CHAPTER 1

Introduction

1.1 Background and rationale

Parkinson's disease is currently regarded as the second most common neurodegenerative disease after Alzheimer's disease, and affects approximately 1% of the world population today (Dorsey *et al*., 2007; Von Campenhausen *et al*., 2005; Dluzen & McDermott, 2000). It is unique among neurodegenerative disorders because of the almost palpable anticipation of an imminent cure (LeWitt & Taylor, 2008). Finding a cure for Parkinson's disease is crucial, as the worldwide increase in human lifespan results in an increase in incidence of age related neurodegenerative disorders, and thus in future, Parkinson's disease can become an extreme socio-economic burden (Dorsey *et al.,* 2007).

This chronic, progressive neurological disease is mainly characterised by a loss of dopamine in the substantia nigra and treatment therefore involves mainly dopamine replacement therapy with agents such as levodopa and dopamine agonists (Jenner *et al.,* 2009). While highly effective in reducing the motor symptoms of the disease, the use of these agents is associated with a range of dopaminergic side effects, both in the acute phase of treatment and as long term complications of therapy. Effective treatment options of Parkinson's disease in the middle to late stages of the illness are therefore limited. As a consequence, novel approaches to treatment are required and non-dopaminergic drugs may offer one such opportunity (Rose *et al.,* 2006).

1.2 Adenosine receptors and Parkinson's disease

Normal movement is controlled by the brain structures located in the basal ganglia. In the last few years, increasing evidence has emerged regarding the importance of the role of adenosine in modulating the functional circuitry of the basal ganglia (Ferré *et al*., 1997; Hauber *et al.,* 2001; Svenningson *et al.*, 1999). Several subtypes of adenosine receptors are involved in motor function, and anatomical studies have demonstrated that adenosine A_{2A} receptors are present in very high densities on striatopallidal neurons, which also tend to co-express dopamine D_2 receptors and enkephalin (Fink *et al*., 1992; Schiffman *et al.,* 1991; Svenningson *et al*., 1999). Adenosine A_{2A} receptors can regulate the functional activity of dopamine D_2 receptors in two ways – firstly by affecting the binding affinity of the D_2 receptor for dopamine, and secondly via the second messenger systems. Stimulation of the A_{2A} receptor results in a decreased affinity of dopamine for the D_2 receptor, and also leads to stimulation of adenylate cyclase and an

increase in cyclic adenosine monophosphate (cAMP) (Morelli *et al.,* 2007). Adenosine A_{2A} receptors and dopamine D_2 receptors thus act in an antagonistic manner, and administering an A_{2A} antagonist to a parkinsonian patient should have similar effects as the administration of D_2 agonists, which are commonly used in the treatment of Parkinson's disease (Mantri *et al.,* 2008). Furthermore, adenosine A_{2A} receptor manipulation can alter the release of GABA, acetylcholine and glutamate within the basal ganglia, thus affecting key transmitters involved in the control of motor behavior (Floran *et al*., 2005; Fuxe *et al*., 2005; Rodrigues *et al*., 2005).

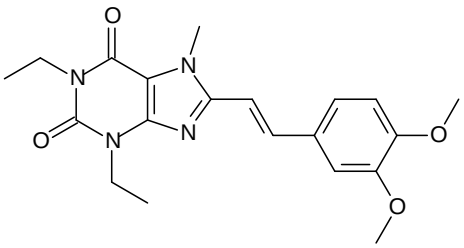
These factors contributed to the recent interest in adenosine A_{2A} receptor antagonists as potential antiparkinsonian agents (Hauser & Schwarzschild, 2005; Jenner, 2005). Data obtained from a number of preclinical studies suggest that A_{2A} antagonists might have numerous applications in the treatment of Parkinson's disease. Furthermore, adenosine A_{2A} antagonists, such as KW 6002 (istradefylline) (**1**), was selected for clinical trials and promising results have been obtained when it was used in combination therapy with drugs like levodopa (Bara-Jimenez *et al.,* 2003; Fernandez *et al.,* 2010; Factor, 2010). In addition to the benefits of A_{2A} antagonists in symptomatic treatment, inhibition of these receptors could also have a neuroprotective action (Cieślak *et al.,* 2008).

Although the aim of many studies is to obtain selective A_{2A} receptor antagonists, dual activity (inhibition of both A_1 and A_{2A} receptors) is not necessarily detrimental, as it has been shown that antagonism of the adenosine A_1 receptor facilitates dopamine release in the striatum and potentiate dopamine-mediated responses (Shook *et al*., 2012). It furthermore can also be beneficial in controlling cognitive side effects that are present in patients with Parkinson's disease (Normile & Barraco, 1991; Costenla *et al*., 1999).

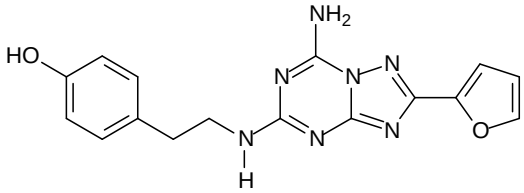
1.3 Adenosine A_{2A} antagonists: Compound classes and design tools

Most adenosine A_{2A} antagonists belong to two different chemical classes namely; the xanthine derivatives and the aminosubstituted heterocyclic compounds, which are derived from adenine or are structurally related to adenine (Müller & Ferré, 2007).

Over the years many xanthine derivatives have been developed and in terms of clinical development, KW 6002 (**1**) was the most successful. There are also a wide range of aminosubstituted heterocyclic compounds like ZM 241385 (**2**), a triazolotriazine, which contains the 2-aminopyrimidine moiety (Müller & Ferré, 2007). The focus of this study will be on a specific subset of aminosubstituted heterocyclic compounds, namely the 2-aminopyrimidines.

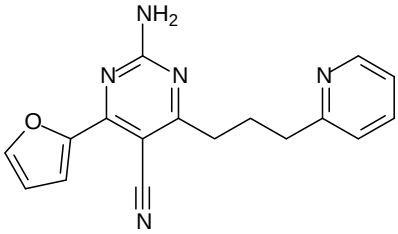


1 KW 6002



2 ZM 241385

Both the 2-aminopyrimidine and furan moieties often occur in potent, selective A_{2A} antagonists, for example compounds **3** and **4** patented by Hoffmann-La Roche (Borroni *et al.,* 2005).



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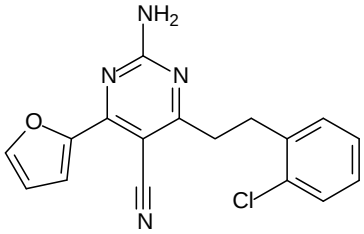

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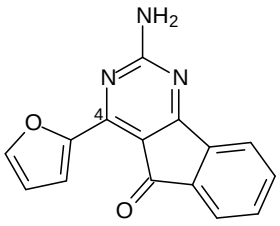
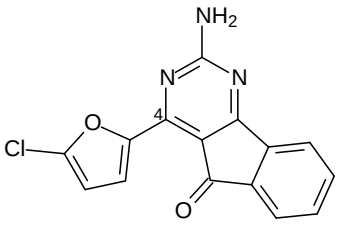
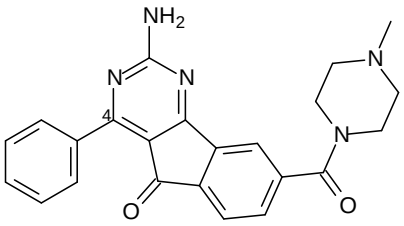
Other examples include several series of arylindenopyrimidines synthesised by Shook and co-workers (2010a, 2010b, 2010c, 2012), (e.g. compounds **5, 6, 7** and **8**), where it was illustrated that although several aryl and heteroaryl substituents were tolerated in position 4, the furan substituent had superior *in vitro* and *in vivo* activity. However, unsubstituted furans are often associated with Ames liability, which can be overcome by substitution of the furan ring (e.g. compound **6**) or by using simple phenyl substitution (e.g. compound **7**) instead. In their methylene amine substituted arylindenopyrimidine series (e.g. compound **8**), metabolic liabilities associated with the benzylic carbon were overcome by synthesising the corresponding amides, as illustrated by compound **7**.



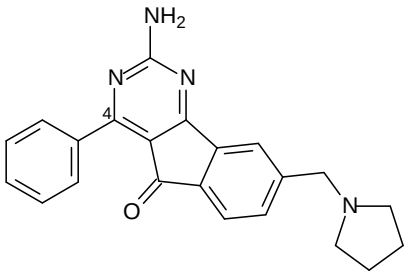
A_{2A} K_i = 0.1 nM
A_1/A_{2A} = 4
5



A_{2A} K_i = 0.2 nM
A_1/A_{2A} = 2.5
6

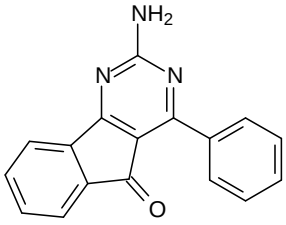


A_{2A} K_i = 8.2 nM
A_1/A_{2A} = 7.12
7

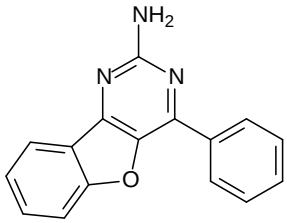


A_{2A} K_i = 4.1 nM
A_1/A_{2A} = 4.15
8

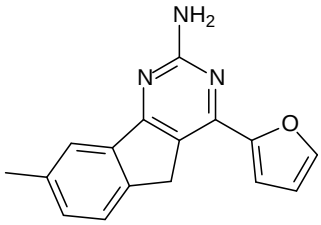
Matasi and co-workers (2005) further synthesised a series of indenopyrimidines (e.g. compounds **9**, **10**, **11** and **12**) and concluded the following: the exocyclic amine group is essential for A_{2A} activity; reduction of the ketone (**9** versus **11**) or its replacement with an ether linkage as in **10** retains affinity without improving selectivity and compounds with phenyl (**10**), furan (**11**) and substituted furan (**12**) moieties in position 4 had similar acitivy, with improved selectivity observed for furan derivatives. These data will be considered in the design of compounds for this study.



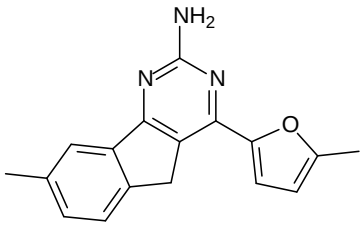
A_{2A} K_i = 11.2 nM
A_1/A_{2A} = 1.2
9



A_{2A} K_i = 9.0 nM
A_1/A_{2A} = 2
10



A_{2A} K_i = 6.7 nM
A_1/A_{2A} = 29.4
11



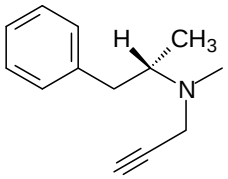
A_{2A} K_i = 7.1 nM
A_1/A_{2A} = 25.7
12

The availability of the crystal structure of the adenosine A_{2A} receptor further offers the unique opportunity of designing A_{2A} antagonists with increased selectivity for this important drug target. Besides the crystal structure, several non-xanthine pharmacophore models exist for A_{2A} antagonists (Muller and Ferré, 2007, Mantri *et al.,* 2008). These pharmacophore models serve as further useful tools in the design of novel adenosine A_{2A} receptor antagonists.

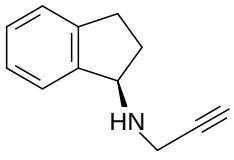
1.4 Monoamine oxidase B inhibitors

Monoamine oxidase B (MAO-B) is responsible for the catabolism of dopamine in the striatum and inhibition of this enzyme therefore presents a promising alternative strategy in the treatment of Parkinson's disease. The MAO-B inhibitors, selegeline (**13**) and rasagiline (**14**) are used clinically in the treatment of Parkinson's disease, and provide symptomatic relief as they conserve dopamine in the brain. These drugs are often administered in combination with the dopamine precurser, levodopa (**15**), seeing that they elevate dopamine (DA) levels derived from levodopa.

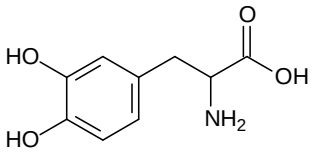
There are also theories that MAO-B inhibitors might posess neuroprotective activity. Hydrogen peroxide is generated in the catalytic cycle of MAO-B and most likely contributes to the progression of Parkinson's disease, as it leads to oxidative stress and apoptotic prosesses. Administering a MAO-B inhibitor will prevent the formation of hydrogen peroxide and therefore act as a neuroprotective agent (Riederer *et al*., 2004).



13 Selegeline



14 Rasagiline

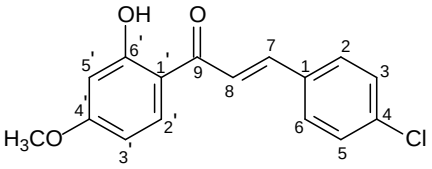


15 Levodopa

Adverse effects, such as psychotoxic and cardiovascular effects, associated with the use of irreversible MAO-B inhibitors, are often problematic as it may take several weeks for enzyme activity to recover. For this reason, new reversible MAO-B inhibitors, which will have a better safety profile, must be developed.

Chalcones (*trans*-1,3-diphenyl-2-propen-1-ones) can be found in edible plants and are considered to be the bio-genetic precursors of all known flavonoids (Go *et al.,* 2005). Chalcones are often intermediates in the syntheses of MAO-A and B inhibitors , however, the MAO

inhibitory activity of chalcones themselves have only been determined in a few instances (Chimenti *et al.,* 2006; Chimenti *et al.,* 2007). Chimenti and co-workers (2009) recently presented the synthesis and MAO-A and MAO-B inhibitory activity of a number of substituted chalcones and showed that these compounds were more active and selective towards the MAO-B isoform than the MAO-A isoform (**16**).



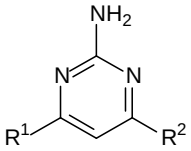
IC_{50} (MAO-A) = Inactive at 50 µM (Highest concentration tested)

IC_{50} (MAO-B) = 0.0044 µM (Chimenti *et al.,* 2009)

16

1.5 Hypothesis of the study:

It is postulated that 2-aminopyrimidines, with the basic structure as in compound **17**, which would fit literature pharmacophore models (e.g Muller and Ferré, 2007; Mantri *et al.,* 2008), and have a similar binding pattern in the adenosine A_{2A} receptor as known adenosine A_{2A} antagonists, should be potentially good inhibitors of the adenosine A_{2A} receptor. It is further hypothesised that selected chalcones, obtained as intermediates in the synthesis of the 2-aminopyrimidines will have activity as MAO inhibitors.

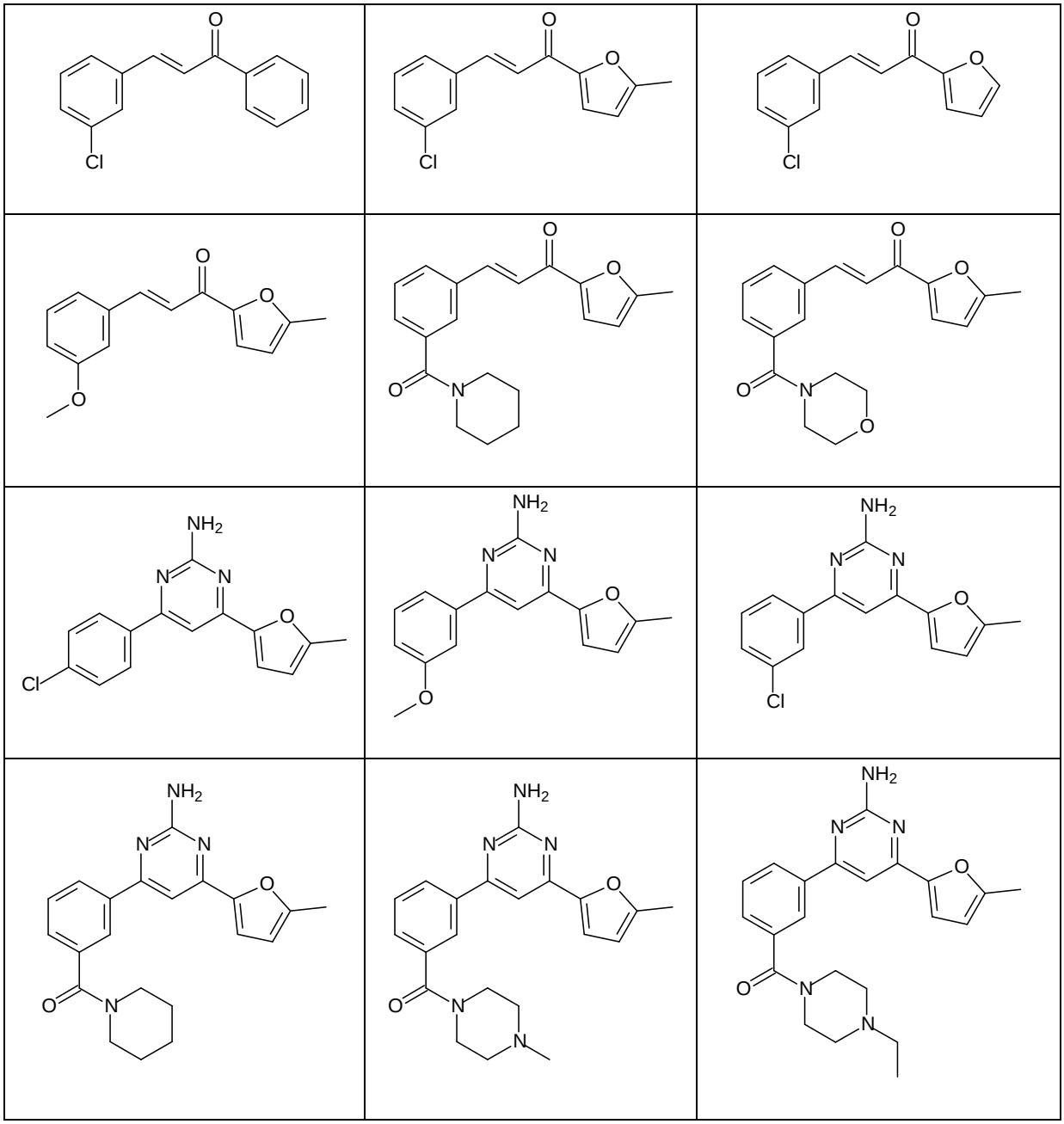


17

1.6 Aim and Objectives

The aim of this study is to design, synthesise and evaluate 2-aminopyrimidine derivatives as adenosine A_{2A} receptor antagonists. Since chalcones are obtained as intermediates during the synthesis of the 2-aminopyrimidines, a secondary aim of this study would be the evaluation of selected chalcones as monoamine oxidase inhibitors. Some examples of proposed chalcones and 2-aminopyrimidines are given in Table 1:

Table 1: Examples of proposed chalcones and 2- aminopyrimidines

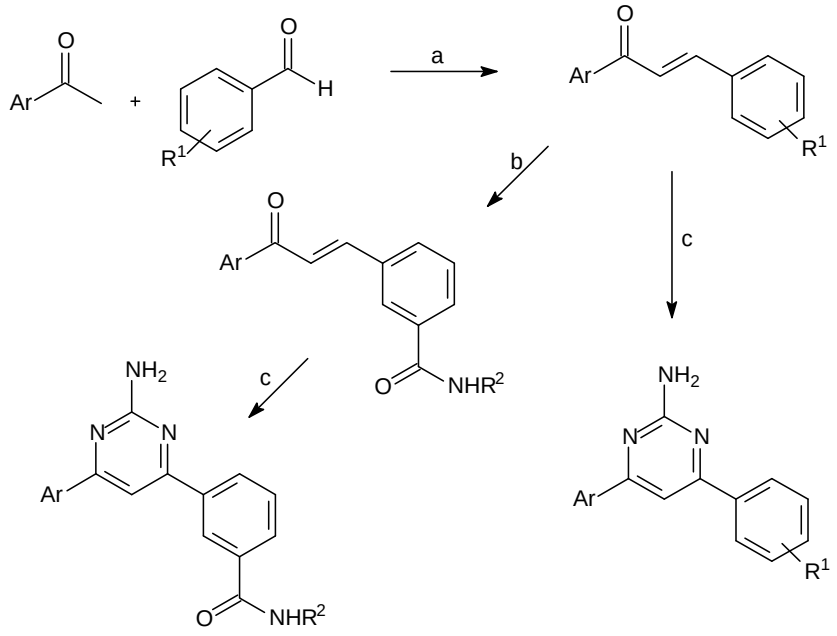


The objectives of the study can be summarised as follows:

a) 2-Aminopyrimidines will be selected for synthesis based on their abilities to fit into current pharmacophore models. Structural features associated with affinity and

selectivity as reported in literature will also be taken into consideration, while molecular modelling will further be used as a design tool.

b) 2-Aminopyrimidines will be synthesised by employing the general synthetic route illustrated in Scheme 1.1, obtaining chalcone intermediates in the process.



Scheme 1.1: General synthesis of aminopyrimidines. Reagents and conditions: (a) NaOH, EtOH/MeOH, rt, 3h; (b) CDI, CH_2Cl_2, NHR^2, rt, 5h; (c) Guanidine hydrochloride, NaH, DMF, 110 °C, 24h. Ar = Phenyl, furan or 5-methylfuran.

c) *In vitro* evaluation of synthesised compounds:
   - The synthesised chalcones will be screened as inhibitors of MAO-A and -B using kynuramine as enzyme substrate in both instances. Mode of binding (reversible or irreversible and competitive or non-competive binding) will also be assessed for the most active compound.
   - The 2-aminopyrimidines will be screened *in vitro* for their adenosine A_{2A} receptor affinities using a radioligand binding assay.

d) *In vivo* evaluation: 2-Aminopyrimidines with promising A_{2A} affinity in the *in vitro* assay will be selected for *in vivo* evaluation in the haloperidol induced catalepsy assay, to determine whether they are adenosine A_{2A} receptor antagonists.

e) Molecular modelling will further be used to determine possible binding orientations of the 2-aminopyrimidines in the adenosine A_{2A} receptor active site to obtain possible explanations for affinities/ or loss of affinities observed.