Chapter 1: Introduction

1.1. Background and rationale

Parkinson's disease is a neurodegenerative disorder of the central nervous system that is characterised by tremor at rest, slowness of movement (bradykinesia) and muscle stiffness. These symptoms are due to the progressive loss of the dopaminergic neurons that project from the substantia nigra pars compacta (SNc) to the striatum (Samii *et al.*, 2004). Extreme disability, which includes gait disturbances, speech dysfunction and dementia, occurs in later stages of the disease (Obeso, 2010). The cause of Parkinson's disease is still unknown although there are several theories pertaining to disease development, such as the environmental exposure to a toxin (Dauer and Przedborski, 2003), excessive amounts of glutamate present in the central nervous system (Lipton and Rosenberg, 1994), genetic predisposition (Lees *et al.*, 2009), damage caused by protein misfolding (McNaught *et al.*, 2001), oxidative stress (Standaert and Young, 2006), and inflammatory damage (Hirsch and Hunot, 2009). Regardless of the causing mechanism, two main pathological features have been correlated with the progression of the disease, namely increased neuronal loss and an accumulation of Lewy bodies (cytoplasmic protein aggregates) (Olanow *et al.*, 2004; Obeso, 2010).

The prevalence of Parkinson's disease is increasing in the ageing world population and consequently, the economic burden associated with this disease will also increase. This is not only due to the expense of medical care, but also due to the loss of productivity of patients and their caregivers (Huse *et al.*, 2005). Since the breakthrough discovery of levodopa as symptomatic treatment of Parkinson's disease it has become the cornerstone of Parkinson's disease therapy. Levodopa therapy however has several shortcomings. The disease still progresses, as the degeneration of neurons continues, and motor fluctuations as well as severe dyskinesia develop with long term levodopa therapy, while non-motor symptoms remain untreated. It is also proposed that levodopa therapy may even contribute to disease progression since its metabolism leads to the formation of reactive oxygen species (ROS), which increases oxidative stress (Samii *et al.,* 2004; Chen and Swope, 2007; Hattori *et al.*, 2009). The golden aim of research is therefore to acquire an effective symptomatic agent (without adverse effects) with neuroprotective abilities that can be employed as disease modifying therapy (Xu *et al.*, 2005).

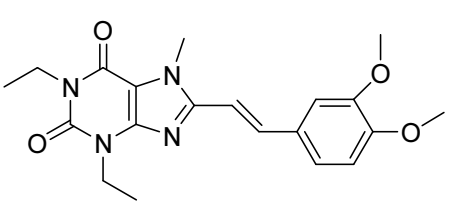
Adenosine receptors

Adenosine modulates the release or effect of other central nervous system neurotransmitters such as dopamine (Cunha, 2005). Adenosine receptors are G-protein coupled receptors and there are four different subtypes, namely A_1,A_{2A}, A_{2B} and A_3 (Schulte and Fredholm, 2003). The adenosine A_1 and A_{2A} receptors are particularly important in basal ganglia function due to their localization in the striatum and their involvement in neuromodulation of the motor pathways. Where A_1 receptor antagonism facilitates dopamine release presynaptically, A_{2A} receptor antagonism enhances postsynaptic responses to dopamine, consequently improving motor symptoms in Parkinson's disease (Ferré *et al*., 1997). The low incidence of Parkinson's disease in coffee drinkers has furthermore been attributed to the neuroprotective properties of caffeine, a non-selective adenosine A_1/A_{2A} receptor antagonist, and in particular, to its adenosine A_{2A} receptor antagonistic activity (Fredholm *et al*., 1999; Hernan *et al*., 2002). Additionally, it was demonstrated in animal models of Parkinson's disease that the development of dyskinesia during treatment with levodopa is also diminished when an adenosine A_{2A} receptor antagonist is administered concomitantly (Tomiyama *et al*., 2004; Jenner *et al.*, 2009).

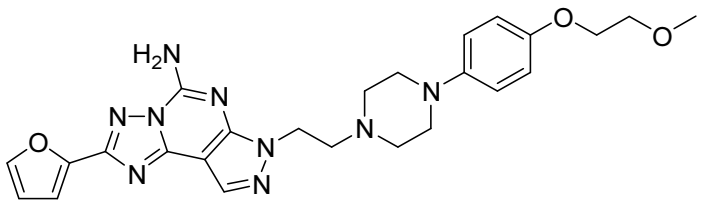
Dual adenosine A_{2A}/A_1 receptor antagonism could not only provide potentiated effects on motor activation, but also improvements in cognitive function. Besides their presence in the basal ganglia, adenosine A_1 receptors are also concentrated in the neocortical and limbic system structures which are important for cognitive function. Adenosine A_1 receptor antagonism improves performance in animal models of learning and memory, and this feature can be an asset in the treatment of Parkinson's disease where cognitive dysfunction is a very important non-motor symptom (Mihara *et al.,* 2007). Hence, dual adenosine A_1/A_{2A} receptor antagonists display positive results in both Parkinson's disease models and cognition models (Shook *et. al*., 2012). Adenosine antagonists have therefore shown promise as both symptomatic and neuroprotective agents and the A_{2A} and A_1 receptors provide validated non-dopaminergic targets as alternative to current therapy.

1.2. Aminopyrimidines as dual adenosine receptor antagonists: Rationale

The adenosine A_{2A} receptor antagonists are structurally divided in two classes, the xanthine derivatives and the amino-substituted heterocyclic compounds (Shook and Jackson, 2011), several of which have progressed into clinical trials. For example, istradefylline (**1**) a xanthine derivative and preladenant (**2**) an amino-substituted heterocyclic compound, both display selective adenosine A_{2A} receptor antagonistic affinity in the nanomolar range.

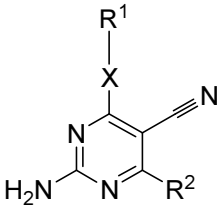


1
$A_{2A}K_i$ = 12 nM
A_1K_i = 9600 nM



2
$A_{2A}K_i$= 1.1 nM
A_1K_i = 1474 nM

The 2-aminopyrimidine motif is of particular importance to this study, and it occurs in several heterocyclic adenosine receptor antagonists with high affinity.



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For example, random screening of the Hoffmann-La Roche proprietary library led to the identification of 2-amino-5-cyano-6-(2-furyl)pyrimidine (**3**) as scaffold with high adenosine A_{2A} receptor affinity (Müller and Ferré, 2007). The structure activity relationships observed for this series are indicated in Figure 1.1.

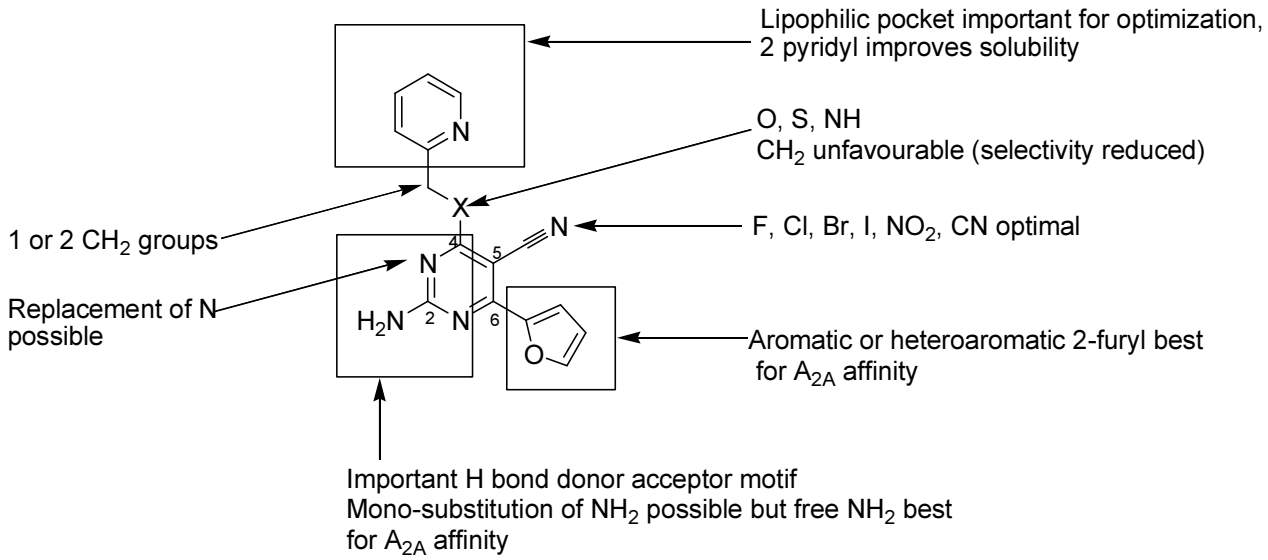

Figure 1.1: Structure activity relationships of 2-amino-5-cyano-6-(2-furyl)pyrimidine adenosine A_{2A} receptor antagonists (Müller and Ferré, 2007).

A series of pyrimidine-4-carboxamides have further been reported to display high affinity for adenosine A_{2A} receptors with good selectivity over other receptor subtypes (Gillespie *et al.*, 2009 a,b,c). These highly compact and efficient derivatives of which **4** (Figure 1.2) is a representative example, exhibited binding affinities in the nanomolar concentration range as well as *in vivo* activity. Interestingly, data suggested that for the amide substituent, *ortho*- and *meta* substitution of the aryl or heteroaryl rings resulted in better affinity and selectivity than *para*-substitution, while the presence of heteroatoms at the 2- or 3-position of the aromatic ring was preferable over a heteroatom in the 4-position (the pyridyl derivatives further had the additional advantage of improved aqueous solubility). Similar to the series of Hoffmann-La Roche the incorporation of the 2-furyl moiety was associated with high affinity, while substitution with a 5-methylfuran instead of a furan group gave compounds with similar levels of potency and selectivity, with a decreased risk of metabolic liability. Data further suggested that as for the previous series, the simple C-2 amino functionality was optimal, as a hydrogen bond donor is required in this region. Related pyridines and triazines have also been synthesised, but they were significantly less potent and selective than the corresponding pyrimidine derivatives (Gillespie *et al.*, 2009 a,b,c).

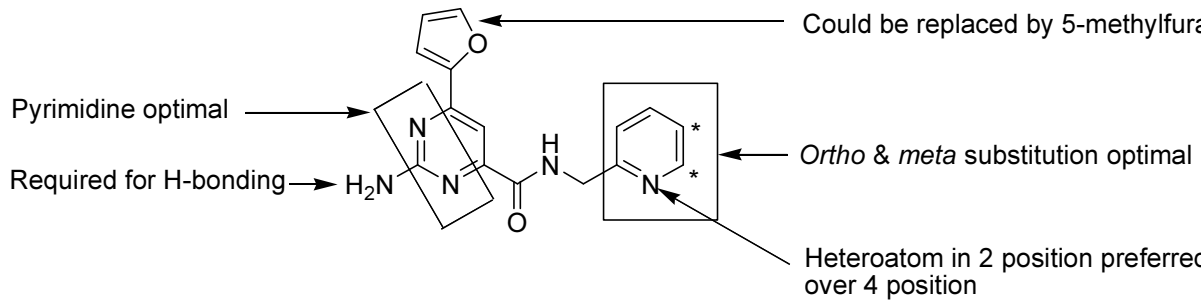

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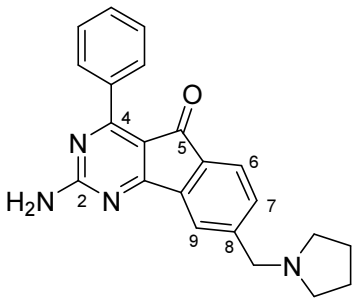
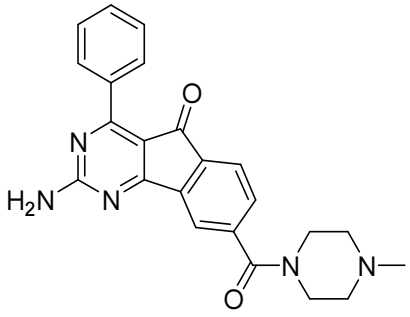
Figure 1.2: Example of a pyrimidine-4-carboxamide derivative with high affinity ($A_{2A}K_i$ = 4.3 nM and A_1K_i = 122 nM) (Gillespie *et al.,* 2009 a, b).

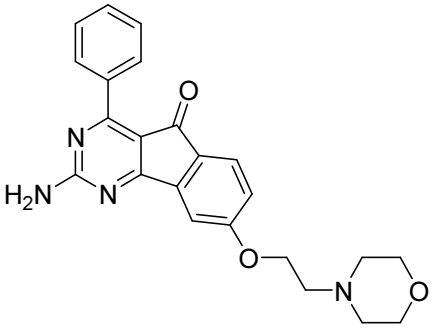
Shook and colleagues illustrated the effectiveness of a series of arylindenopyrimidines as potent dual adenosine A_1/A_{2A} receptor antagonists. Methylamine (**5**), amide (**6**), ether (**7**) and diamine side chains (**8**) were explored, with high affinity derivatives with *in vivo* activity identified in all cases. Again, a furan substituent in the 4 position was associated with superior *in vitro* and *in vivo* activity over other aryl and heteroaryl groups, although replacement with a simple phenyl moiety resulted in derivatives with *in vitro* and *in vivo* activity without the Ames liability associated with the unsubstituted furan moiety. As seen for the previous pyrimidine series, the NH_2 on position 2 of the aminopyrimidine ring had to be unsubstituted since a single methyl substituent completely eliminated any *in vitro* activity (Shook *et al.,* 2010 a,b,c). Interestingly, although derivatives substituted in the 9 position had *in vitro* and *in vivo* activity, related compounds substituted in the 6-position were not active in the A_{2A} functional *in vitro* assay. Furthermore, while substitution on carbon 8 gave good *in vitro* potency for both A_{2A} and A_1 receptors, activity was decreased for analogues substituted on carbon 7.



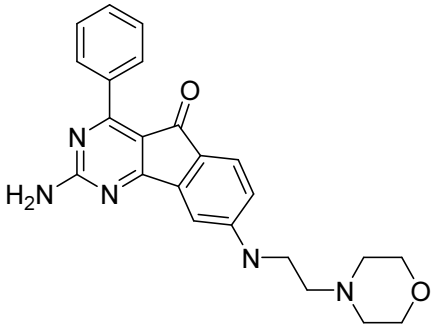
5
$A_{2A}K_i$ = 4.1 nM
A_1K_i = 17.0 nM



6
$A_{2A}K_i$ = 8.2 nM
A_1K_i = 58.4 nM

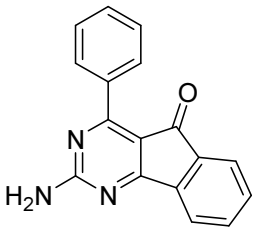


7
$A_{2A}K_i$ = 6.5 nM
A_1K_i = 48.2 nM

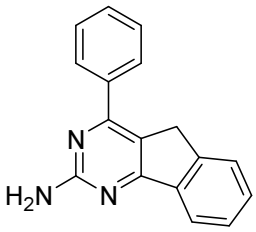


8
$A_{2A}K_i$ = 4.4 nM
A_1K_i= 32.7 nM

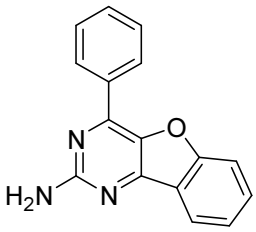
Matasi and co-workers (2005) further illustrated *inter alia* for a very similar series of arylindenopyrimidines (e.g. **9**), that reduction of the central ketone to a methylene (**10**) or its replacement with an ether linkage (**11**) was not detrimental to A_{2A} receptor affinity. Again for this series, the importance of the amino functionality was clear, as alkylation or acylation produced compounds with significantly reduced adenosine A_{2A} receptor affinity.



9
$A_{2A}K_i$ = 1.7 nM

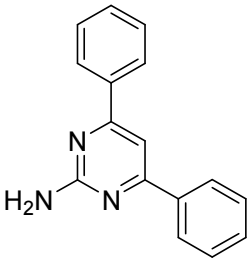


10
$A_{2A}K_i$ = 11.2 nM

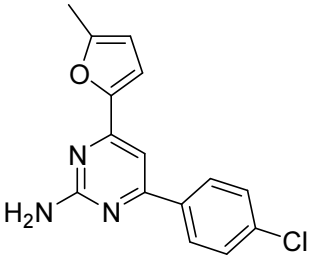


11
$A_{2A}K_i$ = 9.0 nM

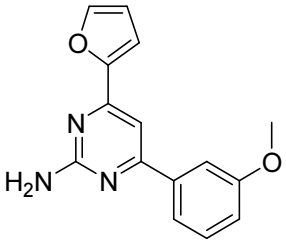
Based on the results above, and the fact that 4,6-diphenyl-2-aminopyrimidine (**12**) had moderate adenosine A_{2A} antagonistic activity and good adenosine A_1 antagonistic activity (Van Veldhoven, *et al.*, 2008), Robinson (2013) synthesised a series of novel, related aryl/heteroaryl substituted aminopyrimidines, exemplified by **13**, **14** and **15** mainly to investigate the effect of omission of the central carbonyl carbon (e.g. **15** in comparison to **6**) on adenosine A_{2A} and A_1 receptor affinity.



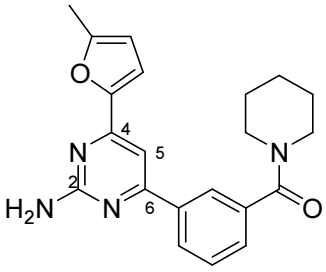
12
$A_{2A}K_i$= 169.0 nM
A_1K_i = 25.2 nM



13
$A_{2A}K_i$ = 3012 nM



14
$A_{2A}K_i$ = 226 nM

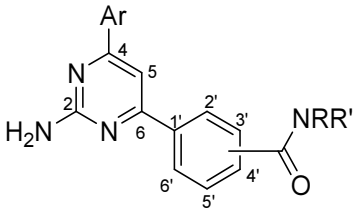


15
$A_{2A}K_i$ = 5.76 nM

While aminopyrimidines such as **13** and **14**, substituted with simple electron withdrawing or donating substituents, showed weak to moderate affinity, aminopyrimidines with a phenylamide substituent on position 6, such as **15**, exhibited promising *in vitro* affinity for adenosine A_{2A} receptors, comparable to the related arylindenopyrimidines, as well as *in vivo* activity in the haloperidol rat catalepsy assay (Robinson, 2013). This compound therefore served as starting point for this study, with the main aim of further exploring the structure activity relationships of this particular aminopyrimidine scaffold.

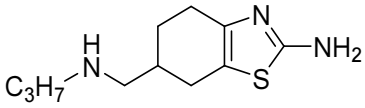
1.3. Aims of this project

The aim of this study is to design and synthesise novel 2-aminopyrimidine derivatives (**16**), as potential adenosine A_1 and A_{2A} receptor antagonists. In order to achieve this, the affinities of synthesised compounds will firstly be determined for the adenosine A_{2A} and A_1 receptors. *In vivo* activity of selected derivatives will furthermore be assessed in the haloperidol induced catalepsy rat model, while the toxicity of these derivatives will be determined using the MTT cell viability assay.



16

Firstly, to investigate the effect of amide substitution in position 4' instead of position 3' (as synthesised by Robinson, 2013) of the phenyl ring, some 4'-amide derivatives will be synthesised. Since the effect of heterocyclic substitution, other than 5-methylfuran on position 4 of this particular scaffold has not previously been investigated, this study will also contribute in this area, by incorporation of different aryl and heteroaryl groups at the 4-position. An aminothiazole moiety will further be incorporated into the phenylamide side chain. This will add extra bulk and rigidity. Thiazoles, which feature both a nitrogen as well as a sulfur atom in an aromatic five-membered ring system, are associated with neuroprotective properties due to their ability to scavenge free radicals (Harnett *et al*., 2004; Siddiqui *et al.,* 2009; Kashyap *et al*., 2012). A thiazole moiety is present in known anti-parkinsonian drugs for example **17** (pramipexole), a registered dopamine agonist therapeutically employed in Parkinson's disease (Van Vliet *et al.,* 2000).



17

1.4. Hypothesis of this study

As discussed above, the affinity of the aminopyrimidine scaffold (**16**) for the adenosine A_{2A} receptor has previously been established. It is thus hypothesised that:

1. The 4'-amide derivatives will have weaker affinity for the adenosine A_1 and A_{2A} receptors than the 3'-amide derivatives, based on the affinities observed for the related arylindenopyrimidines substituted in position 7 (Shook *et al*., 2010b).
2. The incorporation of different heteroaromatic rings will result in compounds with potent adenosine A_1 and A_{2A} affinity.
3. Incorporation of the aminothiazole moiety will give novel compounds with adenosine A_1 and A_{2A} receptor affinity, with a potential additional neuroprotective benefit.

4. The different heteroaryl or aryl groups will have an effect on toxicity and groups with intrinsic toxicity, for example the furan moiety (Boyd, 1981) will be more toxic than other derivatives.

1.5. Objectives

The objectives of the study can be summarised as follows:

a) 2-Aminopyrimidines with the general scaffold (**16**) will be synthesised. Some 4'-amide analogues (Figure 1.3) will be synthesised, a variety of aryl and heteroaryl groups will be incorporated in position 4 (e.g. Figure 1.4), and aminothiazole groups will be incorporated in the phenylamide side chain (Figure 1.5).

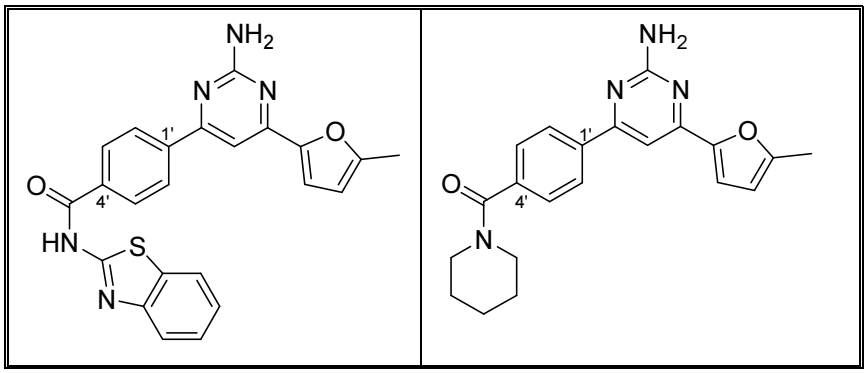


Figure 1.3: Examples of 4'-amide derivatives

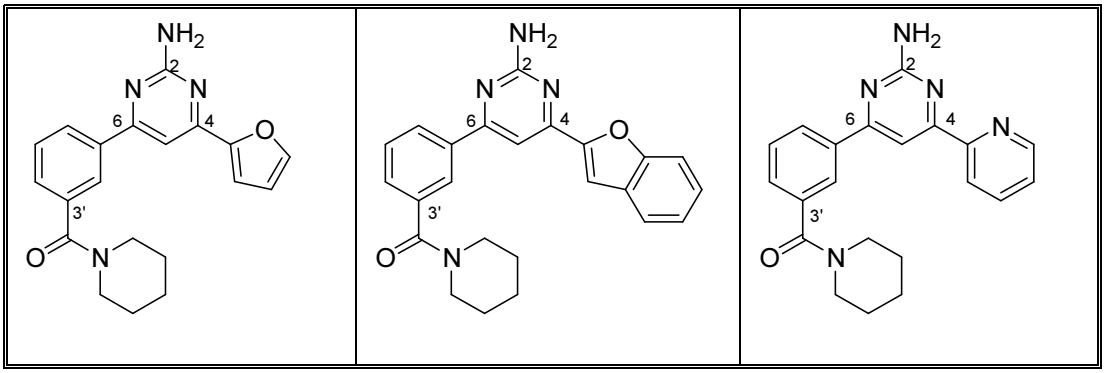


Figure 1.4: Examples of compounds with different heteroaryl and aryl groups in position 4.

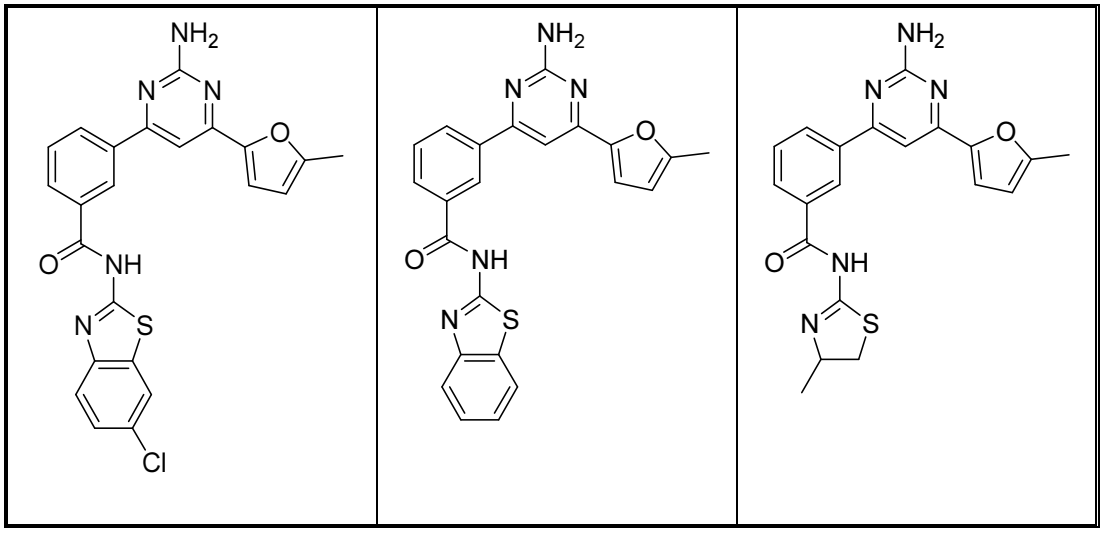
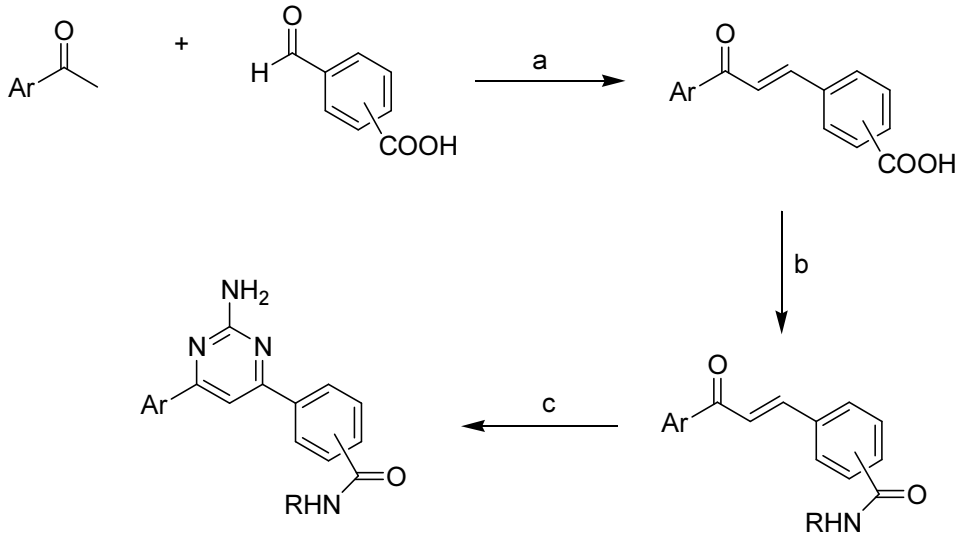


Figure 1.5: Examples of compounds with thiazole substituents

The general synthetic route presented in Scheme 1.1 will be employed to obtain these 2-aminopyrimidines.



Scheme 1.1: General synthetic route towards aminopyrimidine synthesis. Reagents and conditions: a) NaOH, MeOH, rt, 8 h. b) CDI, CH_2Cl_2, NHR, rt, 5 h. c) Guanidine hydrochloride, NaH, DMF, 80 °C – 120 °C. 24 h. Ar = aromatic/heteroaromatic substituent.

b) *In vitro* screening of the aminopyrimidines will be conducted to determine the affinities of synthesised compounds for adenosine A_{2A} and adenosine A_1 receptors using a radioligand binding assay.

c) The *in vivo* activity of selected derivatives will be determined using the haloperidol induced catalepsy assay in rats. This will provide an indication of agonistic/antagonistic activity.
d) The results obtained during the *in vitro* and *in vivo* assays will be rationalised by utilising QSAR and molecular modelling studies.
e) Toxicity of synthesised aminopyrimidines will be assessed in HeLa cells by the MTT cell viability assay.