Animal Care, Health and Safety in Research
Ethics Committee (AnimCare)

Tel: 018 299 2234
Fax: 018 299 2225
Email: Tiaan.Brink@nwu.ac.za

24 March 2017

Dear Prof Terre'Blanche

APPROVAL OF YOUR APPLICATION BY THE ANIMAL CARE, HEALTH AND SAFETY IN RESEARCH ETHICS COMMITTEE (ANIMCARE) OF THE FACULTY OF HEALTH SCIENCES

Ethics Number: NWU-00260-17-A5

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the AnimCare secretariat.

Study Title: 5-Hydroxy-1-tetralone analogues as dual A1/A2A receptor antagonists for the potential treatment of neurological conditions.

Study leader/Supervisor: G Terre'Blanche

Student: HD Janse van Rensburg

Application type: New Application - Category 0

Project Category *(impact on animal wellbeing)*	**Not applicable**	**0**	**1**	**2**	**3**	**4**	**5**
		X					

The abovementioned application has been through the expedited review process and discussed by the AnimCare, Animal Research Ethics Committee Potchefstroom Campus, North-West University, Potchefstroom.

The commencement date for this study is **24th of March 2017** .Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review

The AnimCare, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the AnimCare, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form sent to Ethics-AnimCareIncident-SAE@nwu.ac.za

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at

completion of the study or the AnimCare committee, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za.

The AnimCare, Faculty of Health Sciences has the authority and responsibility to initially approve and subsequently monitor animal activities to confirm on-going compliance with and adherence to the approved protocol in terms of section 5.2.7 of the SANS 10386:2008.The AnimCare, Faculty of Health Sciences reserves the right to visit sites where approved protocols will be conducted and any animal housing facility under the authority of NWU as often as it deem necessary either announced or unannounced.

Please note that for any permits/permission must still be obtained from relevant authorities and provided to the AnimCare, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

The AnimCare Committee, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the SANS 10386:2008 document, the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-AnimCare@nwu.ac.za.

Yours sincerely

Prof Christiaan B Brink
Chair: AnimCare

Prof Minrie Greeff
Head: Ethics Office

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Web: http://www.nwu.ac.za

Institutional Research Ethics Regulatory Committee
Tel: +27 18 299 4849
Email : Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **AnimCare Animal Research Ethics Committee (AREC-130913-015)** on **24/03/2017**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: 5-Hydroxy-1-tetralone analogues as dual A1/A2A receptor antagonists for the potential treatment of neurological conditions.

Study Leader/Supervisor: **G Terre'Blanche**
Student: **HD Janse van Rensburg**

Ethics number:

N	W	U	-	0	0	2	6	0	-	1	7	-	A	5
Institution				Study Number						Year			Status	

Status: S = Submission, E = Re-Submission, P = Provisional Authorisation, A = Authorisation

Application Type: New Application - Category 0

Commencement date: 2017-03-24 **Category:** 0

Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years.

Special conditions of the approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the AnimCare. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via AnimCare:*
  - *annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the AnimCare, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-IRERC and AnimCare retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the AnimCare or that information has been false or misrepresented,*
    - *the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *AnimCare can be contacted for further information or any report templates via Ethics-AnimCare@nwu.ac.za or 018 299 2197.*

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the IRERC or AnimCare for any further enquiries or requests for assistance.

Yours sincerely

Prof LA Du Plessis

Digitally signed by Prof LA Du Plessis
Date: 2017.04.03 08:14:24 +02'00'

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

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Title of Publication: 5-Hydroxy-1-tetralone analogues as dual A1/A2A receptor antagonists for the potential treatment of neurological conditions
Authors/Editors: Author: H.D. Janse van Rensburg

Supervisor: Prof. G. Terre'Blanche
Co-supervisor: Prof. L.J. Legoabe
Co-supervisor: Dr. M.M. Van der Walt

Date of Publication: October 2017
Publisher: North-West University
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Title of the article or chapter the portion is from	Potent Adenosine A1 and A2A Receptors Antagonists: Recent Developments
Editor of portion(s)	N/A
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ACKNOWLEDGEMENTS

Firstly, I wish to express my sincere gratitude to my supervisor Prof. G. Terre'Blanche and co-supervisors Prof. L.J. Legoabe and Dr. M.M. Van der Walt for their assistance and guidance in my *Magister Scientiae* degree at the North-West University (NWU).

Secondly, I owe thanks to Mr. A. Joubert and Dr. J. Jordaan of the SASOL Centre for Chemistrry at the NWU for recording NMR and MS spectra, as well as Prof. J. Du Preez of Pharmaceutics at the NWU for HPLC analyses.

Thirdly, this M Sc degree would not be possible without the financial support of the NWU and National Research Foundation (NRF).

Fourthly, I am grateful to my mother Mrs. M.C. Janse van Rensburg for all she has done in my life — words cannot describe my appreciation. Additionally, I would like to thank my family and friends for their support.

Last, but not least, I thank God for His love and grace in my life and, particularly, during my M Sc studies.