

Chapter 5: Conclusion

Parkinson's disease is a neurodegenerative disorder of the central nervous system, characterised by a progressive loss of the nigrostriatal dopaminergic neurons. This degeneration is well advanced before symptoms appear and diagnosis becomes possible. Classic motor symptoms include bradykinesia, akinesia, tremor at rest and balance disturbances. Non-motor symptoms, which usually develop during the later stages of the disease, typically include depression and cognitive dysfunction. The mainstay of current treatment is still levodopa, which initially provides excellent symptomatic relief, however as the disease progresses, the effectiveness of levodopa eventually declines. Levodopa therapy is also associated with the development of disabling side effects such as dyskinesia. Research aimed at obtaining novel therapies for Parkinson's disease is thus currently focused on finding disease modifying agents that will halt disease progression, while also treating symptoms. The dual antagonism of adenosine A_1/A_{2A} receptors is a promising non-dopaminergic alternative to current therapy, since adenosine A_1/A_{2A} receptor antagonists may, in addition to providing symptomatic relief, also enhance cognition and offer neuroprotective benefits.

The symptomatic relief of motor symptoms, as afforded by these agents, is mediated by antagonism of the adenosine A_{2A} receptor which decreases cAMP levels in the dopaminergic neuron. The second messenger system is therefore normalised and the overactivity of the indirect striatopallidal inhibitory pathway is attenuated, resulting in the normalisation of movement. The antagonism of the adenosine A_1 receptor has a synergistic effect, as affinity of dopamine for its receptor is increased in its presence. Improvement in cognitive function was further demonstrated by the antagonism of adenosine A_1 receptors in rodent models and these agents could thus be beneficial in advanced Parkinson's disease, where cognitive dysfunction is often present. Neuroprotection is thought to be mediated by modulation of neurotransmitter release in the central nervous system (GABA, glutamate, acetylcholine) as well as effects on the inflammatory cycle, which has a great impact on disease progression.

Adenosine A_{2A} receptor antagonists are divided into two chemical classes, namely the xanthine derivatives (structurally related to caffeine) and the amino-substituted heterocyclic compounds (structurally related to adenine), which include several compounds that contain the 2-aminopyrimidine motif. Xanthine adenosine A_{2A} receptor antagonists that have reached clinical trials include istradefylline (KW 6002), while non-xanthines include preladenant and vipadenant. Since the structure-activity relationships of the versatile 2-aminopyrimidine

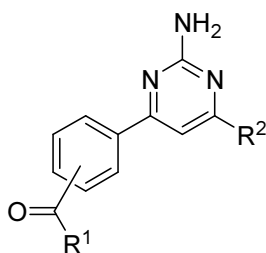
scaffold have not been fully explored with regards to adenosine receptor antagonistic activity, it is in this area that this study aimed to make a contribution.

Aim:

The aim of this study was thus to design, synthesise and evaluate 2-aminopyrimidines as potential dual adenosine A₁/A_{2A} receptor antagonists and to obtain preliminary cytotoxicity data.

Chemistry:

Modifications of the 2-aminopyrimidine scaffold investigated in this study included the synthesis of derivatives substituted in the 4' position of the phenyl ring, varying the aromatic substituent in position 4 of the pyrimidine ring (R²) and the inclusion of a thiazole moiety in the phenylamide side chain (R¹).



Fourteen novel 2-aminopyrimidines were successfully synthesised using standard organic synthetic procedures. Compounds were synthesised in three steps, albeit in low yields. Commercially available aromatic ketones and aldehydes were first reacted by Claisen-Schmidt condensation, followed by an amide coupling reaction (using CDI as coupling reagent) and finally cyclised with guanidine hydrochloride using sodium hydride in DMF. Compounds were characterised by NMR- and IR spectroscopy as well as mass spectrometry. The purity of the final compounds was further assessed by HPLC.

In vitro assays:

Several dual, high affinity 2-aminopyrimidine derivatives were identified in the radioligand binding assays. The radioligands used were [³H]DPCPX and [³H]NECA in the adenosine A₁ and A_{2A} radioligand assays, respectively. After determining that K_i values obtained with male and female brain tissue were similar, whole male rat brain was used as receptor source in the adenosine A₁ assays and female rat striata for the adenosine A_{2A} assays.

Interesting structure activity relationships could be derived for the current series. Firstly, as hypothesised, it was clear that substitution in the 4' position with simple cyclic amines resulted in a loss of both adenosine A₁ and A_{2A} receptor affinity. While the same was also true for thiazole substitution and adenosine A_{2A} receptor affinity, the promising A₁ affinity observed for compound **39i**, a 4' substituted thiazole derivative, warrants further investigation. It is postulated that the highly planar nature of this compound, could contribute to the high degree of A₁ receptor affinity and selectivity observed for this compound. In contrast to previous studies, where 2-furyl substitution resulted in optimal affinity, substitution with a methylthiophene group in this study, by far resulted in the highest adenosine A_{2A} affinity. Several aromatic substituents were however tolerated in the 4 position, since good dual affinity was also obtained for the phenyl (**39c**) and furan (**39d**) derivatives. Furthermore, as illustrated in a preceding study (Robinson, 2013), inclusion of a piperidine substituent resulted in optimal dual adenosine A₁/A_{2A} receptor affinity (e.g. **39f**).

A small QSAR and molecular modelling study was further performed to rationalise the results as obtained in the adenosine A_{2A} radioligand binding studies. The QSAR study indicated the importance of polar surface area, which inversely correlated with the observed affinity. Docking results further showed that hydrogen bonding interactions between either Asn 253 and/or Glu 169, present in the active site, seem to be an important requirement for A_{2A} receptor affinity.

Cell viability in the presence of the synthesised 2-aminopyrimidines was further determined by the MTT cell viability assay in HeLa cells. Most of the compounds (with the exception of compounds **39d** and **39j**) demonstrated no significant toxicity at 1 µM, a concentration several fold higher than the K_i values obtained for most of the high affinity derivatives. It was apparent that both the amine and aromatic substituent in position 4 played a role in the observed cytotoxicity.

In vivo assay:

The *in vivo* activity of the two highest affinity derivatives, **39f** and **39c** was evaluated by the haloperidol induced catalepsy assay, which is used to give a preliminary indication of agonism or antagonism of adenosine receptors. Compound **39f** failed to reduce catalepsy but compound **39c** reversed catalepsy in a statistically significant manner, when compared to the control group, indicating that these compounds are adenosine antagonists. The fact that the highest affinity derivative failed to show *in vivo* activity (under standard assay conditions) highlights the importance of considering the physicochemical properties of compounds in addition to their affinity, when selecting a candidate for animal studies. For

this particular series, while the molecular weights of the synthesised compounds were similar to that of standard CNS drugs, the slightly higher than average log P values, for instance, could affect bioavailability. Improvement of the physicochemical properties of this series should thus be considered in future studies.

Future work could further include the determination of the affinities of the synthesised compounds for other receptors, e.g. D₂ receptors, to exclude the influence of these receptors on *in vivo* activity. Adenosine antagonistic properties should further be confirmed in functional assays where cAMP levels are measured. The potential of these compounds to afford neuroprotection should also be assessed and the neuroprotective mechanisms investigated if present. The effect of these compounds in the reserpine locomotor activity animal model of Parkinson's disease could also be determined to provide further evidence of the *in vivo* effectiveness of this class of compounds as potential treatment of Parkinson's disease.

In conclusion: this study resulted in the successful design, synthesis and biological evaluation of 14 novel 2-aminopyrimidines as dual adenosine receptor antagonists. Several derivatives with highly potent dual affinity for the adenosine receptor subtypes were identified, as hypothesised. Compound **39c** was the most promising candidate identified during this study with promising dual affinity for both adenosine A₁ and A_{2A} receptors, an acceptable preliminary toxicity profile and *in vivo* efficacy.