CHAPTER 5
Conclusion

Parkinson's disease is one of the most common neurodegenerative diseases and is characterised by the loss of dopaminergic neurons in the striatum. This results in pathological features such as loss of movement, dyskinesias, tremor and other non-motor symptoms. Present day treatment does not halt the progression of the disease but only provides symptomatic relief. There are also several other shortcomings associated with these therapies and novel approaches to treatment are urgently required. Adenosine A_{2A} antagonism and MAO-B inhibition therapies are both promising candidates that may improve Parkinson's disease therapy in the future.

The MAO-B enzyme is primarily responsible for the catabolism of dopamine in the striatum and inhibition of this enzyme results in increased levels of dopamine, which will in turn improve the motor symptoms of Parkinson's disease. The inhibition of the MAO-B enzyme may not only result in symptomatic relief, but may also offer neuroprotection strategy, since inhibition of the enzyme is believed to decrease the formation of toxic byproducts associated with the oxidation of dopamine. Irreversible MAO-B inhibitors are associated with certain disadvantages, as enzyme recovery may take up to two weeks after the termination of therapy. In contrast, reversible inhibitors of the enzyme have much better safety profiles as they only inhibit the enzyme temporarily and allow competition with the substrate.

Adenosine A_{2A} antagonism, on the other hand, increases dopaminergic neurotransmission for symptomatic relief by increasing the affinity of the dopamine D_2 receptor for dopamine as well as by decreasing cAMP levels. Adenosine A_{2A} receptor manipulation also affects the release of GABA, glutamate and acetylcholine, which is key transmitters involved in the control of motor behaviour. Several adenosine A_{2A} antagonists have already reached clinical trials and showed promising results. There are also several theories as to why A_{2A} antagonism may be neuroprotective, for example by preventing the death of neurons caused by glutamate excitotoxicity.

Most of the current adenosine A_{2A} antagonists belong to two different chemical classes namely, the xanthine derivatives and the amino-substituted heterocyclic compounds. KW 6002 (istradefylline) is a xanthine derivative and one of the most successful adenosine A_{2A}

antagonists in terms of clinical development, as it has already reached phase III clinical trials for the treatment of Parkinson's disease. Amino-substituted heterocyclic compounds, like ZM 241385, are another promising class of compounds which may provide interesting new leads for the development of novel agents in the treatment of Parkinson's disease. This study focused on a specific type of amino-substituted heterocyclic compound, namely the 2-aminopyrimidines.

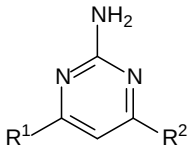
Aim:

The primary aim of this study was to design, synthesise and evaluate 2-aminopyrimidines as adenosine A_{2A} antagonists.

As literature indicated the viability of chalcones as MAO-B inhibitors, and since chalcone intermediates were obtained during the synthesis of the aminopyrimidines, a secondary aim was the evaluation of selected chalcones as MAO-B inhibitors.

Design:

The consideration of literature pharmacophores, as well as the activity of known amino-substituted heterocyclic compounds, led to the design of several compounds (**2a** - **2n**) with the 2-aminopyrimidine core as basic scaffold (**17**).



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Molecular docking was used to determine if the designed 2-aminopyrimidines had the required interactions with important residues (such as a hydrogen bonding with Asn 253 and Glu 169, and a pi interaction with Phe 168) in the active site of the adenosine A_{2A} receptor. It was hypothesised that compounds with these selected interactions would have affinity for the receptor. The designed compounds were successfully docked, suggesting that these compounds would fit into the binding site. Furthmore, since the designed 2-aminopyrimidines all had similar interactions and binding orientations to that observed for ZM 241385, it was concluded that these compounds have the structural features required for interaction with important amino acid residues in the active site. The screening of these 2-aminopyrimidines for adenosine A_{2A} receptor activity, were thus indeed feasible.

Chemistry

Five novel and eight known 2-aminopyrimidines were successfully synthesised using standard laboratory procedures. The synthesis commenced by reacting different commercially available aldehydes and ketones to form the appropriate chalcones. An amide coupling reaction was then done for the phenylamide derivatives, where different amines were coupled to the acid group of the corresponding chalcones using 1,1'-carbonyldiimidazole as coupling reagent. The chalcones were then reacted with guanidine hydrochloride in the presence of NaH to yield the appropriate 4,6-disubstituted 2-aminopyrimidines. All compounds were characterised with 1D and 2D NMR spectroscopy, mass spectrometry and melting points were determined. The purities of compounds were evaluated using HPLC. Purities of above 85% were obtained for all compounds as determined by HPLC.

MAO inhibition studies, reversibility studies and mode of inhibition

The determination of the inhibition of MAO for selected chalcones was performed with a fluorometric assay and activities were expressed as IC_{50} values. IC_{50} values obtained ranged from 0.490 µM to 7.666 µM with compounds **1a**, **1c** and **1g** being the most potent. Most compounds also revealed good selectivity towards the MAO-B isoform. A few preliminary structure-activity relationships could be derived however, this was a very small set of compounds and more derivatives will have to be synthesised before definite conclusions can be made.

The MAO-B catalytic activities were recovered to approximately 80% and 50% respectively during the reversibility studies, indicating that chalcone **1c** is a reversible inhibitor at the concentrations tested. Sets of Lineweaver-Burk plots were also constructed in order to determine the mode of inhibition. The results showed that these Lineweaver-Burke plots intersected on the y-axis, which indicated that compound **1c** is a competitive inhibitor.

Adenosine A_{2A} assays

The binding affinities of 2-aminopyrimidines for the A_{2A} receptor were evaluated with a radioligand binding assay. The assay was performed with the radioligand [^{3}H]NECA in the presence of a 100 nM CPA and the potencies were expressed as IC_{50} and K_i values.

Aminopyrimidines **2a** - **2h** exhibited moderate to weak affinities with K_i values above 200 nM. The phenylamide substituted derivatives (compounds **2j** – **2n**) however, showed promising results with K_i values ranging from 5.76 nM – 52.8 nM. Compound **2m,** for example**,** was the

most potent compound with a K_i value of 5.76 nM. The potency of this antagonist is comparable to that of the known A_{2A} antagonists ZM 241385 (K_i = 2.100 nM) and KW 6002 (K_i = 10.26). In order to rationalise the differences observed in the affinities between the two sets of aminopyrimidines, docking experiments were revisited. The results of the radioligand binding studies were confirmed by the C-DOCKER_INTERACTION energies which illustrated that the energies of the phenylamide derivatives (**2j** - **2n**) were more favourable than those of derivatives **2a** – **2h**. An additional hydrogen bonding interaction observed between the amide carbonyl and Tyr 271 could, at least in part, explain why this particular subset of 2-aminopyrimidines had superior affinities for the adenosine A_{2A} receptor.

To determine whether compounds **2m** and **2k** were antagonists of the adenosine A_{2A} receptor, the reversal of haloperidol induced catalepsy in rats was investigated. Since catalepsy was significantly reduced, it was concluded that these compounds are indeed adenosine A_{2A} antagonists.

This study thus resulted in the successful design, synthesis and evaluation of 2-aminopyrimidines as adenosine A_{2A} antagonists. As hypothesised, 2-aminopyrimidines, particularly the phenylamide substituted derivatives, possess the appropriate structural features to bind to the A_{2A} receptor and act as potent *in vivo* antagonists. Future work would include the determination of adenosine A_1 receptor affinities (in order to determine selectivity), the determination of bioavailability and toxicity studies. In addition, the MAO-B inhibitory activities determined for selected furan substituted chalcones indicate that these compounds are promising leads for the design of future MAO-B inhibitors.