Novel ligands for the human adenosine A1 receptor

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Novel Ligands for the Human Adenosine A_1 Receptor

The Design of a New Pharmacophore and its Subsequent Development through Synthesis and Biological Evaluation

PROEFSCHRIFT

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	Prof. dr. G. J. Mulder

For my mother and in memory of my father

谨以此文献给我敬爱的母亲和缅怀已故的父亲

Contents

Chapter 1

The Adenosine A_1 Receptor

This chapter introduces the concept of the adenosine receptor as a G protein-coupled receptor. The therapeutic potential of the adenosine A_1 receptor is highlighted and the investigated effects of the most well-known adenosine A_1 receptor antagonist, caffeine, are explored. The scope and content of this thesis is then summarised.

General Introduction

Communication within the mammalian body is essential for the regulation of all manner of different physiological functions. The exchange of information from the extracellular environment into the cell is often conducted by membrane receptors. The largest class of cell-surface receptors are the Guanylyl-nucleotide-binding protein-coupled receptors, otherwise known as G protein-coupled receptors or GPCRs. The GPCRs are activated by a diverse assortment of ligands, including peptides, ions, photons and hormones,[1] and is the largest group of current drug targets.[2] This super-family of receptors consists of amino acid chains which are arranged into seven transmembrane (7TM) helices traversing the cell-membrane, linked by intra- and extracellular loops (Figure 1.1). Upon activation of the receptor by the signalling molecule, the G protein binds and provokes a further signal through its association and subsequent dissociation from the receptor. The G protein itself consists of three subunits, α, β and γ. In the inactive state, the Gα subunit has guanosine diphosphate (GDP) in its binding site, when the receptor is activated, a change in the structure occurs causing GDP to leave and be replaced by its triphosphate cousin (GTP). The GTP activates Gα causing it to dissociate from Gβγ. The final effect of this cascade of events is conducted through the activation (or inactivation) of an effector protein, e.g., adenylate cyclase and phospholipase C.[3]

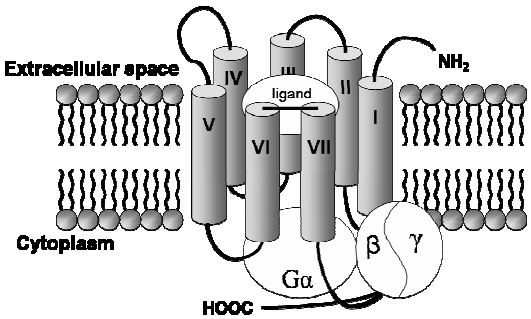

Figure 1.1 The seven transmembrane helices of a G protein-coupled receptor.

The GPCR super-family of receptors can be classified into a number of different categories depending on similar structural features.[4] The largest of these categories is Class A (also called Family 1) characterised by the conservation of certain amino acids.[4,5] Individual examples of Class A receptors are the dopamine receptors, the serotonin receptors and the adenosine receptors.

Adenosine Receptors

The adenosine receptors, hence their name, are subject to activation by the endogenous ligand adenosine (Figure 1.2). In extracellular space adenosine is formed by the breakdown of adenosine triphosphate (ATP). In the body, adenosine has an extremely short half-life (approximately 1 second)[6] and is thus produced when and where it is deemed necessary, acting as a local hormone. There are four categories of adenosine receptor, the A_1, A_{2A}, A_{2B}, and the A_3.[7,8] The nomenclature in current use is based on that proposed by Van Calker *et al.*,[7] who defined the A_1 receptor as being inhibitory to adenylate cyclase and the A_2 receptor as consequently

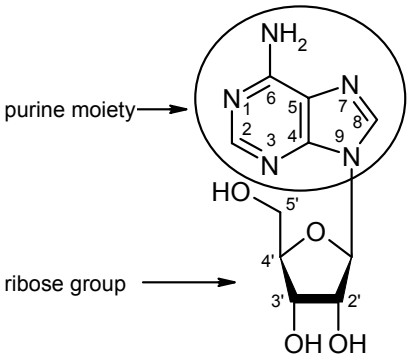

Figure 1.2 Adenosine

stimulatory to this effector protein. Further categorisation of the A_2 receptor was a result of experimental findings describing high and low affinity binding sites.[9,10] The A_3 adenosine receptor was discovered in a 'reversed' manner, where the receptor was first cloned and sequenced before its function and endogenous ligand were discovered.[11] As molecular biological techniques evolved and improved in the early-to-mid 1990s, the receptors were individually cloned and identified for a number of species. The human adenosine A_1 receptor was first characterised in 1992.[12,13] It was revealed to have an amino acid sequence of 326 in length and hold a 95 % sequence homology to the rat, and 94 % to the dog and bovine A_1 receptors.[12] As would be expected, the sequence homology between the different adenosine receptors is quite low, with only the A_{2A} and the A_{2B} receptors retaining more than 60% similarity (73% in the transmembrane domain).[14]

The different adenosine receptors can be found distributed widely in varying levels throughout the physiological system,[15,16] and for this reason, the effects of adenosine are so wide and varied. For example, high expression of the A_1 receptor can be found in such tissue as the brain, the spinal cord and the atria; the A_{2A} receptor is found in good levels of expression in the striatum, the spleen, blood platelets, and in the lung; the A_{2B} receptors are present in fair quantities in tissue such as the bladder and colon, and the A_3 receptors are expressed in somewhat lower levels in human liver and kidney tissues.[15] Although higher levels of expression of certain receptors are found in certain organs, the presence of one particular receptor is not usually exclusive to that organ. As such, highly selective compounds targeted at specific receptors are very much desired.

Having mentioned the almost omnipotent presence of adenosine receptors in the physiological system, the pharmaceutical benefits of compounds targeted at the receptors should be addressed. The two traditional divisions are the agonists and antagonists. Agonists are species that can activate a receptor in its natural state, and replace the need for the presence or generation of the endogenous ligand. Many adenosine receptor agonists have been developed, and most of these mimic the natural ligand closely in terms of structure. Traditionally, the presence of the purine ring and the necessity of the intact ribose moiety (Figure 1.2) allow agonistic properties to prevail, in conjunction, of course, with good affinity for the adenosine receptors. Very recently, agonists have been developed which do not mimic the natural ligand, in that they do not possess the purine ring structure, nor do they contain the ribose group.[17-19] Partial agonism may be desired to overcome side-effects associated with the full agonism of receptors by highly potent compounds. Like most of the full agonists, most partial agonists are adenosine derivatives and created by either substitution at the purine or the sugar group.[20-25]

Antagonists are compounds that occupy the receptor, preventing the binding and thus the action of the endogenous ligand. More recently, the theory that a receptor possesses an intrinsic level of activity has been researched. This has led to a new category of compounds, namely inverse agonists, ligands that lower the level of activity of the receptor. One example

is the well-known adenosine receptor ligand 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX), this was long thought of as being a pure antagonist but it can in fact act as an inverse agonist.[26] No doubt there are many more adenosine receptor 'antagonists' which could be reclassified as inverse agonists.

The Therapeutic Potential of the Regulation of the Adenosine A_1 Receptor

The adenosine A_1 receptor is, as mentioned in the previous section, widely distributed in varying levels of expression about many different tissues in the human body, ranging from the colon to the brain.[15,16] The therapeutic potential of regulating this receptor is thus sizeable. The great expectancy laid upon such a therapeutically attractive target has generated a vast quantity of scientific literature dedicated specifically towards this receptor. Some of the more highlighted aspects that regulation of the adenosine A_1 receptor induce are described below.[27]

The high presence of adenosine A_1 receptors in atrial tissue, suggests its cardiovascular role. To date, the only adenosine-related medicine available is adenosine itself. It is injected directly in cases of supraventricular tachycardia (abnormal heart rhythm) to return to normal sinus rhythm.[28] Other functions that activation of the A_1 receptor preclude are injuries caused by myocardial ischæmia (restriction of blood from the myocardium) and subsequent reperfusion (restoration of blood to an ischæmic area).[29]

One very promising area of research has been the investigation into the use of A_1 receptor antagonists in the treatment of renal disorders in congestive heart failure patients.[30-33] Patients with congestive heart failure often have raised levels of adenosine in the kidneys. The activation of the A_1 receptor in the kidneys mediates vasoconstriction, reducing the glomerular filtration rate (the rate at which the blood is filtered) and thus resulting in fluid retention in the patient.[31] The use of antagonists thus blocks the effect of adenosine and has been shown to increase the urine flow, preventing renal failure.

In the central nervous system (CNS), the activation of the A_1 receptor leads to sedation, decreased locomotor activity, neuroprotection and anticonvulsant effects.[34-36] Although the A_1 and A_{2A} receptors are both present in the brain, the distribution of the two receptors is very different. The A_1 receptors are found in almost all parts of the brain, with higher levels of expression present in the hippocampus and the cerebral cortex.[37] The A_{2A} receptors, in contrast, are located in greater quantities in more specific areas such as the striatum, where dopamine is readily produced.[38]

The depth and levels of sleep were found to be dependent on the amount of adenosine present in the brain and it has become well accepted that the effects of adenosine are mediated through the A_1 receptors,[39] although some recent experiments indicate a role for the A_{2A} receptors too.[40] Adenosine and A_1 agonists have been shown in *in vitro* models of cerebral ischæmia (reduced/inadequate blood flow in the brain) to reduce neuronal damage, providing perhaps hope of its use in such neurological conditions as Parkinson's Disease and Huntington's Disease.[41] At peripheral and spinal sites, activation of the A_1 receptor has also been shown to produce antinociceptive (painkilling) effects.[42] In contrast, compounds that are

able to block the receptor from receiving its endogenous ligand in the CNS may be beneficial in terms of counteracting the sedation and negative locomotor effects. Antagonists have been found to induce cognition enhancement, leading to a general improvement in memory performance.[34-36] This is potentially useful in the treatment of neurological disorders such as Alzheimer's disease.

Caffeine

The potential of adenosine A_1 receptor antagonists as neurological drugs explains the high interest in this type of compound. Undoubtedly the best known and archetypical example is caffeine. The official name of caffeine according to IUPAC convention is 1,3,7-trimethyl-3,7-dihydropurine-2,6-dione and it is also known as trimethylxanthine (Figure 1.3). It is the most widely consumed drug in the world and is found in many forms, from our daily beverages of tea, coffee, soft drinks, to food (chocolate) to formulations of over-the-counter painkillers and coldcures.[40] The average intake of caffeine from tea and coffee for certain regions in the world is displayed below. These statistics were calculated in the same manner as described by Fredholm *et al*.[40] from data published by the UN for 2002 (Table 1.1 and Figure 1.4). It is interesting to note that the average caffeine intake from coffee and tea sources in Europe, a non-tea and coffee producing region, is higher than any other world region. In Europe itself, the caffeine-intake, mostly from coffee, is greatest in northern Europe where filtered coffee rules the hot beverage kingdom. The extraction process and the coffee beans generally used means filtered coffee contains the greatest content of caffeine per mL.[43] The 'average' caffeine content of a cup of coffee is often given as 100 mg implying that the 'average' coffee drinker in Finland, Sweden or the Netherlands consumes at least 5 cups of coffee a day, accounting somewhat for the non-coffee drinkers.

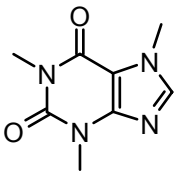

Figure 1.3
Caffeine

At the levels of normal ingestion, the effects of caffeine are a result of its blockade of the adenosine receptors.[44] The affinity of caffeine for the A_{2B} and A_3 receptors is not particularly high ($K_i(hA_{2B})$ = 10.4 μM,[45] K_i (rA_3) > 10 μM)[46] and moreover, the distribution of these two receptors in the CNS is low.[15] Thus, although the affinity of caffeine for the A_1 and A_{2A} receptors is also relatively low ($K_i(rA_1)$ = 8.5 μM, $K_i(rA_{2A})$ = 25 μM),[45] the sheer levels of expression of these receptors in the brain mean that the well-known psychostimulant properties of caffeine are an effect of the antagonism of the adenosine A_1 and A_{2A} receptors.[40] The most well-known effect of caffeine is its power to banish sleepiness.[47] It is also reputed to facilitate cognitive activity, learning, memory and attention span.[48]

Derivatives of caffeine have been made in an attempt to improve the affinity for the individual receptors. However, despite achieving this, compounds based on the xanthine-structure (that caffeine possesses) have been beset by problems, including low solubility and thus bio-availability, and also its ability to interact with other bodily processes, e.g., as an inhibitor of phosphodiesterases.[49] This encouraged the search for ligands not based on the xanthine moiety.

Table 1.1 Caffeine intake from tea and coffee consumption in selected countries and continents in 2002.

	Population 2002 (*1000*)	Coffee consumed (*kton*)	Coffee (*kg/ person/ year*)	Caffeine from coffee (*mg/ person /day*)	Tea consumed (*kton*)	Tea (*kg/ person/ year*)	Caffeine from tea (*mg/ person/ day*)	Total Caffeine from tea and coffee (*mg/ person/ day*)
Asia	3,775,948	1360	0.36	15	2061	0.55	22	37
Africa	832,089	522	0.63	26	301	0.36	15	41
Australasia	31,844	84	2.64	110	18	0.57	23	133
Europe	727,019	2793	3.84	160	456	0.63	26	186
N. & C. America	500,749	1615	3.23	134	168	0.34	14	148
South America	357,329	565	1.58	66	860	2.41	99	165
Belgium	10,296	44	4.27	178	1	0.10	4	182
China	1,302,307	40	0.03	1	511	0.39	16	17
Finland	5,197	57	10.97	457	1	0.19	8	465
France	59,850	335	5.60	233	16	0.27	11	244
Germany	82,414	539	6.54	272	26	0.32	13	285
Italy	57,482	306	5.32	222	6	0.10	4	226
Netherlands	16,067	142	8.84	368	19	1.18	49	417
Norway	4,514	42	9.30	387	1	0.22	9	397
Spain	40,977	169	4.12	172	3	0.07	3	175
Sweden	8,867	87	9.81	409	2	0.23	9	418
UK	58,287	143	2.45	102	134	2.30	94	197
USA	291,038	1159	3.98	166	146	0.50	21	186

Statistics generated as described by Fredholm *et al*.: caffeine content of coffee taken as 1.6% and the extraction efficiency as 95%; caffeine content of tea taken as 3% and the extraction efficiency as 50%.[40] Source data obtained from the UN Food and Agriculture databases.[50]

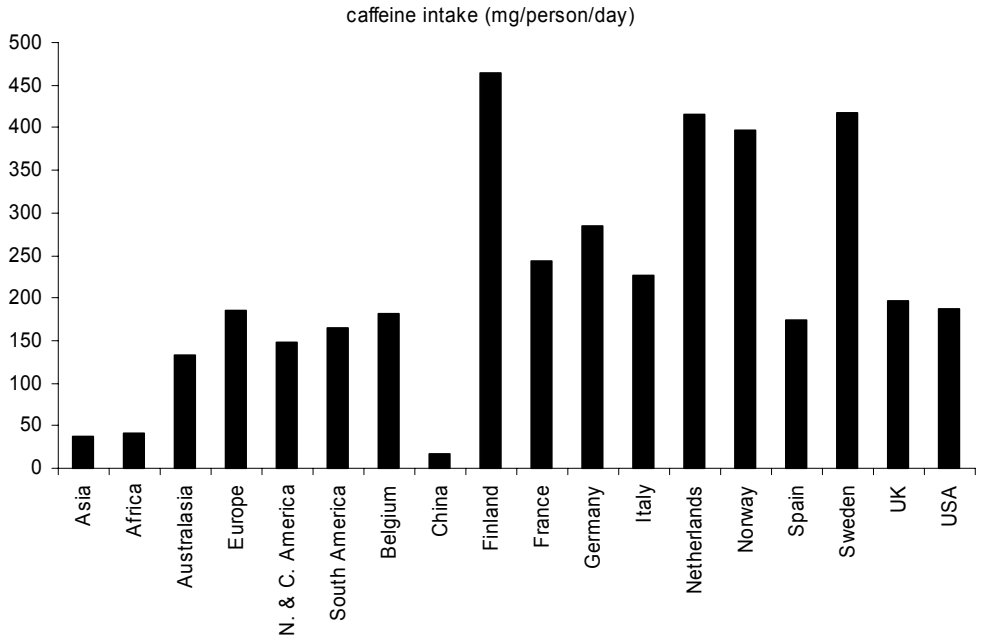

Figure 1.4 Graphical representation of the total caffeine intake derived from coffee and tea in selected countries/continents.

The Scope and Content

The previous sections have outlined the high therapeutic potential of compounds able to regulate the adenosine A_1 receptor. There have been numerous non-xanthine ligands that have been synthesised as antagonists for the adenosine A_1 receptor with varying levels of affinity and selectivity. The diversity of the compounds is quite astounding, but the application of such molecules for medicinal purposes is still lacking. The still relatively limited knowledge of GPCRs and thus the understanding of the active site of the A_1 receptor in terms of size, shape and functionality, contributes to the lack of actual drugs. The potential therefore, for developing novel ligands specifically targeted at this receptor derived from new and different perspectives is still boundless. Thus, the focus of this thesis is the reassessment of the design and development of A_1 receptor ligands with an eye to developing a new understanding of the adenosine A_1 receptor.

In the following chapters, the state of affairs is established with a review of the current literature and the subsequent development of several novel series of ligands for the adenosine A_1 receptor is presented. The basis of this thesis was to generate a pharmacophore by studying computational models of some of the most highly effective ligands. Stemming from this, a novel series of compounds was synthesised and evaluated at the adenosine receptors. The capacity of adenosine receptor ligands as medicines targeted at the CNS requires the incorporation, or at least the consideration, of certain physical molecular characteristics. This is undertaken by taking into account the polar surface area of a compound. Using the initial pharmacophore, a further set of compounds conforming to the set limits was produced. Further refinement of the model was investigated resulting in a new perspective on a familiar ring system. Using the results from this refinement and from the initial two series, consistent sub-nanomolar affinity was produced in a following series. As a consequence of the very recent discovery that non-adenosine compounds could also be agonists of the adenosine receptor, a series of pyridine-3,5-dicarbonitriles with selectivity for the A_1 receptor was investigated. This series of non-adenosine, non-xanthine ligands was shown to display both agonistic and antagonistic behaviour.

A general discussion and a look to the future of adenosine receptor research follow, concluding this thesis.

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Chapter 2

Non-Xanthine Antagonists of the Adenosine A_1 Receptor

Naturally occurring caffeine and theophylline are the most well-known adenosine receptor antagonists. These and other xanthine derivatives have enjoyed a long history of development and investigation into their structure and ability to bind to the adenosine A_1 receptor. However, xanthine-derived compounds have a number of disadvantages and development issues, including the limited creative possibilities about such a rigid core. The scope of non-xanthine ligands as adenosine A_1 receptor antagonists is much broader and also offers more possibilities for exploring and defining the active site of the receptor. This chapter systematically charts the considerable amount of research that has been performed into non-xanthine moieties over the past 25 years since the potential of regulating the adenosine A_1 receptor was highlighted.

Based upon L. C. W. Chang, J. Brussee, A. P. IJzerman, Chemistry and Biodiversity ***2004****, 1, 1591-1626.*

2.1 The Design of Adenosine A_1 Receptor Antagonists

Significant and intensive scientific interest into the adenosine A_1 receptor and its potential as a drug target seemed to be sparked a little more than two decades ago with the publication of a paper by Daly.[1] This was the result of the field-defining publications by Londos *et al*.[2] and Van Calker *et al*.[3] at the end of the 1970s, and the recent search for adenosine receptor ligands by Bruns.[4] At that stage, research into adenosine receptor antagonists only just proffered several non-selective xanthine derivatives, e.g., the naturally occurring compound caffeine (which has an affinity at the rat A_1 receptor of 44 μM)[5] and 8-phenyltheophylline with an IC_{50} value of 1 μM in guinea pig brain.[6] As for non-xanthine derivatives, no systematic study had yet been undertaken, although some research programmes had identified such compounds as etazolate (**2.37**) to be antagonists of the adenosine A_1 receptors.[7]

Since then, a whole host of compounds has been made and tested as antagonists of the adenosine A_1 receptor. Many have been based on the well-documented xanthine-structure that accounts for compounds such as caffeine and theophylline. The associated problems of the xanthine-based compounds, as mentioned in Chapter 1 - poor selectivity over the other receptors (namely $A_{2(A)}$ in the early days), poor solubility and bio-availability encouraged the search for non-xanthine-like compounds. The early papers describe screening programmes to identify potential non-xanthine leads with some success.[8,9] In the mid-1980s rationale was brought into the design process by focussing on the numerously available agonists and their behaviour towards the A_1 receptor.[10,11] This was followed by a flurry of papers in the late 1980s when many of the larger parties in the pharmaceutical industry published their findings in SAR at the adenosine receptors.[12-14] This also coincided appropriately with the growth of computational power (both in the availability of mass-marketed PCs and workstations, and in the computing capacity of these machines), to result in the first models of the A_1 receptor.[15,16] A very good summary of these models was written by Poulsen and Quinn in 1998.[17] Developments in molecular modelling of the A_1 receptor since the Poulsen and Quinn review include a 3-D model of the human A_1 adenosine receptor by Biannucci *et al*. using a bacteriorhodopsin template.[18] In this paper, the His 7.43 residue was proposed to be of great importance for agonist binding to the receptor, confirming earlier biological data.[19] In a return to the ligand-based approach, Doytchinova and Petrova[20] proposed a refinement of the 'N^6-C8' model as proposed by Peet *et al.* in 1990.[21] The 'N^6-N7' model suggested a slight shift in the superimposition of the xanthine and the agonist would give a better electrostatic and steric 'fit'.

Rivkees *et al.* took into account results from mutagenesis experiments and created a model for the A_1 receptor based on mammalian rhodopsin, and demonstrated the importance of TM 3 and 7 for ligand binding.[22] Since then, developments in the field of GPCRs have seen the structural determination of mammalian rhodopsin (the first and only detailed crystal structure of a GPCR to date).[23] Incorporating the co-ordinates of rhodopsin, molecular modelling has, of late, been focussed on the protein, creating a binding site and docking known ligands at this

proposed structure. Unfortunately, although the adenosine receptors are categorised in the same class (Class A) of GPCRs as rhodopsin, the structural similarities are likely to be very limited, based on the function of the receptor, the (lack of) amino acid sequence homology, and the differences in the native ligand. Molecular modelling of the protein based on the rhodopsin template in the search of the agonist binding site, and the docking of agonists in proposed binding sites of models based on the rhodopsin template, must also be viewed with some candour due to the fact that the published structure details an inactive form of rhodopsin.

More recently, Da Settimo *et al.* crossed both regions of molecular modelling in 2001.[24] A series of ligands that was synthesised towards the benzodiazepine receptor was identified as being structurally similar to adenosine A_1 receptor ligands and biological testing confirmed their affinity for the bovine A_1 receptor. Analysing these ligands using molecular modelling techniques showed that they were superimposable with a number of existing adenosine A_1 receptor antagonists and a pharmacophore was developed. This pharmacophore identified three lipophilic regions, and three hydrogen-bonding domains. To rationalise the SARs of the ligands, a model of the bovine A_1 adenosine receptor was built using frog rhodopsin as a template.

Bondavalli *et al*.[25] designed a series of pyrazolo[3,4-*b*]pyridine derivatives with good affinity and selectivity towards bovine A_1 adenosine receptors. The resulting computational studies took eleven structurally different A_1 antagonists from literature and derived a pharmacophoric model. For each conformer selected by the system an electrostatic picture was created and analysed. To confirm these results a second computational system was utilised and the resulting maps pointed to a pharmacophore that comprised of several main features deemed to be necessary for adenosine A_1 receptor antagonists. The first was the presence of a hydrogen-bond acceptor atom in the ligand to correspond with a donor site in the protein, the second alluded to two hydrophobic centres in the bi-cyclic planar nucleus of the antagonist, the next a third hydrophobic domain and lastly another hydrogen-bond acceptor site. The generation of a pseudoreceptor to match this phamacophore was performed by choosing appropriate amino acid residues from information derived from site-directed mutagenesis experiments, and the primary amino acid sequence of the rat A_1 adenosine receptor. The results of this study were in agreement with the proposals by Da Settimo *et al*. although a slight disagreement in the size of one of the pockets of the receptor surfaced.

The receptor was again investigated by Giordanetto *et al*.[26] in 2003, and a model was built for the human A_1 receptor based on the structure of bovine rhodopsin using a set of 22 agonists. The results of several site-directed mutagenesis studies were also taken into account, and the importance of the interactions of synthetic agonists with a number of amino acid residues was confirmed. The docking experiments highlighted the 2-chloro substituent on the purine ring as being especially beneficial for binding to the A_1 receptor. In addition, substitution at the N^6

was suggested to fit both TM 3-5 and TM 5-6-7 lipophilic domains, though more emphasis on the former pocket is vital for good binding affinity.

Bovine rhodopsin was again the basis of a molecular model by Gutiérrez-de-Terán *et al*.,[27] focussing in particular at the A_1 adenosine receptor and its agonist binding site. In this paper, the transmembrane region was the subject of focus, due to the very low homology between the human A_1 adenosine loops and those of bovine rhodopsin. The docking of the natural ligand was performed by searching for a suitable polar binding site for the ribose moiety. The conclusions drawn from this study suggest that certain residues (Ser 1.46, Asp 2.50, His 7.43, and Ser 7.46) are important for receptor activation.

One last area to mention, where very limited attention has been given in adenosine receptor research, is the field of pharmacognosy and phytotherapy. Despite the naturally occurring xanthine derivatives, caffeine and theophylline, which can be found in good quantities in our daily beverages, there is just a handful of papers focussing on products derived from natural sources.[28-30] The active components described in these papers are essentially very different from the (chemically) 'designed' ligands. They are generally structures without nitrogen atoms (see Section 2.3.2.2) and as such offer the possibility of a much more varied and expansive library of non-xanthine adenosine receptor antagonists. The first and most interesting compound discussed in this category was isolated from a traditional Chinese medicine which had been widely used in the treatment of acute myocardiac infarction and angina.[30] The surprisingly high affinity (10 nM at human A_1 receptors) for a compound possessing very different characteristics from the traditional non-xanthine ligand is an indication of the variety and diversity available in the 'natural' world.

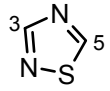
A variety of phytochemicals, amongst which the flavonoids featured, was the focus of a screening programme in 1996.[28] Flavonoids are natural products that are in large abundance in fruit and vegetable matter and have reputed biological properties in all manner of different medical needs, from hypertension and diabetes to allergies and cancers.[31] The most active compounds (e.g., **2.76**) at the adenosine receptors were unfortunately only in the sub-micromolar range, but further optimisation or derivatisation may provide a more selective and active compound.

As a result of the early screening programmes, the computational investigations, molecular biological techniques and the pure intuitiveness of medicinal chemists, the vast selection of differing structural types of adenosine A_1 receptor antagonist is bewildering. This assortment of compounds has been in the past reviewed a little haphazardly according to the latest developments as new types of ligands have been made and tested. In this examination, these compounds are categorised according to the size and type of their central structure. Although there are very many solitary structures that have been shown to have some moderate affinity (usually in the μM range), especially from the screening programmes, only those compounds that have been taken on and developed with clear structure-activity relationships are discussed in detail in this review. There are also very many compounds that have been developed in

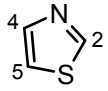
industry. Many of these have only been published in patent literature, and as is common in patents, structure-activity relationships and full biological data are scarcely available. As such, this review is based upon only standard scientific literature. Relevant compounds from the patents, which have some biological data or have outstandingly novel structures, will be mentioned briefly. This discussion splits the published material into three sections depending on the size of the heteroaromatic core of the compounds, i.e., into mono-, bi-, and tri-cyclic (fused) heteroaromatic systems. We begin logically with the mono-cyclic heteroaromatic cores and continue in an increasing order of magnitude, attempting to show how each series was developed historically as more information became available. Early papers test new compounds at the A_1 and what were then known as the A_2 receptors from a variety of different species, including dog, sheep, bovine and rat, the latter being the most common. As pharmacology and molecular biology developed and the (human) A_{2A} and A_3 receptors were identified and made available, these receptors were tested too. The A_{2B} receptor is still an exception in the field; the low affinity nature of the receptor accounts for the lack of good selective ligands and thus, the only recent, existence of appropriate radioligands (e.g., [^{3}H]MRS 1754[32] and [^{3}H]MRE 2029-F20[33]) still excludes its broad assessment in conventional procedures. In the tables, the most recent data is given and selectivity ratios are shown where appropriate.

2.2. Mono-cyclic Heteroaromatic Rings (Non-Fused Rings)

Mono-cyclic heteroaromatic rings are relatively rarely found in adenosine A_1 receptor antagonism. In fact, recent reviews by Hess[34] and by Müller[35,36] state quite clearly that the different structural classes for A_1 adenosine receptor antagonists are bi- and tri-cyclic heterocyclic compounds. The few classes of compounds in this category consist of a bare handful of papers. Mono-cycles were amongst a variety of different compounds screened early on in adenosine receptor research, in the paper by Davies *et al*.[9] The few compounds that could be classed as pyridine, pyrazole, or pyrimidine showed no favourable effects as adenosine receptor antagonists. In 1985, a selection of barbiturates (pyrimidine-2,4,6-triones) were investigated at the A_1 adenosine receptor[37] and although reportedly selective antagonists of the A_1 receptor they only showed affinity in the micromolar range. A screening by Siddiqi *et al*.[38] showed two pyridine derivatives to have micromolar affinity at the A_1 receptor with slightly more affinity for the A_3 receptor. In 1997 Biagi *et al*.[39] compared analogous pyrimidines to their 8-azaadenine series and found them to be of much lower activity, and thus concluded that the bi-cyclic aromatic system was necessary for good affinity at the adenosine A_1 receptor.



2.1-2.5

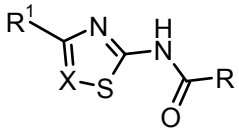


2.6-2.8

Figure 2.1 Mono-cyclic nitrogen heterocycles: thiadiazoles (**2.1-2.5**); thiazoles (**2.6-2.8**).

More recently, an investigation by Van Muilwijk-Koezen *et al*. highlighted the general low affinity of mono-cyclic compounds.[40] The exceptions to this were two 5-membered heterocycles, namely thiazoles and thiadiazoles (Figure 2.1), which were modified to give compounds with affinities in the lower nM range (Table 2.1, compounds **2.1-2.8**) at the A_1 receptor with reasonable selectivity.[40,41] A phenyl group was placed at the 3-position of the thiadiazole in consequence to previously investigated quinolines and quinazolines,[42] and at the 5-position a substituted amido-group was present. Variation of this amido- function resulted in some very potent compounds. The phenyl-substituted compound **2.1** was an encouraging lead with an affinity at the A_1 receptor of 31 nM and a degree of selectivity over the A_{2A} and A_3 receptors. Further substitution of this phenyl group resulted in the discovery of the 4-hydroxy moiety (**2.2**), which showed a gain in affinity over the unsubstituted phenyl group at 7 nM though with a slight loss in selectivity. To assess the interaction of the 4-hydroxy substituent with the receptor, *cis*- and *trans*- 4-hydroxy-cyclohexyl derivatives were tested. Interestingly, the *trans*- substituent (**2.5**) showed a better (2-fold) level of affinity compared to the *cis*- analogue (**2.4**). These 4-hydroxycyclohexyl analogues were less potent than their 4-hydroxyphenyl counterpart, but this was matched by a gain in selectivity for the A_1 receptor over both the A_{2A} and A_3 receptors.

Table 2.1 Biological Data for Compounds **2.1-2.8**



Compound	X	R	R^1	K_i [nM [a])]			A_{2A}/A_1	A_3/A_1	Ref.
				A_1	A_{2A}	A_3			
2.1	N	Ph	Ph	31[a])	4400[b])	410[c])	142	13	[40]
2.2	N	4-HOPh	Ph	7[a])	570[b])	130[c])	81	19	[40]
2.3	N	cC_6H_{11}	Ph	1400[a])	>10000[b)]	16000[c])	>7	11	[40]
2.4	N	*cis*-HO-cC_6H_{10}	Ph	42[a])	>10000[b])	2700[c])	>238	64	[40]
2.5	N	*trans*HO-cC_6H_{10}	Ph	20[a])	>10000[b])	1900[c])	>500	95	[40]
2.6	CH	Ph	2-Py	1700[a])	8700[b])	3400[c])	5	2	[40]
2.7	CH	Ph	Ph	39[a])	>10000[b])	>1000[c])	>256	>26	[41]
2.8	CH	4-ClPh	Ph	18[a])	>10000[b])	>1000[c])	>556	>56	[41]

[a])Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes. [b])Displacement of specific [^{3}H]ZM 241385 binding in rat striatal membranes. [c])Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cell membranes expressing the human adenosine A_3 receptor.

At the thiazoles,[41] a similar substitution pattern was executed, aromatic substitution next to the nitrogen ring (in the 4-position) and amido-substitution between the sulfur and nitrogen atoms (the 2-position) (Figure 2.1). A 2-pyridyl group as the aromatic moiety in the 4-position was an attempt to provide a hydrogen-bond donor at a similar position to the N-2 moiety of the thiadiazoles (**2.6**). The poor results in comparison to the analogous phenyl-thiadiazole (**2.1**) showed that receptor-ligand interactions are not so straightforward. The equivalent

unsubstituted phenyl moiety of the thiazole (**2.7**) however, was much more promising with a K_i value of 39 nM at the A_1 receptor. Further variation at the 5-amido-group resulted in a 2-fold increase in affinity at the A_1 receptor with a 4-chlorophenyl moiety (**2.8**), retaining selectivity for the A_1 receptor.

There are several publications to note in patent literature, which also deal with mono-cyclic (non-fused) heteroaromatics. Although these compounds have very little biological data present and specific A_1 receptor antagonism is often not mentioned, the core heterocycles are as follows (Figure 2.2): pyrazole derivatives (**2.9**) have been claimed by Eisai;[43] pyrimidines (**2.10**) by Fujisawa;[44,45] Boehringer Ingelheim has a series of triazine derivatives (**2.11**) under patent,[46] and Novartis has laid claims on a diaryl thiazole core (**2.12**)[47,48] although the latter patent mentions A_{2B} and A_3 uses above A_1.

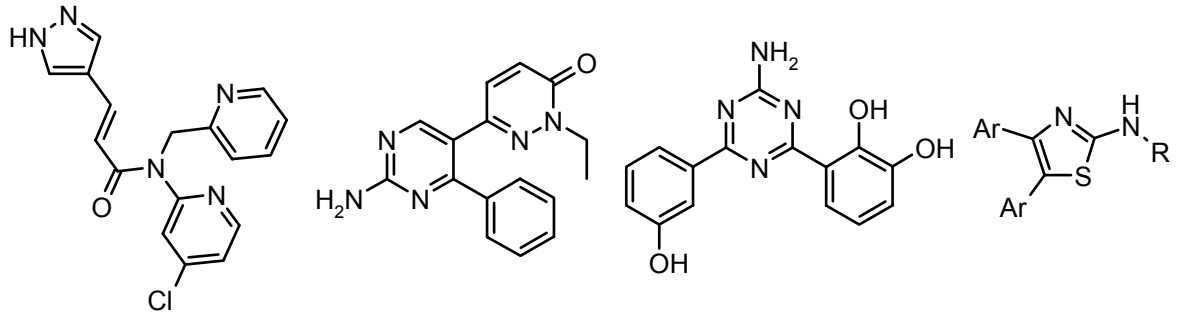


Figure 2.2 Mono-cyclic compounds under patent: pyrazoles (**2.9**), pyrimidines (**2.10**), triazines (**2.11**) and thiazoles (**2.12**).

2.3. Fused Bi-cyclic Heteroaromatic Systems

2.3.1 The 6:5 Fused Nitrogen-Containing Heteroaromatic Systems

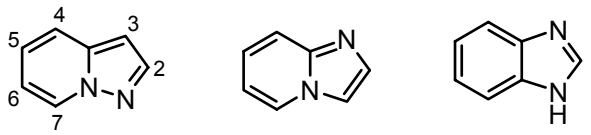
The 6:5 fused nitrogen-containing heteroaromatic compounds make up by far the largest group of published non-xanthine adenosine A_1 receptor antagonists. In this section the core bi-cyclic compounds are discussed in ascending order with respect to the number of nitrogens in the core structure.

2.3.1.1 The 6:5 Fused Heteroaromatic Systems Possessing Two Nitrogen Atoms

Pyrazolo[1,5-*a*]pyridines (**2.13-2.24**), imidazo[1,2-*a*]pyridines (**2.25**) and benzimidazoles (**2.26**) (Figure 2.3) make up the compounds of this category of nitrogen heteroaromatics. By far the most investigated core structure of this class is the pyrazolo[1,5-*a*]pyridine with a number of publications from 1996 to 2001 by the Japanese pharmaceutical concern Fujisawa, detailing the medicinal chemistry of variously substituted compounds.[49-53] The papers describe substitution at the 3-position. Modification at the core, namely at the pyridine ring, creating pyrazolo-pyrimidines and pyrazolo-quinolines, and some variation at the 2-position has also been reported in patent literature, although with little biological data.[54,55]

FK 453 (Figure 2.4, Table 2.2, **2.13**) was one of the most promising of the earlier compounds.[49] The distinguishing features of this compound are a phenyl substituent at the 2-

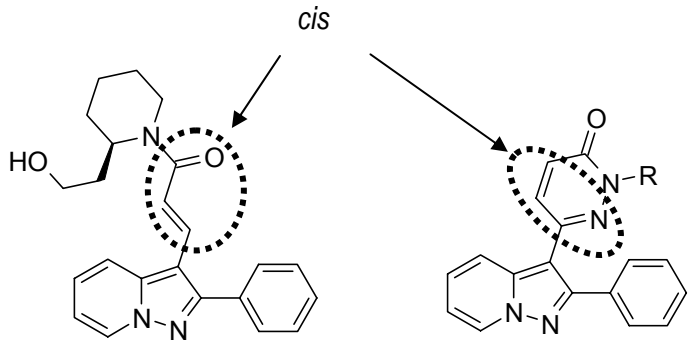
position and the α,β-unsaturated amide motif at the 3-position. Following SAR studies and crystal structural determination of this compound, it was determined that the acryloyl moiety, adopting a *cis* form (the positioning of the carbonyl bond with respect to the double bond (as drawn in Figure 2.4), was of great importance.



2.13-2.24 **2.25** **2.26**

Figure 2.3 The 6:5 Fused Bi-cyclics Possessing Two Nitrogen Atoms: Pyrazolo[1,5-*a*]pyridines (**2.13-2.24**); imidazo[1,2-*a*]pyridines (**2.25**) ; benzimidazoles (**2.26**).

It was reasoned that replacement of this group with rigid heteroaromatics would retain the affinity of FK 453 whilst preventing isomerisation of the double bond. This led to the discovery of a highly potent 2-substituted 3-oxo-2,3-dihydropyridazin-6-yl group (which incidentally was a fragment of one of the compounds screened by Siddiqi *et al*. in 1996 that showed some affinity for the adenosine receptors)[38] (Figure 2.4, **2.14-2.24**). Subsequently, variation at the 2-position of the pyridazinyl group with a number of different functional hydrophilic features resulted in improved affinity and selectivity for the A_1 receptor over the A_{2A} receptor. In Table 2.2 a selection of ligands with varying substitution is shown with their determined affinities. Although the initial lead compound FK 453 already showed good affinity coupled with a good selectivity over the A_2 receptor, there were vast improvements made with the dihydropyridazinyl group.

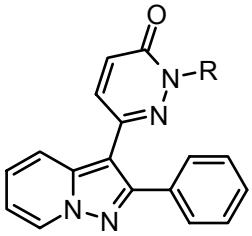


2.13 **2.14-2.24**

Figure 2.4. FK 453 (**2.13**); 3-(2-substituted-3-oxo-2,3-dihydropyridazin-6-yl)-2-phenylpyrazolo[1,5-*a*]pyridines (**2.14-2.24**).

Substitution with a carboxylic acid group (FK 838, **2.14**)[50] reduced the affinity for the A_1 receptor, and also increased the affinity for the A_{2A} receptor significantly. Further manipulation of the dihydropyridazinyl moiety with (2-substituted) cyclohexene derivatives (**2.15-2.19**) was in consequence to the six-membered (2-substituted) piperidine of FK 453. The differing functional groups all performed particularly well in the A_1 receptor binding assays, resulting in affinities in the low nM range.

Table 2.2. Biological Data for Compounds **2.13-2.24**



	R	R^1	R^2	K_i/IC_{50} [56]		A_{2A}/A_1	Ref.
				A_1	A_{2A}		
2.13 (FK 453)	-	-	-	17[a])	11000[b])	650	[52]
2.14 (FK 838)	$(CH_2)_3CO_2H$	-	-	120[a])	5900[b])	50	[52]
2.15		CH_2OH	-	2[a])	2500[b])	1250	[52]
2.16		$CONH_2$	-	2[a])	740[b])	410	[52]
2.17		$CONMe_2$	-	2[a])	530[b])	310	[52]
2.18 (FR166124)		CO_2H	-	15[a])	6200[b])	410	[52]
2.19			-	4[a])	100[b])	25	[51]
2.20	CH_2COR^1	^{i}Pr	-	0.03[c])	140[d])	4700	[53]
2.21	$CH_2CR^1R^2OH$	Me	Me	0.4[c])	160[d])	400	[53]
2.22	CH_2CONHR^1	^{i}Pr	-	0.3[c])	490[d])	1600	[53]
2.23	$(CH_2)_2NR^1$	$-(CH_2)_5-$	-	2[c])	>1000[d])	>400	[53]
2.24		Me	-	7[c])	5400[d])	800	[53]

[a])IC_{50} - Inhibition of [^{3}H]CHA specific binding to rat cortical membranes. [b])IC_{50} - Inhibition of [^{3}H]NECA specific binding to rat striatal membranes. [c])Ki - Inhibition of specific [^{3}H]DPCPX binding in CHO cell membranes expressing the human adenosine A_1 receptor. [d])Ki - Inhibition of specific [^{3}H]CGS 21680 binding in CHO cell membranes expressing the human adenosine A_{2A} receptor.

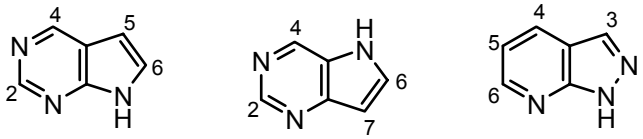
The carboxylic acid derivatives, which to some extent combine features from both FK 453 and FK 838 performed slightly worse in terms of affinity (**2.18**), or in terms of selectivity over the A_{2A} receptor (**2.19**). However, in the search of 'better' physical properties, compound **2.18** showed more than a 20-fold improvement in the water-solubility compared to FK 838 (**2.14**).

The last paper of the series details a number of functional groups attached with methylene spacers to the dihydropyridazinyl group. They all show remarkably high affinity for the A_1 receptor (**2.20-2.22**), indeed three out of the four shown here have subnanomolar affinity, with compound **2.20** being the most potent. Considering the therapeutic target of adenosine A_1 receptor antagonists, these compounds were also investigated for their ability to permeate brain tissue after oral administration. It was concluded that amino substitution (**2.22-2.24**) was most beneficial for blood-brain barrier permeation and compound **2.24** in particular showed

favourable properties leading to its nomination for further pharmacological evaluations. Imidazopyridines (**2.25**) and benzimidazoles (**2.26**) (Figure 2.3) have only been described in patent literature.[57,58] Along with the reported selective adenosine A_1 antagonistic properties, the imidazopyridines are described as being p38 inhibitors useful in the treatment of inflammatory diseases (**2.25**).[57] An interesting feature to note is the presence of the 2-substituted 3-oxo-2,3-dihydropyridazin-6-yl motif seen in an analogous position to that of the pyrazolopyridines described by Fujisawa. The limited biological data describes efficacy at less than 0.01 μM, with functional antagonism in an IC_{50} range of 1-100 nM. In addition, the selectivity over A_{2A} was 500 fold, and >1000-fold over A_{2B} and A_3. The benzimidazoles alluded to are covered by an earlier Japanese patent issued to Toa Eiyoo KK in 1998.[58] In this patent the compounds are said to be A_1 selective with the best in the region of 10 nM.

2.3.1.2 The 6:5 Fused Heteroaromatic Systems Possessing Three Nitrogen Atoms

The bi-cyclic cores containing three nitrogen atoms can be divided into three main categories, namely the 7*H*-pyrrolo[2,3-*d*]pyrimidines (also known as the 7-deazapurines),[59-63] the 5-H-pyrrolo[3,2-*d*]pyrimidines, and the 1*H*-pyrazolo[3,4-*b*]pyridines (Figure 2.5). Amino substitution at the 4-position of the first category, the 7-deazapurines, renders further classification of this series to 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamine, also known as the 7-deazaadenines. It is this series of compounds that have been most widely explored of the bi-cyclics with three N-atoms with publications on this topic spanning more than a decade (Table 2.3). Daly *et al*. published the first biological results from this series, and showed affinity to the A_1 receptor in the low μM range (**2.27**).[59] The most significant finding of this



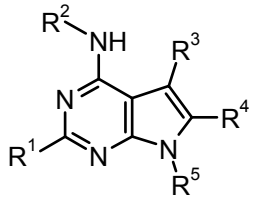
2.27-2.34 **2.36** **2.37-2.43**

Figure 2.5 The 6:5 Fused Bi-cyclics Possessing Three Nitrogen Atoms: 7*H*-pyrrolo[2,3-*d*]pyrimidines (also known as the 7-deazapurines) (**2.27-2.34**) ; 5*H*-pyrrolo[3,2-*d*]pyrimidines (**2.36**); 1*H*-pyrazolo[3,4-*b*]pyridines (**2.37-2.43**).

paper was the importance of the phenyl group in the 7-position of the ring. Further investigations of the 7-phenylpyrrolo[2,3-*d*]pyrimidin-4-ylamines revealed that a phenyl group in the 2-position was also beneficial (**2.29**).[60] Modifying the 7-phenyl group to a chiral moiety showed that the (*R*)-enantiomer (**2.30**) was much preferred to the (*S*) (**2.31**).[60] Incorporating these features also improved the selectivity for A_1 receptors over the $A_{2(A)}$ receptors dramatically. Synthetic ease dictated the presence of a dimethyl substitution at positions 5 and 6, but the only compound evaluated without this di-substitution pattern showed considerable benefits with an almost 6-fold increase in affinity at the A_1 receptor to its analogous dimethyl substituted equivalent (**2.27** vs. **2.28**). Changing the position of the chiral phenylethyl group from the 7-position of the ring to attachment to the exocyclic amine

did not change the affinity for the A_1 receptor significantly, but did improve selectivity dramatically (**2.32**). Campbell *et al*.[62] synthesised a library set of pyrrolo[2,3-*d*]pyrimidines which included the most significant features as described previously by Müller *et al*.,[60] varying only at the exocyclic N. The findings here were tested at the human adenosine receptors and showed compounds in the nanomolar range, although with a loss in selectivity (**2.33**). In the most recent publication in the series the phenyl group was varied at position 2- of the pyrrolopyrimidine ring for different heterocycles, e.g., the 2-, 3-, or 4-pyridyl, 2-thienyl and 2-furyl.[63] Of these, the substitution which retained the highest affinity was the 4-pyridyl species (**2.34**).

Table 2.3 Biological Data for Compounds **2.27-2.34**

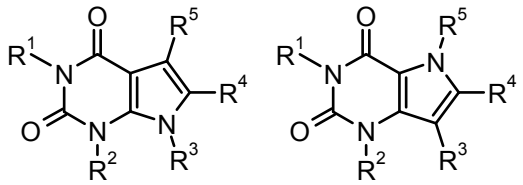


	R^1	R^2	R^3	R^4	R^5	K_i [nM]		A_{2A}/A_1	Ref.
						A_1	A_{2A}		
2.27	H	H	H	H	Ph	3100[a])	17000[b])	5	[59]
2.28	H	H	Me	Me	Ph	18000[a])	123000[b])	7	[60]
2.29	Ph	H	Me	Me	Ph	36[a])	14000[b])	400	[60]
2.30	Ph	H	Me	Me	(*R*)- MeCHPh	5[a])	3700[b])	740	[60]
2.31	Ph	H	Me	Me	(*S*)- MeCHPh	165[a])	80000[b])	490	[60]
2.32	Ph	(*R*)-MeCHPh	Me	Me	H	7[a])	>30000[b])	>4300	[61]
2.33	Ph	$-CH_2CH_2-NHAc$	Me	Me	H	12[c])	23[d])	2	[62]
2.34	4-Py	H	Me	Me	(*R*)- MeCHPh	9[e])	1300[f])	140	[63]

[a])Inhibition of [^{3}H]PIA specific binding to rat cortical membranes. [b])Inhibition of [^{3}H]NECA specific binding to rat striatal membranes. [c])Inhibition of [^{3}H]DPCPX in yeast cells transformed with human A_1 receptor. [d])Inhibition of [^{3}H]CGS 21680 in membranes from HEK293 cells stably expressing the human A_{2A} receptor. [e])Inhibition of [^{3}H]CCPA in human recombinant A_1 adenosine receptors expressed in CHO cells. [f])Inhibition of [^{3}H]CGS 21680 specific binding in rat striatal membranes.

The 5*H*-pyrrolo[3,2-*d*]pyrimidines[64] (Figure 2.5) mentioned here are not strictly pyrrolopyrimidines, but are actually pyrrolopyrimidine diones. They are xanthine derivatives, and are also otherwise known as 7-deazaxanthines (Table 2.4). Similarly, 9-deazaxanthines (2,4-dione variations of the 7*H*-pyrrolo[2,3-*d*]pyrimidines) have also been made and tested for A_1 receptor affinity. The obvious resemblance with the xanthine template affords the logical development of these two series and because of their status as xanthine derivatives only the affinity of the two most promising compounds of each series are shown here. The 7-deazaxanthine derivative (**2.35**) has no great affinity for the A_1 receptor with a K_i value in the μM range, whilst the 9-deazaxanthine compound (**2.36**) shows considerable improvement with an affinity of 13 nM and 35-fold selectivity for the A_1 receptor over the A_{2A} receptor (although the lengthening of the alkyl groups at the 1- and 3- positions may also influence the affinity somewhat).

Table 2.4 Biological data of compounds **2.35-2.36**



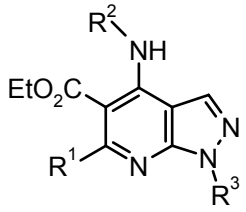
2.35 **2.36**

		R^1	R^2	R^3	R^4	R^5	K_i [nM]		A_{2A}/A_1	Ref.
							rA_1	rA_2		
2.35	7-deazaxanthine	Me	Me	H	Ph	H	3100[a])	12000[b])	4	[64]
2.36	9-deazaxanthine	Pr	Pr	H	Ph	H	13[a])	450[b])	35	[64]

[a])Inhibition of [^{3}H]R-PIA specific binding in rat brain cortex. [b])Inhibition of [^{3}H]NECA specific binding in rat striatum.

The last class of tri-nitrogen bi-cyclic derivatives discussed here are the 1*H*-pyrazolo[3,4-*b*]-pyridines (Figure 2.5). They were first mentioned in connection to the adenosine receptors as early as 1981 in the guise of the putative anxiolytic agents etazolate, cartazolate and tracazolate (**2.37-2.39**, Table 2.5).[7,65] Development of these compounds by Shi *et al*. exploring mostly substitution at the exocyclic amine resulted in only just sub-micromolar affinity towards the A_1 receptor (**2.40**), although selectivity was improved somewhat.[65] New developments at the exocyclic amine and some further variation at the 1-and 6-positions have been detailed only recently by Schenone *et al*.[66,67] and Bondavalli *et al*.,[25] and offer improvements on the previously reported pyrrolopyridines.

Table 2.5 Biological data of compounds **2.37-2.43**



		R^1	R^2	R^3	K_i [nM]			A_{2A}/A_1	Ref.
					A_1	A_{2A}	A_3		
2.37	etazolate	H	N=CMe_2	Me	3400[a])	1200[b])	-	0.4	[65]
2.38	cartazolate	H	Bu	Et	460[a])	1400[b])	-	3	[65]
2.39	tracazolate	Et	Bu	Et	710[a])	1500[b])	-	2	[65]
2.40		H	cC_5H_9	Me	310[a])	5300[b])	-	17	[65]
2.41		H	Pr	$CH_2CH(Cl)Ph$	100[c])	>10000[d])	>10000[e])	>100	[66]
2.42		H	1-pyrrolidinyl	$CH_2CH(Cl)Ph$	98[c])	>10000[d])	>10000[e])	>100	[66]
2.43		H	-CH_2CH_2Ph	$CH_2CH(Cl)Ph$	50[c])	>10000[d])	>10000[e])	>200	[66]

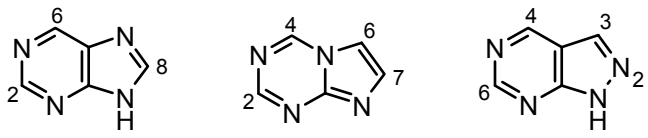
[a])Inhibition of specific [^{3}H]CHA binding to rat cerebral cortical membranes. [b])Inhibition of specific [^{3}H]CGS 21680 binding in rat striatal membranes. [c])Displacement of specific [^{3}H]CHA binding in bovine cortical membranes. [d])Displacement of specific [^{3}H]CGS 21680 binding in bovine striatal membranes. [e])Displacement of specific [^{125}I]AB-MECA binding in bovine cortical membranes.

At the 1-position, a styryl moiety and a chlorophenyl substituent were attempted, of these only the derivatives possessing the chlorophenylethyl substituent gave a positive result.[25] Although no mention of its reactivity is made, this relatively free and reactive halide may possibly react with the receptor (or other materials present in the system) rather than mere covalent interaction with the receptor. At the exocyclic amine, a wider range of substitutions were acceptable, e.g, Pr (**2.41**), pyrrolidinyl (**2.42**), and 2-phenylethyl (**2.43**).[66,67] Though these compounds are fairly well tolerated at the A_1 receptor, no affinity is seen at either the A_{2A} or the A_3 receptors. Substitution at the 6-position with a methylthio-group in analogy to already published material on the pyrazolo[3,4-*d*]pyrimidines (Section 2.3.1.3) did not lead to improvements in affinity.[67]

2.3.1.3 The 6:5 Fused Heteroaromatic Systems Possessing Four Nitrogen Atoms

The three main categories of the four-nitrogen bi-cyclic rings are 9*H*-purine, 8,8*a*-Dihydro-imidazo[1,2-*a*][1,3,5]triazine, and 1*H*-pyrazolo[3,4-*d*]pyrimidine (Figure 2.6).

We begin with the series that holds the greatest resemblance with the endogenous ligand, the purine-based moiety. As perhaps the most logical derivative to be synthesised as antagonists due to its similarities to adenosine itself, this is also one of the series with one of the longest continuing histories in non-xanthine adenosine antagonist research (Table 2.6). Early attempts involved variation at the exocyclic amino group of adenine, along with substitution at the 9-position of the ring. Ukena *et al*. showed that N^6 cyclopentyl was most favourable over other ring systems (phenyl, pyridyls, thienyls).[68] At the 9-position a methyl group showed distinct advantages over the unsubstituted form (**2.44**). In 1991, Thompson *et al*. further examined this category of ligand and showed again the favourable properties of a cyclopentyl group.[69]

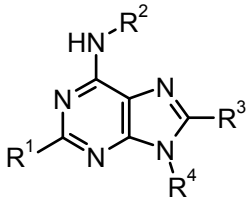


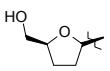
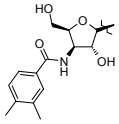
2.44-2.53 **2.54-2.56** **2.57-2.61**

Figure 2.6 The 6:5 Fused Bi-cyclics Possessing Four Nitrogen Atoms: 9*H*-purine (**2.44-2.53**); 8,8*a*-Dihydro-imidazo[1,2-*a*][1,3,5]triazine (**2.54-2.56**); 1*H*-pyrazolo[3,4-*d*]pyrimidine (**2.57-2.61**).

In addition, the 9-ethyl derivatives gave a slight improvement over the 9-methyladenines, and 2-chloro substitution of the ring was not detrimental for affinity (**2.45, 2.46**). In a paper by Peet *et al*. in 1992, chiral substituents at the N^6 position were investigated, the *R*- and the *S*-configurations of 1-(hydroxymethyl)-2-phenylethyl were tested and it seems that the binding pocket of the adenosine receptor has a preference for the (*S*) isomer (**2.47**).[70] In 1998 further investigations into the adenines were undertaken by Camaioni *et al*., systematically placing large substituents at each of the 2-, 6-, and 8-positions of the ring.[71] Though these ligands were generally more effective at the A_{2A} receptor, the paper showed that a large substituent is reasonably well tolerated at the 2-position of the adenine ring at the A_1 receptor with a submicromolar affinity (**2.48**).

Table 2.6 Biological data of compounds **2.44-2.53**



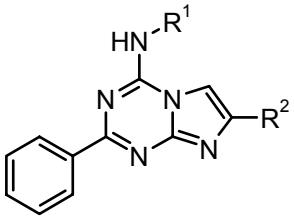
	R^1	R^2	R^3	R^4	K_i [nM]			A_{2A}/A_1	Ref.
					A_1	$A_{2(A)}$	A_3		
2.44	H	cC_5H_9	H	Me	540[a])	11000[b])	-	20	[68]
2.45	H	cC_5H_9	H	Et	440[a])	17000[b])	-	40	[69]
2.46	Cl	cC_5H_9	H	Me	530[a])	9300[b])	-	18	[69]
2.47	OPr	*)	H	Ph	96[a])	5600[b])	-	60	[70]
2.48	$O(CH_2)_2Ph$	H	H	Et	170[c])	120[d])	45000[e])	0.7	[70]
2.49	Ph	cC_6H_{11}	H	CH_2Ph	9[f])	>10000[g])	-	>1000	[72]
2.50	H	H	Ph	Et	27[h])	360[i])	3300[i])	13	[73]
2.51	H	cC_5H_9	NMe^iPr	Me	8[j])	-	14000[k])	-	[74]
2.52	H	cC_6H_{11}	H		4800[l])	160000[m])	-	30	[75]
2.53	H	cC_5H_9	H		24[l])	3680[n])	-	150	[76]

*[a])Inhibition of specific [^{3}H]PIA binding in rat brain membranes. [b])Inhibition of specific [^{3}H]NECA binding in rat striatum. [c])Inhibition of specific [^{3}H]DPCPX binding in CHO cell membranes expressing the human adenosine receptor. [d])Inhibition of specific [^{3}H]CGS 21680 binding in CHO cell membranes expressing the human adenosine receptor. [e])Inhibition of specific [^{125}I]ABMECA binding in CHO cell membranes expressing the human adenosine receptor. [f])Inhibition of specific [^{3}H]CHA binding in bovine brain cortical membranes. [g])Inhibition of specific [^{3}H]CGS 21680 binding in bovine brain striatal membranes. [h])Inhibition of specific [^{3}H]CCPA binding in CHO cell membranes expressing the human adenosine receptor. [i])Inhibition of specific [^{3}H]NECA binding in CHO cell membranes expressing the human adenosine receptor. [j])Displacement of specific [^{3}H]DPCPX binding from CHO-A_1^{++} membranes. [k])Displacement of specific [^{125}I]IBMECA from HEK 293-A_3 membranes. [l])Inhibition of specific [^{3}H]DPCPX binding to rat brain membranes. [m])Inhibition of specific [^{3}H]NECA binding to solubilised $A_{2(A)}$ receptors of human platelet membranes. [n])Inhibition of specific [^{3}H]CGS 21680 binding to rat striatal membranes. *) (S)-1-(hydroxymethyl)-2-phenylethyl*

Bianucci *et al*. described yet more variations at the 4-amino and 9-positions and discovered that a benzyl group at the 9-position improved affinity significantly over the 9-alkyl-adenines.[72] Cyclopentyl at the N^6 position was still found to be positive, but the cyclohexyl group showed slightly more affinity (**2.49**). 2- and 8-substitution of 9-ethyladenines was the subject of a recent study by Klotz *et al*., 8-substitution proved more favourable towards A_1 receptor binding and in particular the 8-phenyl derivative (**2.50**).[73] The most recent addition to this series of compounds retains the N^6-cyclopentyl group and the N9-methyl adduct as described by Ukena *et al*. (**2.44**), and explores the 8-position with amino-derivatives.[74] The most promising compound (**2.51**) in terms of affinity at the A_1 adenosine receptor possesses an isopropylmethylamine substituent at the C8 position. Since the ribose ring has been found necessary for agonistic activity, modification of this moiety results in compounds with a range of effects from partially agonistic to antagonistic. In 1988, Lohse *et al*. published the

properties of 2',3'-dideoxy-N^6-cyclohexyl adenosine, and showed it to possess antagonistic properties (**2.52**).[75] More significantly, Van Calenberg *et al*. demonstrated nanomolar affinity with ribose modified compounds.[76] Cyclopentyl adenosine (CPA) was altered at the 3' position with various amide derivatives. The compounds which showed the most affinity were the 3,4-disubstituted-benzamides, and in particular the 3,4-dimethyl-benzamide (**2.53**) with an affinity of 24 nM at the A_1 receptor.

Table 2.7 Biological data of compounds **2.54-2.56**



	R^1	R^2	K_i [nM]			A_{2A}/A_1	A_3/A_1	Ref.
			A_1[a])	A_{2A}[b])	A_3[c])			
2.54	cC_5H_9	Me	41	4100	2250	100	55	[77]
2.55	CO-cC_5H_9	Me	4	4300	410	1000	100	[77]
2.56	CO-cC_5H_9	Et	3	2600	20	870	7	[77]

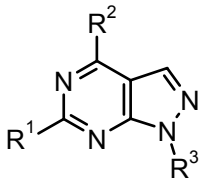
[a])Displacement of specific [^{3}H]CHA binding in bovine cortical membranes. [b])Displacement of specific [^{3}H]CGS 21680 binding in bovine striatal membranes. [c])Displacement of specific [^{125}I]AB-MECA binding in bovine cortical membranes.

The 8,8*a*-Dihydro-imidazo[1,2-*a*][1,3,5]triazines (Figure 2.6, Table 2.7, **2.54-2.56**) have only recently been discovered and exploited as adenosine receptor antagonists.[77] The compounds were designed according to a pharmacophore reported in an earlier paper.[24] In consequence to the discoveries in the adenine series, a phenyl group in the 2-position is retained, and an exo-cyclic amine is present in the 4-position of the ring. Substitution at the 4-amino group with a cycloalkyl group had a positive influence upon the binding affinity of the species towards the A_1 receptor (**2.54**). Inserting a CO spacer between the heterocyclic ring and the cycloalkyl group further enhanced affinity for the A_1 receptor (**2.55**), and subsequent lengthening of the alkyl moiety at the 7-position was yet more positive for A_1 affinity (**2.56**). However, this also had a generally beneficial effect on the binding affinity at the A_{2A} and A_3 receptors, and thus lowered the overall selectivity of the compounds for the A_1 receptor.

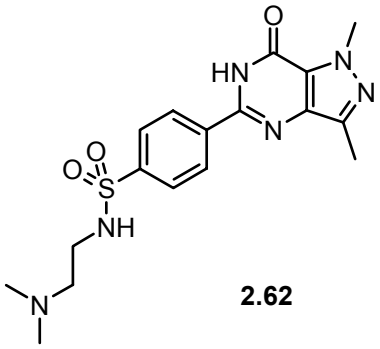
Pyrazolo[3,4-*d*]pyrimidines (Figure 2.6, Table 2.8, **2.57-2.61**) were examined early on in adenosine receptor research. In 1983 Davies *et al*. identified pyrazolo[3,4-*d*]pyrimidines as having affinity to the adenosine A_1 receptor (**2.57**).[78] The 1992 paper by Peet *et al*. investigating chiral substituents also looked at pyrazolopyrimidines and showed affinity for both the A_1 and A_2 receptors without much selectivity (**2.58**).[70] Quinn and co-workers followed up on the discoveries by Davies and Peet to systematically explore the pyrazolo[3,4-*d*]pyrimidines, explaining in detail the effects of varying the substituents at the adenosine A_1 and A_{2A} receptors.[79-85] The initial lead (**2.57**) was quickly dissected at the 4- and 6-positions. The first examination determined the necessity of the symmetrical substituents and found that

the 6-substituent was essential for good binding affinity at the A_1 receptor.[79] Following this, variation of the thio-substituent at the 4-position with an amino moiety incorporating the essence of the discovery by Davies *et al*. improved affinity significantly (**2.59**),[80] and further modification of the 6-substituent with a branched alkyl chain introduced a 16-fold improvement to sub-nanomolar affinity (**2.60**).[83]

Table 2.8 Biological data of compounds **2.57-2.62**



2.57-2.61



2.62

	R^1	R^2	R^3	K_i [nM]		A_{2A}/A_1	Ref.
				A_1	A_{2A}		
2.57	$SCHMeCONH_2$	$SCHMeCONH_2$	H	370[a])	-	-	[9]
2.58	OPr	NH*)	Ph	350[a])	370[b])	1	[70]
2.59	$SCH_2CONHEt$	NH_2	Ph	12[a])	131[c])	11	[80]
2.60	$SCH(Bu)CONH_2$	NHMe	Ph	0.8[a])	247[c])	300	[82]
2.61	$NHCH(Me)CONH_2$	NH_2	Ph	49[a])	648[c])	13	[85]
2.62		-		39[d])	-	-	[86]

*[a])Displacement of specific [^{3}H]PIA bound to rat membranes. [b])Inhibition of specific [^{3}H]NECA binding in rat striatum. [c])Inhibition of specific [^{3}H]CGS 21680 binding to rat brain striatum membranes. [d])Inhibition of specific [^{3}H]CHA binding in rat brain membranes. *) (R)-1-(hydroxymethyl)-2-phenylethyl*

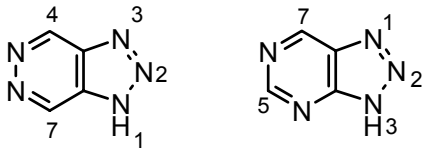
Later variations of the 6-thio substituent for an amino-analogue displayed less though still good and selective affinity for the A_1 receptor (49 nM, 13-fold selectivity over A_{2A}) (**2.61**).[85] Also of note to mention here are the related structures of the pyrazolo[4,3-*d*]pyrimidin-7-ones. The most interesting compound to note is that published by Hamilton *et al*., which showed a K_i of 39 nM at the A_1 receptor vs. [^{3}H]CHA (**2.62**).[86]

3-Deaza-8-azaadenines (1*H*-[1,2,3]triazolo[4,5-*c*]pyridines) were examined recently by Biagi *et al*. in relation to the 8-azaadenines (see Section 2.3.1.4).[87] It seems that this variant of the adenine ring can also show good affinity for the A_1 receptor depending upon its substituents. A norbonyl moiety was the most positive with a K_i of 11 nM at the A_1 receptor (**2.63**, Table 2.9).

2.3.1.4 The 6:5 Fused Heteroaromatic Systems Possessing Five Nitrogen Atoms

The five-nitrogen bi-cyclic rings have been explored in detail by Biagi *et al*. A whole selection of 1*H*-[1,2,3]triazolo[4,5-*d*]pyridazines and 3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidines have been published over the past decade. Early work on the [1,2,3]triazolo[4,5-*d*]pyridazines (Figure 2.7, Table 2.9) looked at substitution at the 1- and 4-positions of the ring.[88] The 4-

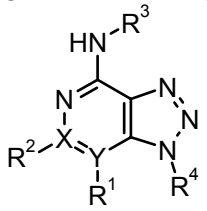
substituted exocyclic amino group was shown yet again to be the most beneficial variation on the bi-cyclic core (**2.64**).[88,89] Modification at the 1-benzyl group resulted in 30-70 nM affinity.[90] Swapping the benzyl group for a methyl-thienyl moiety (**2.65**) retained a similar affinity for the A_1 receptor.[91]



2.64-2.65 **2.66-2.71**

Figure 2.7 The 6:5 Fused Bi-cyclics Possessing Five Nitrogen Atoms: 1*H*-[1,2,3]triazolo[4,5-*d*]pyridazines (**2.64-2.65**); 3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidines (8-azapurines) (**2.66-2.71**).

Table 2.9 Biological data of compounds **2.63-2.71**



	X	Y	R^1	R^2	R^3	R^4	K_i [nM]		A_{2A}/A_1	Ref.
							A_1	A_{2A}		
2.63	C	C	H	Ph	norbornyl	CH_2Ph	11[a])	>1000[b])	>90	[87]
2.64	N	C	OH	-	3-tolyl	CH_2Ph	7[c])	3000[d])	430	[88]
2.65	N	C	OH	-	c-C_5H_9	$CH_2$2-thienyl	47[a])	895[b])	19	[91]
2.66	C	N	-	Ph	c-C_5H_9	CH_2Ph	11[c])	>1000[d])	>90	[92]
2.67	C	N	-	H	c-C_5H_9	CH_2(4-FPh)	11[a])	3422[b])	300	[93]
2.68	C	N	-	Ph	c-C_5H_9	*erythro*-CH(Hex)CH(OH)Me	4[a])	>1000[b])	>250	[94]
2.69	C	N	-	Ph	H	*erythro*-CH(Hex)CH(OH)Me	3[a])	>1000[b])	>300	[94]
2.70	C	N	-	Ph	(*trans*-4-HO-cC_6H_{11})	CH_2Ph	3[a])	>1000[b])	>300	[95]
2.71	C	N	-	Ph	c-C_5H_9	(*S*)-CH_2CH(OH)Me	2[a])	>1000[b])	>500	[95]

[a])Displacement of specific [^{3}H]CHA binding in bovine cortical membranes. [b])Displacement of specific [^{3}H]CGS 21680 binding in bovine striatum. [c])Displacement of specific [^{3}H]CHA binding in sheep cortical membranes. [d])Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes.

The 1,2,3-triazolo[4,5-*d*]pyrimidines (8-azapurines) (Figure 2.7, Table 2.9) were mentioned early on by Escher *et al*.[96] They constitute a series based upon the experiences of Quinn *et al*. in the area of the pyrazolo[3,4-*d*]pyrimidines.[79,97] However, the same substitution pattern imposed upon the [1,2,3]triazolo[4,5-*d*]pyrimidines did not result in a similar potency. Further exploration of this series seems to have been abandoned by the Australian group. In the mid-1990s the series was picked up on with much more success by Biagi and co-workers. A large number of publications from 1994-2003 detail the exploration of various positions. Fairly early on in their endeavours an exocyclic amino group in the 7-position was found to be beneficial for adenosine receptor affinity. At this exocyclic amine a cyclopentyl substituent was found repeatedly to have a positive influence upon binding affinity. Generally, a phenyl

group in the 5-position of the ring brought about good binding affinity to the A_1 receptor too (**2.66**),[92] although compound **2.67**, without this substituent, also displayed good affinity.[93] Substitution at the 3-position of the heterocyclic ring seemed also to be of importance for affinity,[93,98] the first variants being substitutions on the benzyl moiety (**2.67**). More recently, a number of different substituents have been attempted to show the stereoselective nature of the A_1 receptor and in the pursuit of water solubility. An *erythro*-CHRCH(OH)Me group (R = alkyl) was shown to retain the good binding affinity of the azaadenines, though in this case the unsubstituted exocyclic amino variant was the best compound - showing a lack of additivity in substitution (**2.68-2.69**).[94] To attain better water solubility several hydroxyl-variants were made, both at the exocyclic amino group and at the 3-positions.[95] Compounds **2.70** and **2.71** both show very good selective affinity for the A_1 receptor and water solubility.

2.3.2 Other Fused Bi-cyclic Heteroaromatic Systems

2.3.2.1 Other Nitrogen-containing Heteroaromatic Systems

1,8-Naphthyridine derivatives consist of two 6:6 fused rings. With regard to the adenosine receptors, the first publication by Müller *et al*. came about based on its similarities to the adenines.[99] Substitution in the 3-position and 4-amino positions only yielded micromolar affinities with not much selectivity over the $A_{2(A)}$ receptors (Figure 2.8, Table 2.10, **2.72**). Siddiqi *et al*. screened many compounds and found some affinity for naphthyridine derivatives.[38] More recently, Ferrarini *et al*. published more lucrative substitution about the 1,8-naphthyridine ring.[100] At the 7-position a number of halides were used, showing an almost equal effect across the board. At the 4-position, an amino group was favourable, as was a mono-substituted amine. A chloro-substituent had very little effect, and a hydroxy moiety was the most positive. The compound with the highest affinity for the A_1 receptor was 7-chloro-4-hydroxy-2-phenyl-1,8-naphthyridine at 0.15 nM at the bovine A_1 receptor (**2.73**). Further examination of the compound and its derivatives showed that the hydroxyl function on the heterocycle was necessary to allow ring tautomerism, resulting in the availability of a hydrogen-bond donor and acceptor adjacent at the 1- and 8-positions of the ring (Figure 2.8). Although the amino acid sequence homology for the A_1 adenosine receptors is more than 90% between a number of different mammalian species, a subsequent paper by Ferrarini *et al*. highlights the great differences in affinity achieved by compounds at the human and bovine receptors.[101] 7-Chloro-4-hydroxy-2-phenyl-1,8-naphthyridine (**2.73**) has a K_i value of 300 nM at the human adenosine A_1 receptor, and also reports a drop in selectivity over the A_{2A} receptor.

Other nitrogen-based heteroaromatic fused ring systems have generally a low affinity, mainly in the micromolar range. For the sake of completeness they are mentioned only briefly here. Another 6:6 fused nitrogen heterocyclic system are the pyridopyrimidines (Figure 2.8, **2.74**).[99] They were investigated by Müller *et al*. in conjunction with the 1,8-naphthyridines mentioned previously. The pyridopyrimidines have a xanthine-like structure in the sense of the pyrimidinone moiety of the system, but show better affinity and selectivity for the A_1 receptor

compared to the prototypic caffeine and theophylline. Two examples were made and tested, and both showed better affinity and selectivity than the 1,8-naphthyridines featured in that particular publication. Substitution of these pyridopyrimidines in a similar manner to the Ferrarini naphthyridines may also have a positive influence on the affinity.

An example of a 5:7 fused nitrogen heterocycle that has been synthesised and tested as adenosine receptor antagonists are the imidazodiazepinediones (Figure 2.8, **2.75**).[102] As with many of the earlier publications they are again xanthine-like derivatives, incorporating the 7-membered ring in the place of the 6-membered ring of xanthine. 1-Benzyl substitution was seen to be essential for affinity to the adenosine receptors and substitution at the N4- and N7-positions with Pr groups (analogous to DPCPX) gave rise to the best compound in terms of affinity.

2.3.2.2 Nitrogen-Free Heteroaromatic Systems

This class of ligands contains the compounds isolated from natural products. There are just two categories of natural products that have been explored with respect to adenosine receptor research, namely the benzofuran derivatives and the flavonoids. The former category was derived from the extraction and isolation of an active component of a natural product used frequently in traditional Chinese medicine (TCM). The dried root of *Salvia miltiorrhiza* Bunge (Danshen) has been widely used in TCM to treat coronary heart disease, particularly angina pectoris and myocardial infarction, and has been a subject of great interest throughout the 20th century.[103] It has been reported to have a host of medicinal properties, ranging from antibacterial to anti-inflammatory activity, and more than 40 separate components have been isolated.[30,103,104] The compound of interest to the adenosine receptor field was reported in 1991 (Figure 2.8, Table 2.10, **2.76**).[103] It was the first nitrogen-free ligand to show good affinity to the adenosine A_1 receptor with an IC_{50} of 17 nM. Some subsequent variations and derivatives have been reported, but the original compound remains the most attractive.[105] Unfortunately, no further investigations have been undertaken and the exploration of similar phytomaterials may warrant further attention.

The identification of the flavonoids came as a result of a report that showed 5,7-dihydroxy-4'-hydroxyisoflavone to have an affinity of 5 μM at the adenosine A_1 receptor.[106] Flavonoids are plant pigments ubiquitous to green plant cells. They are highly diverse and the daily intake is estimated to be 1-2 g of a normal human diet.[31] Following the publication mentioned above, a broad screening effort of phytochemicals ensued and identified the potential of several flavones.[28] However, the highest affinity attained was only in the sub-micromolar range (**2.77**). Some sporadic reports of further flavones have been reported since, an affinity of 3 μM (at the rat A_1 receptor) was published in 1997 for an extract of *Microtea debilis*, a plant from Suriname used against proteinuria.[107]

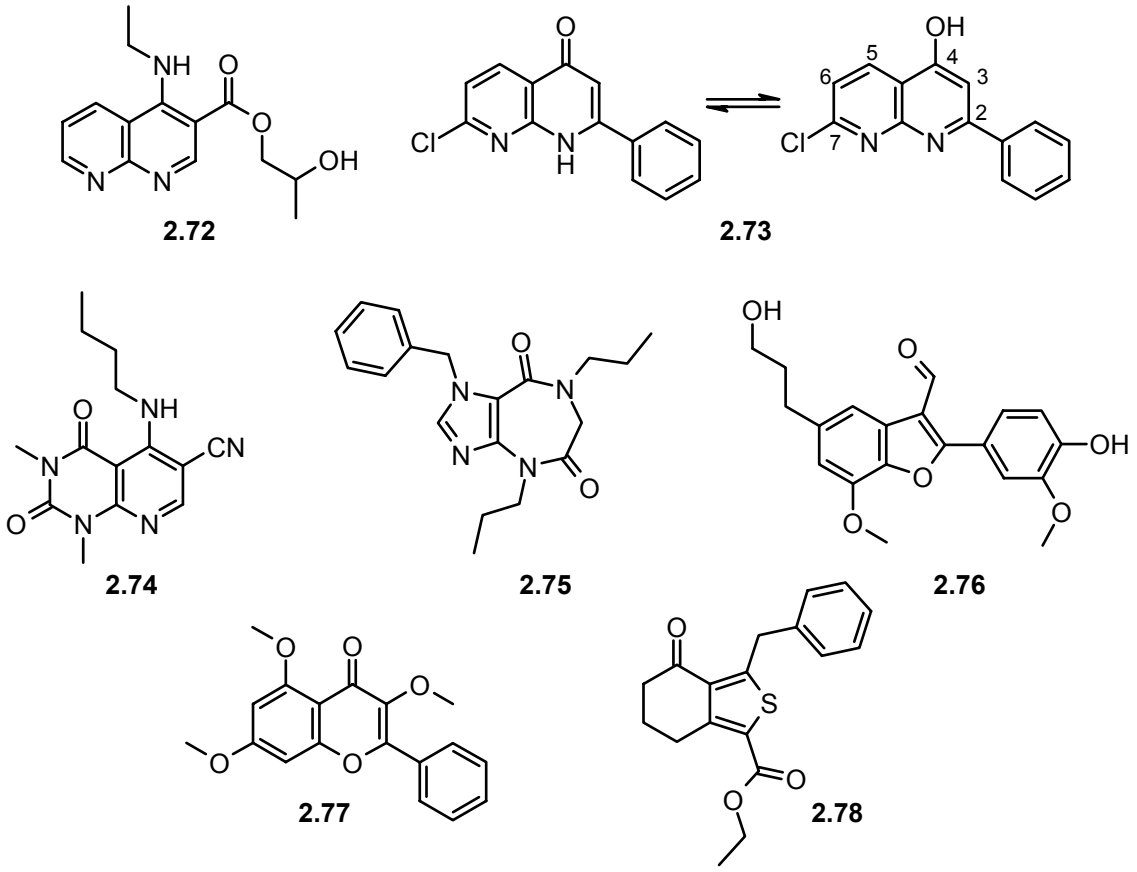


Figure 2.8 Other Fused Bi-cyclic Heteroaromatic Systems. 1,8-Naphthyridines (**2.72-2.73**), pyridopyrimidines (**2.74**), imidazodiazepinediones (**2.75**), benzo[*b*]furan derivatives (**2.76**), flavones (**2.77**), tetrahydrobenzothiophenone (**2.78**).

Table 2.10 Biological data of compounds **2.72-2.78** (see Figure 2.8)

	K_i [nM]			A_{2A}/A_1	A_3/A_1	Ref.
	A_1	A_{2A}	A_3			
2.72	7500[a])	44000[b])	-	6	-	[99]
2.73	300[c])	450[d])	2100[e])	1.5	7	[100,101]
2.74	1800[a])	18900[b])	-	11	-	[99]
2.75	11000[f])	52000[g])	-	5	-	[102]
2.76	17[h])[*])	-	-	-	-	[30]
2.77	509[a])	6450[i])	1210[j])	13	2	[28]
2.78	567[a])	>10000[b])	-	>18	-	[108]

[a])Displacement of specific [^{3}H]R-PIA binding in rat brain cortical membranes. [b])Displacement of specific [^{3}H]NECA binding in rat striatal membranes. [c])Displacement of specific [^{3}H]DPCPX binding to human brain cortical membranes. [d])Inhibition of specific [^{3}H]CGS 21680 binding to human striatal membranes. [e])Inhibition of specific [^{3}H]R-PIA binding to rat testis membranes in the presence of 150 nM DPCPX. [f])Inhibition of specific binding of 1 nM [^{3}H]R-PIA to rat cerebral cortical membranes. [g])Inhibition of 1 nM [^{3}H]NECA specific binding to rat striatal membranes. [h])Inhibition of [^{3}H]R-PIA binding to bovine cerebral cortical membranes. [i])Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes. [j])Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in HEK-293 cell membranes. [*])IC_{50}

In 2000, the leaves of *Senna siamea*, a widely used plant in Thailand were extracted and its components were isolated and tested at the adenosine A_1 receptor. One compound, luteolin, was shown to have an affinity for the rat A_1 receptor of 1 μM.[29] However, the compounds revealed by the screening programme by Ji *et al*. are still the most potent in this area of

phytochemicals.[28] Although there are (probably) many flavonoids still to be examined, the only moderate activity and the lack of any distinct structure-activity relationship for those that have been studied does not encourage an extensive search.

The last non-nitrogen series to mention are the tetrahydrobenzothiophenones. Following up on a lead generated by the screening programme by Siddiqi *et al*.,[38] the tetrahydrobenzothiophenones (Figure 2.8, **2.78**) were explored further by Van Rhee *et al*.[108] Although reasonable selectivity for the A_1 receptor was achieved, the best compound only just displayed micromolar affinity.

2.4. Fused Tri-cyclic Heteroaromatic Systems

2.4.1 The 6:6:5 Fused Heteroaromatic Systems

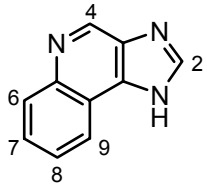
The 6:6:5 fused nitrogen heteroaromatics can be grouped together to form the largest collection of the tri-cyclic antagonists (Figure 2.9). They are in fact a collection of chemically very different structures, with either three or four nitrogens in the rings, different substitution patterns and very different methods of synthesis. However, as ligands towards the adenosine A_1 receptors, they have a remarkable number of similarities and some are just further developments on the bi-cyclic heteroaromatics described previously.

The main similarities between the different classes of 6:6:5 fused nitrogen heteroaromatic systems are: 1) the peripheral 6-membered ring (as opposed to the sandwiched 6-membered ring) contains no heteroatoms; 2) the position of the rings in relation to each other; 3) the patterns of substitution in the most favourable compounds (numbered positions 2- and 8- for the majority); and 4) an exocyclic amino group (numbered as the 4-position of the ring for the majority of the structures). Another similarity of the most potent examples is the favourable presence of a cyclopentyl group at this exocyclic amine. The difference between all these structures can be considered as the number and the arrangement of the nitrogen atoms about the 5-membered ring.

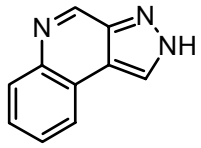
The first compound in the table (Table 2.11, **2.79**) is an example of an 1*H*-imidazo[4,5-*c*]quinolin-4-amine (Figure 2.9). It was designed and synthesised by Van Galen *et al*.[109] according to a predicted pharmacophore.[15] The 2- and 4-amino positions were explored with the hypothesis that hydrophobic substituents would enhance affinity. The most potent and selective compound incorporated a cyclopentyl group at the 4-amino-group and a phenyl substituent at the 2-position, showing a 10 nM affinity at the rat A_1 receptor and 45-fold selectivity over the $A_{2(A)}$ receptor. These substituents were regio-specific in terms of the affinity for the adenosine receptors; when the position of the cyclopentyl and phenyl groups were swapped, a drastic drop in affinity was observed (**2.80**).

The next two series to mention with three nitrogen atoms in the fused tri-cyclic system are the pyrazolo[3,4-*c*]quinolines (**2.81-2.82**) and the imidazo[1,2-*a*]quinoxalines (**2.83-2.85**) published by Colotta *et al*. and Ceccarelli *et al*. The pyrazoloquinolines were explored at the 2-position with a number of substituted phenyl groups; at the 4-position with amino-derivatives or a hydroxyl-function; and at the 8-position with a chloro-moiety.[110] The most

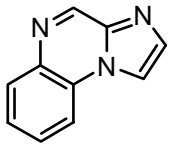
effective compound (**2.81**) at the A_1 receptor consisted of a 4-cyclopentylamino group, an unsubstituted 2-phenyl moiety and no substitution at the 8-position. Selective A_3 affinity could also be attained with this type of structure by the inclusion of a carbonyl moiety at the 4-position (**2.82**).



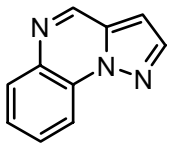
2.79-2.80



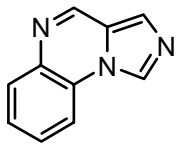
2.81-2.82



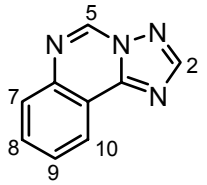
2.83-2.85



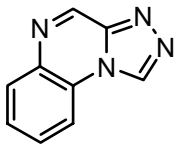
2.86



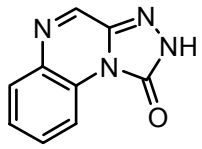
2.87-2.88



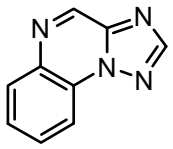
2.89-2.90



2.91-2.92



2.93-2.95



2.96-2.98

Figure 2.9 The 6:6:5 Fused Tri-cyclic Systems; 1*H*-imidazol[4,5-*c*]quinolines (**2.79-2.80**), 2*H*-pyrazolo[3,4-*c*]quinolines (**2.81-2.82**), imidazo[1,2-*a*]quinoxaline (**2.83-2.85**), pyrazolo[1,5-*a*]-quinoxaline (**2.86**), imidazo[1,5-*a*]quinoxaline (**2.87-2.88**), [1,2,3]triazolo[1,5-*c*]quinazoline (**2.89-2.90**), [1,2,4]triazolo[4,3-*a*]quinoxaline (**2.91-2.92**), 2*H*-[1,2,4]triazolo[4,3-*a*]quinoxalin-1-one (**2.93-2.95**), [1,2,4]triazolo[1,5-*a*]quinoxaline (**2.96-2.98**). Atom numbering about the rings are as given for compounds **2.79-2.80**, except where stated.

Imidazoquinoxalines were explored briefly by Colotta *et al*. in 1995,[111] these compounds however, only just displayed sub-micromolar affinity and were not pursued further. In this paper they showed that C2 phenyl substitution was beneficial for A_1 affinity and that the presence of the amino group was also necessary for A_1 receptor affinity (**2.83**). Ceccarelli *et al*. revisited this series and showed that cycloalkyl substitution at the 4-amino group was attractive and that variation at the 7- and 8-positions of the fused phenyl ring was possible whilst retaining affinity (**2.84**).[112] In a further variation, a methyl moiety at the 1-position resulted in an improvement in affinity to low nanomolar values (**2.85**).

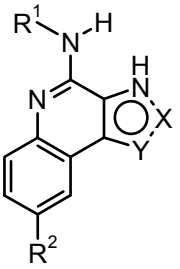
The Colotta group in 1995 also investigated two more types of fused tri-cyclic structures containing three nitrogen atoms, namely pyrazolo[1,5-*a*]quinoxalines and imidazo[1,5-*a*]quinoxalines.[111] Of the former structure, only one example was given (**2.86**), and this showed only micromolar affinity. The two examples of the imidazo[1,5-*a*]quinoxalines both incorporated the 2-phenyl group, and the 4-amino moiety was also present. Cyclopentyl substitution at the 4-amino group did not improve the affinity over the unsubstituted form for the A_1 receptor, but affinity for the A_{2A} receptor did drop off significantly (**2.87-2.88**).

The largest sub-section of the fused tri-cyclic category is that with four nitrogen atoms incorporated into the rings. Francis *et al*. showed the triazoloquinazolines to be adenosine receptor antagonists as early as 1988,[12] in consequence to the discovery of CGS 15943 (**2.89**) (a potent, non-selective adenosine receptor antagonist, discovered in 1983) and patent

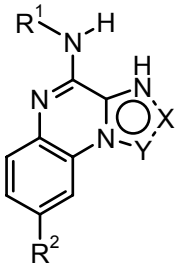
material which indicated these compounds as substances to treat depression and fatigue.[113] Substitution with an aromatic group in the 2-position in analogy to CGS 15943 showed that the 2-furyl substituent was the most effective, and some selectivity for the A_1 receptor was obtained with alkyl-substitution at the 4-amino group (**2.90**).

Also in 1988 Trivedi and Bruns published the triazolo[4,3-*a*]quinoxalines as selective A_1 receptor antagonists.[13] Several alkyl groups were investigated at the exocyclic amine, and the

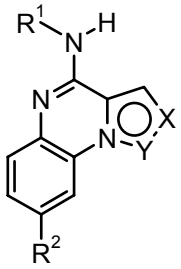
Table 2.11 Biological data of compounds **2.79-2.98**



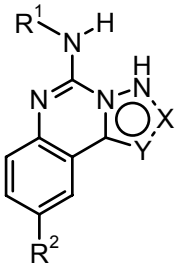
2.79-2.82



2.83-2.85,2.91-2.98



2.86-2.88



2.89-2.90

	X	Y	R^1	R^2	K_i [nM]			A_{2A}/A_1	A_3/A_1	Ref.
					A_1	A_{2A}	A_3			
2.79	CPh	N	cC_5H_9	H	10[a])	450[b])	-	45	-	[109]
2.80	C(cC_5H_9)	N	Ph	H	230[a])	>1000[b])	-	>4	-	[109]
2.81	NPh	CH	cC_5H_9	H	3[c])	849[d])	61[e])	300	20	[110]
2.82	NPh	CH	COPh	H	>20000[c])	>20000[d])	2[e])	-	-	[110]
2.83	CPh	CH	H	H	230[f])	720[g])	-	3	-	[111]
2.84	CH	CH	cC_5H_9	Cl	24[a])	>2500[g])	>10000[e])	>100	>400	[112]
2.85	CH	CMe	cC_5H_9	H	8[a])	2500[g])	>10000[e])	300	>1250	[112]
2.86	CH	N	H	H	3050[f])	3000[g])	-	1	-	[111]
2.87	N	CPh	H	H	130[f])	310[g])	-	2	-	[111]
2.88	N	CPh	cC_5H_9	H	810[f])	>10000[g])	-	>12	-	[111]
2.89	C(2-furyl)	N	H	Cl	21[f])[*])	3[b])[*])	-	0.3	-	[12]
2.90	C(2-furyl)	N	^{i}Pr	Cl	22[f])[*])	179[b])[*])	-	8	-	[12]
2.91	N	CCF_3	cC_5H_9	H	7[f])	1000[b])	-	140	-	[13]
2.92	N	CCF_3	cC_5H_9	Cl	36[h])	710[i])	-	20	-	[14,114]
2.93	NPh	CO	cC_5H_9	H	0.4[c])	986[d])	55[j])	2500	140	[115]
2.94	NPh	CO	H	Cl (6-NO_2)	0.2[c])	256[d])	112[e])	1300	560	[116]
2.95	NPh	CO	cC_5H_9	NO_2	0.4[c])	>20000[d])	212[e])	>50000	530	[117]
2.96	CPh	N	H	Cl	50[f])	161[g])	-	3	-	[111]
2.97	C(2-F-Ph)	N	H	Cl	13[f])	>10000[g])	-	>770	-	[111]
2.98	C(2-thienyl)	N	H	Cl	12[f])	>10000[g])	-	>800	-	[111]

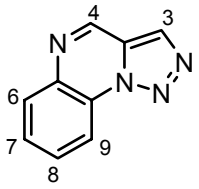
[a])Displacement of specific [^{3}H]DPCPX binding in rat brain cortical membranes. [b])Displacement of [^{3}H]NECA in rat striatal membranes. [c])Displacement of specific [^{3}H]CHA binding in bovine brain membranes. [d])Displacement of specific [^{3}H]CGS 21680 binding in bovine striatal membranes. [e])Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in CHO cells. [f])Displacement of specific [^{3}H]CHA binding in rat cerebral cortex membranes. [g])Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes. [h])Inhibition of specific binding of 1.1 nM [^{3}H]CHA to A_1 receptors in guinea pig forebrain membranes. [i])Inhibition of specific [^{3}H]NECA binding in the presence of 50 nM CPA in rat striatal membranes. [j])Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in HEK293 cell membranes. [*])IC_{50}

cyclopentyl was shown as the most beneficial substituent. At the 2- and 3-positions of the ring were situated cyclic nitrogen atoms, thus the only possible place of variance was explored with short alkyl derivatives, with the most effective being the trifluoromethyl moiety (**2.91**). In consequence to this set of compounds, Sarges *et al*.[14] showed further development describing another 138 derivatives. The variations involved mainly new 4-amino substituents and new substitutions at the 6-, 7- and 8-positions of the fused phenyl ring. The most effective compound (**2.92**) possessed chloro-substitution at the 8-position on the fused phenyl ring, an analogy to the series published by Francis *et al*. (**2.89, 2.90**). The reported value in Table 2.11 is the K_i as reported by Suzuki *et al*.[114]

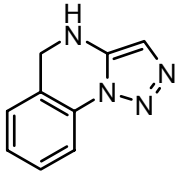
A variation of the triazolo[4,3-*a*]quinoxalines by Trivedi and Bruns and Sarges *et al*. incorporates a carbonyl oxygen in the 1-position allowing for substitution in the 2-position. These compounds were first reported by Colotta *et al*. in 2000, and were shown to be very effective at the A_1 receptor (**2.93**).[115] Further development in 2003 showed that the cyclopentyl group was not necessary for good affinity at the A_1 receptor, when compensated with 6- and 8- substitution at the fused phenyl ring (**2.94**).[116] The most recent developments of this series re-incorporate the cycloalkyl group at the 4-amino moiety and also incorporate the substitutions at the 6- and 8- positions.[117] The most effective compound in terms of overall affinity and selectivity has cyclopentyl as the 4-amino-substituent and a nitro group in the 8-position (**2.95**).

The [1,2,4]triazolo[1,5-*a*]quinoxalines as reported by Colotta *et al*. in 1995[111] investigated the receptor pocket that allows 2-substitution of these 6:6:5 fused tri-cyclic systems. Leaving the 4-amino-group unsubstituted, good affinity was obtained with a 2-phenyl moiety (**2.96**). Replacing this with a 2-fluorophenyl moiety (**2.97**) gave improvements in both affinity and selectivity over the A_{2A} receptor and a 2-thienyl group showed slightly better affinity for the A_1 receptor (**2.98**).

Another variation of the four-nitrogen tri-cyclic fused rings incorporates a hydroxyl function at the 4-position in the place of the exocyclic amino group. Tautomerism dictates that this moiety exists also in the amido form, and substitution at the (5-)nitrogen locks the molecule in this form. The first compounds to mention are the triazolo[4,3-*a*]quinoxalin-1-ones with this moiety in place. They were published in 2003 by Colotta *et al*. in analogy to the 4-NH_2 series.[116] No substitutions were shown at the N5 group, and as such these compounds could also exist in the iminol form. The affinity and selectivity was not better than the 4-amino-variations and are thus not shown here.



2.99-2.101

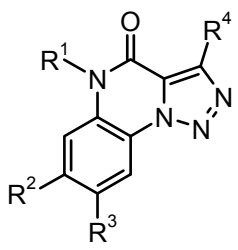


2.102-2.103

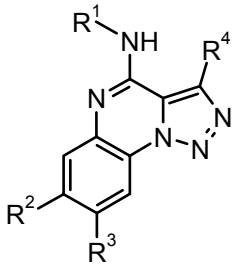
Figure 2.10 The 6:6:5 Fused Tri-cyclic Systems; [1,2,3]triazolo[1,5-*a*]quinoxaline (**2.99-2.101**), 4*H*-[1,2,3]triazolo[1,5-*a*]-quinazolines (**2.102-2.103**).

[1,2,3]Triazolo[1,5-*a*]quinoxalines are a relatively new series of compounds which have been reported by Bertelli *et al*. and Biagi *et al*.[118,119] The 4-hydroxy variants were reported first and display good affinity with the A_1 receptor, especially where the N5-position was left unsubstituted (Figure 2.10, Table 2.12, **2.99** vs. **2.100**). An ethoxy-carbonyl group was found in the 3-position of the system, and substitution of the fused phenyl ring at the 7-position favoured a methoxy- group. In the second paper of this series, some other variations at the 3- and 5-positions were attempted, but these led to a complete loss of affinity for the A_1 receptor. The 4-hydroxy function was also exchanged for an amino group, again leading to a significant loss of affinity at 5 μM (**2.101**).[119]

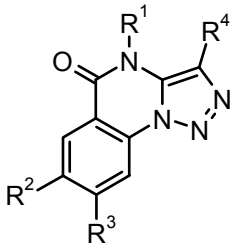
Table 2.12 Biological Data for compounds **2.99-2.103**



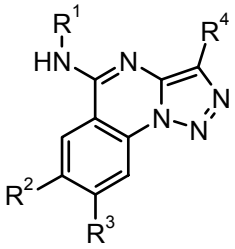
2.99-2.100



2.101



2.102



2.103

	R^1	R^2	R^3	R^4	K_i [nM]		A_{2A}/A_1	Ref.
					A_1[a])	A_{2A}[b])		
2.99	H	MeO	H	CO_2Et	29	>1000	>35	[118]
2.100	Me	MeO	H	CO_2Et	389	>1000	>3	[118]
2.101	MeCHPh	H	H	CO_2Et	5139	>10000	>2	[119]
2.102	H	H	Cl	Ph	148	>10000	>70	[120]
2.103	H	H	H	Ph	239	>10000	>40	[120]

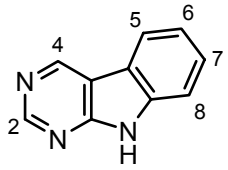
[a])Displacement of specific [^{3}H]CHA binding in bovine brain membranes. [b])Displacement of specific [^{3}H]CGS 21680 binding in bovine striatal membranes.

The last in this series of four-nitrogen tri-cyclic fused aromatics we report on are the [1,2,3]triazolo[1,5-*a*]quinazolines (Figure 2.10, Table 2.12).[120] They show great similarities with the above [1,2,3]triazolo[1,5-*a*]quinoxalines, but differ in the position of the nitrogen atom in the central 6-membered ring. In analogy to the triazoloquinoxalines, the 4-hydroxy group showed the best biological results, however substitution in the 3-position with the ethoxy-carbonyl group was not beneficial to this ring system and a 3-phenyl group was found to be much more potent (**2.102**). Exchange of the 4-hydroxy for a 4-amino group again led to a drop in affinity at the A_1 receptor (**2.103**).

2.4.2 The 6:5:6 Tri-cyclic Heteroaromatic Systems

These compounds published by Müller *et al*. are derivatives of the deazapurine series (Section 2.3.1.2).[59] The fusion of a 6-membered ring at the 7- and 8-positions of the deazapurine gives rise to this series. Two varieties were explored, involving a partially saturated and an unsaturated 6-membered ring. The most potent of the partially saturated 6-membered ring

derivatives published in 1990, showed the signature exocyclic 4-amino moiety, and a phenyl group at the 9-position (Figure 2.11, Table 2.13, **2.104**). Follow-up work on this series was reported in 2000 and substitution at the 2-position was explored, the most effective of which was 4-pyridyl (**2.105**).[63]



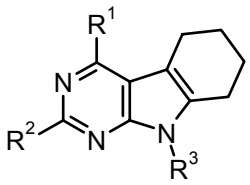
2.104-2.107

Figure 2.11 Tri-cyclic deazapurine derivatives (**2.104-2.107**).

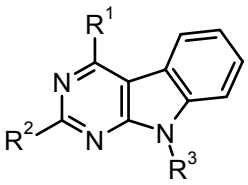
Where a benzene ring was fused onto the deazapurine, the initial lead (**2.106**) contained a hydroxyl group at the 4-position (tautomerism with purinone structure). This compound had very little selectivity over the A_{2A} receptor.[60] Later work showed the 4-amino group to be favourable, with the inclusion of an aromatic substituent at the 2-position and a chiral moiety at the N9 position (**2.107**). [61]

The last compound in this class to mention is also based on the tri-cyclic deazapurine moiety. The difference is at the 4-amino domain, a chiral substituent attaches this in a 5-membered ring formation to the N1 of the purine ring (Table 2.13, **2.108**).[70]

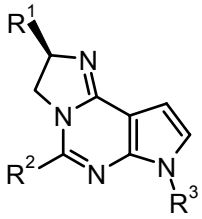
Table 2.13 Biological data of compounds **2.104-2.108**



2.104-2.105



2.106-2.107



2.108

	R^1	R^2	R^3	K_i [nM]			A_{2A}/A_1	A_3/A_1	Ref.
				A_1	A_{2A}	A_3			
2.104	NH_2	H	Ph	3240[a])	10900[b])	-	3	-	[60]
2.105	NH_2	4-Py	Ph	600[c])	>10000[d])	-	>17	-	[63]
2.106	OH	H	Ph	880[a])	1440[b])	-	2	-	[60]
2.107	NH_2	Ph	(*R*)-MeCHPh	3[a])	6200[b])	>1000[e]	2000	>330	[61]
2.108	CH_2Ph	OPr	Ph	620[a])	>100000[b])	-	>160	-	[70]

[a])Inhibition of [^{3}H]PIA specific binding to rat cortical membranes. [b])Inhibition of [^{3}H]NECA specific binding to rat striatal membranes. [c])Inhibition of specific [^{3}H]CCPA binding in human recombinant A_1 adenosine receptors expressed in CHO cells. [d])Inhibition of [^{3}H]CGS 21680 specific binding in rat striatal membrane. [e])Inhibition of specific [^{3}H]NECA binding in human recombinant receptors expressed in CHO cell membranes.

A very different 6:5:6 heterocycle was proposed and investigated by Da Settimo *et al*.[24] These were the aryl[1,24]triazino[4,3-a]benzimidazol-4-(10*H*)-ones and were first reported as ligands towards the benzodiazepine receptor and later were also shown to behave as

adenosine receptor antagonists. Substitution was seen in two positions about the main ring; at N10 – the nitrogen in the central 5-membered ring; and in the 3-position. The most effective compound incorporated phenyl substituents at these two positions and had an affinity of 18 nM at the A_1 receptor with very good selectivity over the A_{2A} and A_3 receptors (Figure 2.12, Table 2.14, **2.109**). Some tri-cyclic xanthine derivatives also fall into the class of 6:5:6 fused ring systems. These were published by Geis *et al*. in 1995, and are direct derivatives of theophylline, with the third fused ring formed about the N7 and C8 atoms.[121]

The compounds in general only showed micromolar affinity at the A_1 receptor with poor selectivity over the $A_{2(A)}$ adenosine receptors (as for theophylline). One example however, displayed a 15-fold selectivity for the A_1 over the $A_{2(A)}$ receptor. Along with the theophylline base, the fused 6-membered ring formed a tetrahydro-pyrimidin-4-one, and further substitution at the nitrogen accounted for the selectivity (**2.110**).

2.4.3 The 5:6:5 Tri-cyclic Heteroaromatic Systems

Some other tri-cyclic xanthine derivatives of note feature in patent literature and have been developed by Boehringer Ingelheim. The first to mention has a linear arrangement of the three rings, and can be thought of as a xanthine with a third 5-membered ring fusing at the N1-C2 bond. The most potent example consists of a cyclopentyl group substituted at C8 (**2.111**).[122] The two last tri-cyclic xanthine derivatives mentioned here have the third fused ring between C2 and N3 of the xanthine core. The first example is a fairly compact molecule in the sense that the most effective example has small alkyl subsituents about the tri-cyclic core (**2.112**).[123] The second example contains a larger phenyl substituent on the third fused ring and also sees substitution at the C8 position (**2.113**).[124]

Table 2.14 Biological data for Compounds **2.109-2.118** (Figure 2.12)

	K_i [nM]			A_{2A}/A_1	A_3/A_1	Ref.
	A_1	A_{2A}	A_3			
2.109	18[a])	>10000[b])	>1000[c])	550	55	[24]
2.110	17000[d])	>250000[e])	-	>15	-	[121]
2.111	1[f])	422[g])	-	400	-	[122]
2.112	2[f])	-	-	-	-	[123]
2.113	2[f])	-	-	-	-	[124]
2.114	283[h])	420[i])	-	1.5	-	[125]
2.115	90[h])	1370[i])	-	15	-	[126]
2.116	47[h])	>20000[i])	-	>425	-	[126]
2.117	240[h])	2960[i])	-	12	-	[126]
2.118	84[h])	2280[i])	-	27	-	[127]

[a])Displacement of specific [^{3}H]CHA binding in bovine brain membranes. [b])Displacement of specific [^{3}H]CGS 21680 binding in bovine striatal membranes. [c])Displacement of specific [^{125}I]AB-MECA binding in bovine cortical membranes. [d])Inhibition of [^{3}H]PIA specific binding to rat cortical membranes. [e])Inhibition of [^{3}H]NECA specific binding to rat striatal membranes. [f])tested in radioligand binding assays at the human A_1 receptor, further details (e.g., radioligand) not given. [g])tested in radioligand binding assays at the rat A_{2A} receptor, further details (e.g., radioligand) not given. [h])Displacement of specific [^{3}H]CHA binding in rat cerebral cortex membranes. [i])Inhibition of [^{3}H]CGS 21680 specific binding in rat striatal membrane.

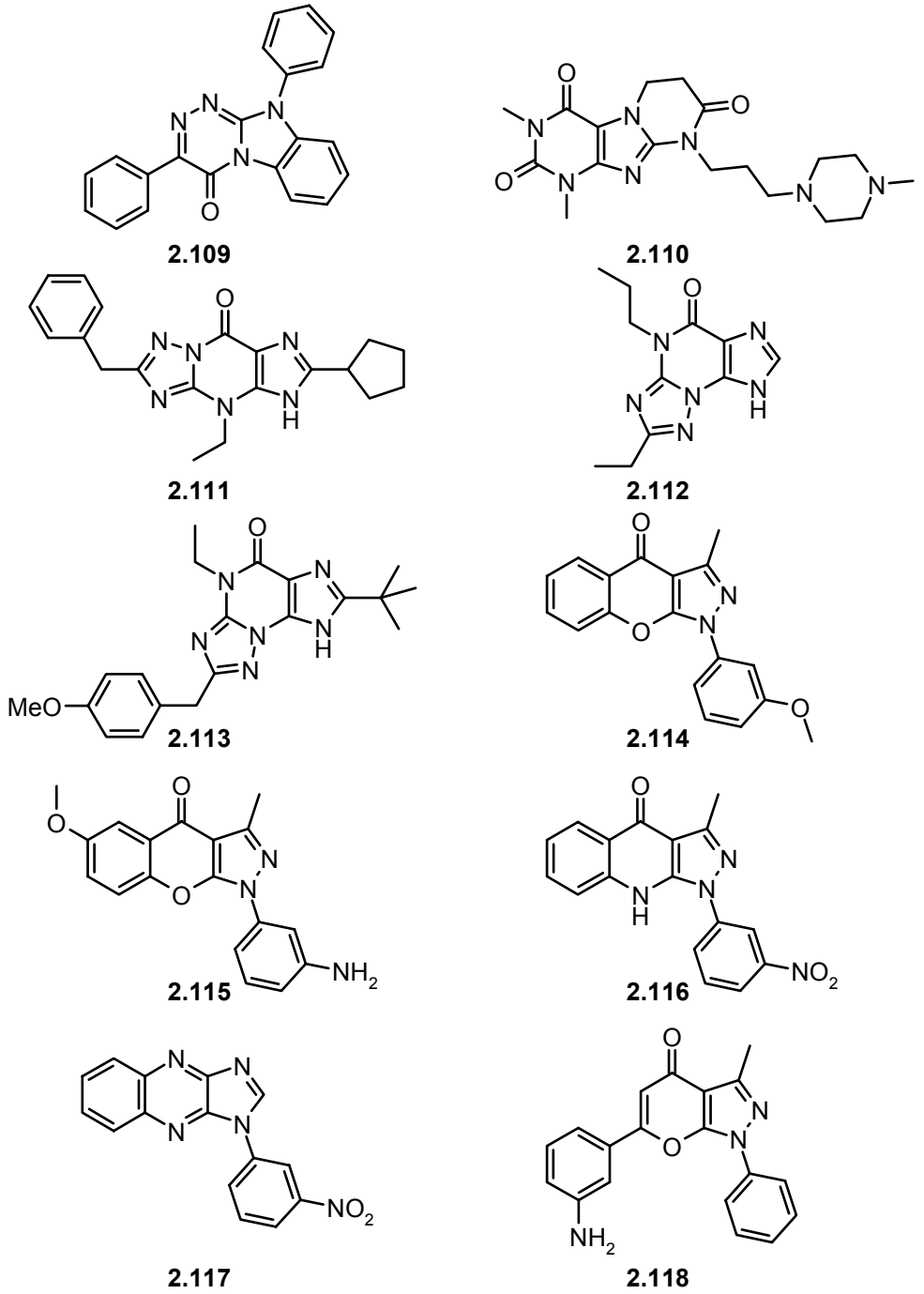


Figure 2.12 Other Fused Tri-cyclic Systems: [3,10]diphenyl-10*H*-benzo[4,5]imidazo[2,1-*c*][1,2,4]triazin-4-one (**2.109**), 1,3-dimethyl-8-[3-(4-methylpiperazin-1-yl)-propyl]-5,6-dihydro-[1*H*,8*H*-1,3,4*b*,8,9]-pentaaza-fluorene-2,4,7-trione (**2.110**), 6-benzyl-2-cyclcopentyl-4-ethyl-3,4-dihydro-[1,2,4]triazolo[1,5-*a*]purin-9-one (**2.111**), 2-ethyl-4-propyl-4*H*,8*H*-[1,2,4]triazolo[5,1-*b*]purin-5-one (**2.112**), 7-t-butyl-4-ethyl-2-(4-methoxybenzyl)-4*H*,8*H*-[1,2,4]triazolo[5,1-*b*]purin-5-one (**2.113**), 1-(3-methoxyphenyl)-3-methyl-1*H*-chromeno[2,3-*c*]pyrazol-4-one (**2.114**), 1-(3-aminophenyl)-6-methoxy-3-methyl-1*H*-chromeno[2,3-*c*]pyrazol-4-one (**2.115**), 3-methyl-1-(3-nitrophenyl)-1,9-dihydro-pyrazolo[3,4-*b*]quinolin-4-one (**2.116**), 1-(3-nitrophenyl)-1*H*-imidazo[4,5-*b*]quinoxaline (**2.117**), 6-(3-aminophenyl)-3-methyl-1-phenyl-1*H*-pyrano[2,3-*c*]pyrazol-4-one (**2.118**).

2.4.4 The 6:6:5 Tri-cyclic Heteroaromatic Systems

In 1993 Colotta *et al*. reported on a novel mixed heteroatomic tri-cyclic system in the search of A_{2A} selective ligands.[125] These were the benzopyrano[2,3-*c*]pyrazolo-4-ones, and although on the whole more selective for the A_{2A} receptor, some derivatives showed favour, albeit very slightly, for the A_1 receptor (**2.114**). Development of the series in 1995 with further substitution at the fused benzo-ring increased the affinity and selectivity for the A_1 receptor (**2.115**).[126] In a variation of this, the oxygen was replaced with a nitrogen atom creating 1,9-dihydro-pyrazolo[3,4-*b*]quinolin-4-ones. Improved selectivity for the A_1 receptor over the A_{2A} receptor was achieved with the amino substitution at the non-fused phenyl ring (**2.116**).[126] The third series in this paper is another 6:6:5 fused tri-cyclic nitrogen heteroaromatic system. It is an imidazo[4,5-*b*]quinoxaline and of the three examples that were synthesised and tested only one showed sub-micromolar affinity at the A_1 receptor (**2.117**).[125] The last compound to mention is actually a fused bicyclic compound, but is included in this section due to its logical development from the 6:6:5 compounds **2.114-2.115**. Instead of a fused phenyl ring on the heterocyclic system, the phenyl ring was placed in the 1- or the 2-positions.[127] The most positive influence was the phenyl in the 1-position (**2.118**), attaining better or comparable affinity and better selectivity than compounds **2.114** and **2.115**.

2.5 Summary

This review of the current state of non-xanthine A_1 adenosine receptor antagonists shows that research in this area has been highly active and imaginative, despite the restrictions of general GPCR research, i.e., the lack of a fully disclosed binding site.

Although there are numerous ligands with reportedly good potencies and selectivity for the A_1 receptor, many of them may still lack a complete data set. Those made in the 1980s and early 1990s only document binding data at the A_1 and $A_{2(A)}$ receptors, making suggestions of selectivity highly speculative. In addition, although the cloning of the human adenosine A_1 receptor was reported in 1992, many even very recently developed compounds have not been tested at this receptor. One particularly relevant and very recent example are the naphthyridines reported by Ferrarini *et al*.[100,101] The quoted 94% amino-acid homology between the human and bovine A_1 receptors concealed the discrepancies in affinity that was experienced by these compounds. At the bovine adenosine A_1 receptor, the most potent compound possessed an affinity of 0.15 nM, but retesting this at the human adenosine A_1 receptor resulted in a K_i value of 300 nM. To be thoroughly consistent and comprehensive, all previously reported compounds should be tested at all human adenosine receptors. However, the value of the data available should not be underestimated and despite the discrepancies and the absent information, we can still draw on the general themes and conclusions offered by over two decades of research.

2.6 Concluding Remarks

This large collection of non-xanthine adenosine A_1 receptor antagonists is the result of more than twenty years of intensive research. The most developed and only openly available (non-selective) adenosine receptor antagonists on the market are xanthine derivatives, e.g., theophylline and enprofylline used in the treatment of asthma, or caffeine widely used (recreationally) for its cognition enhancing properties. Although the therapeutic potential of a highly selective and potent adenosine A_1 receptor antagonist is great, we still seem to be some way off this goal. Thus the scope of research into non-xanthine ligands remains a broad and (almost) infinite field.

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Chapter 3

Substituted Pyrimidines as a New Class of Selective Adenosine A_1 Receptor Antagonists

Part I: 4-Amido-2,6-Diphenyl-Pyrimidines

Adenosine receptor antagonists usually possess a bi- or tri-cyclic heteroaromatic structure at their core with varying substitution patterns to achieve selectivity and/or greater affinity. Taking into account molecular modelling results from a series of potent adenosine A_1 receptor antagonists, a pharmacophore was derived from which we show that a monocyclic core can be equally effective. As a result, a novel series of 4-amido-2,6-diphenyl-pyrimidines was synthesised. The compounds were all tested at the human A_1, A_{2A}, and A_3 receptors, showing in many cases low nanomolar affinity for the adenosine A_1 receptor. In particular, compound **3.16** (LUF 5764) displayed, in terms of both affinity (K_i = 9 nM) and selectivity (displacement of the radioligand at the A_{2A} and A_3 receptors at 1 μM <32% and <39%, respectively), some of the most favourable characteristics.

Based upon L. C. W. Chang, R. F. Spanjersberg, J. K. von Frijtag Drabbe Künzel, T. Mulder-Krieger, G. van den Hout, M. W. Beukers, J. Brussee, A. P. IJzerman, J. Med. Chem. ***2004****, 47, 6529-6540.*

3.1 Introduction

The widespread purpose and presence of adenosine has led to substantial research into the individual adenosine receptors as pharmaceutical targets. Over the years, there have been many attempts to design and develop adenosine receptor antagonists, and over the past decade, the search for ligands that show selectivity towards individual receptors has intensified as the role of the receptors in many therapeutic areas expands.[1] As detailed in Chapter 2, there have been many ligands developed for the adenosine A_1 receptor. These compounds have been identified as a result of serendipity, screening programmes, rational design and computer models.

One of the first computer-derived models for the adenosine A_1 receptor binding site was published by Van Galen *et al.* in 1990.[2] This was based on the superimposition of theophylline and the endogenous agonist adenosine. The 'best' orientation of the xanthine, in terms of better electrostatic fit with respect to adenosine was suggested to be at a rotation 180º about the longer axis of the molecule (Figure 3.1). This was subsequently labelled as the 'flipped' model. In this same paper, certain features were highlighted to form the basis of a pharmacophore for adenosine A_1 receptor antagonists (Figure 3.2). The overlap of a selection of ligands suggested that one of the most important features was a large Y-shaped area of negative electrostatic potential that arose from the aromatic 6:5 fused N-heterocycles.

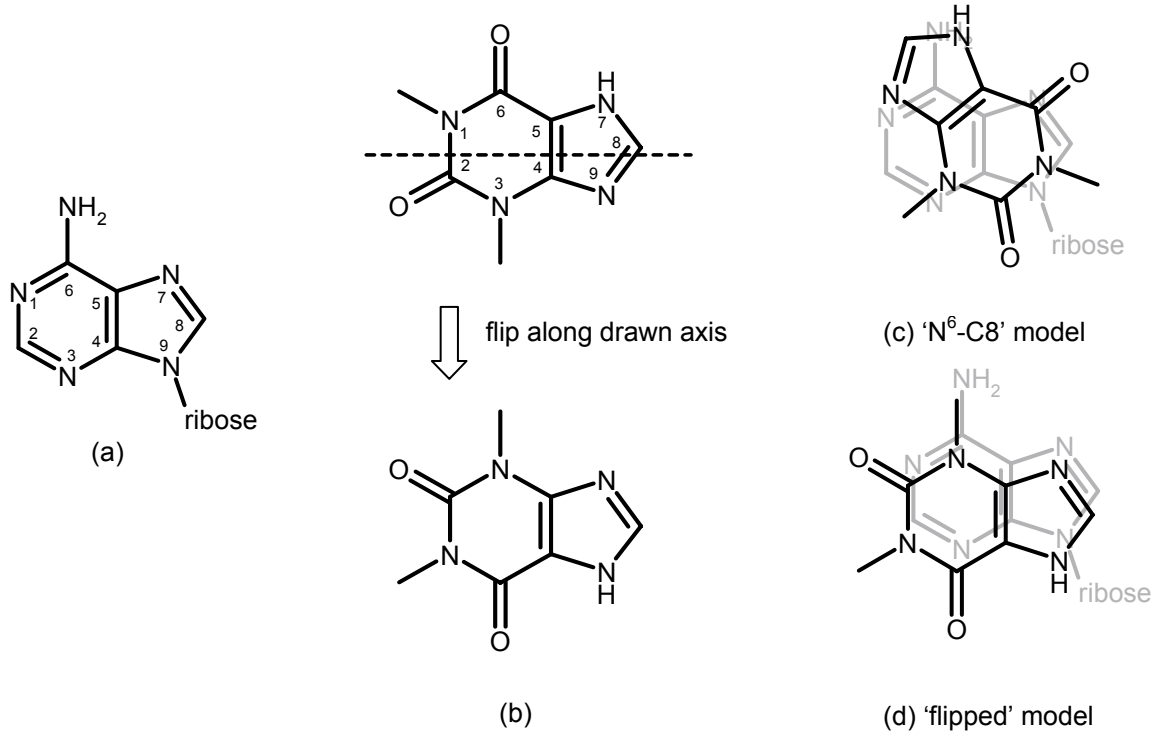


Figure 3.1. a) adenosine, b) above the standard depiction of theophylline and below the 'flipped' depiction of theophylline, c) the N^6-C8 model, and d) the 'flipped' model.

Two further areas of relatively positive electrostatic potential were also suggested. Months later, a different model was proposed by Peet *et al.* which suggested that N^6 of the purine derivative was more compatible with the C8 position of the xanthine derivative, hence the model acquiring the name N^6-C8 (Figure 3.1).[3] An analysis of these models by Van der Wenden *et al.*[4] suggested that the latter model was more appropriate based on better spatial compatibility. However, the suggested pharmacophore by Van Galen *et al.*[2] describes only adenosine A_1 receptor antagonists and thus the relevancy of the 'flipped' vs. the 'N^6-C8' models is negligible. This pharmacophore noted the start of a number of structurally very different compounds, encompassing a variety of bi- and tri-cyclic heteroaromatic systems. Analysis of these compounds in subsequent reviews revealed that they almost all fulfill certain criteria: they possess (i) a planar central structure, (ii) aromatic or π-electron rich and (iii) nitrogen-containing 6:5 fused heterocycles.[5,6]

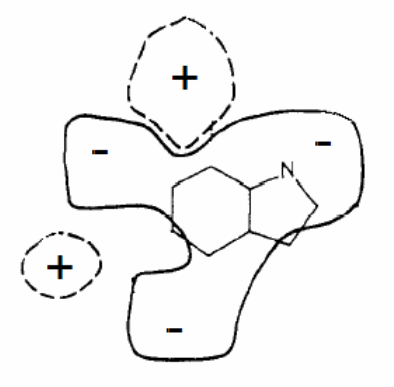


Figure 3.2. Depiction of the pharmacophore as described by Van Galen *et al.*[2] The 6:5 fused heteroaromatic core gives rise to areas of negative electrostatic potential, seen as (-) in the diagram. Two common areas of positive electrostatic potential (+) are also described.

Further modelling studies based on the superimposition of the antagonist with the agonist[7,8] have been published and more recently the protein itself has been modelled, identifying the potential importance of specific amino acids (see Chapter 2). However, actual suggestions that update the criteria mentioned above have been lacking, despite the identification of new compounds that no longer conform, e.g., the 6:6 fused naphthyridines[9] and the mono-cyclic thiazoles/thiadiazoles.[10,11]

In this chapter, molecular modelling of a number of ligands further refined the criteria for ligands that act as adenosine A_1 receptor antagonists. Subsequent appraisal of this model was performed by the development of a set of ligands that fulfilled the requirements, yet were significantly different from compounds that had been previously synthesised and tested.

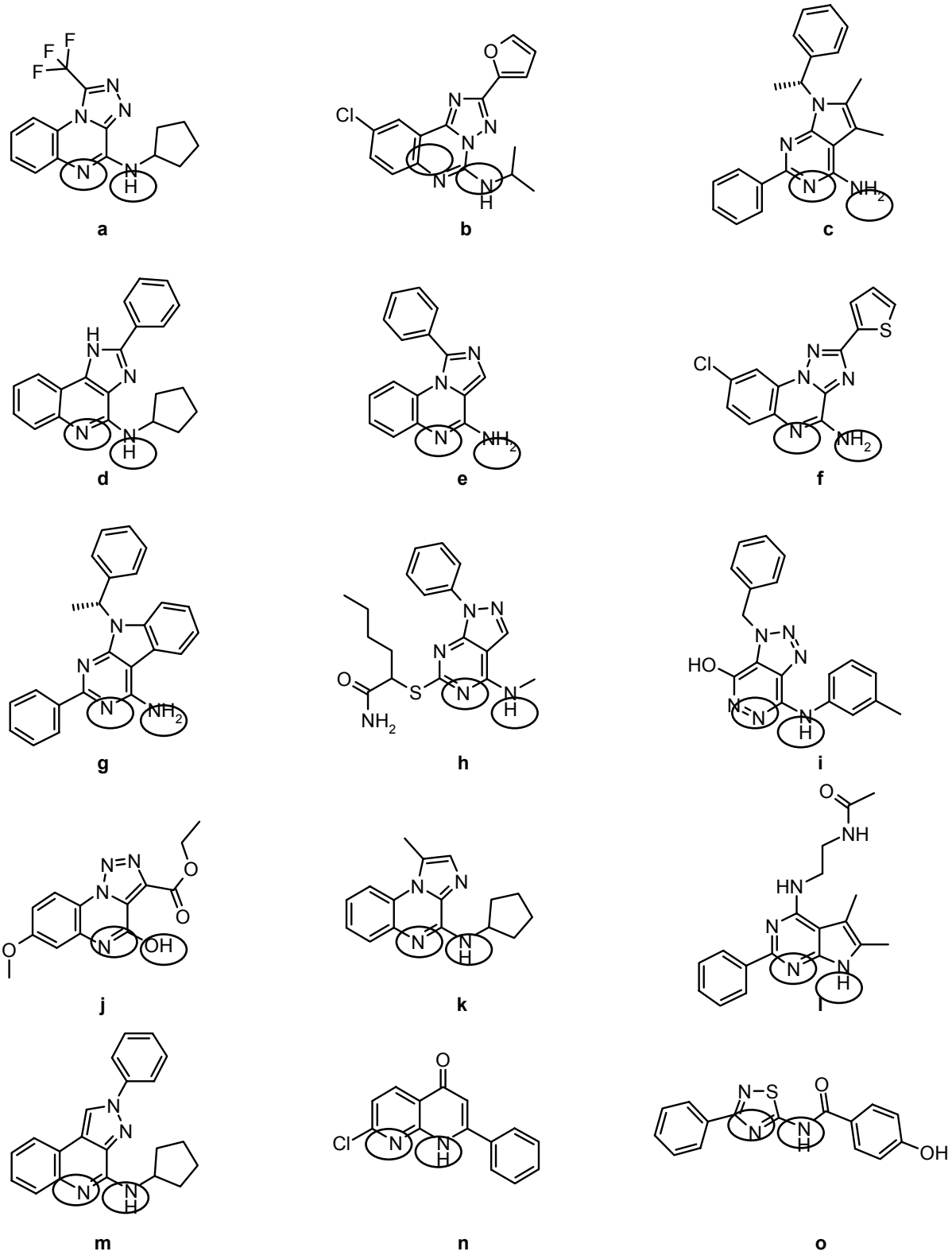


Figure 3.3. The 15 A_1 adenosine receptor antagonists modelled in this study. Of the two areas marked on each molecule, the left ellipse signifies electronically rich regions corresponding to hydrogen-bond accepting regions. The area marked with the right-hand ellipse on each molecule depicts electron-poor regions corresponding to hydrogen-bond donating regions. **a** $K_i(rA_1)$ = 7.3 nM;[12] **b** $IC_{50}(rA_1)$ = 22 nM;[13] **c** $K_i(rA_1)$ = 5 nM;[14] **d** $K_i(rA_1)$ = 10 nM;[15] **e** $K_i(rA_1)$ = 130 nM;[16] **f** $K_i(rA_1)$ = 12 nM;[16] **g** $K_i(rA_1)$ = 3 nM;[17] **h** $K_i(rA_1)$ = 0.8 nM;[18] **i** $K_i(sA_1)$ = 7 nM;[19] **j** $K_i(sA_1)$ = 29 nM;[20] **k** $K_i(rA_1)$ = 8 nM;[21] **l** $K_i(hA_1)$ = 12 nM;[22] **m** $K_i(bA_1)$ = 3 nM;[23] **n** $K_i(bA_1)$ = 0.15 nM;[9] **o** $K_i(rA_1)$ = 7 nM.[10]

3.2 Results and Discussion

3.2.1 Molecular Modelling.

Molecular modelling work was conducted on a number of ligands with high affinity and selectivity for the A_1 adenosine receptor as shown in Figure 3.3. These compounds were selected, based on the available literature at the end of 2000 and their diversity with respect to each of their central cores, with the only repetition being examples **c** and **l**, which possess very different substituents about the central structure. The actual ligand depicted and used in the modelling was that which displayed the most affinity for the A_1 receptor of each series. The molecules were drawn in the SPARTAN[24] molecular modelling package, minimised, and only the lowest energy conformer was taken into consideration. This conformer was then subjected to surface calculations from which an electrostatic potential energy was drawn, and upon which the electron density was mapped. Relatively electronegative and electron-poor regions were denoted by colour-coded regions. These areas were mapped upon each of the compounds shown in Figure 3.3. It became clear that each molecule had in common an electron-rich and poor area at the 'bottom', as denoted by the ellipses in Figure 3.3. These areas correspond to hydrogen-bond accepting and donating regions, respectively. Superimposition of the compounds using these regions as a basis resulted in the illustration seen in Figure 3.4a. Figure 3.4b shows the electron density mapped upon the electrostatic potential of compound f overlayed upon the superimpostitions from Figure 3.4a.

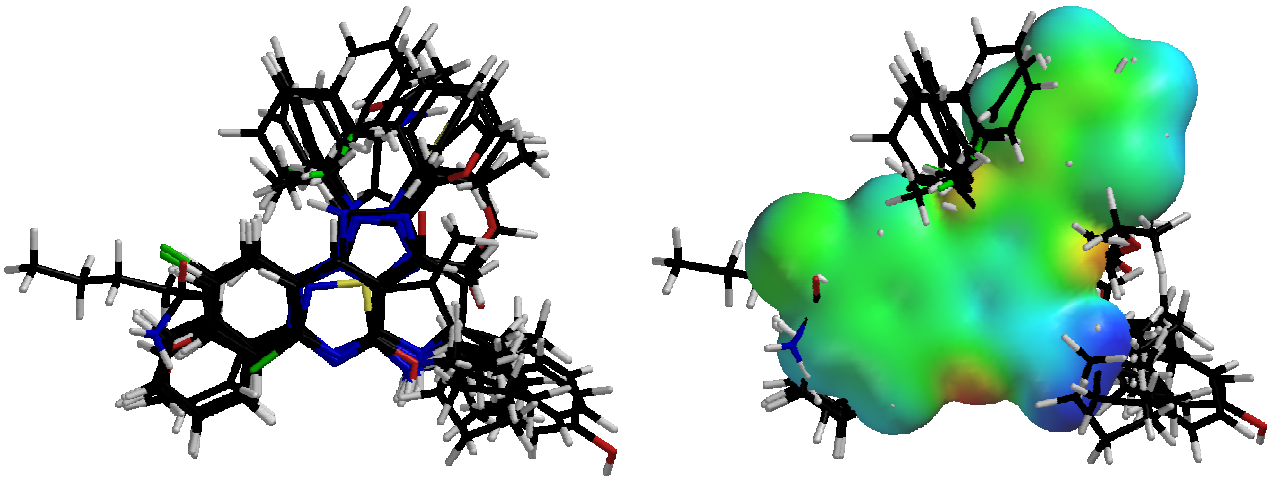


Figure 3.4. a) The 15 compounds mapped upon the adjacent electron-rich and electron-poor regions at the 'bottom' of each molecule, b) The inclusion of just one electrostatic potential map (of compound f) typical of this selection of compounds to illustrate the relative degrees of electronegativity seen in the molecular modelling study.

This resulting pharmacophore derived from the molecular modelling is illustrated with a schematic diagram in Figure 3.5. The criteria to create ligands with good affinity at the adenosine A_1 receptor seem to include the following aspects: a requirement for neighbouring electronically rich and poor regions at the 'bottom' of the molecule (labelled regions A and B in Figure 3.5), corresponding to hydrogen-bond accepting and donating regions, respectively.

At the 'top' of the figure (region C) there is a requirement for another hydrogen-bond acceptor. Furthermore, three lipophilic entities about the central planar core, labelled L1, L2 and L3, are desirable for good binding properties.

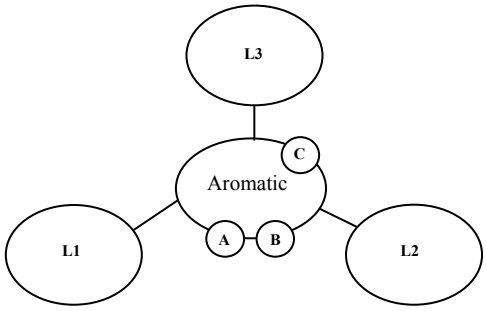


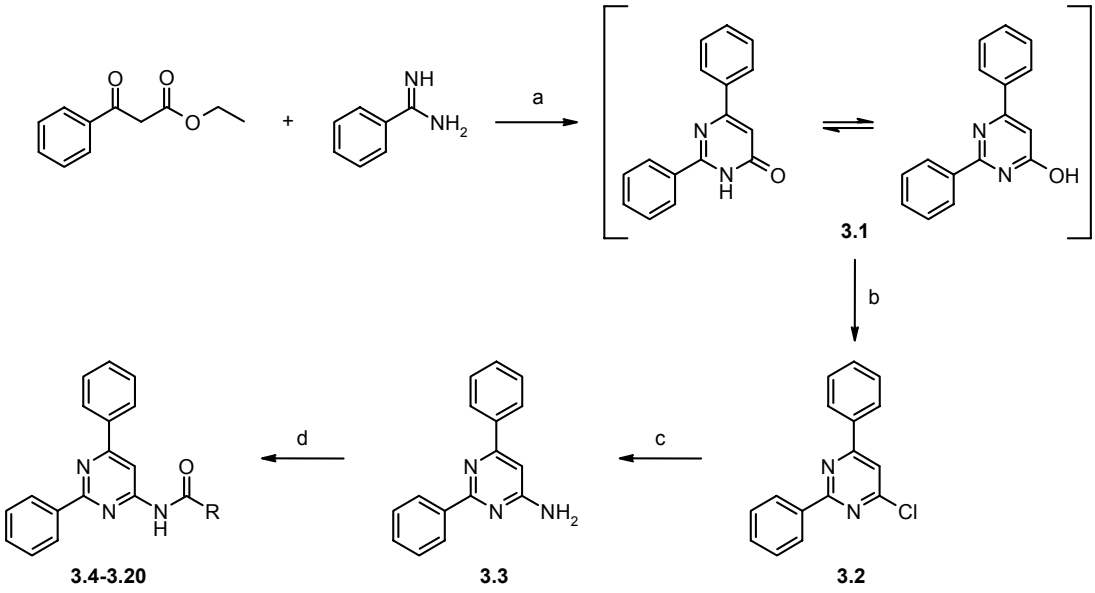
Figure 3.5. Schematic diagram of the resulting pharmacophore attained from the modelling study. **A** and **B** represent H-bond accepting and donating regions at the 'bottom' of the molecule, respectively. An H-bond acceptor is denoted by **C**. Three lipophilic domains are represented by L_1, L_2 and L_3.

Since the design of this pharmacophore, several papers have been published which draw similar conclusions. A model for the bovine A_1 adenosine receptors was published by Da Settimo *et al.* to rationalise the SARs of a set of aryl-triazino-benzimidazolones.[25] This model was further exploited by the same group to provide a series of imidazotriazines with good affinity for the bovine A_1 receptor.[26] In 2002, Bondavalli and co-workers[27] carried out some computational work on a collection of eleven different A_1 antagonists using different methods to achieve a similar pharmacophore to that of Da Settimo *et al*. These models however were all based on bi- and tri-cyclic heteroaromatic cores, fuelling new antagonists which were again bi-cyclic - the pyrazolopyridines of Bondavalli, or tri-cyclic – the triazinobenzimidazoles by Da Settimo. In our case, the model proposed only suggests an aromatic core with the given characteristics, to be substituted accordingly to fill the lipophilic pockets. Thus, it seemed that to fulfil the requirements of this model, a singular non-fused heterocycle as the central core would be sufficient. Singular non-fused heterocycles have seldom been seen as adenosine receptor antagonists as detailed in Chapter 2. Of the few exceptions to this were the thiazoles and thiadiazoles as published in 2001.[10,11] Examining the model in more detail, a single aromatic group containing a nitrogen atom would fit the hydrogen-bond accepting region. An amido group in an adjacent position would fulfil hydrogen-bond donating and accepting regions B and C. The two lipophilic pockets L1 and L3 could be 'filled' with phenyl groups to give an almost symmetric core, leaving the pocket L2 to be explored thoroughly. Thus the 2,6-diphenyl-4-amido-pyrimidines were conceived.

3.2.2 Chemistry

The pyrimidinone ring was created by the reaction of the commercially available β-ketoester, ethyl benzoate, with benzamidine hydrochloride in the presence of a base (**3.1**). The first attempts to achieve the condensation using sodium ethoxide rendered only poor yields; changing the base to sodium hydroxide in accordance to a script by De Valk and Van der

Plas[28] improved the reaction significantly, resulting in a yield of 59%. Displacement of the hydroxide function was achieved with an excess of phosphoryl chloride, containing phosphorous pentachloride, according to a preparation by Brown *et al*.[29] Subsequent substitution with ammonia in a sealed vessel gave the key 4-amino-2,6-diphenyl pyrimidinyl intermediate (**3.3**). The final step to give compounds **3.4-3.20** involved the formation of the amide bond. Attempts using the carboxylic acid with standard coupling agents, for example EDC and HOBt, gave poor or no yields. As an alternative, the amine was reacted with acid chlorides in the presence of triethylamine to give, in general, good to excellent yields.

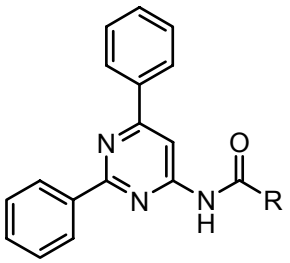


Scheme 1. Synthetic route to the 4-amido-2,6-diphenyl-pyrimidines. (a) NaOH, EtOH; (b) $POCl_3$, PCl_5; (c) NH_3, EtOH; (d) RCOCl, Et_3N, 1,4-dioxane.

3.2.3 Structure Activity Relationships

The results of the binding assays are represented in Table 3.1. There are distinct and vital differences in affinity for the compounds across the receptors, thus highlighting the variation in the electronic and spatial requirements necessary to create good ligands for the adenosine receptors. In consequence to previously published material by our group,[30] the first amides prepared were phenyl derivatives based on the Topliss system of substitution,[31] and assumed the L2 pocket to be similar to L1 and L3 in size and shape (Figure 3.5) . The initial compound (the unsubstituted phenyl derivative) shows fair affinity for the A_1 adenosine receptor at 670 nM with a two-fold selectivity over the A_3 receptor (K_i = 1300 nM). Substituting following the Topliss system gave little or no improvement in affinity, with only 4-methoxyphenyl derivative **3.6** showing a very slight gain in affinity at the A_3 receptor, in agreement with our previous publications. With the alkyl amides, significant improvements were made over the phenyl substituents at the L2 pocket. In the straight-chained alkyls, from methyl to pentyl (**3.7-3.10**) a distinct optimum is apparent for a two-carbon chain (compound **3.8**) at the A_1 receptor, with an affinity of 9.5 nM.

Table 3.1. Affinities of the 4-Amido-2,6-Diphenyl-Substituted-Pyrimidines **3.4-3.20** in Radioligand Binding Assays of Human Adenosine Receptors.



	R	K_i (nM) or % displacement[a]		
		A_1[b]	A_{2A}[c]	A_3[d]
3.4	Ph	671 ± 110	17%	1300 ± 300
3.5	4-Cl-Ph	35%	0%	33%
3.6	4-MeO-Ph	30%	0%	1170 ± 150
3.7	Me	38 ± 8	489 ± 140	6.9 ± 2
3.8	Et	9.5 ± 5	82 ± 14	22 ± 9
3.9	Pr	18 ± 5	124 ± 17	167 ± 51
3.10	Bu	109 ± 15	48%	392 ± 65
3.11	iPr	11 ± 6	376 ± 76	267 ± 39
3.12	2-MePr	15 ± 3	157 ± 42	447 ± 62
3.13	1-EtPr	6.4 ± 0.4	381 ± 33	57%
3.14 (LUF 5767)	1-MePr	2.2 ± 1	899 ± 130	147 ± 22
3.15	tBu	28 ± 6	18%	49%
3.16 (LUF 5764)	2,2-diMePr	8.8 ± 4	32%	39%
3.17	c-Pr	7.6 ± 1	189 ± 44	25 ± 2
3.18	c-Bu	6.5 ± 2	179 ± 58	178 ± 57
3.19 (LUF 5740)	c-Pent	2.1 ± 0.1	196 ± 66	170 ± 60
3.20	c-Hex	16 ± 8	208 ± 77	32%

[a]K_i ± SEM (n = 3), % displacement (n = 2). [b]Displacement of specific [^{3}H]DPCPX binding in CHO cell membranes expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations. [c]Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cell membranes expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations. [d]Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cell membranes expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations.

This pattern is repeated at the A_{2A} receptor, with a K_i of 82 nM (**3.8**). At the A_3 receptor however, the methyl-substituted derivative (**3.7**), with an affinity of 7 nM was far and away the better compound. In terms of selectivity, compound **3.7** was 5-fold more selective for the A_3 receptor than for the A_1 receptor, whilst in a slight reversal of this compound **3.8** showed a 2-fold selectivity in favour of the A_1 adenosine receptor over the A_3 receptor. Better selectivity for the A_1 adenosine receptor came with the n-propyl derivative (**3.9**) with an almost 7-fold better affinity over the A_{2A} receptor (K_i = 17.6 nM vs. K_i = 124 nM) and by almost a factor of ten with the A_3 adenosine receptor (K_i = 167 nM). From these results, it seems that the lipophilic pocket (L2) is not quite as 'deep' for the A_1 adenosine receptor as expected.

To explore the 'breadth' versus 'depth' hypothesis of the L2 pocket thrown up by the results of the straight-chained alkyls, a number of branched alkyl derivatives (**3.11-3.15**) were made. Though all showed good to fair affinity across the adenosine receptors, the overall bias was

towards the A_1 receptor. The isopropyl compound (**3.11**) showed a 24-fold selectivity for the A_1 receptor over the A_3 with an affinity of 11 nM vs. 267 nM. Improving upon this, the isopentyl (**3.13**) showed a K_i value of 6 nM at the A_1 receptor, whilst losing a significant amount of affinity at the A_3 receptor. Compound **3.14** was overall the best of the branched alkyl group in terms of affinity to the A_1 receptor with an affinity of 2.2 nM. The large, sterically hindered t-butyl group only seems to be tolerated at the A_1 receptor with compounds **3.15** and **3.16** showing little affinity for either the A_{2A} or the A_3 receptors, making these compounds highly selective for the A_1 adenosine receptor.

The cycloalkyl derivatives (**3.17-3.20**) were made to combine and confirm the length and breadth hypothesis of the L2 pocket of the A_1 receptor drawn from the branched and straight alkyl derivatives. It is notable that the cyclopentyl compound (**3.19**) shows the best affinity with a K_i of 2.1 nM at the A_1 receptor, suggesting an optimal two-three carbon chain length of the pocket. It can also be compared favourably to the isopropyl, isobutyl, and isopentyl in terms of the width. The affinity of the compounds at the A_{2A} and A_3 adenosine receptors were only fair (the only exception being the good affinity of the cyclopropyl derivative, K_i = 25 nM for the A_3 receptor), and thus showing an overall marked selectivity for the A_1 receptor. It is notable that the cyclopentyl group occurs more frequently as the compound in a series with the highest affinity for the A_1 receptor; namely, in the structurally different compounds of the imidazoquinolines,[15] the imidazoquinozalinamines,[21] the triazoloquinoxalines,[12] and the xanthines (DPCPX).[32] This is however, the first thorough examination of this particular area of the receptor site in such a manner. We have found that in agreement with Da Settimo *et al*.[25] the L2 pocket is relatively small and compact. This hypothesis was disputed by the Bondavalli group[27] due to the good affinity at the bovine A_1 receptor of one of their compounds, which incorporated a rather large side chain. We can see clearly in our results that there is some affinity for a phenyl substituent, i.e., that the pocket can accommodate a phenyl ring, but that this is not optimal.

It is also interesting to note the different requirements across the receptors. Although it has not been optimised in this paper, there is a distinct trend to be seen in the results at the A_{2A} and A_3 receptors. Affinity at the A_{2A} peaked with a relatively short narrow chain - the ethyl group at this pocket, and the A_3 affinity increased with the very smallest substituents, namely the methyl and the cyclopropyl groups.

3.3 Conclusion

Presented in this chapter is a novel series of adenosine receptor antagonists which have been designed and synthesised on the basis of a pharmacophore derived from the modelling of a number of previously published ligands. The idea of a mono-cyclic heteroaromatic core as the basis of an antagonist has been proven to be compatible with the adenosine receptor site, and one of the proposed lipophilic pockets of the site has been thoroughly investigated in terms of spatial requirements.

3.4 Experimental Section

3.4.1 Molecular Modelling

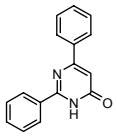
Molecular modelling work was performed with the SPARTAN molecular modelling package version 5.0 (Wavefunction Inc.)[24] running on a Silicon Graphics O_2 workstation. Default values in the Merck Force Field were used in Molecular Mechanics minimisations. Conjugate gradient energy minimisations were continued until the rms energy derivative was less than 0.001 kcal·mol^{-1}Å^{-1}. The conformers were generated using the systematic search method. The energy and molecular electrostatic potential was calculated using the semi-empirical molecular orbital program AM1. The electrostatic potentials were sampled over the entire accessible surface of the molecules (equal to a Van der Waals contact surface). The most negative electrostatic potential is depicted as red and the most positive as blue.

3.4.2 Chemistry

Materials and Methods

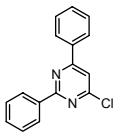
All reagents used were obtained from commercial sources and all solvents were of an analytical grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad and ar = aromatic protons. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Leiden Institute of Chemistry and are within 0.4% of the theoretical values unless otherwise stated. Reactions were routinely monitored by TLC using Merck silica gel F_{254} plates.

2,6-Diphenyl-3H-pyrimidin-4-one (3.1).



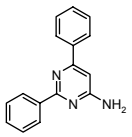
Benzamidine hydrochloride (3.0 g, 19.2 mmol) was dissolved in a minimal amount of H_2O (10 mL), to this was added NaOH (0.8 g, 19.2 mmol, 1 eq.) dissolved in H_2O (2 mL), followed by ethylbenzoate (3.5 mL, 20.2 mmol, 1.05 eq.). Ethanol was then added until a clear solution was obtained. The reaction mixture was allowed to stir at room temperature overnight yielding a thick suspension, which was filtered to give a white solid. After washing with diethyl ether to remove unreacted/excess β-ketoester the solid was dried *in vacuo* to give 59% of the desired product. ^{1}H NMR δ (DMSO-d6): 8.31-8.18 (m, 5H, phenyl-*H*), 7.60-7.54 (m, 5H, phenyl-*H*), 6.92 (s, 1H, pyrimidine-*H*).

4-Chloro-2,6-diphenyl-pyrimidine (3.2).



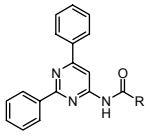
Phosphorous oxychloride (9.30 mL, 99.8 mmol, 7.5 eq.) was added dropwise to 2,6-diphenyl-3H-pyrimidin-4-one (3.3 g, 13.3 mmol) in a vigorous reaction. To this mixture was added cautiously and portionwise phosphorous pentachloride (2.77 g, 13.3 mmol, 1 eq.) and the reaction mixture was stirred at reflux for 3 hours. The reaction mixture was quenched by pouring into ice-water, and extracted with ethyl acetate (3 x 150 mL). The combined organic layers were washed with water, dried ($MgSO_4$) and then concentrated to give a yellow solid. This was recrystallised from ethanol to give fine white needles (65%). ^{1}H NMR δ ($CDCl_3$): 8.60-8.18 (m, 5H, phenyl-*H*), 7.63 (s, 1H, pyrimidine-*H*), 7.51-7.57 (m, 5H, phenyl-*H*).

2,6-Diphenyl-pyrimin-4-ylamine (3.3).



Ethanol (50 mL) was saturated with $NH_{3(g)}$ at 0 °C and added to 4-chloro-2,6-diphenyl-pyrimidine (2.30 g, 8.63 m mol) in a sealed vessel. This was stirred at 140 °C for 24 h. Upon cooling and concentrating, the residue was extracted with hot chloroform (3 x 50 mL) and the solvent evaporated *in vacuo*. The crude product was purified by column chromatography on SiO_2 eluting with CH_2Cl_2 to give an off-white solid (80%). ^{1}H NMR δ (DMSO-d6): 8.47-8.42 (m, 2H, phenyl-*H*), 8.16-8.13 (m, 2H, phenyl-*H*), 7.57-7.5 (m, 6H, phenyl-*H*), 7.02 (br s, 2H, NH_2), 6.88 (s, 1H, pyrimidine-*H*).

General Procedure for the Preparation of 4-Amido-2,6-diphenylpyrimidines (3.4-3.20).



To a solution of 4-amino-2,6-diphenylpyrimidine (0.202 mmol, 1 eq.) in 1,4-dioxane (5 mL) was added triethylamine (0.223 mmol, 1.1 eq.), followed by the appropriate acid chloride (0.304 mmol, 1.5 eq.). This was then stirred at reflux until no starting material was visible by TLC. Upon completion, the reaction mixture was separated between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was further extracted with ethyl acetate (2 × 20 mL) and the combined organics washed with water. After drying over $MgSO_4$ and evaporation under reduced pressure, the crude product was purified by column chromatography, eluting with a petroleum ether-ethyl acetate or a dichloromethane-methanol solvent system. Recrystallisation from ethanol or petroleum ether-ethyl acetate gave the corresponding amide in crystalline form.

N-(2,6-Diphenyl-pyrimidin-4-yl)-benzamide (3.4). Yield 48%; white solid; mp 120-123 °C; ^{1}H NMR δ($CDCl_3$): 8.78 (br s, 1H, N-*H*), 8.72 (s, 1H, pyrimidine-*H*), 8.58-8.54 (m, 2H, phenyl-*H*), 8.34-8.29 (m, 2H, phenyl-*H*), 7.99-7.96 (m, 2H, phenyl-*H*), 7.64-7.48 (m, 9H, phenyl-*H*). ^{13}C-NMR δ($CDCl_3$): 166.2, 165.9, 164.0, 158.4, 137.3, 137.1, 133.4, 132.6, 130.8, 130.7, 128.9, 128.7, 128.3, 128.1, 127.4, 127.2, 103.3. MS (ES^+): 351.57, 373.55 Da. Anal. ($C_{23}H_{17}N_3O.0.3H_2O$) C, H, N.

4-Chloro-N-(2,6-diphenyl-pyrimidin-4-yl)-benzamide (3.5). Yield 57%; white solid; mp 182 °C; ^{1}H NMR δ($CDCl_3$): 8.70 (br s, 1H, N-*H*), 8.68 (s, 1H, pyrimidine-*H*), 8.57-8.52 (m, 2H, phenyl-*H*) 8.33-8.28 (m, 2H, phenyl-*H*), 7.93-7.89 (m, 2H, aromatic-*H*), 7.56-7.49 (m, 8H, phenyl-*H*). ^{13}C-NMR δ($CDCl_3$): 165.2, 164.0, 158.3, 157.1, 139.2, 137.1, 136.2, 131.9, 131.0, 130.9, 129.3, 128.9, 128.8, 128.7, 128.5, 128.2, 127.5, 127.2, 103.4. MS (ES^+): 385.85, 407.9 Da. Anal. ($C_{23}H_{16}ClN_3O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-4-methoxy-benzamide) (3.6). Yield 12%; white solid; mp 155-156 °C; ^{1}H NMR δ($CDCl_3$): 8.73 (s, 1H, pyrimidine-*H*), 8.67 (br s, 1H, N-*H*), 8.59-8.54 (m, 2H, phenyl-*H*) 8.34-8.29 (m, 2H, phenyl-*H*), 8.00-7.96 (m, 2H, aromatic-*H*), 7.55-7.51 (m, 6H, phenyl-*H*), 7.06-7.02 (m, 2H, aromatic-*H*), 3.91 (s, 3H, C*H*$_3$). MS (ES^+): 381.85 Da. Anal. ($C_{24}H_{19}N_3O_2.0.4H_2O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-acetamide (3.7). Yield 43%; white solid; mp 140 °C; ^{1}H NMR δ($CDCl_3$): 8.54-8.49 (m, 3H, phenyl-*H* + pyrimidine-*H*) 8.45 (s, 1H, N-*H*), 7.55-7.49 (m, 6H, phenyl-*H*), 2.20 (s, 3H, C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 165.9, 158.1, 154.3, 140.7, 130.74, 130.68, 128.7, 128.4, 128.0, 127.4, 103.0, 35.7. MS (ES^+): 289.89 Da. Anal. ($C_{18}H_{15}N_3O.0.5EtOH$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-propionamide (3.8). Yield 77%; white solid; mp 125-126 °C; ^{1}H-NMR δ($CDCl_3$): 8.58 (s, 1H, pyrimidine-*H*), 8.55-8.50 (m, 2H, phenyl-*H*), 8.36 (br s, 1H, N*H*), 8.30-8.25 (m, 2H, phenyl-*H*), 7.54-7.49 (m, 6H, phenyl-*H*), 2.41(q, 2H, J= 7.3 Hz, C*H*$_2$CH$_3$), 1.23 (t, 2H, -CH$_2$C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 173.2, 165.8, 163.9, 137.3, 137.0, 130.7, 128.7, 128.0, 127.4, 121.5, 103.1, 30.7, 8.87. MS (ES^+): 303.8 Da. Anal. calc. for $C_{19}H_{17}N_3O$ (C 75.23; H 5.65; N 13.85) found (C 75.32; H 6.23; N 14.04) %.

N-(2,6-Diphenyl-pyrimidin-4-yl)-butyramide (3.9). Yield 45%; white solid; mp 102-103 °C; ^{1}H-NMR δ($CDCl_3$): 8.60 (br s, 2H, pyrimidine-*H* + N*H*), 8.56-8.51 (m, 2H, phenyl-*H*), 8.31-8.26 (m, 2H, phenyl-*H*), 7.45-7.50 (m, 6H, phenyl-*H*), 2.29 (t, 2H, J = 7.48 Hz, C*H*$_2$CH$_2$CH$_3$), 1.71 (sextet, 2H, J = 7.39 Hz, CH$_2$C*H*$_2$CH$_3$), 0.95 (t, 3H, J = 7.30 Hz, CH$_2$CH$_2$C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 172.9, 165.8, 163.8, 158.5, 137.4, 137.0, 130.8, 130.7, 128.6, 128.4, 128.1, 127.3, 103.3, 39.2, 18.3, 13.5. MS (ES^+): 317.87 Da. Anal. ($C_{20}H_{19}N_3O.\ 0.1H_2O$) C, H, N.

Hexanoic acid (2,6-diphenyl-pyrimidin-4-yl)-amide (3.10). Yield 53%; white solid; mp 40 °C; ^{1}H-NMR δ($CDCl_3$): 8.53 (s, 1H, pyrimidine-*H*), 8.52-8.49 (m, 2H, phenyl-*H*,) 8.30-8.23 (m, 3H, phenyl-*H* + N-*H*), 7.55-7.47 (m, 6H, phenyl-*H*), 2.43 (t, 2H, J = 7.30 Hz, C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.82-1.67 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.41-1.21 (m, 4H, CH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_3$), 0.96-1.89 (m, 3H, CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 172.8, 165.9, 164.0, 158.4, 137.4, 137.1, 130.8, 128.6, 128.4, 128.1, 127.4, 103.2, 37.2, 31.1, 24.6, 22.2, 13.8. MS (ES^+): 345.88, 690.58 Da Anal. ($C_{22}H_{23}N_3O.\ 1.7\ H_2O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-isobutyramide (3.11). Yield 39%; white solid; mp 116-117 °C; ^{1}H-NMR δ($CDCl_3$): 8.59 (s, 1H, pyrimidine-*H*), 8.55-8.50 (m, 2H, phenyl-*H*), 8.30-8.25 (m, 2H, phenyl-*H*), 8.05 (br s, 1H, N*H*), 7.54-7.49 (m, 6H, phenyl-*H*), 2.64 (septet, 1H, J = 6.85 Hz, C*H*(CH$_3$)$_2$), 1.33 (d, 6H, J = 6.94 Hz, CH(C*H*$_3$)$_2$). ^{13}C-NMR δ($CDCl_3$): 176.5, 165.8, 158.3, 137.4, 137.1, 130.7, 128.7, 128.4, 128.0, 127.4, 103.4, 36.8, 19.2, 19.1. MS (ES^+): 317.94, 634.75 Da. Anal. ($C_{20}H_{19}N_3O.0.1H_2O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-3-methyl-butyramide (3.12). Yield 52%, white solid; mp 127°C; ^{1}H-NMR δ($CDCl_3$): 8.59 (s, 1H, pyrimidine-*H*), 8.56-8.51 (m, 2H, phenyl-*H*), 8.35 (br s, 1H, N*H*), 8.31-8.26 (m, 2H, phenyl-*H*), 7.56-7.49 (m, 6H, phenyl-*H*), 2.25-2.24 (m, 3H, C*H*$_2$C*H*(CH$_3$)$_2$), 1.02-0.99 (d, 6H, CH$_2$CH(C*H*$_3$)$_2$). ^{13}C-NMR δ($CDCl_3$): 172.1, 165.9, 158.2, 137.4, 137.1, 130.7, 130.6, 128.6, 128.4, 128.0, 127.4, 113.5, 103.2, 46.8, 25.8, 22.3ppm. MS (ES^+): 331.8 Da. Anal. ($C_{21}H_{21}N_3O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-2-ethyl-butyramide (3.13). Yield 66%, white solid; mp 137-138 °C; ^{1}H-NMR δ($CDCl_3$): 8.64 (s, 1H, pyrimidine-*H*), 8.55-8.50 (m, 2H, phenyl-*H*), 8.31-8.26 (m, 2H, phenyl-*H*), 8.09 (br s, 1H, N*H*), 7.54-7.49 (m, 6H, phenyl-*H*), 2.23-2.11 (m, 1H, C*H*(CH$_2$CH$_3$)$_2$), 1.86-1.56 (m, 4H, CH(C*H*$_2$CH$_3$)$_2$), 0.99 (t, 6H, J = 7.31 Hz, CH(CH$_2$C*H*$_3$)$_2$). ^{13}C-NMR δ($CDCl_3$): 175.8, 165.9, 158.3, 130.8, 130.7, 128.7, 128.4, 128.1, 127.4, 121.6, 103.2, 52.2, 25.5, 11.8. MS (ES^+): 345.86, 690.56 Da. Anal. ($C_{22}H_{23}N_3O.\ 0.1H_2O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-2-methyl-butyramide (3.14), Yield 89%, white solid; mp; 102 °C; ^{1}H-NMR δ($CDCl_3$): 8.71 (br s, 1H, N-*H*), 8.67 (s, 1H, pyrimidine-*H*), 8.59-8.54 (m, 2H, aromatic-*H*), 8.33-8.28 (m, 2H, aromatic-*H*), 7.53-7.50 (m, 6H, aromatic-*H*), 2.29-2.19 (m, 1H, CH), 1.82-1.86 (m, 1H, 0.5*C*H*$_2$), 1.55-1.41 (m, 1H, 0.5*C*H*$_2$), 1.16 (d, J=6.58Hz, 3H, C*H*$_3$), 0.90 (t, J=7.30Hz, 3H, C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 176.4, 165.9, 163.9, 158.5, 137.4, 137.1, 130.8, 130.7, 128.7, 128.4, 128.1, 127.4, 103.3, 44.0, 27.0, 16.9, 11.6. MS (ES^+): 331.8 (MH^+) Da. Anal. ($C_{21}H_{21}N_3O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-2,2-dimethyl-propionamide (3.15). Yield 66%, white solid; mp 52 °C; ^{1}H-NMR δ($CDCl_3$): 8.63 (s, 1H, pyrimidine-*H*), 8.58-8.51 (m, 2H, phenyl-*H*), 8.30-8.27 (m, 2H, phenyl-*H*), 8.21 (s, 1H, N-*H*), 7.54-7.51 (m, 6H, phenyl-*H*), 1.40 (s, 9H, C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 178.0, 165.8, 163.8, 158.4, 137.3, 137.1, 130.7, 130.6, 128.6, 128.3, 128.1, 127.4, 103.2, 40.0, 27.2. MS (ES^+): 331.92 Da. Anal. ($C_{21}H_{21}N_3O$) C, H, N.

N-(2,6-Diphenyl-pyrimidin-4-yl)-3,3-dimethyl-butyramide (3.16). Yield 62%, white solid; mp 134 °C; ^{1}H-NMR δ($CDCl_3$): 8.73 (br s, 1H, N-*H*), 8.64 (s, 1H, pyrimidine-*H*), 8.55-8.50 (m, 2H, aromatic-*H*), 8.32-8.27 (m, 2H, aromatic-*H*), 7.54-7.49 (m, 11H, aromatic-*H*), 2.20 (s, 2H, CH$_2$), 1.08 (s, 9H, 3*CH$_3$). ^{13}C-NMR

δ($CDCl_3$): 171.7, 165.9, 163.9, 158.4, 137.4, 137.1, 130.8, 130.7, 128.7, 128.4, 128.2, 127.4, 103.2, 51.0. 31.2, 30.0. MS (ES^+): 367.6 (MNa^+), 345.9 (MH^+) Da. Anal. ($C_{22}H_{23}N_3O$) C, H, N.

Cyclopropanecarboxylic acid (2,6-diphenyl-pyrimidin-4-yl)-amide (3.17). Yield 86% white solid; mp 127-128 °C; ^{1}H-NMR δ($CDCl_3$): 8.99 (br s, 1H, N*H*), 8.58 (s, 1H, pyrimidine-*H*), 8.56-8.52 (m, 2H, phenyl-*H*), 8.29-8.24 (m, 2H, phenyl-*H*), 7.53-7.49 (m, 6H, phenyl-*H*), 1.47-1.43 (m, 1H, -C*H*CH_2CH_2-), 1.11-1.05 (m, 2H, -CHC*H*$_2$$CH_2$-), 0.86-0.76 (m, 2H, -CHC*H*$_2$$CH_2$-). ^{13}C-NMR δ($CDCl_3$): 173.5, 165.7, 163.8, 158.4, 137.4, 137.0, 130.7, 130.6, 128.6, 128.3, 128.0, 127.2, 113.5, 103.2, 58.2, 15.7, 8.84. MS (ES^+): 315.8 Da. Anal. ($C_{20}H_{17}N_3O$) C, H, N.

Cyclobutanecarboxylic acid (2,6-diphenyl-pyrimidin-4-yl)-amide (3.18). Yield 90% white solid; mp; 121-122 °C; ^{1}H-NMR δ($CDCl_3$): 8.62 (s, 1H, pyrimidine-*H*), 8.56-8.51 (m, 2H, phenyl-*H*), 8.32-8.27 (m, 3H, phenyl-*H* + N-*H*), 7.54-7.48 (m, 6H, phenyl-*H*), 3.13 (pentet, 1H, -C*H*$CH_2CH_2CH_2$-), 2.45-1.90 (m, 6H, -CHC*H*$_2$C*H*$_2$C*H*$_2$-). ^{13}C-NMR δ($CDCl_3$): 174.6, 165.8, 163.9, 158.4, 137.1, 130.7, 128.7, 128.4, 128.0, 127.4, 103.2, 86.9, 40.7, 24.9, 17.9. MS (ES^+): 329.7 Da. Anal. ($C_{21}H_{19}N_3O$. 0.1H_2O) C, H, N.

Cyclopentanecarboxylic acid (2,6-diphenyl-pyrimidin-4-yl)-amide (3.19). Yield 69%; white solid; mp 126.5-127 °C. ^{1}H-NMR δ($CDCl_3$): 8.60 (s, 1H, pyrimidine-*H*), 8.56-8.51 (m, 2H, phenyl-*H*), 8.32-8.26 (m, 3H, phenyl-*H* + N*H*), 7.53-7.50 (m, 6H, phenyl-*H*), 2.77-2.65 (m, 1H, -C*H*$CH_2CH_2CH_2CH_2$-), 1.98-1.60 (m, 8H, -CHC*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$-). ^{13}C-NMR δ($CDCl_3$): 175.9, 165.8, 158.4, 137.4, 137.1, 130.7, 130.6, 128.7, 128.4, 128.0, 127.4, 103.2, 46.8, 30.2, 25.9. MS (ES^+): 343.7 Da. Anal. ($C_{22}H_{21}N_3O$. 0.1H_2O) C, H, N.

Cyclohexanecarboxylic acid (2,6-diphenyl-pyrimidin-4-yl)-amide (3.20). Yield 87%; white solid; mp 142-143 °C. ^{1}H-NMR δ($CDCl_3$): 8.60 (s, 1H, pyrimidinel-*H*), 8.57-8.52 (m, 2H, phenyl-*H*), 8.34 (br s, 1H, N*H*), 8.30-8.25 (m, 2H, phenyl-*H*), 7.53-7.49 (m, 6H, phenyl-*H*), 2.31-2.18 (m, 1H, -C*H*$CH_2CH_2CH_2CH_2CH_2$-), 1.97-1.30 (m, 10H, -CHC*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$-). ^{13}C-NMR δ($CDCl_3$): 175.7, 165.8, 163.8, 158.4, 137.1, 130.7, 130.6, 128.6, 128.3, 127.3, 113.6, 103.2, 46.4, 29.2, 25.3. MS (ES^+): 357.7, 358.7 Da. Anal. ($C_{23}H_{23}N_3O$. 0.2H_2O) C, H, N.

3.4.3 Biology

Materials and Methods

[^{3}H]DPCPX and [^{125}I]AB-MECA were purchased from Amersham Biosciences (NL). [^{3}H]ZM241385 was obtained from Tocris Cookson, Ltd. (UK). CHO cells expressing the human adenosine A_1 receptor were provided by Dr. Andrea Townsend-Nicholson, University College London, UK. HEK 293 cells stably expressing the human adenosine A_{2A} and A_3 receptor were gifts from Dr. Wang (Biogen, USA) and Dr. K.-N. Klotz (University of Würzburg, Germany), respectively. All compounds made were tested in radioligand binding assays to determine their affinities at the human adenosine A_1, A_{2A} and the A_3 receptors.

Adenosine A_1 Receptor

Affinity at the A_1 receptor was determined on membranes from CHO cells expressing the human receptors, using [^{3}H]DPCPX as the radioligand. Membranes containing 40 µg of protein were incubated in a total volume of 400 µL of 50 mM Tris/HCl (pH 7.4) and [^{3}H]DPCPX (final concentration 1.6 nM) for 1 h at 25 °C in a shaking water bath. Non-specific binding was determined in the presence of 10 µM CPA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell Harvester. Filters were washed three times with ice-cold buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 2 h radioactivity was counted in an LKB rack β scintillation counter.

Adenosine A_{2A} Receptor

At the A_{2A} receptor, affinity was determined on membranes from HEK 293 cells stably expressing this receptor, using [^{3}H]ZM 241385 as the radioligand. Membranes containing 25-30 µg of protein were incubated in a total volume of 200 µL of 50 mM Tris/HCl (pH 7.4) and [^{3}H]ZM 241385 (final concentration 2.0 nM) for 2 h at 25 °C in a shaking water bath. Non-specific binding was determined in the presence of 100 µM CPA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell Harvester. Filters were washed three times with ice-cold buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 2 h radioactivity was counted in an LKB rack β scintillation counter.

Adenosine A_3 Receptor

The affinity at the A_3 receptor was measured on membranes from HEK 293 cells stably expressing the human A_3 receptor, using [^{125}I]AB-MECA as the radioligand. Membranes containing 20-40 µg of protein were incubated in a total volume of 100 µL of 50 mM Tris/HCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.01% CHAPS (pH 7.4), and [^{125}I]AB-MECA (final concentration 0.10 nM) for 1 h at 37 °C in a shaking water bath. Non-specific binding was determined in the presence of 100 µM R-PIA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell Harvester. Filters were washed three times with ice-cold buffer and placed in scintillation vials. Radioactivity was counted in a γ counter.

Data Analysis

K_i values were calculated using a non-linear regression curve-fitting program (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA). K_D values of the radioligands were 1.6 nM, 1.0 nM and 5.0 nM for [^{3}H]DPCPX, [^{3}H]ZM 241385 and [^{125}I]AB-MECA, respectively.

3.5 References

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Chapter 4

Substituted Pyrimidines as a New Class of Selective Adenosine A_1 Receptor Antagonists

Part II: 2-Amido-4,6-Diphenyl-Pyrimidines

Adenosine mediates a number of responses in the central nervous system (CNS) via cell membrane receptors. The four known adenosine receptors are present in varying degrees throughout the human body, and A_1 and A_{2A} receptors are particularly prevalent in the brain. The blockade of adenosine A_1 receptors in the brain may be useful in therapeutic areas such as cognition enhancement and sleep disorders. Designing a selective compound that is able to cross the blood-brain barrier is therefore of great importance. In this chapter a restriction is proposed in relation to the polar surface area of a compound in the quest to design and synthesise an adenosine A_1 receptor antagonist that may act at the CNS. We show that a novel series of 2-amido-4,6-diphenyl-pyrimidines are selective A_1 antagonists with a polar surface area within the desired range for a compound to be able to cross the blood-brain barrier. In particular, compound **4.23** (LUF 5735) shows excellent affinity (K_i = 4 nM) and selectivity (displacement of the radioligand at the A_{2A} and A_3 receptors at 1 μM <7% and <38% respectively), and has a polar surface area of 53 $Å^2$.

Based upon L. C. W. Chang, R. F. Spanjersberg, J. K. von Frijtag Drabbe Künzel, T. Mulder-Krieger, G. van den Hout, M. W. Beukers, J. Brussee, A. P. IJzerman, J. Med. Chem. ***2004****, 47, 6529-6540.*

4.1 Introduction

As described in Chapter 1, adenosine receptors are localised throughout the human body in varying levels of expression and facilitate different reactions in accordance to physiological needs. Adenosine A_1 receptors are in abundance in the mammalian brain,[1] and the role that they play in important functions, such as in the modulation of neurotransmitter release, sleep regulation and cognition enhancement, has been thoroughly investigated.[2-4] For this reason it is essential that a compound targeted at these therapeutic areas is able to cross the blood-brain barrier (BBB). Research into the BBB and the ability of a compound to cross it has become a highly investigated topic in recent years. A recent review[5] highlighted some 'rules of thumb' which have emerged from numerous research articles from the past few years. Amongst these was the almost qualitative example that the sum of the nitrogen and oxygen atoms in a molecule should be five or less for that molecule to have a greater chance of entering the brain.[6] Of the more quantitative prediction techniques, the rule that for good brain permeation, as for good intestinal absorption, the polar surface area (PSA) of a molecule should be below a certain limit has been very thoroughly investigated.[7-11] The PSA is defined as the surface area of a molecule occupied by nitrogen and oxygen atoms, and hydrogen atoms that are attached to these atoms.[11] It has become one of the most convenient and reliable parameters to calculate, and the limits for brain penetration according to research, e.g., by Kelder *et al*.[9] have been proposed to be in the region of 60-70 $Å^2$.

In the preceding chapter, the design and synthesis of a series of pyrimidines as a new class of A_1 adenosine antagonists was described. With the notion of the importance of the BBB in mind, we performed PSA calculations on this series, and realised that the compounds are within the limits described by Kelder *et al*.[9] This chapter describes the verification of our methods of calculating the PSA and the development of a new series of ligands related to that in Chapter 3 displaying similar levels of affinity and a notable increase in selectivity for the A_1 adenosine receptor over the A_{2A} and A_3 receptors, whilst conforming to the PSA limits detailed above.

4.2 Results and Discussion

4.2.1 Polar Surface Area Calculations

The polar surface area values of the molecules were calculated using Spartan 5.0 for SGI,[12] in combination with an in-house developed application called PolSurf 1.0. [A copy of PolSurf and its (C) source code can be obtained via the author]. First Spartan was used to build the molecule, optimise its 3D-structure and calculate its property data; subsequently PolSurf was applied to convert the raw data from the Spartan "input" and "proparc" files into the polar surface area of the molecule. The resulting PSA values were verified by comparison with data already available in literature for calculations based on a single conformer and based upon Van der Waals surface areas (R^2 = 0.98 for 75 compounds, Figure 4.1), as described by Clark.[7,8] In the initial study,[7] Clark took a training set of 20 compounds as described by Palm *et al*.,[11] and showed that the use of a single-conformer based calculation was sufficient for

determining molecules that were likely to exhibit poor intestinal absorption. In further investigations, 74 drugs were taken, the PSA values calculated and compared to experimental percentage fractional absorption data, and the 'rule-of-five'.[13] He concluded that poor passive intestinal absorption occurred when a molecule has a PSA value of ≥ 140 Å^2. Likewise, Kelder *et al*.[9] published a similar paper stating that orally active drugs, when transported passively, should not exceed a PSA of 120 Å^2.

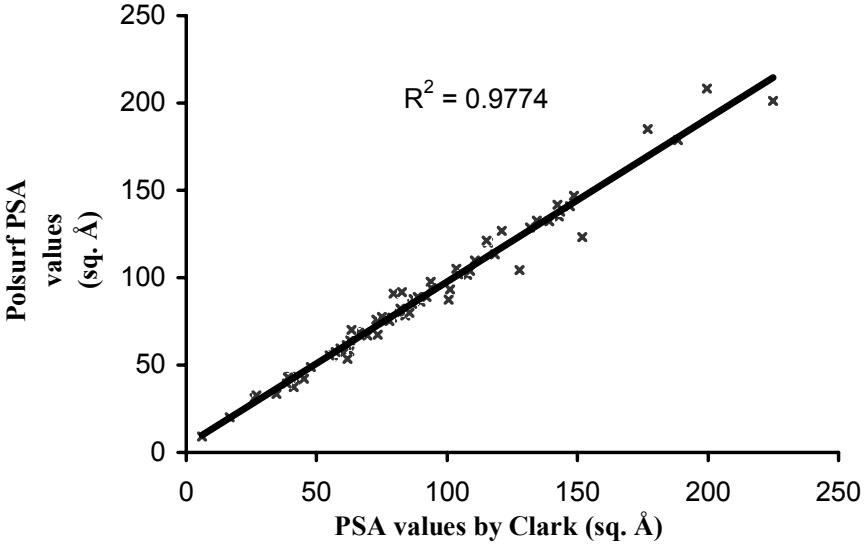


Figure 4.1. PSA values as calculated by Polsurf vs. values from Clark.[7,8]

Furthermore, the Kelder investigation also studied BBB penetration, and concluded that by limiting the PSA to 60-70 Å^2, brain penetration could be achieved. As in our calculations, both parties use Van der Waals surface areas, and only consider the heteroatoms N, O and any hydrogens that may be attached to them. This significant value of 60-70 Å^2 was therefore proposed as the relevant limit for PSA to favour brain penetration.

The PSA values of the compounds described in the previous chapter were calculated to be under the set limit, with a range of 49 (R = ^{t}Bu) - 60 (R = Me) Å^2. With reference to the preceding chapter, the current set of ligands was prepared with the model and PSA limits in mind.

Again, the hydrogen-bond accepting and donating regions at the 'bottom' of the model can be satisfied by an aromatic nitrogen atom and an amido-hydrogen atom, respectively. At the 'top' of the model a carbonyl oxygen will account for the hydrogen-bond accepting region (see Figure 4.2). This 'fulfilment' of the requirements leads directly to the concept of 2,4,6-substituted pyridines, especially considering the benefits to PSA of only 4 atoms to include in the calculations (the two N atoms, the O atom and the hydrogen atom attached to amide nitrogen). One example, namely N-(4,6-diphenyl-pyridin-2-yl)-benzamide (PSA = 40 Å^2) was made and tested, yielding an affinity at the human A_1 adenosine receptor of 657 nM and displacement of the radioligand of 16% and 38% for the hA_{2A} and hA_3 receptors at 1 μM concentrations, respectively. These figures compare favourably with the analogous pyrimidine

(**3.4**) in Chapter 3 ($K_i(hA_1)$ = 670 nM, hA_{2A} = 17% displacement at 1 μM, $K_i(hA_3)$ = 1700 nM; PSA = 50 $Å^2$), and similar substitution at the amide with small alkyl groups would have no doubt improved affinity dramatically. However, synthetic ease directed further developments towards the 2-amido-4,6-disubstituted pyrimidines. For this type of compound, the calculated PSA values are almost identical to the series described in Chapter 3, and thus within the 60-70 $Å^2$ limit proposed earlier. The distinction between the 2-amido-4,6-disubstituted pyrimidines and the 4-amido-2,6-disubstituted pyrimidines can be most simply seen as the rearrangement of the nitrogen atoms within the core heterocycle.

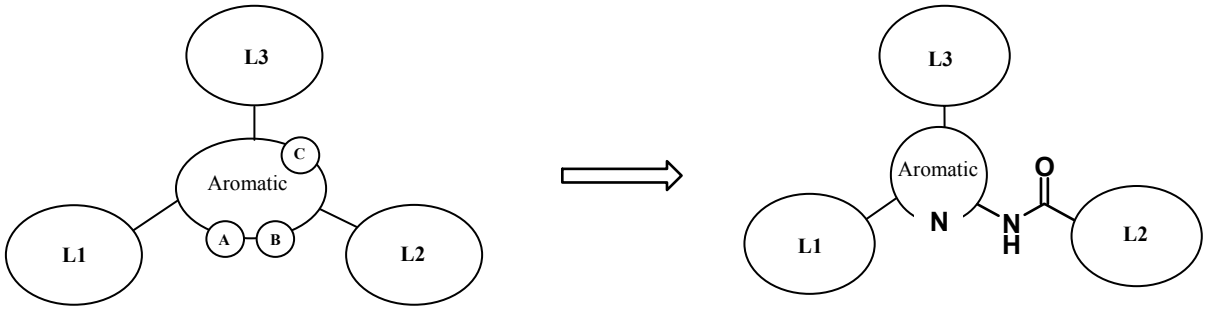


Figure 4.2 The fulfilment of the various hydrogen-bonding regions of the pharmacophore described in Chapter 3.

The two lipophilic groups described as L1 and L3 in the model were initially set again as phenyl groups in analogy to the previous series, and the exploration of the L2 pocket deemed to be of most importance. Some further investigation into the L1 and L3 pockets was realised with the synthesis of compounds with various substituted phenyl groups at the 4- and 6-positions of the 2-amido-pyrimidines. Two non-aromatic examples were also made.

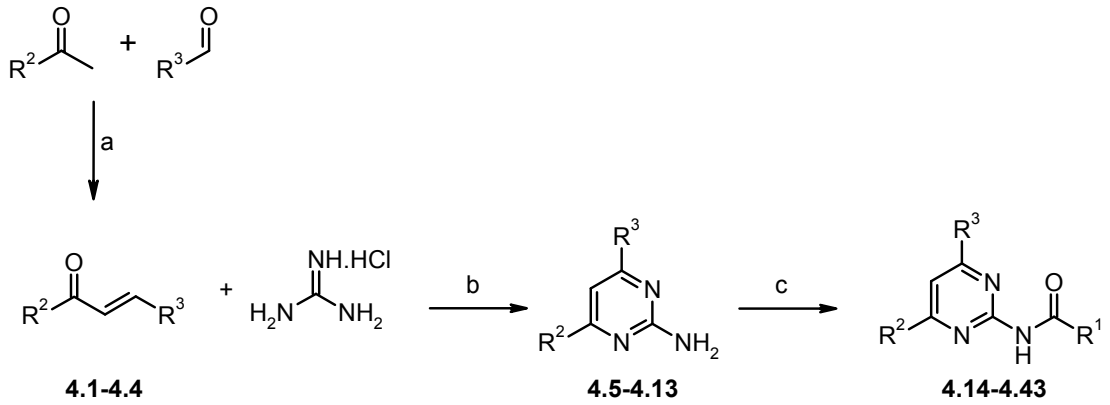
4.2.2 Chemistry

The pyrimidines **4.14-4.43** were synthesised according to the route depicted in Scheme 4.1. The chalcones **4.1-4.4** were synthesised in a single step via an aldol condensation in the presence of $TiCl_4$[14] whilst the other chalcones used were commercially available. Guanidine was freed from its hydrochloride salt form with $NaOH_{(aq.)}$ and reacted with the appropriate chalcone according to a method described by Al-Hajjar and Sabri.[15] The primary amines **4.5-4.13** were obtained in 15-51% yields. This 2-aminopyrimidine was reacted with the respective acid chlorides in the presence of triethylamine to give the desired 2-amido compounds (**4.14-4.19, 4.21-4.43**). These were obtained in 13-98% yields, where the reaction conditions for each substrate were not optimised. The formamide derivative (**4.20**) was synthesised according to a preparation by Krein and Lowary using formic acid and acetic anhydride.[16]

4.2.3 Structure Activity Relationships

Table 4.1 summarises the results of the binding assays. The (substituted) benzamides were the first compounds to be prepared and tested. We found that the unsubstituted compound (**4.14**) showed relatively good affinity at the A_1 receptor. In consequence to the Topliss[17] system of substitution, the 4-position of the phenyl ring was varied first. This generally lowered the

affinity of the pyrimidines at all the adenosine receptors. In previous publications, it has been shown that the A_3 adenosine receptor requires a 4-methoxy substitution to increase affinity.[18,19] However, in this series of benzamides the 4-chloro substituent (**4.15**) showed the highest potency. The only 3-substituent (**4.19**) we made showed only fair affinity at the A_1 adenosine receptor, comparable to the unsubstituted phenyl compound.



Scheme 4.1. Synthetic route to the 2-amido-4,6-diphenyl-pyrimidines **4.14-4.43**. (a) $TiCl_4$, Et_3N; (b) NaOH, EtOH, H_2O; (c) R^1COCl, Et_3N, 1,4-dioxane; (d) benzaldehyde, $NaBH(OAc)_3$.

As with the 4-amido-2,6-diphenyl-pyrimidines (Chapter 3), the alkyl substituents caused a substantial increase in affinity when compared to the phenyl counterparts, suggesting perhaps that these molecules bind in a similar way (at the A_1 receptor), albeit with a different electronic pattern in the central heterocyclic core. At an affinity of 4 nM, compound **4.23** (LUF 5735) with an n-propyl substituent, was the most active ligand.

The A_3 receptor is favoured by the smaller methyl and ethyl derivatives, improving on the affinity achieved by the 4-chlorobenzamide derivative substantially. The extent of this resulted in a two-fold selectivity for A_3 over A_1; compound **4.21** K_i (hA_1) = 480 nM vs K_i (hA_3) = 240 nM.

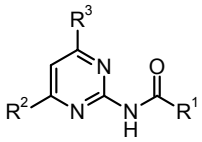
The branched alkyl derivatives (**4.26-4.29**) showed a remarkably high selectivity for the A_1 receptor over the A_{2A} and the A_3 receptors. With K_i values ranging between 9-30 nM for most of these derivatives, it can be said that they are very well suited as ligands for the A_1 adenosine receptor.

The cycloalkyl analogues were similarly good at the A_1 receptor, with the cyclopentyl substituent (**4.32**, LUF 5751) having an affinity of 11 nM and good selectivity over the other two receptors. Like the small methyl and ethyl derivatives (**4.21**, **4.22**), the smallest cycloalkyl group (**4.30**) had the best affinity to the other two receptors. The formamide derivative (**4.20**) displayed good affinity and surprisingly good selectivity for the A_1 receptor, showing that although small substituents are more suitable for the A_{2A} and A_3 receptors, the lack of a substituent at this position does not favour these receptors.

In an attempt to explore the maximal size and any specific hydrogen-bonding aspects of the two lipophilic areas labelled L1 and L3 in Figure 4.2, some phenyl derivatives were made, again following the Topliss scheme of substitution. The compounds (**4.34-4.40**) remarkably

displayed a drastic loss in their affinity to bind to the A_1 receptor. This was, however, matched by a general increase in the affinity of the compounds to bind to the A_3 receptor, culminating in the oft-predicted 4-methoxyphenyl substituent (**4.36** and **4.39**). The symmetrical nature of this series of molecules prevents our knowing whether the singularly substituted phenyl resides in the L1 or the L3 pocket.

Table 4.1. Affinities and PSA values of the 2-amido-4,6-disubstituted-pyrimidines **4.14-4.43** in radioligand binding assays at the human adenosine receptors.



	R^1	R^2	R^3	PSA values ($Å^2$)	K_i (nM) or % displacement[a]		
					A_1[b]	A_{2A}[c]	A_3[d]
4.14	Ph	Ph	Ph	53	309 ± 73	5%	38%
4.15	4-Cl-Ph	Ph	Ph	53	0%	0%	3280 ± 1700
4.16	4-MeO-Ph	Ph	Ph	64	31%	45%	41%
4.17	4-Me-Ph	Ph	Ph	53	37%	0%	13%
4.18	3,4-diCl-Ph	Ph	Ph	53	0%	0%	30%
4.19	3-Cl-Ph	Ph	Ph	53	368 ± 66	22%	41%
4.20	H	Ph	Ph	53	20 ± 2	21%	0%
4.21	Me	Ph	Ph	53	483 ± 90	31%	237 ± 150
4.22	Et	Ph	Ph	53	46 ± 3	893 ± 160	547 ± 47
4.23 (LUF 5735)	Pr	Ph	Ph	53	3.7 ± 2	7%	38%
4.24	Bu	Ph	Ph	53	28 ± 10	0%	23%
4.25	Pent	Ph	Ph	52	28%	0%	24%
4.26 (LUF 5737)	iPr	Ph	Ph	51	8.9 ± 4	44%	45%
4.27	tBu	Ph	Ph	50	224 ± 120	0%	4%
4.28	2-MePr	Ph	Ph	51	25 ± 7	42%	23%
4.29	1-EtPr	Ph	Ph	51	27 ± 6	11%	27%
4.30	c-Pr	Ph	Ph	52	25 ± 5	228 ± 95	676 ± 120
4.31	c-Bu	Ph	Ph	53	107 ± 36	33%	18%
4.32 (LUF 5751)	c-Pent	Ph	Ph	51	11 ± 2	11%	39%
4.33	c-Hex	Ph	Ph	51	119 ± 42	31%	9%
4.34	Ph	4-ClPh	4-ClPh	49	2%	0%	6630 ± 1350
4.35	Ph	4-MePh	4-MePh	51	0%	6%	36%
4.36	Ph	4-MeOPh	4-MeOPh	73	6%	2%	1030 ± 308
4.37	Ph	4-ClPh	Ph	51	0%	15%	2110 ± 1310
4.38	Ph	4-MePh	Ph	51	6%	0%	1090 ± 340
4.39	Ph	4-MeOPh	Ph	62	7%	16%	825 ± 36
4.40	Me	4-MeOPh	Ph	64	314 ± 54	11%	73 ± 10
4.41	Ph	cHex	Ph	53	34%	0%	30%
4.42	Ph	iPr	Ph	53	19%	0%	30%
4.43	Ph	Ph	H	53	8%	0%	2%

*[a]K_i ± SEM (n = 3), % displacement (n = 2). [b]Displacement of specific [^{3}H]DPCPX binding in CHO cell membranes expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations. [c]Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cell membranes expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations. [d]Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cell membranes expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations.*where the group is a direct substituent on the amine.*

However, there is a clear distinction in the benefits of substitution at just one of the phenyl groups for the A_3 receptor, e.g., **4.34** (K_i = 6.6 μM) vs. **4.37** (K_i = 2 μM); **4.35** (36% displacement at 1 μM) vs. **4.38** (K_i = 1 μM); **4.36** (K_i = 1 μM) vs. **4.39** (K_i = 0.8 μM). Combining this single 4-methoxyphenyl substituent with the 'best' R^1 substituent (Me), in terms of affinity for the A_3 receptor, led to our most potent compound at this receptor (**4.40**), possessing also a more favourable selectivity for the A_3 over the A_1 receptor than compound **4.21**. There is certainly great potential to optimise this series to provide better ligands for the A_3 receptor.

To check the necessity of an aromatic substituent in either the L1 or the L3 pocket (Figure 4.2), a cyclohexyl variety was synthesised (**4.41**). The loss of affinity with this compound suggests that either the lipophilic pocket is relatively narrow or that the π-electrons form favourable interactions with the amino acids of the receptor and thus increasing the affinity of the compound significantly. The further drop in affinity when a much smaller isopropyl group is introduced in this position (**4.42**), suggests that the π-electron interaction is very important, although some steric obstruction may still occur. The complete removal of one of the two groups, i.e. compound **4.43** shows that the dramatic loss in affinity results from the lack of π-electron interaction rather than steric hindrance in the receptor pocket.

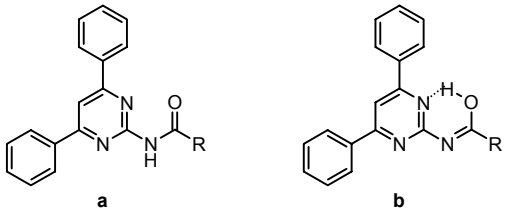


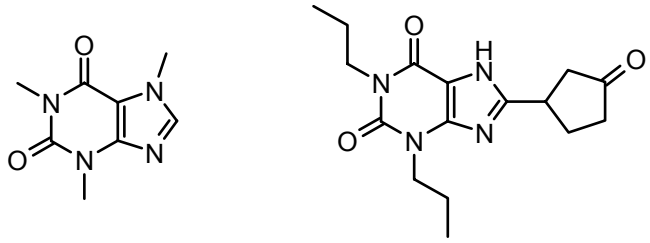
Figure 4.3 Two possible tautomers of the amide-bond (**a**) the amide-form; (**b**) the iminol form.

If we assume that this set of ligands binds to the A_1 receptor in the same manner as the 4-amido-2,6-diphenylpyrimidines (Chapter 3) based on the comparably good affinities, there is one major difference between the two series of compounds that must therefore account for the enhanced selectivity. This is the position of the nitrogens in the ring with respect to the amide bond. The positioning of both nitrogen atoms adjacent to the amide functionality now highlights the issue of electronic interaction between the pyrimidine ring and the amide group. The close proximity of the two systems may lead to a forced conformation of the amide substituent, twisting the carbonyl group out of plane with the ring. This of course assumes that the molecule sits in the normally energetically more favourable amide form. It is also possible that there is a shift in electrons/protons forcing a preferred iminol-tautomer (see Figure 4.3), leaving the substituent still in the plane of the ring. This tautomerism may also be relevant to the 4-amido-2,6-diphenyl-pyrimidines of the preceding chapter, but due to the close proximity of not only one, but two nitrogen atoms (from the pyrimidine ring) to the amide group, we believe that it is of much more importance to the current series. This type of tautomerism question has been dealt with previously in our group on a set of isoquinolines.[20] In these compounds, it was suggested that a phenyl substituent on the amide in the same plane

as that of the isoquinoline might contribute to conjugation, promoting the iminol form. In the ^{1}H NMR ($CDCl_3$) spectra, a signal seen in the 16 ppm region was attributed to the iminol-form and a peak present in the 10 ppm region to the amide form. This was further corroborated with IR spectral evidence using the presence or the absence of the characteristic carbonyl shift in the 1600-1700 cm^{-1} region. The IR spectra of the solid compounds showed that the compounds were primarily in the amide form. The similarities of the isoquinolines to the pyrimidines with respect to the positioning of the amide group adjacent to the nitrogen of the heterocycle led us also to examine the spectroscopic data of one representative compound in more detail. Compound **4.26** (LUF 5737) was chosen as the representative ligand due to its high affinity and good selectivity for the A_1 adenosine receptor. Although the phenyl-substituted compounds (**4.14-4.19**) are more similar to the isoquinoline derivatives, these were not examined in further detail due to their relatively poor affinities. The ^{1}H NMR ($CDCl_3$) of compound **4.26** showed clearly a signal in the 8 ppm region which corresponds to the N-H of the traditional amide form of the molecule. The use of the more polar DMSO as a NMR solvent gave a comparable spectrum. The ligand was further examined with IR spectroscopy, where a sharp carbonyl signal was present at 1680 cm^{-1} in the solid phase, shifting to a broader signal at 1683 cm^{-1} in chloroform. Examining compound **4.26** *in silico*, the molecules were drawn in Spartan, minimised and conformers were then generated using the systematic search method. To determine a representative and comparable energy of each tautomer, the lowest energy conformer was subjected to *ab initio* calculations using 3-21G* as the basis set. The resulting ΔG between the lowest energy conformers of the two tautomeric forms was 17 $kcal \cdot mol^{-1}$ in favour of the amide. It is therefore proposed that the alkyl-substituted 2-amido-pyrimidines reside in the amide tautomeric form. It should be noted though, that the receptor-bound conformation could still be different despite the unambiguous experimental and computational evidence for the amide tautomer. The ability of this particular hydrogen in question to shift between the heteroatoms, causing the molecule to adopt different tautomers, may also be a key reason why the 2-amido- or indeed the 4-amido-pyrimidines possess a high affinity for the adenosine A_1 receptor.

The compounds designed, synthesised and tested in this chapter show PSA values within the proposed limits for BBB penetration by Kelder.[9] To relate our compounds to examples of adenosine A_1 antagonists that already display CNS activity, we calculated the PSA of two well-documented molecules (see Figure 4.4). The first example was caffeine, an adenosine antagonist with well-known CNS effects, and was calculated by our methods to have a PSA value of 59 $Å^2$. Another xanthine, which was published with thorough *in vivo* data, is KFM 19 (the more active *S*(-) enantiomer is known as ampaxifylline).[21] This was administered orally to a number of different species and found to demonstrate good bioavailability. Moreover, its pharmacological profile suggested a high therapeutic potential for dementia and other cognitive deficits. We calculated the PSA value of KFM 19 to be 82 $Å^2$. Since the PSA values

for the ligands detailed in this paper lie within or under these two values we believe that this is further evidence to suggest that they possess the potential to be active at the CNS.



a b

Figure 4.4 a) Caffeine (PSA = 59 Å^2), b) KFM 19 (PSA = 82 Å^2)

4.3 Conclusion

Presented here is a new series of ligands with good affinity at the adenosine A_1 receptor and excellent selectivity over the A_{2A} and A_3 adenosine receptors. Moreover, they have a PSA of approximately 60 Å^2, and as such meet our first simple cut-off for BBB penetration. In particular compound **4.23** (LUF 5735) combined high affinity (K_i = 4 nM) with high selectivity for the A_1 adenosine receptor and a PSA value of only 53 Å^2.

4.4 Experimental Section

4.4.1 Molecular Modelling

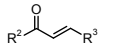
Molecular modelling work was performed on the SPARTAN molecular modelling package version 5.0 (Wavefunction Inc.)[12] running on a Silicon Graphics O_2 workstation. Default values in the Merck Force Field were used in Molecular Mechanics minimisations. Conjugate gradient energy minimisations were continued until the rms energy derivative was less than 0.001kcal·mol^{-1}Å^{-1}. Conformers were then generated using the systematic search method and the lowest energy conformer was used for further calculations. The energy and molecular electrostatic potential was calculated using the semi-empirical molecular orbital program AM1. The electrostatic potentials were sampled over the entire accessible surface of the molecules (equal to a Van der Waals contact surface). The polar surface areas of the molecules were then calculated by applying Polsurf 1.0 to convert the raw data from the Spartan "input" and "proparc" files into the polar surface area of the molecule. [A copy of PolSurf and its (C) source code can be obtained from the author.] Further energy calculations on compound **4.26** were performed by taking the lowest energy conformer (as generated by the procedure described above) and subjecting this to *ab initio* calculations using 3-21G* as the basis set.

4.4.2 Chemistry

Materials and Methods

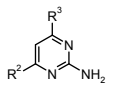
All reagents used were obtained from commercial sources and all solvents were of an analytical grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad and ar = aromatic protons. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Leiden Institute of Chemistry and are within 0.4% of the theoretical values unless otherwise stated. Reactions were routinely monitored by TLC using Merck silica gel F_{254} plates. Solid phase infrared spectra were measured with a Perkin-Elmer FT-IR Paragon 1000 spectrometer equipped with a Golden Gate Diamond ATR, using reflectance technique (neat, 4000-300 cm^{-1}, res. 4 cm^{-1}). Solution phase infrared was recorded on a Bruker 330V IR spectrophotometer equipped with a circle liquid analyzer from Spectra Tech. (4000-300 cm^{-1}, res. 4 cm^{-1})

General Procedure for the synthesis of the substituted chalcones (4.1-4.4).

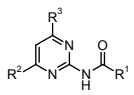


$TiCl_4$ (5.17 mL, 47 mmol, 1.1 eq) was added carefully to CH_2Cl_2 (120 mL) at 0 ºC. To this was added a solution of aldehyde (43 mmol) and ketone (43 mmol) in CH_2Cl_2 (50 mL) and the reaction mixture stirred for 5 mins. A solution of Et_3N (6.9 mL, 49 mmol, 1.14 mmol) in CH_2Cl_2 (20 mL) was then added and the reaction mixture then stirred at 0 ºC for a further 45 mins before pouring onto crushed ice. The organic layer was separated and washed with H_2O and brine, dried ($MgSO_4$) and the solvent evaporated. Chromatography on SiO_2 eluting with a petroleum ether and ethyl acetate mixture (10:1).

4,4'-Dimethylchalcone (4.1). Yield 32%. ^{1}H NMR δ ($CDCl_3$): 7.96-7.91 (m, 2H, Ar-*H*), 7.83-7.75 (m, 1H, C*H*), 7.57-7.45 (m, 3H, Ar-*H* + C*H*), 7.32-7.20 (m, 4H, Ar-*H*), 2.44, 2.40 (2 × s, 6H, 2 × CH_3).
4-Methylchalcone (4.2). Yield 16%. ^{1}H NMR δ ($CDCl_3$): 8.05-7.99 (m, 2H, Ar-*H*), 7.84-7.77 (d, 1H, J = 16 Hz, C*H*), 7.59-7.46 (m, 7H, Ar-*H*), 7.25-7.21 (m, 1H, C*H*), 2.40 (s, 3H, CH_3).
Cyclohexyl-1-phenyl-propenone (4.3). Yield 25%. ^{1}H NMR δ ($CDCl_3$): 7.94-7.90 (m, 2H, Ar-*H*), 7.54-7.41 (m, 3H, Ar-*H*), 7.07-6.96 (m, 1H, C*H*), 6.87-6.78 (m, 1H, C*H*), 2.40-2.21 (m, 1H, C*H*), 1.86-1.18 (m, 10H, cHex).
Isopropyl-1-phenyl-propenone (4.4). Yield 26%. ^{1}H NMR δ ($CDCl_3$): 7.95-7.90 (m, 2H, Ar-*H*), 7.53-7.41 (m, 3H, Ar-*H*), 7.12-6.82 (m, 2H, 2 × C*H*), 3.52-3.41 (m, 1H, C*H*), 0.94-0.91 (m, 6H, 2 × CH_3).
General procedure for the synthesis of 2-amino-4,6-disubstitutedpyrimidines (4.5-4.13).



A mixture of chalcone (benzylidenacetophenone) (38.6 g, 0.185 mol, 1.1 eq.) and guanidine hydrochloride (16 g, 0.168 mol, 1 eq.) were refluxed in ethanol (150 mL). Sodium hydroxide (21.6 g, 0.539 mol, 3.2 eq.) was dissolved in a minimum amount of water (40 mL), and added dropwise to the refluxing mixture. The reaction mixture was then stirred at reflux for a further 6 h. Upon cooling, the reaction mixture was concentrated and then separated between ethyl acetate (200 mL) and water (200 mL). The aqueous layer was then extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with water (200 mL) and brine (200 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by column chromatography on SiO_2, eluting with dichloromethane. Recrystallisation from ethyl acetate gave clear colourless crystals.
2-Amino-4,6-diphenylpyrimidine (4.5). Yield 31%. ^{1}H NMR δ ($CDCl_3$): 8.09-8.04 (m, 4H, phenyl-*H*), 7.54-7.48 (m, 7H, phenyl-*H* + pyrimidinyl-*H*), 5.25 (br s, 2H, NH_2).
2-Amino-4,6-di(4-chlorophenyl)-pyrimidine (4.6). Yield 44%. ^{1}H NMR δ ($CDCl_3$): 8.04-7.99 (m, 4H, phenyl-*H*), 7.50-7.46 (m, 4H, phenyl-*H*), 7.40 (s, 1H, pyrimidinyl-*H*), 5.38 (br s, 2H, NH_2).
2-Amino-4,6-di(4-tolyl)-pyrimidine (4.7). Yield 27%. ^{1}H NMR δ ($CDCl_3$): 7.99-7.94 (m, 4H, Ar-*H*), 7.43 (s, 1H, pyrimidinyl-*H*), 7.32-7.27 (m, 4H, Ar-*H*), 5.21 (br s, 2H, NH_2).
2-Amino-4,6-di(4-methoxyphenyl)-pyrimidine (4.8). Yield 35%. ^{1}H NMR δ ($CDCl_3$): 8.06-8.02 (m, 4H, Ar-*H*), 7.37 (s, 1H, pyrimidinyl-*H*), 7.03-6.99 (m, 4H, Ar-*H*), 5.19 (br s, 2H, NH_2), 3.88 (s, 6H, 2 × OCH_3).
2-Amino-4-(4-chlorophenyl)-6-phenylpyrimidine (4.9). Yield 51%. ^{1}H NMR δ ($CDCl_3$): 8.09-7.99 (m, 4H, Ar-*H*), 7.53-7.44 (m, 5H, Ar-*H* + pyrimidinyl-*H*), 5.36 (br s, 2H, NH_2).
2-Amino-4-(4-tolyl)-6-phenylpyrimidine (4.10). Yield 28%. ^{1}H NMR δ ($CDCl_3$): 8.09-8.04 (m, 2H, Ar-*H*), 8.00-7.96 (m, 2H, Ar-*H*), 7.52-7.48 (m, 2H, Ar-*H*), 7.46 (s, 1H, pyrimidinyl-*H*), 7.33-7.26 (m, 2H, Ar-*H*), 5.23 (br s, 2H, NH_2), 2.44 (s, 3H, OCH_3).
2-Amino-4-(4-methoxyphenyl)-6-phenylpyrimidine (4.11). Yield 23%. ^{1}H NMR δ ($CDCl_3$): 8.08-8.04 (m, 4H, Ar-*H*), 7.51-7.48 (m, 3H, Ar-*H*), 7.43 (s, 1H, pyrimidinyl-*H*), 7.03-6.99 (m, 4H, Ar-*H*), 5.23 (br s, 2H, NH_2), 3.89 (s, 6H, OCH_3).
2-Amino-4-(cyclohexyl)-6-phenylpyrimidine (4.12). Yield 15%. ^{1}H NMR δ ($CDCl_3$): 7.97-7.92 (m, 2H, phenyl-*H*), 7.45-7.42 (m, 3H, phenyl-*H*), 6.88 (s, 1H, pyrimidinyl-*H*), 5.52 (br s, 2H, NH_2), 2.57-2.45 (m, 1H, C*H*), 1.97-1.17 (m, 10H, cHex).
2-Amino-4-(isopropyl)-6-phenylpyrimidine (4.13). Yield 36%. ^{1}H NMR δ ($CDCl_3$): 7.99-7.92 (m, 2H, phenyl-*H*), 7.47-7.41 (m, 3H, phenyl-*H*), 6.86 (s, 1H, pyrimidinyl-*H*), 5.46 (br s, 2H, NH_2), 2.18-2.08 (m, 1H, C*H*), 0.98-0.95 (m, 6H, 2 × CH_3).
General Procedure for the Preparation of 2-Amidopyrimidines (4.14-4.42).



To a solution of 2-amino-4,6-diphenylpyrimidine (0.202 mmol, 1 eq.) in 1,4-dioxane (5 mL) was added triethylamine (0.223 mmol, 1.1 eq.), followed by the appropriate acid chloride (0.304 mmol, 1.5 eq.). This was then stirred at reflux until no starting material was visible by TLC. Upon completion, the reaction mixture was separated between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was further extracted with ethyl acetate (2 × 20 mL) and the combined organic layers washed with water and brine. After drying over $MgSO_4$ and evaporation under reduced pressure, the crude product was purified by column chromatography, eluting with a petroleum ether-ethyl acetate or a dichloromethane-methanol solvent system. Recrystallisation with ethanol or petroleum ether-ethyl acetate gave the corresponding amide in crystalline form.
N-(4,6-Diphenyl-pyrimidin-2-yl)-benzamide (4.14). Yield 34%; white solid; mp 169-170 °C. ^{1}H NMR δ ($CDCl_3$): 8.75 (br s, 1H, N-*H*), 8.22-8.17 (m, 4H, phenyl-*H*), 8.02-7.98 (m, 2H, phenyl-*H*), 7.90 (s, 1H, pyrimidine-*H*), 7.56-7.52 (m, 9H, phenyl-*H*). ^{13}C NMR δ ($CDCl_3$): 166.1, 136.5, 132.1, 131.0, 128.9, 128.7, 127.2, 108.1. MS (ES^+): 351.50 Da. Anal. calc. for ($C_{23}H_{17}N_3O$) (C 78.61; H 4.88; N 11.96) found (C 78.20; H 4.88; N 12.38) %.
N-(4,6-Diphenyl-pyrimidin-2-yl)-4-chloro-benzamide (4.15). Yield 82%; white solid; mp 185 °C. ^{1}H NMR δ ($CDCl_3$): 8.86 (br s, 1H, N-*H*), 8.15-8.11 (m, 2H, phenyl-*H*), 7.88-7.85 (m, 3H, phenyl-*H*), 7.53-7.41 (m, 8H,

phenyl-*H*). ^{13}C-NMR δ ($CDCl_3$): 166.1, 164.5, 157.9, 138.3, 136.4, 136.1, 133.1, 131.1, 128.9, 127.2, 113.4, 108.1. MS (ES^+): 385.84 Da. Anal. ($C_{23}H_{16}ClN_3O.0.2H_2O$) C, H, N.

N-(4,6-diphenyl-pyrimidin-2-yl)-4-methoxy-benzamide (4.16). Yield 27%; white solid; mp 155 °C. ^{1}H NMR δ ($CDCl_3$): 8.82 (br s, 1H, N*H*), 8.22-8.13 (m, 4H, phenyl-*H*), 7.99-7.92 (d, 2H, phenyl-*H*), 7.85 (s, 1H, pyrimidinyl-*H*), 7.53-7.48 (m, 6H, phenyl-*H*), 7.00-6.93 (d, 2H, phenyl-*H*), 3.86 (s, 3H, C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 165.9, 158.1, 136.5, 130.8, 129.3, 128.7, 127.1, 113.7, 107.8, 55.2. MS (ES^+): 382.1 Da. Anal ($C_{24}H_{19}N_3O_2.3H_2O$) C, H, N.

N-(4,6-diphenylpyrimidin-2-yl)-4-methyl-benzamide (4.17). Yield 22%; white solid; mp 190 °C. ^{1}H NMR δ ($CDCl_3$): 8.76 (br s, 1H, N*H*), 8.23-8.18 (m, 4H, phenyl-*H*), 7.94 (d, 2H, phenyl-*H*), 7.90 (s, 1H, pyrimidinyl-*H*), 7.56-7.53 (m, 6H, phenyl-*H*), 7.36-7.32 (d, 2H, phenyl-*H*), 2.46 (s, 3H, C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 166.2, 165.2, 158.2, 142.9, 142.9, 136.7, 131.3, 131.1, 129.5, 128.9, 128.8, 127.6, 127.3, 108.1, 21.5. MS (ES^+): 366.0 Da. Anal. ($C_{24}H_{19}N_3O.0.2H_2O$) C, H, N.

3,4-Dichloro-N-(4,6-diphenyl-pyrimidin-2-yl)-benzamide (4.18). Yield 13%; white solid; mp 146-147 °C. ^{1}H NMR δ ($CDCl_3$): 8.67 (br s, 1H, N*H*), 8.18-8.11 (m, 4H, phenyl-*H*), 8.08-8.07 (m, 1H, phenyl-*H*), 7.93 (s, 1H, pyrimidinyl-*H*), 7.83-7.78 (m, 1H, phenyl-*H*), 7.64-7.51 (m, 7H, phenyl-*H*). ^{13}C NMR δ ($CDCl_3$): 166.2, 157.8, 136.3, 134.4, 133.1, 131.1, 130.6, 129.6, 128.9, 127.2, 126.5, 108.4. MS (ES^+): 419.5 Da. Anal. ($C_{23}H_{15}Cl_2N_3O.1.3H_2O$) C, H, N.

N-(4,6-Diphenyl-pyrimidin-2-yl)-3-chloro-benzamide (4.19). Yield 98%; white solid; mp 147-148 °C. ^{1}H NMR δ ($CDCl_3$): 8.96 (br s, 1H, N*H*), 8.16-8.11 (m, 4H, phenyl-*H*), 7.91-7.89 (m, 1H, phenyl-*H*), 7.85 (s, 1H, pyrimidinyl-*H*), 7.81-7.77 (m, 1H, phenyl-*H*), 7.54-7.45 (m, 6H, phenyl-*H*), 7.42-7.35 (m, 2H, phenyl-*H*). ^{13}C NMR δ ($CDCl_3$): 166.2, 164.2, 136.4, 134.8, 131.1, 129.9, 128.9, 127.7, 127.2, 125.5, 108.2, 98.0. MS (ES^+): 385.69 Da. Anal. ($C_{23}H_{16}ClN_3O.0.2H_2O$) C, H, N.

N-(4,6-Diphenyl-pyrimidin-2-yl)-formamide (4.20).[16] Formic acid (0.37g, 8.1 mmol, 10 eq.) was cooled to 0 ºC, 2-amino-4,6-diphenylpyrimidine (0.2g, 0.81 mmol) and acetic anhydride (0.125 g, 1.2 mmol, 1.5 eq.) were added carefully and the mixture brought slowly to RT and stirred overnight. Ethyl acetate (20 mL) and H_2O (5 mL) were added and the layers separated. The organic layer was then further washed with H_2O, saturated $NaHCO_{3(aq.)}$, brine and then dried ($MgSO_4$) and the solvents evaporated *in vacuo*. Recrystallisation in hot EtOH rendered the pure product. Yield 81%; white solid; mp 186 °C. ^{1}H NMR δ ($CDCl_3$): 11.06 (br s, 1H, N*H*), 9.66 (s, 1H, C*H*O), 8.36-8.30 (m, 5H, phenyl-*H* + pyrimidinyl-*H*), 7.59-7.56 (m, 6H, phenyl-*H*). ^{13}C NMR δ ($CDCl_3$): 165.2, 158.1, 136.0, 131.4, 128.8, 127.4, 107.9. MS (ES^+): 275.9 Da. Anal. ($C_{17}H_{13}N_3O$) C, H, N.

N-(4,6-Diphenyl-pyrimidin-2-yl)-acetamide (4.21). Yield 43%; white solid; mp 217-218 °C. ^{1}H NMR δ ($CDCl_3$): 8.15-8.10 (m, 5H, phenyl-*H* + N-*H*), 7.83 (s, 1H, pyrimidinyl-*H*), 7.56-7.53 (m, 6H, phenyl-*H*), 2.76 (s, 3H, C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 166.0, 148.5, 136.1, 131.1, 128.9, 127.1, 107.3, 25.5. MS (ES^+): 289.91 Da. Anal ($C_{18}H_{17}N_3O.0.2H_2O$) C, H, N.

N-(4,6-Diphenyl-pyrimidin-2-yl)-propionamide (4.22). Yield 21%; white solid; mp 165 °C. ^{1}H NMR δ ($CDCl_3$): 8.17-8.08 (m, 5H, phenyl-*H* + N*H*), 7.81 (s, 1H, pyrimidinyl-*H*), 7.56-7.50 (m, 6H, phenyl-*H*), 3.05 (q, 2H, J = 7.30 Hz, C*H*$_2$CH$_3$), 1.32 (t, 3H, J = 7.31 Hz, CH$_2$C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 174.8, 157.7, 136.5, 131.0, 128.8, 127.0, 107.2, 30.7, 11.5. MS (ES^+): 303.86 Da. Anal ($C_{19}H_{17}N_3O.0.2H_2O$) C, H, N.

N-(4,6-Diphenyl-pyrimidin-2-yl)-butyramide (4.23). Yield 47%; white solid; mp 157 °C. ^{1}H NMR δ ($CDCl_3$): 8.16-8.09 (m, 4H, phenyl-*H*), 8.08 (br s, 1H, N*H*), 7.83 (s, 1H, pyrimidinyl-*H*), 7.55-7.52 (m, 6H, phenyl-*H*), 3.02 (t, 2H, J = 7.30 Hz, C*H*$_2$CH$_2$CH$_3$), 1.86 (sextet, 2H, J = 7.30 Hz, CH$_2$C*H*$_2$ CH$_3$), 1.08 (t, 3H, J = 7.30Hz, CH$_2$CH$_2$C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 174.2, 166.0, 157.7, 131.0, 128.8, 127.1, 107.3, 39.3, 18.3, 11.8. MS (ES^+): 317.95 Da. Anal ($C_{20}H_{19}N_3O$) C, H, N.

Pentanoic acid (4,6-diphenyl-pyrimidin-2-yl)-amide (4.24). Yield 32%; white solid; mp 157 °C. ^{1}H NMR δ ($CDCl_3$): 8.16-8.11 (m, 4H, phenyl-*H*), 8.09 (br s, 1H, N*H*), 7.82 (s, 1H, pyrimidinyl-*H*), 7.56-7.52 (m, 6H, phenyl-*H*), 3.05 (t, 2H, J = 7.67 Hz, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.86-1.74 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.58-1.40 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 0.99 (t, 3H, J = 7.30 Hz, CH$_2$CH$_2$CH$_2$C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 165.9, 157.8, 136.6, 131.0, 128.8, 127.1, 121.3, 113.6, 107.2, 37.2, 22.4, 11.8. MS (ES^+): 331.86 Da. Anal. ($C_{21}H_{21}N_3O.0.2H_2O$) C, H, N.

Hexanoic acid (4,6-diphenyl-pyrimidin-2-yl)-amide (4.25). Yield 86%; white solid; mp 164-165 °C. ^{1}H NMR δ ($CDCl_3$): 8.30 (br s, 1H, N*H*), 8.14-8.09 (m, 4H, phenyl-*H*), 7.79 (s, 1H, pyrimidinyl-*H*), 7.54-7.50 (m, 6H, phenyl-*H*), 3.00 (t, 2H, J = 7.67 Hz, C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 1.86-1.78 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.46-1.41 (m, 4H, CH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_3$), 0.93 (m, 3H, CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 174.1, 165.9, 157.8, 136.6, 131.0, 128.8, 127.1, 107.2, 37.2, 31.4, 24.4, 22.4, 13.9. MS (ES^+): 345.95 Da. Anal. ($C_{22}H_{23}N_3O$) C, H, N.

N-(4,6-Diphenyl-pyrimidin-2-yl)-isobutyramide (4.26). Yield 39%; white solid; mp 160-161 °C. ^{1}H NMR δ ($CDCl_3$): 8.17-8.10 (m, 4H, phenyl-*H*), 8.09 (br s, 1H, N*H*), 7.84 (s, 1H, pyrimidinyl-*H*), 7.56-7.51 (m, 6H, phenyl-*H*), 3.41-3.38 (m, 1H, C*H*(CH$_3$)$_2$), 1.35 (d, 6H, J = 6.94 Hz, CH(C*H*$_3$)$_2$). ^{13}C NMR δ ($CDCl_3$): 166.0, 157.7, 136.6, 136.0, 131.0, 128.8, 127.1, 107.5, 35.2, 19.2. MS (ES^+): 317.94 Da. Anal ($C_{20}H_{19}N_3O$) C, H, N.

N-(4,6-diphenyl-pyrimidin-2-yl)-2,2-dimethyl-propionamide (4.27). Yield 64%; white solid; mp 128 °C. ^{1}H NMR δ ($CDCl_3$): 8.29 (br s, 1H, N*H*), 8.20-8.14 (m, 4H, phenyl-*H*), 7.82 (s, 1H, pyrimidinyl-*H*), 7.51-7.48 (m,

6H, phenyl-*H*), 1.39 (s, 9H, 3x C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 175.7, 158.0, 136.6, 130.9, 128.8, 127.2, 107.8, 40.2, 27.4. MS: 332.3 Da. Anal. ($C_{21}H_{21}N_3O.0.2H_2O$) C, H, N.

N-(4,6-diphenyl-pyrimidin-2-yl)-3-methylbutyramide (4.28). Yield 48%; white solid; mp 134-135 °C. ^{1}H NMR δ ($CDCl_3$): 8.27 (br s, 1H, N*H*), 8.15-8.07 (m, 4H, phenyl-*H*), 7.79 (s, 1H, pyrimidinyl-*H*), 7.55-7.47 (m, 6H, phenyl-*H*), 2.87 (d, 2H, J = 7.30, C*H*$_2$), 2.40-2.26 (m, 1H, C*H*), 1.06 (d, 6H, J = 6.94, 2x C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 173.1, 166.0, 157.8, 136.6, 131.1, 128.9, 127.2, 107.4, 46.3, 25.3, 22.6. MS: 345.9,367.6 Da. Anal. ($C_{21}H_{21}N_3O.0.3H_2O$) C, H, N.

N-(4,6-Diphenyl-pyrimidin-2-yl)-2-ethyl-butyramide (4.29). Yield 22%; white solid; mp 120-121 °C. ^{1}H NMR δ ($CDCl_3$): 8.18-8.13 (m, 4H, phenyl-*H*), 8.11 (br s, 1H, N*H*), 7.84 (s, 1H, pyrimidinyl-*H*), 7.56-7.51 (m, 6H, phenyl-*H*), 2.97 (m, 1H, C*H*(CH_2CH_3)$_2$), 1.94-1.59 (m, 4H, CH(C*H*$_2$CH$_3$)$_2$), 1.02 (t, 6H, J = 7.30 Hz, CH(CH_2C*H*$_3$)$_2$). ^{13}C NMR δ ($CDCl_3$): 166.0, 157.7, 136.6, 130.9, 128.8, 127.1, 121.6, 107.6, 50.1, 24.9, 11.8. MS (ES^+): 324.86, 690.57 Da. Anal. ($C_{22}H_{23}N_3O$) C, H, N.

Cyclopropane carboxylic acid (4,6-diphenyl-pyrimidin-2-yl)-amide (4.30). Yield 57%; white solid; mp 158 °C. ^{1}H NMR δ ($CDCl_3$): 8.77 (br s, 1H, N*H*), 8.13-8.08 (m, 4H, phenyl-*H*), 7.78 (s, 1H, pyrimidinyl-*H*), 7.51-7.47 (m, 6H, phenyl-*H*), 2.67 (m, 1H, -C*H*CH_2CH_2-), 1.29-1.21 (m, 2H, CHC*H*$_2$CH$_2$-), 0.99-0.90 (m, 2H, -CHC*H*$_2$CH$_2$-). ^{13}C NMR δ ($CDCl_3$): 174.1, 165.9, 157.9, 136.5, 130.9, 128.8, 127.1, 107.4, 14.8, 9.4. MS (ES^+): 337.75 Da. Anal ($C_{20}H_{17}N_3O$) C, H, N.

Cyclobutane carboxylic acid (4,6-diphenyl-pyrimidin-2-yl)-amide (4.31). Yield 98%; white solid; mp 146-147 °C. ^{1}H NMR δ ($CDCl_3$): 8.35 (br s, 1H, N*H*), 8.13-8.08 (m, 4H, phenyl-*H*), 7.76 (s, 1H, pyrimidinyl-*H*), 7.52-7.48 (m, 6H, phenyl-*H*), 4.05-3.96 (m, 1H, -C*H*$CH_2CH_2CH_2$-), 2.57-2.21 (m, 4H, -CHC*H*$_2$CH$_2$C*H*$_2$-), 2.09-1.92 (m, 2H, -CHC*H*$_2$CH$_2$C*H*$_2$-). ^{13}C NMR δ ($CDCl_3$): 175.2, 165.9, 157.7, 136.5, 130.9, 128.8, 127.0, 113.5, 107.2, 40.3, 24.8, 18.0. MS (ES^+): 330, 331 Da. Anal. ($C_{21}H_{19}N_3O.0.1H_2O$) C, H, N.

Cyclopentane carboxylic acid (4,6-diphenyl-pyrimidin-2-yl)-amide (4.32). Yield 98%; white solid; mp 145-146 °C. ^{1}H NMR δ ($CDCl_3$): 8.16-8.11 (m, 4H, phenyl-*H*), 7.82 (s, 1H, pyrimidinyl-*H*), 7.55-7.51 (m, 6H, phenyl-*H*), 2.04-1.65 (m, 9H, cyclopentyl). ^{13}C NMR δ ($CDCl_3$): 176.2, 165.9, 157.8, 131.0, 128.8, 127.1, 107.4, 45.7, 30.1, 26.0. MS: 344.2 Da. Anal. ($C_{22}H_{21}N_3O.0.1H_2O$) C, H, N.

Cyclohexanecarboxylic acid-(4,6-diphenyl-pyrimidin-2-yl)-amide (4.33). Yield 26%; white solid; mp 172 °C. ^{1}H NMR δ ($CDCl_3$): 8.11-8.19 (m, 4H, phenyl-*H*), 7.83 (s, 1H, pyrimidinyl-*H*), 7.50-7.57 (m, 6H, phenyl-*H*), 0.83-2.10 (m, 11H, C*H*$_2$ + C*H*). ^{13}C NMR δ ($CDCl_3$): 166.1, 157.8, 136.6, 131.0, 128.8, 127.2, 022.7, 121.7, 107.5, 44.9, 29.1, 25.6. MS: 357.8 Da. Anal. ($C_{23}H_{23}N_3O.0.2H_2O$) C, H, N.

N-[4,6-Bis(4-chlorophenyl)-pyrimidin-2-yl]-benzamide (4.34). Yield 65%; white solid; mp 193 °C. ^{1}H NMR δ ($CDCl_3$): 8.19-8.15 (m, 4H, Ar-*H*), 8.08-8.04 (m, 2H, Ar-*H*), 7.83 (s, 1H, pyrimidinyl-*H*), 7.60-7.51(m, 7H, Ar-*H*). ^{13}C NMR δ ($CDCl_3$): 165.2, 165.0, 158.7, 158.0, 137.4, 132.3, 129.1, 128.7, 128.5, 127.4, 107.3. MS: 419.5, 421.5 Da. Anal. ($C_{23}H_{15}Cl_2N_3O.0.25H_2O$) C, H, N.

N-[4,6-Di-4-tolyl-pyrimidin-2-yl]-benzamide (4.35). Yield 97%; white solid; mp 210 °C. ^{1}H NMR δ ($CDCl_3$): 8.69 (br s, 1H, N-*H*), 8.07 (d, 4H, J = 8.4 Hz, Ar-*H*), 7.99-7.95 (m, 2H, Ar-*H*), 7.83 (s, 1H, pyrimidinyl-*H*), 7.59-7.46 (m, 3H, Ar-*H*), 7.32 (d, 2H, J = 8.4 Hz, Ar-*H*), 2.43 (s, 6H, 2 × C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 166.0, 141.5, 133.9, 132.1, 129.6, 128.7, 127.5, 127.2, 121.8, 107.4, 21.4. MS: 379.7 Da. Anal. ($C_{25}H_{21}N_3O.0.15H_2O$) C, H, N.

N-[4,6-Bis(4-methoxyphenyl)-pyrimidin-2-yl]-benzamide (4.36). Yield 43%; white solid; mp 159-160 °C. ^{1}H NMR δ ($CDCl_3$): 8.81 (br s, 1H, N-*H*), 8.13-8.07 (m, 4H, Ar-*H*), 7.95-7.90 (m, 2H, phenyl-*H*), 7.69 (s, 1H, pyrimidinyl-*H*), 7.55-7.41 (m, 3H, phenyl-*H*), 7.00-6.96 (m, 4H, Ar-*H*), 3.84 (s, 6H, 2 × OC*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 165.4, 165.2, 161.9, 157.9, 134.8, 131.9, 129.1, 128.7, 128.6, 127.4, 121.5, 114.1, 106.2, 55.3. MS: 411.7 Da. Anal. ($C_{25}H_{21}N_3O_3.0.3H_2O$) C, H, N.

N-[4-(4-Chlorophenyl)-6-phenyl-pyrimidin-2-yl]-benzamide (4.37). Yield 81%; white solid; mp 175 °C. ^{1}H NMR δ ($CDCl_3$): 8.72 (br s, 1H, N-*H*), 8.19-8.12 (m, 4H, Ar-*H*), 8.02-7.97 (m, 2H, Ar-*H*), 7.86 (s, 1H, pyrimidinyl-*H*), 7.62-7.43 (m, 8H, Ar-*H*). ^{13}C NMR δ ($CDCl_3$): 166.3, 165.3, 164.7, 158.1, 136.4, 134.9, 132.2, 131.1, 129.0, 128.9, 128.7, 128.5, 127.4, 127.2, 107.7. MS: 385.7 Da. Anal. ($C_{23}H_{16}ClN_3O.0.25H_2O$) C, H, N.

N-[4-(4-Tolyl)-6-phenyl-pyrimidin-2-yl]-benzamide (4.38). Yield 77%; white solid; mp 171-172 °C. ^{1}H NMR δ ($CDCl_3$): 8.82 (br s, 1H, N-*H*), 8.19-7.93 (m, 6H, Ar-*H*), 7.84 (s, 1H, pyrimidinyl-*H*), 7.57-7.44 (m, 6H, Ar-*H*), 7.33-7.29 (m, 2H, Ar-*H*), 2.43 (s, 3H, C*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 166.0, 165.9, 165.3, 158.0, 141.5, 136.7, 134.7, 133.7, 132.0, 130.9, 129.6, 128.8, 128.6, 127.4, 127.2, 127.1, 107.6, 21.4. MS: 365.9 Da. Anal. ($C_{24}H_{19}N_3O.0.25H_2O$) C, H, N.

N-[4-(4-Methoxyphenyl)-6-phenyl-pyrimidin-2-yl]-benzamide (4.39). Yield 29%; white solid; mp 159-161 °C. ^{1}H NMR δ ($CDCl_3$): 8.68 (br s, 1H, N-*H*), 8.19-8.14 (m, 4H, Ar-*H*), 8.01-7.96 (m, 2H, Ar-*H*), 7.82 (s, 1H, pyrimidinyl-*H*), 7.63-7.48 (m, 6H, Ar-*H*), 7.06-6.99 (m, 2H, Ar-*H*), 3.90 (s, 3H, OC*H*$_3$). ^{13}C NMR δ ($CDCl_3$): 165.5, 165.4, 162.1, 157.8, 132.0, 130.9, 128.9, 128.8, 128.6, 127.4, 127.1, 114.2, 107.1, 55.3. MS: 381.8 Da. Anal. ($C_{24}H_{19}N_3O_2$) C, H, N.

N-[4-(4-Methoxyphenyl)-6-phenyl-pyrimidin-2-yl]-acetamide (4.40). Yield 58%; white solid; mp 164 °C. ^{1}H NMR δ ($CDCl_3$): 8.19 (br s, 1H, N-*H*), 8.10-8.06 (m, 4H, Ar-*H*), 7.71 (s, 1H, pyrimidinyl-*H*), 7.53-7.50 (m, 3H,

phenyl-*H*), 7.03-6.99 (m, 2H, Ar-*H*), 3.88 (s, 3H, CH_3), 2.72 (s, 3H, CH_3). ^{13}C NMR δ ($CDCl_3$): 171.8, 165.5, 165.3, 162.1, 157.6, 142.3, 136.6, 130.9, 128.8, 128.6, 127.0, 114.1, 106.2, 55.3, 25.5. MS: 319.9 Da. Anal. ($C_{19}H_{17}N_3O_2$) C, H, N.

N-(4-Cyclohexyl-6-phenyl-pyrimidin-2-yl)-benzamide (4.41). Yield 50%; white solid; mp 98 °C. ^{1}H NMR δ ($CDCl_3$): 8.71 (br s, 1H, N-*H*), 8.13-8.08 (m, 2H, Ar-*H*), 7.97-7.93 (m, 2H, Ar-*H*), 7.56-7.44 (m, 6H, Ar-*H*), 7.35 (s, 1H, pyrimidinyl-*H*), 2.76-2.63 (m, 1H, C*H*), 2.03-0.86 (m, 10H, cHex). ^{13}C NMR δ ($CDCl_3$): 177.1, 165.3, 157.7, 136.7, 132.1, 130.8, 128.8, 128.7, 127.5, 127.3, 109.6, 46.1, 32.0, 26.2. MS: 358.2 Da. Anal. ($C_{23}H_{23}N_3O.0.2H_2O$) C, H, N.

N-(4-Isopropyl-6-phenyl-pyrimidin-2-yl)-benzamide (4.42). Yield 18%; white solid; mp 95-96 °C. ^{1}H NMR δ ($CDCl_3$): 8.81 (br s, 1H, N-*H*), 8.15-7.99 (m, 2H, Ar-*H*), 7.98-7.94 (m, 2H, Ar-*H*), 7.53-7.47 (m, 6H, Ar-*H*), 7.30 (s, 1H, pyrimidinyl-*H*), 2.24-2.04 (m, 1H, C*H*), 0.98 (d, 6H, J = 7.8 Hz, 2 × CH_3). ^{13}C NMR δ ($CDCl_3$): 172.3, 165.1, 132.2, 131.0, 128.9, 128.7, 127.5, 127.3, 112.0, 47.0, 28.6. MS: 332.3 Da. Anal. ($C_{20}H_{19}N_3O.0.1H_2O$) C, H, N.

N-(4-Phenyl-pyrimidin-2-yl)-benzamide (4.43). To 4-phenyl-pyrimidin-2-ylamine (20 mg, 0.117 mmol) in pyridine (0.5 mL) was added benzoyl chloride (16.3 μL, 0.140 mmol, 1.2 eq.) and the reaction mixture stirred at RT for 2 h. The reaction mixture was then separated between H_2O and CH_2Cl_2, and the organic layer then further washed with brine and dried ($MgSO_4$). Chromatography of the crude product on SiO_2 eluting with CH_2Cl_2 gave the title product as a white solid. Yield 47% ^{1}H NMR δ ($CDCl_3$): 8.74-8.71 (m, 2H, pyrimidinyl-*H*), 8.12-8.07 (m, 2H, phenyl-*H*), 7.99-7.95 (m, 2H, phenyl-*H*), 7.55-7.46 (m, 7H, Ar-*H* + N-*H*). MS: 276.1 Da.

4.4.3 Biology

Materials and Methods

[^{3}H]DPCPX and [^{125}I]AB-MECA were purchased from Amersham Biosciences (NL). [^{3}H]ZM 241385 was obtained from Tocris Cookson, Ltd. (UK). HEK 293 cells stably expressing the human adenosine A_{2A} and A_3 receptor were gifts from Dr. Wang (Biogen, USA) and Dr. K.-N. Klotz (University of Würzburg, Germany), respectively.

All compounds made were tested in radioligand binding assays to determine their affinities at the human adenosine A_1, A_{2A} and the A_3 receptors as described in Chapter 3.

Data Analysis

K_i values were calculated using a non-linear regression curve-fitting program (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA). K_D values of the radioligands were 1.6 nM, 1.0 nM, and 5.0 nM for [^{3}H]DPCPX, [^{3}H]ZM 241385, and [^{125}I]AB-MECA, respectively.

4.5 References

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Chapter 5

2,6-Disubstituted and 2,6,8-Trisubstituted Purines as Adenosine Receptor Antagonists

Purines have long been exploited as adenosine receptor antagonists. The substitution pattern about the purine ring has been well investigated and certain criteria have become almost a prerequisite for good affinity at the adenosine A_1 receptor. The adaptation of the pharmacophore and the initial series of pyrimidines developed earlier in this thesis resulted in a series of purines with an entirely new substitution pattern. One compound in particular, 8-cyclopentyl-2,6-diphenylpurine (**5.31**, LUF 5962) has been shown to be very promising with an affinity of 0.29 nM at the adenosine A_1 receptor.

5.1 Introduction

In Chapter 3 it was shown that a rudimentary pharmacophore (see Figure 5.3) can lead to new types of adenosine A_1 receptor antagonists.[1] The resulting 2,4,6-trisubstituted pyrimidines were shown to be both potent and very selective for the A_1 receptor (Chapters 3 and 4). In an attempt to judge the relative location of the hydrogen-bond acceptor close to the 'top' of a central aromatic group and a more precise orientation of the L2 lipophilic group in relation to the central ring, fixation of this group was considered. Figure 5.1 shows the logical development of the 4-amido-2,6-diphenylpyrimidines into the purines explored in this chapter. Fixing the hydrogen-bond accepting group at the 'top' of the central group prompted the change of the heteroatom, for both synthetic ease and to preserve the C=Heteroatom unsaturated bond.

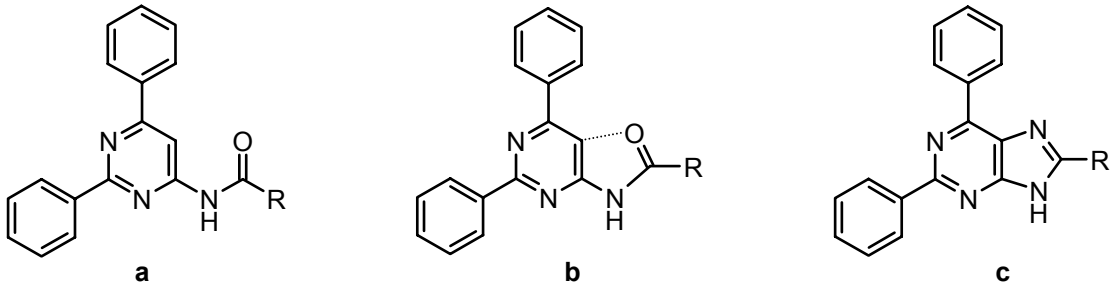


Figure 5.1 a) 2,6-diphenyl-4-amidopyrimidine; b) the fixation of the hydrogen-bond acceptor at the 'top' of the molecule; c) the change of the heteroatom to accomplish this fixation.

Purines have been explored at length as adenosine receptor antagonists as detailed in Chapter 2. However, these have mainly been direct analogues of adenine, in the sense that the N^6 group has always been preserved. This N^6 amino group has also usually been substituted with a cyclopentyl group to attain good affinity for the A_1 receptor.[2-7] Further exploration of this central core also deems the necessity of N9 substitution for potency, with both large benzyl derivatives (compound **2.49**)[5] and small methyl (**2.51**)[7] or ethyl substituents (**2.50**)[6] showing good affinity for the A_1 receptor. De Ligt *et al*. postulated certain features to enhance the selectivity of the basic adenine-ring (Figure 5.2).[7] The resulting series of compounds displayed good affinity for the adenosine A_1 receptor, with N^6 - cyclopentyl-8-(N-methylisopropylamino)-9-methyladenine showing the best K_i value at 7.7 nM. These types of compounds, with the presence of the N^6 group and with N9 substitution show good affinity at the A_1 receptor, corroborating with the model detailed in Chapter 3 in a manner shown in Figure 5.3. The hydrogen-bond donating group (denoted B) is represented by the available hydrogen on the N^6 and the hydrogen-bond acceptor groups A or C may be one of the two ring nitrogens (N1 or N7), depending on the

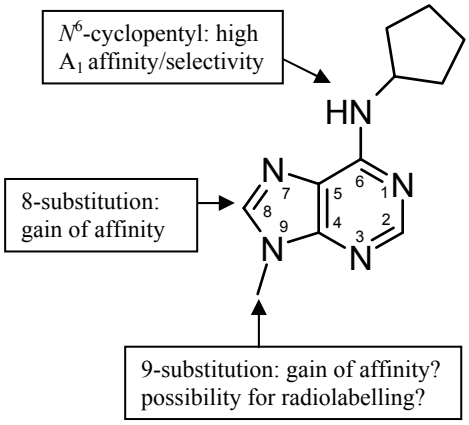


Figure 5.2 The features proposed by De Ligt *et al*.[7] to enhance affinity and selectivity at the A_1 receptor and the numbering about the purine ring.

orientation and the size of possible substitutents on the other positions of the purine ring. The lipophilic pocket denoted L2 may be filled with the substituent labelled R^1 and either L1 or L3 are satisfied by the R^2 substituent. Optimal receptor interaction may be provided by substitution in the C2 position, as found by Bianucci *et al*. in their investigations.[5]

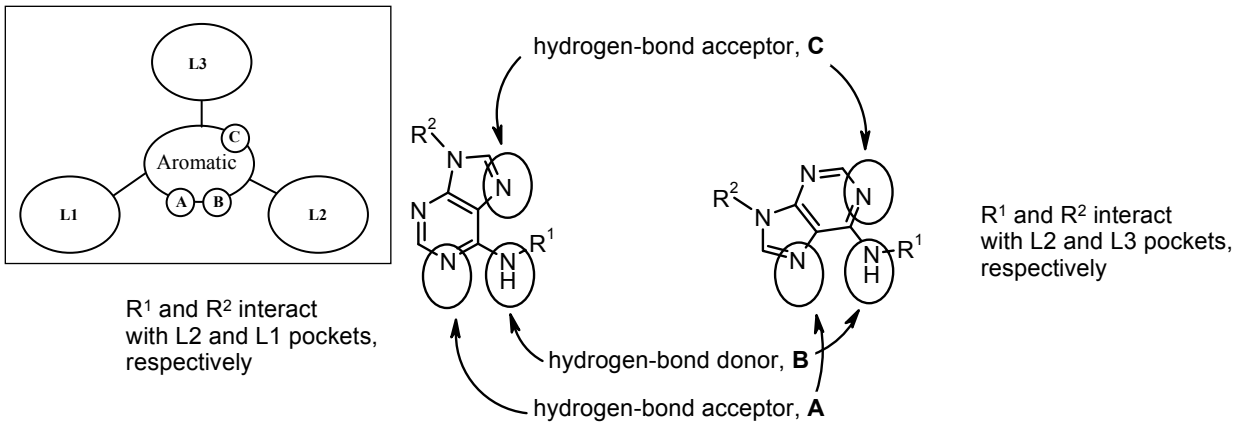


Figure 5.3 The model detailed in Chapter 3 (in the upper left corner) and the two possible orientations of the 'traditionally' substituted adenines to fit this model.

In this chapter, a new perspective upon the purines is realised according to the model proposed in Chapter 3, where excellent affinity for the A_1 adenosine receptor is achieved despite the lack of the seemingly essential N^6 and the N9 groups/substituent proposed in earlier papers. Direct aromatic substitution at the C2 and C6 positions (labelled R^1 and R^2 in Figure 5.4) provide analogy to the 2,6-aromatic substituents on the 4-amino-pyrimidines (Chapter 3). C8 substitution (R^3) explores the 'L2' pocket, and R^4 should be, according to the model, left unsubstituted to achieve high affinity at the A_1 adenosine receptor. In accordance to the PSA requirement, as detailed in Chapter 4, the value for these purines lies in the region of 50 $Å^2$.

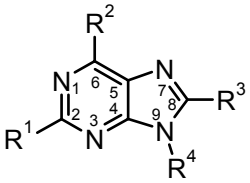


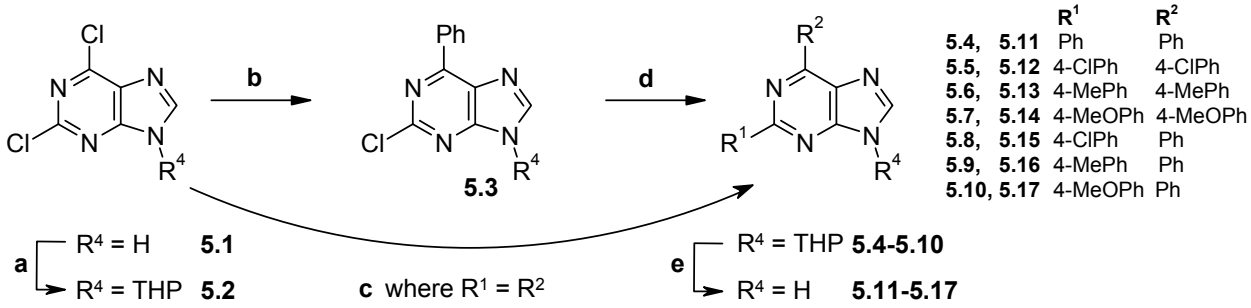
Figure 5.4 Proposed substitution about the purine structure.

5.2 Results and Discussion

5.2.1 Chemistry

The purines were made via two routes. The 2,6-disubstituted purines were synthesised from commercially available 2,6-dichloropurine as described in Scheme 5.1. Substitution of the chlorines was possible through metal-mediated cross-coupling reactions. Both Stille and Suzuki-Miyaura couplings of purines have been reported in literature.[8-15] To facilitate the Suzuki cross-coupling, protection of the nitrogen at N9 was necessary. This was successfully achieved with tetrahydropyran (THP). Initial attempts with a benzylic group rendered the product far too stable, and removal of the protecting group was impossible under usual hydrogenation or transfer hydrogenation conditions. Substitution of both chlorines with the same phenyl derivative occurred under standard Suzuki conditions as detailed by Hocek *et al*. (compounds **5.11-5.14**).[11] However, microwave heating to temperatures of 150 °C were

employed instead of conventional heating methods, reducing reaction time from 8 hours to approximately 20 minutes. An excess of the boronic acid (3 equivalents) encouraged the reactions to completion. Where the 2- and 6-substituents differed, one equivalent of the boronic acid provided initial singular substitution at the 6-position (compounds **5.15-5.17**), confirmed by X-ray crystallography. Further reaction with an excess of the second boronic acid gave the desired 2,6-diphenyl derivatives.



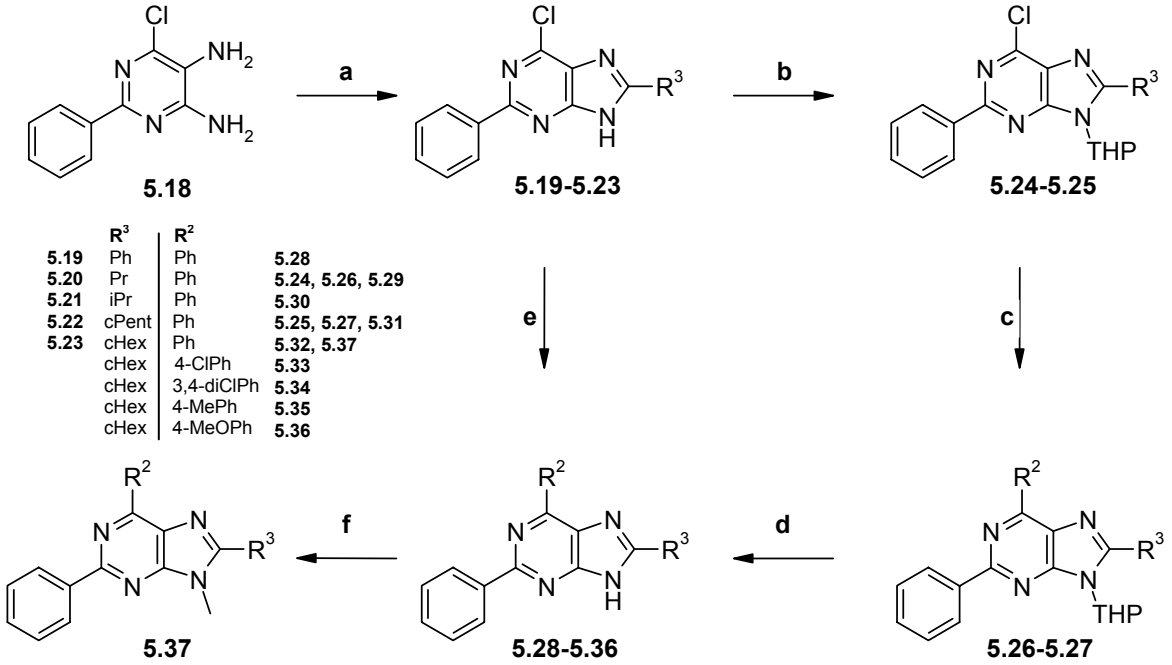
Scheme 5.1 a) dihydropyran, pTSA, THF; b) 1 eq. $PhB(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; c) 3 eq. $R^1B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; d) 1.2 eq $R^1B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; e) dowex, EtOH.

For substitution at the 8-position, bromination of a protected 2,6-dichloropurine to provide a more versatile intermediate was attempted following the procedure described by De Ligt *et al*.[7] in their evaluation of N0840 analogues. Unfortunately, this was unsuccessful, and the 8-substituent was introduced into the purine frame at an earlier stage, as described in Scheme 5.2. To make the vital intermediate **5.18**, a four step procedure described in a number of papers by Biagi *et al*. was broadly exploited.[16-18] Reaction of commercially available benzamidine with diethyl malonate created a pyrimidine ring, after which various substitution steps resulted in intermediate **5.18**. Ring closure was attempted by several different methods that are detailed in literature, e.g., using trimethylorthoformate, triethylorthoformate or PPA as the reaction medium.[19,20] These methods were either poor-yielding or were difficult to work up or purify. Finally, a two-step reaction incorporating the addition of the appropriate acid chloride followed by cyclisation under basic conditions provided a more ideal synthetic pathway.[21] The 6-chloro-2-phenyl-8-substituted purine was then subjected to metal-catalysed cross-coupling reactions as described above, furnishing compounds **5.28-5.36**. It was discovered that protection at N9 was no longer necessary, probably due to the increased steric bulk around this position preventing the complexation of the catalyst and/or the boronic acid. Methylation of compound **5.32** following standard procedures with methyl iodide in basic conditions rendered compound **5.37**.

5.2.2 Structure Activity Relationships

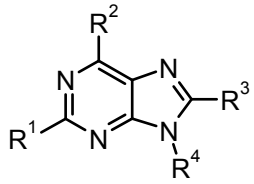
The results of the radioligand binding assays performed on these purines are shown in Table 5.1. Compounds **5.11-5.17** are unsubstituted in the C8 position and are varied at C2 and C6. 2,6-Diphenyl-9*H*-purine (**5.11**) has an affinity of 4 nM for the hA_1 receptor, already matching the 'best' ligands from the two pyrimidine series (Chapters 3 and 4). The selectivity is in

general however, somewhat lower, with good affinities registered at both the A_{2A} and the A_3 receptors (52 and 38 nM, respectively). An identical substitution at the 4-position of both of the phenyl groups (**5.12-5.14**) is to the great detriment of the affinity for the A_1 and A_{2A} receptors, in contrast to the improved affinity (9 nM) at the A_3 receptor by the bis-4-methyl substitution (**5.13**). The single substitution of just the C2 phenyl group shows no enhancement for the A_1 receptor, with both the 4-Cl (**5.15**) and the 4-MeO (**5.17**) moieties reducing the affinity of the ligand for this receptor, whilst compound **5.16** possessing the 4-Me group retains the affinity of the unsubstituted diphenylpurine at 4 nM. The great beneficiary of this particular substitution pattern is the A_3 receptor, where both the 4-Me (**5.16**) and the 4-MeO (**5.17**) compounds display significantly enhanced affinity (K_i values of 9 and 3 nM, respectively) when compared to compound **5.11**. 4-Methoxy-substitution of phenyl groups have usually been shown to enhance the affinity for the A_3 receptor in a number of different series of adenosine antagonists,[22,23] thus it may seem surprising that the bis-4-Me substituted variety (**5.13**) displayed significantly higher affinity for the A_3 receptor than the analogous bis-4-MeO ligand (**5.14**). However, the results of the mono-substituted compounds (**5.16**, 9 nM and **5.17**, 3 nM) confirm that the 4-MeO- group is still preferred by the A_3 receptor. Bis-4-MeO-substitution is probably just too large for optimal binding in the A_3 receptor pocket, accounting for its poorer affinity compared to the slightly smaller bis-4-Me variation. It seems that for the A_1 receptor, unsubstituted phenyl groups may be the optimal R^1 and R^2 groups for the affinity of 9*H*-purines.



Scheme 5.2 a) (i) R^3COCl, pyridine, DCM; (ii) 2M NaOH; b) dihydropyran, pTSA, THF; c) 1.5 eq. $R^2B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; d) dowex, EtOH; e) 1.5 eq. $R^2B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; f) NaH, MeI, DMF.

Table 5.1. Affinities of the 2,6,8-Trisubstituted-Purines **5.11-5.37** in Radioligand Binding Assays of Human Adenosine Receptors.



	R^1	R^2	R^3	R^4	K_i (nM) or % disp.[a]		
					hA_1[b]	hA_{2A}[c]	hA_3[d]
5.11	Ph	Ph	H	H	4.1 ± 0.5	52.5 ± 14	38 ± 12
5.12	4-ClPh	4-ClPh	H	H	20.8%	11%	71 ± 12
5.13	4-MePh	4-MePh	H	H	235 ± 25	4%	9.6 ± 2
5.14	4-MeOPh	4-MeOPh	H	H	40%	37%	163 ± 39
5.15	4-ClPh	Ph	H	H	27 ± 6	315 ± 96	93 ± 11
5.16	4-MePh	Ph	H	H	4.1 ± 0.6	289 ± 19	9.7 ± 4
5.17	4-MeOPh	Ph	H	H	32 ± 6	47%	3.3 ± 2
5.28	Ph	Ph	Ph	H	21 ± 12	66 ± 4	50 ± 18
5.29	Ph	Ph	Pr	H	4.4 ± 0.7	167 ± 25	17 ± 8
5.30 (LUF 5956)	Ph	Ph	iPr	H	0.82 ± 0.08	148 ± 27	9.3 ± 0.9
5.31 (LUF 5962)	Ph	Ph	cPent	H	0.29 ± 0.07	55 ± 3	34 ± 14
5.32 (LUF 5957)	Ph	Ph	cHex	H	0.73 ± 0.07	118 ± 17	195 ± 43
5.33	Ph	4-ClPh	cHex	H	25 ± 5	24%	239 ± 88
5.34	Ph	3,4-diClPh	cHex	H	37 ± 10	20%	37.2%
5.35	Ph	4-MePh	cHex	H	36 ± 4	9%	41%
5.36	Ph	4-MeOPh	cHex	H	3.7 ± 0.5	27%	135 ± 41
5.37	Ph	Ph	cHex	Me	24%	0%	25%

[a]K_i ± SEM (n = 3), % displacement (n = 2). [b]Displacement of specific [^{3}H]DPCPX binding in CHO cell membranes expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations. [c]Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cell membranes expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations. [d]Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cell membranes expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations.

Exploration of the C8 position of the purine led in general to significant improvements in the affinity for the A_1 receptor. A phenyl group in the 8-position (**5.28**) is evidently too large for the A_1 receptor binding pocket with a drop in affinity to 21 nM. Both the A_{2A} and A_3 receptors seem to tolerate this much larger substituent well, on the whole retaining the affinity achieved by the comparable unsubstituted compound **5.11**. This indicates, perhaps, more space in this part of the respective A_{2A} and A_3 receptor pockets than in the A_1 receptor site. The single straight-chained alkyl group (nPr, **5.29**) showed a K_i value of 4 nM, improving on the selectivity over the A_{2A} receptor when compared to the unsubstituted form (**5.11**) by a factor of 3. However, the affinity at the A_3 receptor also showed an improvement to 16.5 nM. The most active compounds at the adenosine A_1 receptor registered sub-nanomolar affinity. These were the C8- isopropyl (**5.30**), cyclopentyl (**5.31**) and cyclohexyl (**5.32**) derivatives at 0.82, 0.29, 0.73 nM, respectively. In particular, the cyclopentyl moiety (**5.31**) displayed an impressive gain in affinity at the A_1 receptor, whilst retaining the same degree of affinity at the A_{2A} and A_3 receptors in comparison to ligand **5.11**. In comparison to the cyclopentyl derivative, the cyclohexyl compound (**5.32**) was only slightly lower in affinity at the A_1

receptor, yet showed a significant drop in affinity at the A_{2A} and A_3 receptors. To see whether both C8 substitution and substitution at one of the phenyl groups would be well tolerated, compounds **5.33**-**5.36** were synthesised and tested. Again, a significant loss in affinity is noted across the three receptors, although the 4-MeO moiety seems to interfere the least, retaining an affinity of 4 nM for the A_1 receptor. Compound **5.37** highlights the importance of the free N9 proton to act as a hydrogen-bond donor**,** since the methyl substitution results in a complete loss of affinity at the adenosine receptors.

Comparing the most potent compound at the A_1 receptor of this series, **5.31** (0.29 nM), to the most potent purine published to-date, compound **2.51** (Chapter 2, 8 nM), it may be speculated that the affinity to the A_1 receptor is much improved (almost 30-fold) because both the L1 and the L3 pockets are now subject to interaction with the ligand. In Figure 5.5, the two possible ways of superimposing these compounds is depicted, following the proposed model, as mentioned in Figure 5.3. Superimposition **a** suggests that there are no interactions with the L1 pocket, as provided by the C2-phenyl group of **5.31**, whilst **b** suggests that there likewise is little interaction with the L3 pocket, provided by the C6-phenyl of **5.31**. In the lowest energy state of the compound, the 8-substituent of **2.51** is also most probably orientated out of the plane of the core heterocycle, although this may not be a significant factor upon the final ligand-receptor complex.

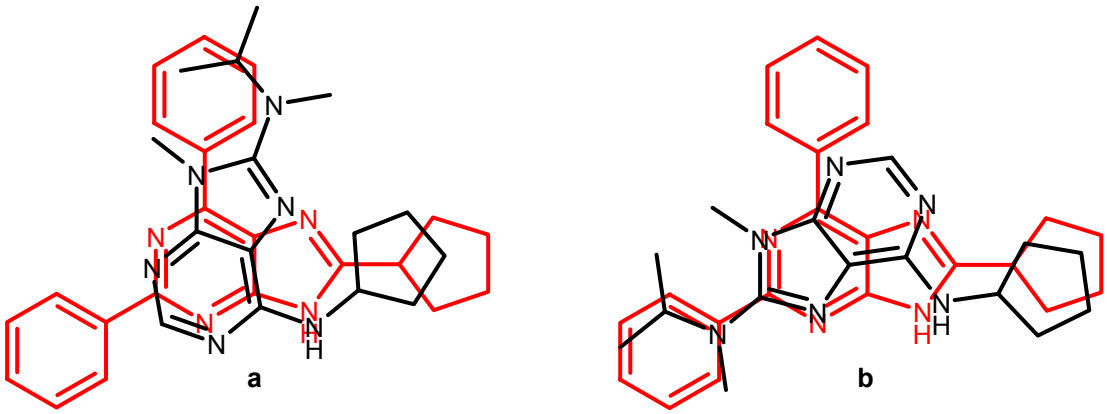


Figure 5.5 The two possible ways of superimposing compounds **5.31** and **2.51**.

By immobilising the H-bond acceptor at the 'top' of the molecule to form the fused heterocyclic ring, the position, and thus the orientation towards the receptor, of the L2 group is shifted slightly higher than predicted in the model detailed in Chapter 3. This slight relocation of the L2 group has brought about substantial improvements in the affinity for the A_1 receptor. This is best illustrated by comparing the analogous compounds **3.19** (cyclopentanecarboxylic acid (2,6-diphenyl-pyrimidin-4-yl)-amide) and **5.31** (8-cyclopentyl-2,6-diphenyl-9*H*-purine). In both series these two compounds displayed the highest affinity for the A_1 receptor, yet **5.31** was, at 0.29 nM, significantly more active than **3.19** (2.14 nM). The selectivity for the A_1 receptor over the A_{2A} and A_3 receptors was also somewhat better for the purine compound than for the pyrimidine. It is therefore appropriate to take into

consideration these results and refine the pharmacophoric model given in Chapter 3. Figure 5.6 displays schematically the new refinements. It has previously been discovered and discussed in both of the pyrimidines series (Chapters 3 and 4) and in this current chapter that the optimal L2 group seems to be somewhat smaller than the L1 and L3 groups and the lipophilic pocket that this group fills lacks the ability to interact with π-electrons. The ideal L2 group is therefore an alkyl group that consists of an alkyl chain of 2-3 carbons in length (i.e., ethyl or propyl) and a secondary-branched moiety (i.e., isopropyl, cyclopentyl, etc). Although this chapter details the development of a fused bicyclic ring, the central aromatic region was sufficiently covered by a single ring, as detailed in Chapters 3 and 4. Thus, it is appropriate to assume that it is not the size of the aromatic ring that is particularly important, but the relative positioning to the aromatic centre of the hydrogen-bond donor, B and the acceptor C.

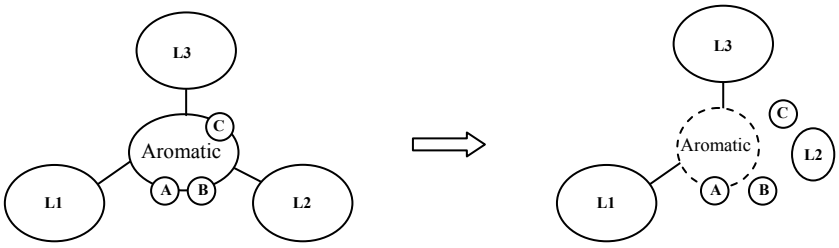


Figure 5.6 Refinement of the model proposed in Chapter 3.

5.3 Conclusions

This chapter describes a series of 2,6,8-trisubstituted purines synthesised as a consequence of the refinement of the model proposed in Chapter 3. The fixation of the H-bond acceptor at the 'top' of the molecule repositions the relative location of the L2 group. Exchanging the heteroatom for a nitrogen creates the imidazole ring. The benefits of these modifications are highlighted by compound **5.31**, LUF 5962, 8-cyclopentyl-2,6-diphenyl-9*H*-purine, which has an affinity of 0.29 nM at the human adenosine A_1 receptor.

5.4 Experimental

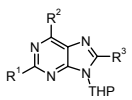
5.4.1 Chemistry

Materials and Methods

All reagents used were obtained from commercial sources and all solvents were of an analytical grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad and ar = aromatic protons. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Leiden Institute of Chemistry and are within 0.4% of the theoretical values unless otherwise stated. Reactions were routinely monitored by TLC using Merck silica gel F_{254} plates. Microwave reactions were performed on an Emrys Optimizer (Biotage AB, formerly Personal Chemistry). Wattage was automatically adjusted so as to maintain the desired temperature.

To protect the purines with the THP group a procedure reported in literature by Cassidy *et al*. was followed.[24] Compounds **5.2**[24] and **5.18**[18] have been reported previously.

General Preparation for the Suzuki-Miyaura Cross-Coupling Under Microwave Conditions



THP-protected-2,6-dichloropurine **5.2** (1 eq.) was dissolved in dry toluene (5 mL). To this was added the appropriate boronic acid (3 eq. if double substitution required or 1 eq. if single substitution desired), K_2CO_3 (1.5 eq. or 1 eq., respectively) and $Pd(PPh_3)_4$ (0.052 eq.). The reaction

vial was then sealed and heated at 150 °C for 20 minutes. Upon completion of the reaction (monitored by TLC), the solvents were evaporated and the crude product pre-absorbed on silica. Purification with column chromatography gave the desired product.

2-Chloro-6-phenyl-9-(tetrahydro-pyran-2-yl)-9*H*-purine (5.3).

White solid, 71%. ^{1}H-NMR δ($CDCl_3$): 8.80-8.75 (m, 2H, phenyl-*H*), 8.32 (s, 1H, purine-*H*), 7.57-7.51 (m, 3H, phenyl-*H*), 5.84 (m, 1H, THP), 4.22-4.17 (m, 1H, THP), 3.86-3.75 (m, 1H, THP), 2.20-1.57 (m, 6H, THP).

2,6-Diphenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.4).

White solid, 80%. ^{1}H-NMR δ($CDCl_3$): 9.05-8.94 (m, 2H, phenyl-*H*), 8.73-8.69 (m, 2H, phenyl-*H*), 8.32 (s, 1H, purine-*H*), 7.66-7.4 (m, 4H, phenyl-*H*), 5.93 (dd, 1H, J = 6.6, 2.9 Hz, THP), 4.23-4.16 (m, 1H, THP), 3.88-3.75 (m, 1H, THP), 2.24-1.67 (m, 6H, THP).

2,6-Bis(4-chlorophenyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (5.5).

White solid, 91%. ^{1}H-NMR δ($CDCl_3$): 8.85 (d, 2H, J = 8.0 Hz, Ar-*H*), 8.54 (d, 2H, J = 8.0 Hz, Ar-*H*), 8.29 (s, 1H, purine-*H*), 7.53-7.43 (m, 4H, Ar-*H*), 5.89 (dd, 1H, J = 6.6, 2.9 Hz, THP), 4.24-4.17 (m, 1H, THP), 3.89-3.76 (m, 1H, THP), 2.18-1.71 (m, 6H, THP).

2,6-Bis(4-tolyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (5.6).

White solid, 77%. ^{1}H-NMR δ($CDCl_3$): 8.83 (d, 2H, J = 8.0 Hz, Ar-*H*), 8.55 (d, 2H, J = 8.0 Hz, Ar-*H*), 8.29 (s, 1H, purine-*H*), 7.41-7.30 (m, 4H, Ar-*H*), 5.95 (dd, 1H, J = 5.8, 3.6 Hz, THP), 4.25-4.19 (m, 1H, THP), 3.92-3.81 (m, 1H, THP), 2.46, 2.45 (2× s, 6H, 2×CH_3), 2.22-1.72 (m, 6H, THP).

2,6-Bis(4-methoxyphenyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (5.7).

White solid, 82%. ^{1}H-NMR δ($CDCl_3$): 8.93 (d, 2H, J = 9.5 Hz, Ar-*H*), 8.61 (d, 2H, J = 8.8 Hz, Ar-*H*), 8.25 (s, 1H, purine-*H*), 7.11-7.01 (m, 4H, Ar-*H*), 5.91 (dd, 1H, J = 6.6, 2.2 Hz, THP), 4.24-4.18 (m, 1H, THP), 3.92-3.83 (m, 1H, THP), 3.91 (s, 6H, 2×OCH_3), 2.18-1.67 (m, 6H, THP).

2-(4-Chlorophenyl)-6-phenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.8).

White solid, 87%. ^{1}H-NMR δ($CDCl_3$): 8.91-8.86 (m, 2H, phenyl-*H*), 8.60-8.55 (m, 2H, Ar-*H*), 8.29 (s, 1H, purine-*H*), 7.62-7.44 (m, 5H, phenyl-*H*+Ar-*H*), 5.87 (dd, 1H, J = 6.6, 2.9 Hz, THP), 4.21-4.15 (m, 1H, THP), 3.87-3.76 (m, 1H, THP), 2.17-1.68 (m, 6H, THP).

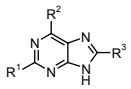
2-Tolyl-6-phenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.9).

White solid, 90%. ^{1}H-NMR δ($CDCl_3$): 8.94-8.88 (m, 2H, phenyl-*H*), 8.55 (d, 2H, J = 8.8 Hz, Ar-*H*), 8.28 (s, 1H, purine-*H*), 7.63-7.49 (m, 3H, phenyl-*H*), 7.31 (d, 2H, J = 8.0 Hz, Ar-*H*) 5.92 (dd, 1H, J = 7.3, 2.9 Hz, THP), 4.21-4.15 (m, 1H, THP), 3.88-3.75 (m, 1H, THP), 2.43 (s, 3H, CH_3), 2.17-1.68 (m, 6H, THP).

2-(4-Methoxyphenyl)-6-phenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.10).

Oil, 97%. ^{1}H-NMR δ($CDCl_3$): 8.93-8.89 (m, 2H, phenyl-*H*), 8.63-8.58 (m, 2H, Ar-*H*), 8.26 (s, 1H, purine-*H*), 7.62-7.51 (m, 3H, phenyl-*H*), 7.03-6.99 (m, 2H, Ar-*H*) 5.88 (dd, 1H, J = 6.6, 2.9 Hz, THP), 4.19-4.12 (m, 1H, THP), 3.85 (s, 3H, CH_3), 3.79-3.75 (m, 1H, THP), 2.15-1.64 (m, 6H, THP).

General Procedure for the Removal of the THP Protecting Group[25]



2,6-Bis(4-chlorophenyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (0.67 mmol) and Dowex® (50WX2-100) ion exchange resin (300 mg) were refluxed in EtOH (20 mL) and H_2O (0.7 mL) until no starting material was evident by TLC. The mixture was then filtered whilst hot and the resin further washed with aliquots of hot EtOH. The combined filtrates were evaporated and then co-distilled with toluene to remove the last traces of H_2O to yield the free purine.

2,6-Diphenyl-9*H*-purine (5.11).

Recrystallised from EtOAc/PE. White solid, 53%. mp: >252 ºC dec. ^{1}H-NMR δ(DMSO): 11.44 (br s, 1H, N*H*), 9.04-9.01 (m, 2H, phenyl-*H*), 8.67-8.58 (m, 2H, phenyl-*H*), 7.64-7.56 (m, 6H, phenyl-*H*). ^{13}C-NMR δ(DMSO): 157.2, 154.6, 152.1, 145.3, 138.1, 136.0, 131.0, 130.1, 129.5, 128.7, 127.8. MS (ESI): 273.0. Anal. ($C_{17}H_{12}N_4$·0.4EtOAc) C, H, N.

2,6-Bis(4-chlorophenyl)-9*H*-purine (5.12).

Recrystallised from CH_2Cl_2. White solid, 43%. mp: >290 ºC dec. ^{1}H-NMR δ(DMSO): 8.98-8.94 (m, 2H, 4-chlorophenyl-*H*), 8.66 (s, 1H, purine-*H*), 8.54-8.50 (m, 2H, 4-chlorophenyl-*H*), 7.70-7.57 (m, 4H, 4-chlorophenyl-*H*). ^{13}C-NMR δ(DMSO): 156.0, 145.6, 136.7, 135.8, 134.9, 134.5, 131.0, 129.4, 128.7, 128.6. MS (ESI): 340.7. Anal. ($C_{17}H_{10}N_4Cl_2$·0.1H_2O.0.05CH_2Cl_2) C, H, N.

2,6-Bis(4-tolyl)-9*H*-purine (5.13).

Recrystallised from CH_2Cl_2. White solid, 52%. mp: >288 ºC dec. ^{1}H-NMR δ(DMSO): 8.90-8.86 (m, 2H, 4-tolyl-*H*), 8.61 (s, 1H, purine-*H*), 8.48-8.44 (m, 2H, 4-tolyl-*H*), 7.47-7.35 (m, 4H, 4-tolyl-*H*), 2.44, 2.41 (2s, 6H, 2×CH_3). ^{13}C-NMR δ(DMSO): 157.2, 151.9, 144.9, 141.8, 140.8, 139.6, 135.5, 133.2, 131.7, 129.4, 129.2, 129.0, 127.7, 21.2, 21.0. MS (ESI): 300.9. Anal. ($C_{19}H_{16}N_4$·0.06CH_2Cl_2) C, H, N.

2,6-Bis(4-methoxyphenyl)-9*H*-purine (5.14).

Recrystallised several times from various solvents, including CH_2Cl_2, EtOH, MeOH and EtOAc/petroleum ether mixtures. White solid. mp: 282 ºC. ^{1}H-NMR δ(MeOD): 8.66-8.61 (m, 4H, 4-methoxyphenyl-*H*), 8.41 (s, 1H, purine-*H*), 7.13-7.09 (m, 4H, 4-methoxyphenyl-*H*), 3.91 (s, 6H, 2×OCH_3). MS (ESI): 332.0.

2-(4-chlorophenyl)-6-phenyl-9*H*-purine (5.15).
Recrystallised from MeOH. White solid, 46%. mp: 262 ºC. ^{1}H-NMR δ(DMSO): 8.97-8.93 (m, 2H, phenyl), 8.69 (s, 1H, purine-*H*), 8.56 (d, 2H, J = 8.8 Hz, Ar-*H*), 7.63-7.59 (m, 5H, phenyl-*H*). ^{13}C-NMR δ(DMSO): 156.2, 155.0, 151.9, 145.7, 137.0, 135.8, 134.9, 131.0, 128.5, 128.7. MS (ESI): 306.8. Anal. ($C_{17}H_{11}ClN_4$) C, H, N.
2-Tolyl-6-phenyl-9*H*-purine (5.16).
Recrystallised from MeOH. White solid, 43%. mp: 251 ºC. ^{1}H-NMR δ(DMSO): 8.99-8.96 (m, 2H, phenyl), 8.65 (s, 1H, purine-*H*), 8.50-8.46 (d, 2H, J = 8.04 Hz, 4-tolyl-*H*), 7.66-7.57 (m, 3H, phenyl-*H*), 7.39-7.35 (d, 2H, J = 8.04 Hz, 4-tolyl-*H*), 2.41 (s, 3H, C*H*$_3$). ^{13}C-NMR δ(DMSO): 157.3, 151.9, 145.2, 139.7, 136.0, 135.5, 130.9, 129.3, 128.7, 127.8, 21.1. MS (ESI): 286.8. Anal. ($C_{18}H_{14}N_4$·0.16MeOH) C, H, N.
2-(4-methoxyphenyl)-6-phenyl-9*H*-purine (5.17).
Recrystallised from MeOH. White solid, 55%. mp: 269 ºC. ^{1}H-NMR δ(DMSO): 8.98-8.96 (m, 2H, phenyl), 8.63 (s, 1H, purine-*H*), 8.54 (d, 2H, J = 8.8 Hz, Ar-*H*), 7.67-7.62 (m, 3H, phenyl-*H*), 7.13 (d, 2H, J = 8.8 Hz, Ar-*H*), 3.88 (s, 3H, OC*H*$_3$). ^{13}C-NMR δ(DMSO): 161.0, 157.3, 155.0, 151.8, 145.0, 136.0, 130.9, 130.7, 129.4, 128.7, 114.0, 55.3. MS (ESI): 302.8. Anal. ($C_{18}H_{14}N_4$) C, H, N.
General Ring-Closing Procedure to Form the Purine Moiety[21]
The appropriate acid chloride (4.53 mmol) in CH_2Cl_2 (10 mL) was added to 6-chloro-4,5-diamino-2-phenylpyrimidine (1 g, 4.53 mmol) in dry pyridine (5 mL) and stirred at room temperature overnight. H_2O (15 mL) was then added and the mixture separated. The solvents were evaporated and the intermediate purified by column chromatography on SiO_2, eluting with a CH_2Cl_2 and MeOH mixture (99:1). This intermediate was then dissolved in 2M NaOH (15 mL), adding MeOH until a clear solution was obtained and refluxed overnight. The cooled reaction mixture was then adjusted to pH 7 using 1M HCl forming a white precipitate. This solid was then filtered and dried and purified where necessary to give the respective 8-substituted-purine.
6-Chloro-2,8-diphenyl-9*H*-purine (5.19).
White solid, 66%. ^{1}H-NMR δ(MeOD/DMSO): 8.44-8.42 (m, 2H, phenyl-*H*), 8.27-8.16 (m, 2H, phenyl-*H*), 7.63-7.39 (m, 6H, phenyl-*H*).
2-Phenyl-6-chloro-8-propyl-9*H*-purine (5.20).
White solid, 51%. ^{1}H-NMR δ(MeOD): 8.40-8.36 (m, 2H, phenyl-*H*), 7.44-7.34 (m, 3H, phenyl-*H*), 2.89 (t, 2H, J = 7.3 Hz, C*H*$_2$), 1.97-1.79 (m, 2H, C*H*$_2$), 1.02 (t, 3H, J = 7.3 Hz, C*H*$_3$).
2-Phenyl-6-chloro-8-isopropyl-9*H*-purine (5.21).
White solid, 78%. ^{1}H-NMR δ(MeOD): 8.40-8.35 (m, 2H, phenyl-*H*), 7.45-7.39 (m, 3H, phenyl-*H*), 3.33-3.19 (m, 1H, C*H*), 1.42 (d, 6H, J = 7.3 Hz, 2×C*H*$_3$).
2-Phenyl-6-chloro-8-cyclopentyl-9*H*-purine (5.22).
White solid, 44%. ^{1}H-NMR δ(MeOD): 8.38-8.33 (m, 2H, phenyl-*H*), 7.42-7.39 (m, 3H, phenyl-*H*), 3.42-3.30 (m, 1H, C*H*), 2.27-1.70 (m, 8H, 4×C*H*$_2$).
2-Phenyl-6-chloro-8-cyclohexyl-9*H*-purine (5.23).
White solid, 99%. ^{1}H-NMR δ(MeOD): 8.39-8.34 (m, 2H, phenyl-*H*), 7.45-7.41 (m, 3H, phenyl-*H*), 3.00-2.88 (m, 1H, C*H*), 2.09-1.42 (m, 10H, 5×C*H*$_3$).
2-Phenyl-6-chloro-8-propyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.24).
White solid, 53%. ^{1}H-NMR δ($CDCl_3$): 8.51-8.46 (m, 2H, phenyl-*H*), 7.52-7.46 (m, 3H, phenyl-*H*), 5.78 (dd, 1H, J = 8.8, 2.2 Hz, THP), 4.26-4.19 (m, 1H, THP), 3.85-3.70 (m, 2H, THP), 3.53-3.37 (m, 1H, THP), 3.10-3.00 (m, 2H, C*H*), 2.90-2.73 (m, 1H, THP), 2.06-1.70 (m, 5H, THP + C*H*$_2$), 1.09 (t, 3H, J = 7.3 Hz, C*H*$_3$).
2-phenyl-6-chloro-8-cyclopentyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.25).
White solid, 75%. ^{1}H-NMR δ($CDCl_3$): 8.50-8.46 (m, 2H, phenyl-*H*), 7.53-7.45 (m, 3H, phenyl-*H*), 5.76 (dd, 1H, J = 8.8, 2.2 Hz, THP), 4.25-4.18 (m, 1H, THP), 3.79-3.68 (m, 1H, THP), 3.51 (pent, 1H, J = 8.0 Hz, C*H*), 3.03-2.83 (m, 1H, THP), 2.18-1.52 (m, 13H, THP+4×C*H*$_2$).
2,6-diphenyl-8-propyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.26).
White solid, 46%. ^{1}H-NMR δ($CDCl_3$): 9.06-8.93 (m, 2H, phenyl-*H*), 8.68-8.63 (m, 2H, phenyl-*H*), 7.60-7.44 (m, 6H, phenyl-*H*), 5.85 (dd, 1H, J = 8.8, 2.2 Hz, THP), 4.23-4.16 (m, 1H, THP), 3.79-3.68 (m, 1H, THP), 3.14-2.96 (m, 2H, C*H*$_2$), 2.79-2.71 (m, 1H, THP), 2.10-1.61 (m, 7H, THP + C*H*$_2$), 1.11 (t, 3H, J = 7.3 Hz, C*H*$_3$).
2,6-diphenyl-8-cyclopentyl-9-(tetrahydropyran-2-yl)-9*H*-purine (5.27).
White solid, 32%. ^{1}H-NMR δ($CDCl_3$): 9.05-9.00 (m, 2H, phenyl-*H*), 8.69-8.64 (m, 2H, phenyl-*H*), 7.59-7.43 (m, 6H, phenyl-*H*), 5.78 (dd, 1H, J = 8.8, 2.2 Hz, THP), 4.20-4.14 (m, 1H, THP), 3.75-3.62 (m, 1H, THP), 3.54 (pent, 1H, J = 8.0 Hz, C*H*), 2.98-2.86 (m, 1H, THP), 2.18-1.56 (m, 13H, THP+4×C*H*$_2$).
2,6,8-Triphenyl-9*H*-purine (5.28).
Recrystallised from MeOH. White solid, 87%. mp: 233 ºC. ^{1}H-NMR δ($CDCl_3$): 9.14-9.11 (m, 2H, phenyl-*H*), 8.61-8.56 (m, 2H, phenyl-*H*), 7.98-7.94 (m, 2H, phenyl-*H*), 7.65-7.26 (m, 9H, phenyl-*H*). ^{13}C-NMR δ($CDCl_3$): 137.9, 135.8,130.4, 130.0, 129.2, 128.3, 127.9, 127.7, 127.5, 126.7. MS (ESI): 348.7. Anal. ($C_{23}H_{16}N_4$·0.12 MeOH) C, H, N.

8-Propyl-2,6-diphenyl-9*H*-purine (5.29).
Recrystallised from MeOH. White solid, 52%. mp: 149 ºC. ^{1}H-NMR δ($CDCl_3$/MeOD): 8.60-8.52 (m, 4H, phenyl-*H*), 7.63-7.47 (m, 6H, phenyl-*H*), 3.01 (t, 2H, J = 7.30 Hz, CH_2), 2.00-1.89 (m, 2H, CH_2), 1.08 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C-NMR δ($CDCl_3$): 155.6, 135.4, 130.2, 129.5, 128.8, 128.1, 127.9, 127.6, 30.4, 20.9, 12.8. MS (ESI): 314.8. Anal. ($C_{20}H_{18}N_4$) C, H, N.
8-Isopropyl-2,6-diphenyl-9*H*-purine (5.30).
Recrystallised from MeOH. White solid, 36%. mp: 214 ºC. ^{1}H-NMR δ($CDCl_3$): 8.94-8.89 (m, 2H, phenyl-*H*), 8.53-8.49 (m, 2H, phenyl-*H*), 7.62-7.38 (m, 6H, phenyl-*H*), 3.34-3.27 (m, 1H, C*H*), 1.48 (d, 6H, J = 7.3 Hz, 2×CH_3). ^{13}C-NMR δ($CDCl_3$/MeOD): 162.7, 157.6, 152.4, 138.3, 136.0, 133.3, 132.9, 132.1, 131.8, 131.6, 130.2, 129.5, 128.5, 128.1, 127.8, 127.3, 29.2, 20.6. MS (ESI): 314.8. Anal. ($C_{20}H_{18}N_4$·0.11EtOH) C, H, N.
8-Cyclopentyl-2,6-diphenyl-9*H*-purine (5.31).
Recrystallised from DCM. White solid, 45%. mp: 224 ºC. ^{1}H-NMR δ($CDCl_3$): 8.53-8.65 (m, 4H, phenyl-*H*), 7.48-7.63 (m, 6H, phenyl-*H*), 3.33-3.50 (m, 1H, C*H*), 1.79-2.28 (m, 8H, 4×CH_2). ^{13}C-NMR δ($CDCl_3$/MeOD): 162.5, 157.7, 156.0, 151.0, 137.7, 135.4, 129.8, 129.1, 128.7, 127.8, 127.6, 127.3, 126.3, 125.2, 39.2, 31.5, 24.9. MS (ESI): 341.0. Anal. ($C_{22}H_{20}N_4$·0.04CH_2Cl_2) C, H, N.
8-Cyclohexyl-2,6-diphenyl-9*H*-purine (5.32).
Recrystallised from EtOH. White solid, 49%. mp: 209 ºC. ^{1}H-NMR δ($CDCl_3$): 9.07-9.04 (m, 2H, phenyl-*H*), 8.70-8.63 (m, 2H, phenyl-*H*), 7.61-7.57 (m, 6H, phenyl-*H*), 2.66-2.54 (m, 1H, C*H*), 1.92-1.29 (m, 10H, 5×CH_2). ^{13}C-NMR δ($CDCl_3$): 161.6, 157.7, 155.4, 152.9, 138.6, 136.2, 133.2, 130.7, 130.0, 129.9, 128.7, 128.5, 128.4, 127.6, 38.1, 25.5. MS (ESI): 354.8. Anal. ($C_{23}H_{22}N_4$·0.1H_2O) C, H, N.
8-Cyclohexyl-6-(4-chlorophenyl)-2-phenyl-9*H*-purine (5.33).
Recrystallised from MeOH. White solid, 86%. mp: 156 ºC. ^{1}H-NMR δ($CDCl_3$): 8.78-8.70 (m, 2H, 4-chorophenyl-*H*), 8.54-8.50 (m, 2H, phenyl-*H*), 7.64-7.41 (m, 5H, (4-chloro)phenyl-*H*), 3.07-2.92 (m, 1H, C*H*), 2.11-1.30 (m, 10H, 5×CH_2). ^{13}C-NMR δ($CDCl_3$): 164.2, 158.4, 154.5, 138.0, 136.0, 134.4, 130.6, 129.3, 128.6, 128.1, 127.9, 127.5, 30.8, 30.8, 25.4. MS (ESI): 388.9, 390.2. Anal. ($C_{23}H_{21}ClN_4$·0.7H_2O.0.5MeOH) C, H, N.
8-Cyclohexyl-6-(3,4-dichlorophenyl)-2-phenyl-9*H*-purine (5.34).
Recrystallised from EtOH. White solid, 64%. mp: 201 ºC. ^{1}H-NMR δ($CDCl_3$): 12.50 (br s, 1H, NH_2), 9.09-9.08 (m, 1H, 3,4-dichorophenyl-*H*), 8.91-8.86 (m, 1H, 3,4-dichorophenyl-*H*), 8.57-8.52 (m, 2H, phenyl-*H*), 7.64-7.57 (m, 3H, phenyl-*H*), 7.24-7.14, 6.78-6.77, 6.59-6.53 (3×m, 1H, 3,4-dichorophenyl-*H*), 2.60-2.49 (m, 1H, C*H*), 1.89-0.98 (m, 10H, 5×CH_2). ^{13}C-NMR δ($CDCl_3$): 162.2, 157.9, 155.5, 150.3, 138.3, 136.1, 134.9, 132.8, 131.4, 130.5, 130.1, 129.2, 128.9, 128.5, 117.5, 115.4, 38.5, 31.1, 25.5. MS (ESI): 422.7, 425.2. Anal. ($C_{23}H_{20}Cl_2N_4$·0.7EtOH) C, H, N.
8-Cyclohexyl-6-(4-tolyl)-2-phenyl-9*H*-purine (5.35).
Recrystallised from MeOH. White solid, 98%. mp: 149 ºC. ^{1}H-NMR δ($CDCl_3$): 8.93-8.88 (d, 2H, J = 8.04 Hz, 4-tolyl-*H*), 8.61-8.56 (m, 2H, phenyl-*H*), 7.59-7.51 (m, 3H, phenyl-*H*), 7.39-7.35 (d, 2H, J = 8.04 Hz, 4-tolyl-*H*), 2.57-2.50 (m, 1H, C*H*), 2.46 (s, 1H, CH_3), 1.89-0.98 (m, 10H, 5×CH_2). ^{13}C-NMR δ($CDCl_3$): 161.3, 157.7, 155.2, 153.0, 141.0, 138.7, 133.3, 130.6, 130.0, 129.8, 129.3, 128.7, 128.4, 38.5, 31.1, 25.5, 21.6. MS (ESI): 368.5. Anal. ($C_{24}H_{24}N_4$·0.9H_2O.0.35MeOH) C, H, N.
8-Cyclohexyl-6-(4-methoxyphenyl)-2-phenyl-9*H*-purine (5.36).
Recrystallised from MeOH. White solid, 29%. mp: 141 ºC. ^{1}H-NMR δ($CDCl_3$): 9.05-9.01 (d, 2H, J = 8.78 Hz, 4-methoxyphenyl-*H*), 8.61-8.57 (m, 2H, phenyl-*H*), 7.58-7.54 (m, 3H, phenyl-*H*), 7.11-7.07 (d, 2H, J = 8.78 Hz, 4-methoxyphenyl-*H*), 3.90 (s, 1H, OCH_3), 2.57-2.50 (m, 1H, C*H*), 1.89-0.98 (m, 10H, 5×CH_2). ^{13}C-NMR δ($CDCl_3$): 161.8, 161.1, 157.7, 155.1, 138.8,131.6, 130.0, 129.0, 128.7, 128.4, 113.9, 55.35, 38.49, 31.18, 25.5. MS (ESI): 384.9. Anal. ($C_{24}H_{24}N_4O$·0.95H_2O.0.3MeOH) C, H, N.
8-Cyclohexyl-9-methyl-2,6-diphenyl-9*H*-purine (5.37).
Recrystallised from $CHCl_3$. White solid, 30%. mp: 200-202 °C. ^{1}H-NMR δ($CDCl_3$): 9.03-9.00 (m, 2H, phenyl-*H*), 8.65-8.62 (m, 2H, phenyl-*H*), 7.63-7.53 (m, 6H, phenyl-*H*), 3.92 (s, 3H, CH_3), 3.25-3.31 (m, 1H, C*H*), 2.10-1.44 (m, 10H, 5×CH_2). ^{13}C-NMR δ($CDCl_3$): 161.0, 157.3, 55.0, 151.9, 140.9, 138.7, 136.5, 130.3, 129.7, 129.6, 128.3, 128.2, 128.0, 36.7, 30.8, 28.3, 25.9, 25.6. MS (ESI): 369.1. Anal. ($C_{24}H_{24}N_4$·0.09$CHCl_3$) C, H, N.

5.4.2 Biology

Materials and Methods
[^{3}H]DPCPX and [^{125}I]AB-MECA were purchased from Amersham Biosciences (NL). [^{3}H]ZM 241385 was obtained from Tocris Cookson, Ltd. (UK). CHO cells expressing the human adenosine A_1 receptor were provided by Dr. Andrea Townsend-Nicholson, University College London, UK. HEK 293 cells stably expressing the human adenosine A_{2A} and A_3 receptor were gifts from Dr. Wang (Biogen, USA) and Dr. K.-N. Klotz (University of Würzburg, Germany), respectively.
All compounds were tested in radioligand binding assays to determine their affinities at the human adenosine A_1, A_{2A} and the A_3 receptors as described previously in Chapter 3, with the exception of non-specific binding on the A_{2A} receptor was determined in the presence of 10 μM CGS21680 instead of 100 μM CPA. The human A_1

receptors were expressed in CHO cells, and [^{3}H]DPCPX used as the radioligand. The A_{2A} and A_3 receptors were expressed in HEK 293 cells, and [^{3}H]ZM 241385 and [^{125}I]AB-MECA were used as the respective radioligands.

Data Analysis

K_i values were calculated using a non-linear regression curve-fitting program (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA). K_D values of the radioligands were 1.6 nM, 1.0 nM and 5.0 nM for [^{3}H]DPCPX, [^{3}H]ZM 241385 and [^{125}I]AB-MECA, respectively.

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Chapter 6

2,6,8-Trisubstituted-1-Deazapurines as Adenosine Receptor Antagonists

Exploration of the pyrimidine and purine series in the preceding three chapters led to a refinement of the pharmacophore defined (in Chapter 3) for antagonists of the adenosine A_1 receptor. This chapter details the adoption of these new criteria to produce a series of 1-deazapurines with consistently high affinity for the adenosine A_1 receptor. 1-Deazapurines (otherwise known as 3*H*-imidazo[4,5-*b*]pyridines) are structurally very similar to the purines, however, in a synthetic sense they pose an array of difficulties, mainly as a result of the reduced reactivity about the 6-membered ring. The desired double aromatic substituents at the 2- and 6-positions were amongst the most troublesome features to incorporate. An eventual adaptation of a known route resulted in a series with five of the derivatives displaying K_i values in the sub-nanomolar region. The most potent of these, compound **6.10** (LUF 5978), displays an affinity of 0.55 nM at the human adenosine A_1 receptor.

6.1 Introduction

In Chapter 5, the re-analysis of the purine ring and its substitution pattern in accordance to the pharmacophore developed in Chapter 3 produced a very good series of ligands, with particular examples displaying sub-nanomolar affinity at the A_1 receptor. The subsequent analysis of the results led to a refinement of the original model, highlighting the relative position of the L2 lipophilic group to the stipulated H-bond acceptors and donors.

The purine derivatives that were devised in Chapter 5 came about as a direct result of their structural similarity to 4-amido-2,6-diphenylpyrimidines (Chapter 3) (Figure 5.1). However, the 4-amido-2,6-diphenylpyrimidines were eclipsed by the analogous 2-amido-4,6-diphenylpyrimidines (Chapter 4) in terms of displaying similar levels of affinity and better selectivity for the adenosine A_1 receptor. Extrapolating the 2-amido-4,6-diphenylpyrimidines in the same manner as the 4-amido-2,6-diphenylpyrimidines, 5,7-diphenyl-3,3*a*-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidines are fashioned (Figure 6.1). The specifications of the refined model still only demand the fulfilment of three hydrogen-bonding regions, thus it is reasonable to assume that the extra nitrogen atom in the triazolopyrimidine ring will not play an important role in the affinity of the compound for the A_1 receptor, in terms of hydrogen-bonding. This nitrogen also disturbs the planarity of the central core; therefore, its replacement with a carbon is more compliant with the model. The removal of this nitrogen also reduces the PSA value from approximately 46 $Å^2$ to 41 $Å^2$. The resulting 3*H*-imidazo[4,5-*b*]pyridine (Figure 6.2) bears comparison to the 2-amido-4,6-diphenylpyridines originally planned in Chapter 4, of which one example was synthesised displaying similar K_i values to the earlier pyrimidines.

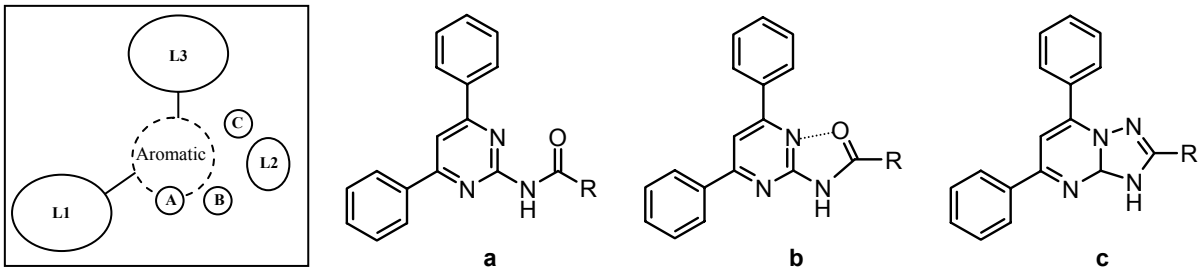


Figure 6.1 On the left the refined model detailed in Chapter 5; **a**) 2-amido-4,6-diphenylpyrimidine; **b**) the fixation of the hydrogen-bond acceptor at the 'top' of the molecule; **c**) the change of the heteroatom to accomplish this fixation resulting in a 5,7-diphenyl-3,3*a*-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine.

Trivially, 3*H*-imidazo[4,5-*b*]pyridine is also known as 1-deazapurine (and 4-azabenzimidazole). Figure 6.2 shows the numbering about the ring for the systematic nomenclature (**a**) and the numbering system adopted from the purines (**b**). In this chapter, the trivial name and numbering system are used for the purpose of easy comparison to the purines (Chapter 5). The new stipulations of the model disclosed in Chapter 5 require the L2 lipophilic group (here labelled as R^3) to be a branched alkyl group, with a chain length of 2-3

carbons. In this chapter, the further refinements to the pharmacophore outlined in Chapter 5 are investigated and confirmed.

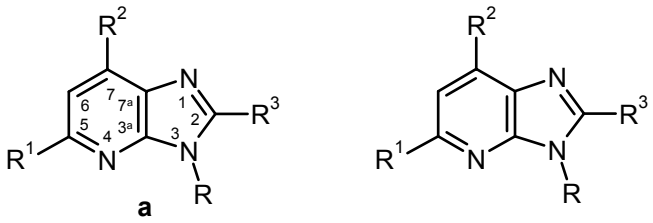


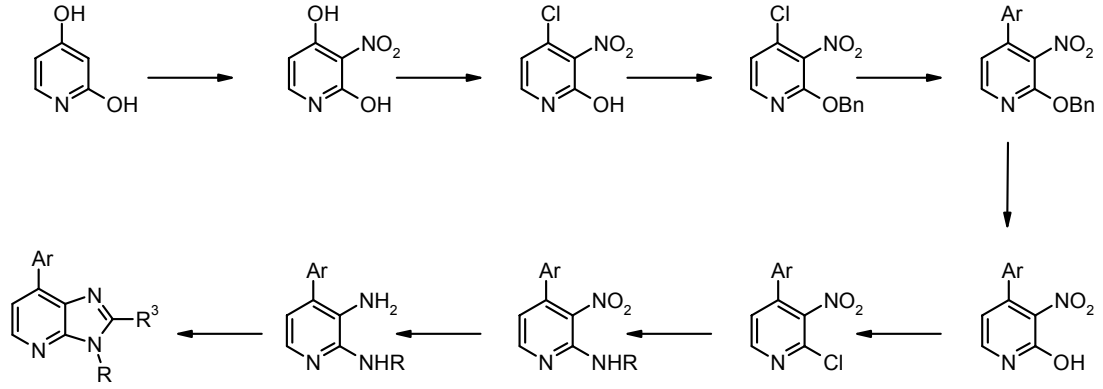
Figure 6.2 a) The systematic (IUPAC) name: 3*H*-imidazo[4,5-*b*]pyridine with the corresponding numbering system; **b**) 1-deazapurine and the purine numbering system.

6.2 Results and Discussion

6.2.1 Chemistry

Synthesis of the 1-deazapurines was less than straightforward, with little or no precedence for creating compounds with two aromatic substituents in the 2- and 6- positions of the ring. In this section, a brief description of the known routes to compounds similar to the target ligands is described, along with the potential variations to produce the target structures.

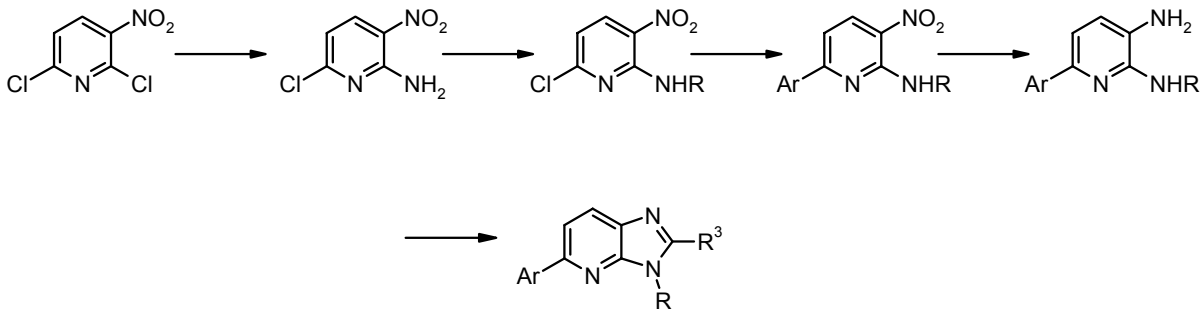
A series of 1-deazapurines were published in 2003 as corticotropin releasing factor receptor ligands.[1] The compounds featured direct aromatic substitution at the 6-position, with further derivatisation at N9. The synthetic procedure numbered 9 steps from commercially available starting materials and is described in Scheme 6.1. Functional group interconversions allowed various substitutions to create a functionalised pyridine ring that could be ring-closed to form the second fused (imidazole) ring.



R = alkyl, OMe, R^1 = branched alkyl derivatives

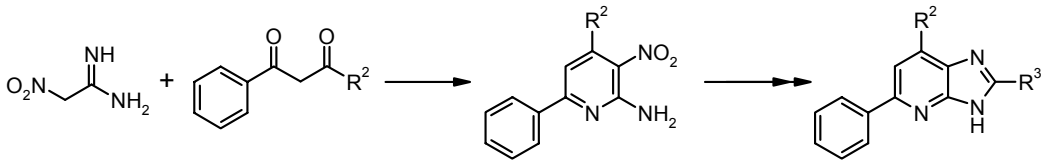
Scheme 6.1 The synthetic route described by Arvanitis *et al*.[1] to 6-,8-disubstituted-1-deazapurines.

Compounds featuring direct aromatic substitution at the C2 position of the 1-deazapurine ring have been explored by Oguchi *et al*. (Scheme 6.2).[2] A primary amine reacted preferentially at the 2-position of the commercially available 2,6-dichloro-3-nitropyridine. This intermediate then underwent substitution under Suzuki conditions to allow an aromatic group to reside at the 6-position. Subsequent ring closure furnished a C8-substituent on the 1-deazapurine.



Scheme 6.2 The synthesis of a series of 1-deazapurine substituted directly with an aromatic group at the C2 position, as described by Oguchi *et al*.[2]

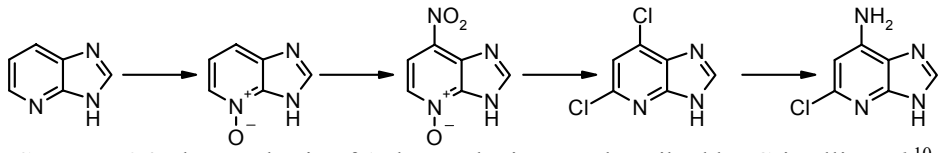
One example where an aromatic substituent at C2 and another substituent at C6 exist concurrently is the series of NK-3 receptor antagonists by Giardina *et al*.[3] A phenyl group and a carboxylate substituent were incorporated into the pyridine ring by the reaction of a diketone and nitroacetamidine in a Guareshi condensation following a procedure by Batt and Houghton.[4] Subsequent ring closure yielded the respective 1-deazapurines (Scheme 6.3). One compound that would have been an extremely interesting intermediate for the target compounds in this chapter was attempted by Batt and Houghton. This was 2-amino-3-nitro-4,6-diphenylpyridine. However, dibenzoylmethane could not be induced to react with nitroacetamide at all, even 4-methoxy-4'-nitrodibenzoylmethane, a relatively activated form of the diketone could not be induced to react.



Scheme 6.3 The synthetic route towards 2,6,8-trisubstituted-1-deazapurines as described by Batt and Houghton.[4]

Further explorations of direct carbon-carbon bonds at the 2- and 6-positions of 1-deazapurines generally seem to be restricted to simple alkyl groups. The majority of these compounds are angiotensin II receptor ligands and require further substitution at N9.[5-7] The synthesis of these compounds is relatively straightforward, in that 2-amino-4,6-dimethylpyridine is commercially available. Nitration of this ring, followed by reduction creates a 2,3-diamino species that can be ring-closed to form the 5-membered imidazole part of the deazapurine system.

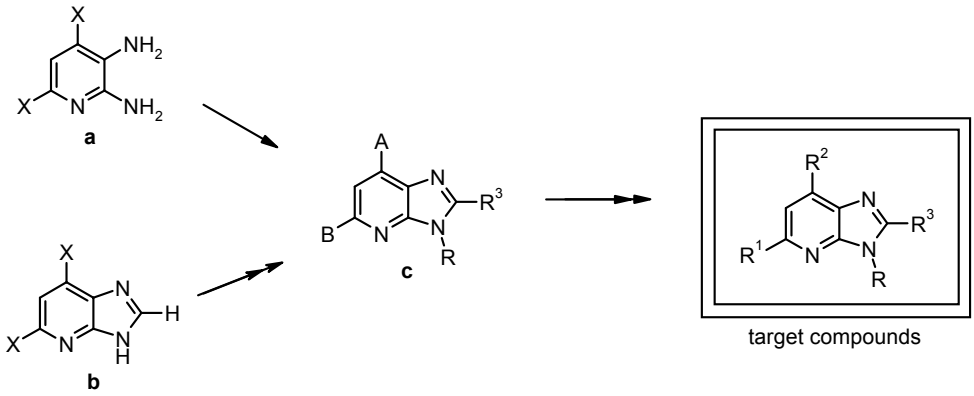
All of these routes detailed above require the ring closure of a 2,3-diaminopyridine and as in the synthesis of the purines (Chapter 5) there are a variety of procedures to accomplish this. An unsubstituted C8 position can be made by refluxing the pyridine in tri(m)ethylorthoformate.[8] Substitution is generally introduced by the use of a carboxylic acid at elevated temperatures, either neat[2] or in the presence of a medium such as PPA (polyphosphoric acid).[6] As an alternative, a two-step technique using an acid chloride in the presence of a base followed by base-catalysed hydrolysis, as described in Chapter 5, can be successful.[9]



Scheme 6.4 The synthesis of 1-deazaadenines as described by Cristalli *et al*.[10]

There is precedent to form 2,6-disubstituted 1-deazapurines where the substituents are often amines,[11] sulfides,[12] halogens[13,14] or mixtures of these. Cristalli *et al*. have reported on the synthesis of 1-deaza analogues of 2-chloroadenosine from the commercially available unsubstituted intact 1-deazapurine species (Scheme 6.4).[10] This procedure starts with the manipulation of the reactivity about the pyridine ring allowing chloro-substitution of C2 and C6 leading to the possibility of further functional group interconversions.

Thus, it seems that the majority of the synthetic routes to the singly, doubly, or triply 2-, 6-, or 8-substituted deazapurines can be split into two different branches. The first option focuses on incorporating groups on the pyridine part of the 1-deazapurine system as the initial phase of the synthesis, i.e. creating substituted 2,3-diaminopyridines. Ring-closure of this intermediate with a carboxylic acid derivative provides an 8-substituent. The second route requires the introduction of the 8-substituent in an intact functional deazapurine ring. The first method leads to an intermediate of the form **a** in Scheme 6.5, the latter method leading to intermediate **b**. Both methods should, ideally provide intermediates that allow late-stage variation of the three positions of interest, C2, C6 and C8.



Scheme 6.5 The two general routes detailed in literature to form 2,(6),(8)-(di)(tri)substituted-1-deazapurines.

Numerous attempts were made to create the 2,3-diaminopyridine intermediate (Scheme 6.5**a**); these are summarised in Scheme 6.6. The routes chosen mirror the various synthetic routes described earlier in this section. The main problems encountered were based upon the reactive nature and polarity of the functional groups and the reactivity of the positions about the pyridine ring. The first issue could probably be solved using protecting groups on the free heteroatoms (i.e., the amine and hydroxyl moieties), however using protecting groups incorporates a minimal of two further steps into an already long, multi-stepped synthesis. The latter problem is much more difficult to overcome. The electronegative nature of the nitrogen atom in the pyridine ring withdraws electron density both inductively and by resonance. The

resulting electron-poor ring is then several orders of magnitude less reactive than benzene and electrophilic substitution occurs only under relatively extreme conditions.[15] Activation of the ring with electron-donating substituents or the use of catalysts is a way of promoting these reactions. From Figure 6.3**a** it can also be seen that the positions susceptible to electrophilic substitution are the 3- and 5- positions. On the contrary, N-oxide derivatives allow 2-, 4- and 6-substitution (Figure 6.3**b**), although the rather unreactive nature of the pyridine ring still requires strong conditions to fulfil this.

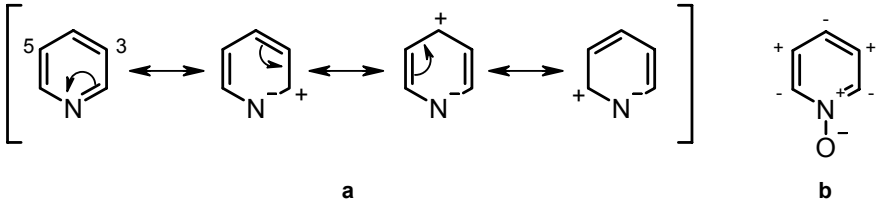
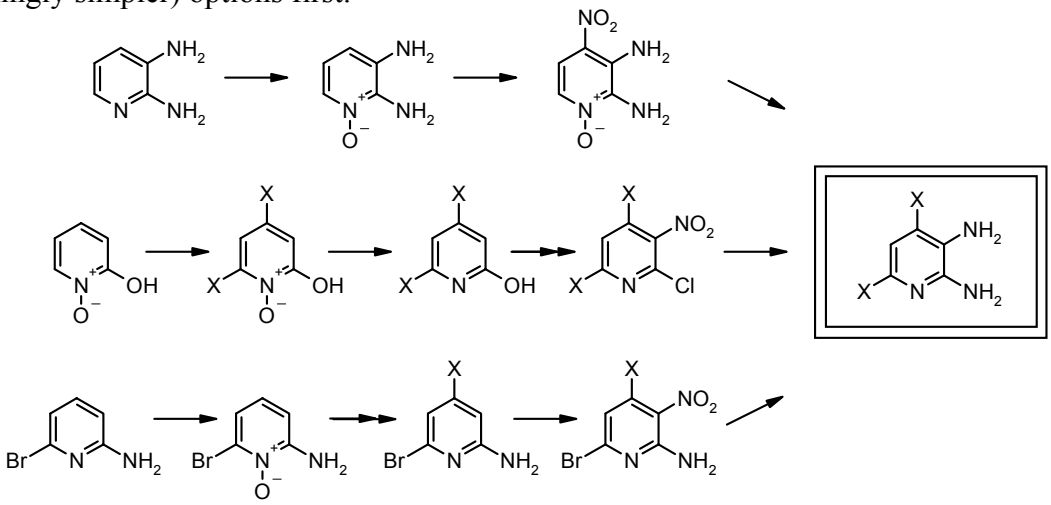


Figure 6.3 (**a**) The resonance forms of pyridine showing that electrophilic substitution occurs at the 3- and 5- positions, and (**b**) the relative partial charges about an N-oxide derivative.

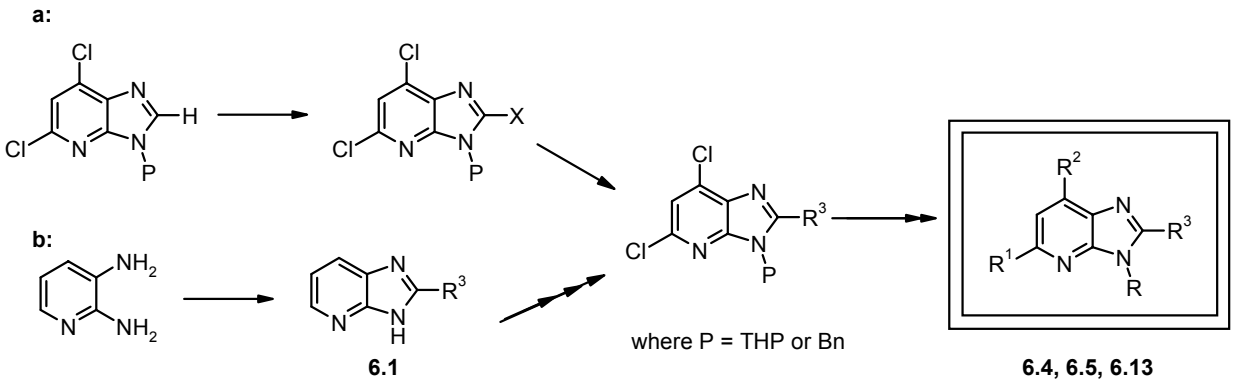
The synthesis of 2,3-diamino-4,6-dihalopyridines has been reported in literature. De Roos and Salemink showed that the reduction of 2-amino-3-nitro-4-chloropyridine with stannous chloride sporadically yields small quantities of 2,3-diamino-4,6-dichloropyridine.[16] Schelling and Salemink converted 2-amino-4,6-dichloropyridine through nitration and reduction steps into the desired diamino derivative.[13] The 2-amino-4,6-dichloropyridine was however in turn synthesised following a preparation by Graf.[17] This was a 5 step reaction sequence, starting from picolinic acid in a 10-day reflux period to create 4,6-dichloropicolinic acid. Although it has been shown to be possible to make this vital intermediate, the number of steps, the harsh conditions and problematical work-up procedures favoured the exploration of alternative (seemingly simpler) options first.



Scheme 6.6 A summary of some of the various routes to create a 2,3-diamino-4,6-disubstituted pyridine intermediate.

The alternative route exploiting the commercially available and intact 1-deazapurine was also undertaken (Schemes 6.5 and 6.7**a**). The 2,6-dichloro-1-deazapurine intermediate was synthesised according to the procedure by Cristalli *et al.* described in Scheme 6.4.[10] Attempts

were then made to functionalise the 8-position of the 1-deazapurine ring. The relatively acidic N9 proton was found to disrupt the intended reactions and thus protection of this group was necessary (studies have also shown that N3 and N7 substitution occurs as minor isomers in these reactions).[18] This was initially performed with a benzyl moiety. Unfortunately, the stability of this benzyl group presented difficulties in isolating the target compounds; standard catalytic hydrogenation using H_2 was sluggish, as was microwave-assisted catalytic transfer hydrogenation, despite reaching pressures of more than 20 bar at 150 ºC. Tetrahydropyran (THP) was later found to be much more suitable. Straightforward bromination techniques as described by De Ligt *et al*. for purine moieties,[19] using bromine and a phosphate buffer gave preferred substitution at the C1 position to the C8 position, a detail confirmed by NMR (seen both in ^{1}H and ^{13}C). Bromination at C8 was more successful using nBuLi and NBS. This product was accomplished by the lithiation at this position by nBuLi at -78 ºC, according to a procedure by Leonard and Bryant as applied to purines.[20] Lithiation of purine nucleosides regiospecifically at C8 has also been achieved in literature using LDA at low temperatures.[21] Further reaction of this species with an alkyl boronic acid to form an 8-alkyl substituent failed, possibly due to the unreactive nature of the alkyl boronic acid.



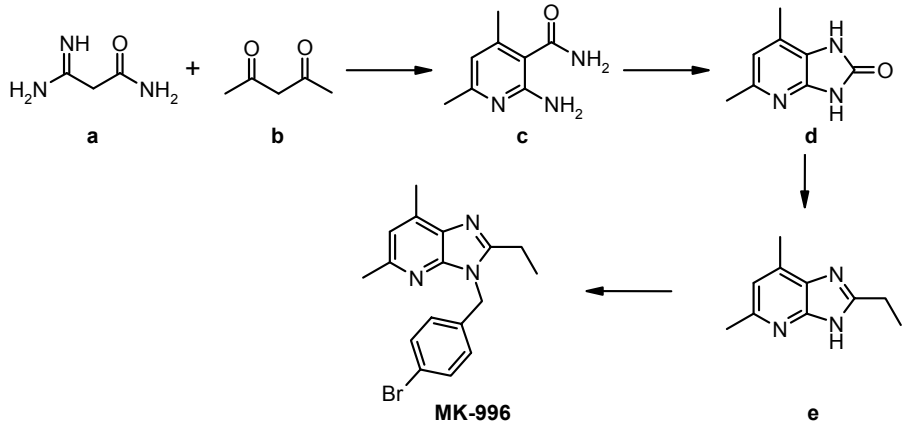
Scheme 6.7 The manipulation of an intact 1-deazapurine to create 2-, 6- and 8- substitution.

To increase the chances of a successful coupling reaction at this position, a better leaving group in the form of iodine was introduced. Iodination of the THP-protected deazapurine using NIS was, according to analysis of its NMR spectrum, successfully achieved at C8. The iodo compound underwent Suzuki-coupling with phenylboronic acid to provide 2,6-dichloro-8-phenyl-THP-protected-1-deazapurine in a 75% yield. Unfortunately, again attempts to carry out alkyl substitution under these conditions were not successful probably due to the relatively inactivated state of the boronic acid substrate. According to literature,[20,22] the quenching of the lithiated species with other electrophiles is also possible and this was successfully achieved with acetone, although not with an alkyl iodide.

The SAR revealed from the series detailed in Chapters 3-5 meant that the most favourable target compounds at C8 were directly linked alkyl groups. Therefore, the failure of the Suzuki reaction on the 8-iodo-1-deazapurine and the failure of the alkyl iodides as the electrophile of

choice to quench the 8-lithiated-deazapurine led to the simultaneous examination of the route incorporating the 8-derivative as one of the first steps of the synthesis (Scheme 6.7**b**).

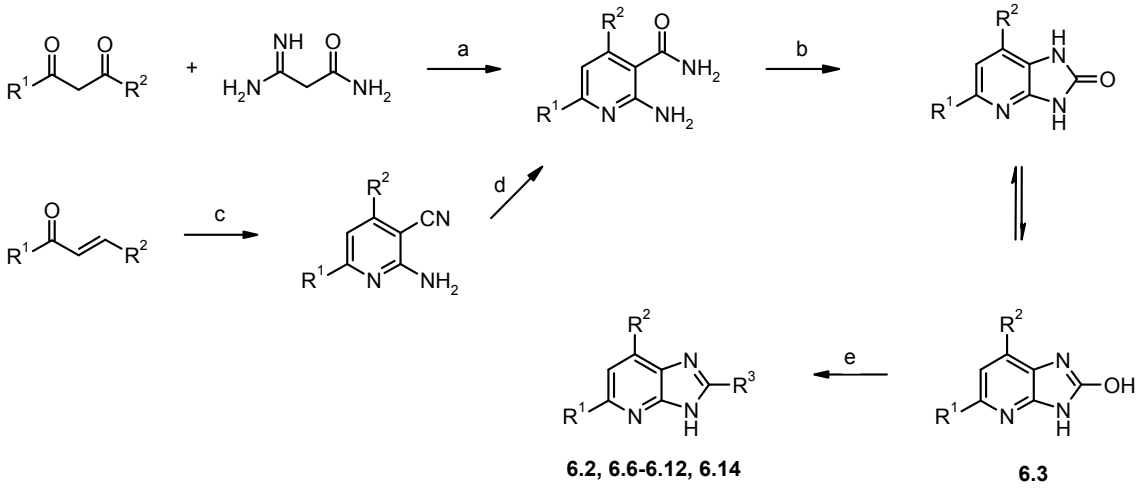
Three compounds were synthesised following the method detailed in Scheme 6.7, the unsubstituted forms (**6.4** and **6.5**), where the deazapurine was already commercially available and 8-cyclopentyl-2,6-diphenyl-deazapurine (**6.13**). The intermediate 2,6-dichloro-1-deazapurine was benzyl protected and subsequent aryl coupling gave **6.5** and deprotection yielded compound **6.4**. The third derivative was made by reacting 2,3-diaminopyridine with cyclopentyl carboxylic acid in the presence of PPA. The product, 8-cyclopentyl-1-deazapurine, was then subjected to the first three steps described in Scheme 6.4. The inclusion of a C8 substituent dramatically changed the reactivity of the species and each of the subsequent steps was significantly worse than the unsubstituted equivalent, in terms of yields and ease of handling. Another negative aspect of this route is the number of reactions involved to produce a series from which further SAR can be drawn. Incorporating the 8-derivative at the initial step in this manner leads to a 7-step synthetic sequence for each target product. With an aim to make more than 10 different compounds to be able to explore the SAR somewhat, this would lead to at least 70 reactions.



Scheme 6.8 The process development route to angiotensin II antagonist MK-996.[23,24]

Attention was thus turned on to another alternative procedure that seemed to offer a more encouraging pathway. In the process development of angiotensin II antagonists at Merck, a new approach to the key intermediate MK-996, benzylated imidazolutidine (Scheme 6.8), was investigated.[23,24] Malonamamidine (Scheme 6.8**a**) was condensed with a β-diketone (**b**) to provide the nicotinamide (**c**). The Hoffman rearrangement of this nicotinamide resulted in the isocyanate, which then reacted intramolecularly with the 2-amino group to give the urea (**d**). This compound was ingeniously found to react with a mixture of a carboxylic acid and anhydride in the presence of $MgCl_2$ to give 8-alkyl-2,6-dimethyl-1-deazapurines. Our fears for utilising this reaction pathway, similarly to many of the previous attempts to make 2,6-diaryl-1-deazapurine analogues, was that the desired diaryl-substituents would have a major (negative) influence on the reactivity of firstly the ketone, followed by each of the subsequent steps. As mentioned earlier (Scheme 6.3), the condensation of dibenzoylmethane with

nitroacetamide to produce 2-amino-3-nitro-4,6-diphenylpyridine failed due to the unreactive nature of the diketone.[4] In this case the ring-closure of dibenzoylmethane with malonamamidine was extremely low-yielding (6%). We thus sought the construction of the nicotinamide in a different manner. A 2-step approach employing a chalcone and malononitrile in the presence of ammonium acetate formed a cyanopyridine, which upon hydrolysis gave the target nicotinamide (Scheme 6.9).[25] This route also allows regioselective substitution about the pyridine ring if so desired, a feature not possible in the original route with the diketone. The Hoffman rearrangement proceeded without any significant problems and a substantial amount of the 2,6-diphenyl-8-hydroxy-1-deazapurine (**6.3**) could be made.



Scheme 6.9 The synthesis of 8-alkyl-2,6-diphenyl-1-deazapurine. a) KOH, MeOH, RT; b) KOH, $PhI(OAc)_2$, MeOH, RT; c) NH_4OAc, EtOH, Δ; d) KOH, EtOH, Δ; e) $MgCl_2$, R^3CO_2H, μw.

Substitution of the 8-OH was performed as described by Senanayake *et al*. [23] using a mixture of an acid and the corresponding anhydride in the presence of $MgCl_2$. The one main difference introduced was the use of the microwave for this procedure. Aromatic substituents at the 2- and 6-positions of the 1-deazapurine ring significantly lower its reactivity, and the employment of conventional heating methods resulted in very low yields and considerable quantities of by-products, which made the final product difficult to isolate. Using the microwave, rapid heating of the sealed vessel created very high temperatures quickly and elevated pressures. This method improved the synthesis dramatically, leading to better yields and easier isolation of the final target products.

The carboxylic acid anhydrides used were all commercially available, except for the cyclohexyl variant. This was synthesised according to a method as described by Kazemi *et al*. employing tosyl choride and potassium carbonate with a minimal use of solvents.[26] To remove the excess of the acid/anhydride reagents the crude material was distilled azeotropically with water. Removal of the last traces of water was subsequently performed by azeotropic distillation with dry toluene.

6.2.2 Structure Activity Relationships

The compounds **6.1-6.14** were tested in radioligand binding assays and the results of these assays are presented in Table 6.1. It is clear that the substitution at C2 (R^1) and C6 (R^2) of the 1-deazapurines is vital for affinity at the adenosine receptors. Compound **6.1**, lacking in substituents at these positions displays no affinity for the A_1, A_{2A}, or A_3 adenosine receptors. The π-electrons of the phenyl groups at these two positions provide seemingly essential interactions with the receptor pocket, although alkyl groups may afford some contact points for interaction, i.e., **6.2** ($K_i(hA_1)$ = 101 nM). Further substitution at the phenyl rings was not conducted due to the negative outcome obtained in the pyrimidine series (Chapter 4) and the purine series (Chapter 5).

Table 6.1. Affinities of the 2,6,8-Trisubstituted-1-Deazapurines **6.1-6.14** in Radioligand Binding Assays of Human Adenosine Receptors.

	R^1	R^2	R^3	R	K_i (nM) or % disp.[a]		
					hA_1[b]	hA_{2A}[c]	hA_3[d]
6.1	H	H	cPent	H	6%	3%	0%
6.2	Me	Me	Et	H	101 ± 26	8%	49%
6.3	Ph	Ph	OH	H	8.6 ± 3	192 ± 97	262 ± 25
6.4	Ph	Ph	H	H	1.2 ± 0.3	39 ± 9.0	180 ± 70
6.5	Ph	Ph	H	Bn	38%	2%	15%
6.6	Ph	Ph	Me	H	14 ± 5	375 ± 28	61 ± 14
6.7	Ph	Ph	Et	H	2.4 ± 0.4	177 ± 13	8.5 ± 6
6.8	Ph	Ph	Pr	H	2.5 ± 0.2	114 ± 34	69 ± 22
6.9 (LUF 5980)	Ph	Ph	iPr	H	0.61 ± 0.04	230 ± 40	7.3 ± 2
6.10 (LUF 5978)	Ph	Ph	2-MePr	H	0.55 ± 0.3	189 ± 23	25 ± 16
6.11 (LUF 5983)	Ph	Ph	1-EtPr	H	0.87 ± 0.1	247 ± 61	171 ± 49
6.12	Ph	Ph	tBu	H	5.5 ± 2	1055 ± 129	115 ± 15
6.13 (LUF 5816)	Ph	Ph	cPent	H	0.62 ± 0.3	49%	6.9 ± 1
6.14 (LUF 5981)	Ph	Ph	cHex	H	0.90 ± 0.2	194 ± 57	637 ± 71

[a]K_i ± SEM (n = 3), % displacement (n = 2). [b]Displacement of specific [^{3}H]DPCPX binding in CHO cell membranes expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations. [c]Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cell membranes expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations. [d]Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cell membranes expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations.

Of the 2,6-diphenyl compounds, the derivative with the unsubstituted C8 position (**6.4**) was an encouraging start with an affinity of 1 nM at the A_1 receptor. The N9-substituted compound (**6.5**) again showed that the free proton is necessary as a hydrogen-bond donor from the ligand to the receptor (cf. **5.37**). The C8-substituent was shown to have a drastic influence on the affinity (and selectivity) for the A_1 receptor. The intermediate compound of

the final route, **6.3**, shows that although an alkyl group is more tolerated at this position, the hydrogen-bonding potential of the hydroxyl group does not disturb too greatly the binding affinity of the deazapurines. Ethyl (**6.7**, $K_i(hA_1)$ = 2 nM) and propyl (**6.8**, $K_i(hA_1)$ = 2 nM) substitution confirmed the requisites of the model, in that as groups with a chain length of either 2 or 3 carbons, they were more effective than the methyl variant (**6.6**, $K_i(hA_1)$ = 14 nM). Fortifying the claims of the refined model, the predicted secondary-branched substituents possessed by far the most affinity for the adenosine A_1 receptor (**6.9-6.11**, **6.13** and **6.14**) with sub-nanomolar K_i values. The single tertiary-branched variety, the t-butyl derivative (**6.12**, K_i = 5.5 nM) displayed a 9-fold loss of potency at the A_1 receptor compared to the isopropyl derivative (**6.9**).

The selectivity of the 1-deazapurines for the A_1 over the A_{2A} receptor was better than the analogous purine compounds. Derivatives **6.4**, **6.9**, **6.13** and **6.14** had selectivity ratios of 33, 377, >1600 and 215 respectively, compared to 13, 180, 190 and 161 from the analogous purines **5.11** and **5.30-5.32**. The binding affinity of the deazapurines at the adenosine A_3 receptor was also shown to be worse than those measured at the A_1 receptor. Namely, compounds **6.11** and **6.14** with selectivity ratios of 199 and 718, respectively, were significant examples. Analysing the A_3 affinity values of the whole series, it seems that there is an obvious optimal two carbon-chain length. Of the simple unbranched alkyl groups (**6.6-6.8**), the ethyl moiety was easily the most potent at this receptor. Similarly, in the branched alkyl series (**6.9-6.11**) the iPr with an affinity of 7 nM was the best compound compared to the 2-methylpropyl (**6.10**, K_i = 25 nM) and 1-ethylpropyl (**6.11**, K_i = 173 nM). Of the two cycloalkyl variants made, the cyclopentyl (**6.13**) was also more influential at the A_3 receptor, with an affinity of 7 nM, than the cyclohexyl group (**6.14**) with its three-carbon chain length (K_i = 646 nM).

6.3 Conclusions

This series of 1-deazapurines confirms that the subtle refinements to the model in the previous chapter are substantial enough to produce a series with very good affinity for the adenosine A_1 receptor. In particular, compound **6.10** (LUF 5978) with a K_i value of 0.55 nM at the A_1 receptor is noteworthy in terms of affinity. However, compound **6.14** with a K_i value of 0.90 nM and selectivity ratios over the A_{2A} and A_3 receptors of 216 and 718, respectively, is overall the most interesting 1-deazapurine derivative presented in this chapter.

6.4 Experimental Section

6.4.1 Chemistry

Materials and Methods

All reagents used were obtained from commercial sources and all solvents were of an analytical grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad and ar = aromatic protons. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Leiden Institute of Chemistry and are within 0.4% of the theoretical values unless otherwise stated. Reactions were routinely monitored by TLC using Merck silica gel F_{254} plates.

Microwave reactions were performed in an Emrys Optimizer (Biotage AB, formerly Personal Chemistry). Wattage was automatically adjusted so as to maintain the desired temperature.

1-Deazapurine-N-oxide[27]

To a solution of 4-azabenzimidazole (1-deazapurine) (1.3 g, 10.9 mmol) in acetic acid (6.5 mL) was added H_2O_2 (35% in H_2O) (1.2 mL) and the mixture stirred at 70 ºC for 3 h. A further aliquot of H_2O_2 was then added (0.9 mL) and the reaction heated at 60 ºC for 16 h. The reaction mixture was allowed to cool to RT, upon which a precipitate formed. This was filtered to give a crystalline product (96%). ^{1}H NMR δ(MeOD): 8.45 (s, 1H, C8-*H*), 8.34 (d, 1H, J = 7.3 Hz, C6-*H*), 7.87 (d, 1H, J = 7.3 Hz, C2-*H*), 7.34-7.29 (m, 1H, C1-*H*).

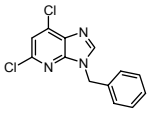
6-Nitro-1-deazapurine-N-oxide[27]

1-Deazapurine-N-oxide (1.4 g, 10.4 mmol) was dissolved in TFA (9.7 mL, 125 mmol, 12 eq.) at 0 ºC and to this was added fuming HNO_3 (6.6 mL, 157 mmol, 15 eq.). The reaction mixture was then heated at 90 ºC for 3 h and then left to cool to RT. Only slight precipitation occurred, thus the mixture was neutralised carefully with cNH_4OH, revealing further product. This was then filtered and the yellow solids were dried *in vacuo* at 40 ºC (67%). ^{1}H NMR δ(DMSO): 8.27 (s, 1H, C8-*H*), 8.10 (d, 1H, J = 7.3 Hz, C2-*H*), 7.89 (d, 1H, J = 6.58 Hz, C1-*H*).

2,6-Dichloro-1-deazapurine[10]

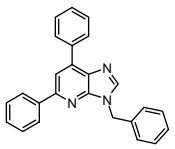
6-Nitro-1-deazapurine-N-oxide (1.2 g, 6.66 mmol) was added portionwise to an ice-cooled solution of $POCl_3$ (20 mL) in DMF (10 mL). The reaction mixture was then refluxed for 2 h, allowed to cool then added slowly to crushed ice. After neutralising carefully with 2M NaOH soln. the product was extracted with EtOAc (3 × 150 mL). The combined organic phases were then washed with H_2O (3 × 100 mL), dried over $MgSO_4$ and concentrated to give an off-white solid (80%). ^{1}H NMR δ(MeOD): 8.45 (s, 1H, C8-*H*), 7.46 (s, 1H, C1-*H*).

2,6-Dichloro-9-benzyl-1-deazapurine



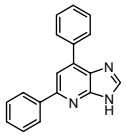
2,6-Dichloro-1-deazapurine (0.34 g, 1.80 mmol) was dissolved in DMF (15 mL). K_2CO_3 (0.77 g, 5.60 mmol, 3.1 eq.) was added and stirred for 30 mins. Benzyl chloride (0.29 mL, 2.53 mmol, 1.4 eq.) was then added and the reaction mixture stirred for 16 h at RT. The solids were filtered off and the filtrate separated between EtOAc (100 mL) and H_2O (100 mL). The aqueous layer was then further extracted with EtOAc (2 × 50 mL) and the combined organic layers then washed with H_2O (3 × 150 mL), dried over $MgSO_4$ and the solvents evaporated *in vacuo* to give a yellow oil. This crude product was chromatographed on SiO_2 eluting with a mixture of petroleum ether (bp 40-60 ºC) and EtOAc (gradient elution 5:1 to 2:1) to give the title compound (42 %). ^{1}H NMR δ($CDCl_3$): 8.02 (s, 1H, C8-*H*), 7.35-7.30 (m, 6H, Ar), 5.42 (s, 2H, CH_2).

2,6-diphenyl-9-benzyl-1-deazapurine (6.5)



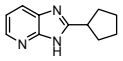
2,6-Dichloro-9-benzyl-1-deazapurine (150 mg, 0.54 mmol), phenyl boronic acid (197 mg, 1.62 mmol, 3 eq.), $Pd(PPh_3)_4$ (32 mg, 0.03 mmol, 0.05 eq.), K_2CO_3 (112 mg, 0.81 mmol, 1.5 eq.) and toluene (5 mL) were heated in a sealed vessel in the microwave for 30 mins at 150 ºC. The reaction mixture was then filtered and the filtrate pre-absorbed onto SiO_2 and chromatographed, eluting with petroleum ether (bp 40-60 ºC) and EtOAc (gradient elution 5:1 to 2:1). A white solid was obtained (91 %), which was further recrystallised from acetonitrile. mp 143 ºC; ^{1}H NMR δ($CDCl_3$): 8.17-8.13 (m, 4H, Ph), 8.04 (s, 1H, C8-*H*), 7.89 (s, 1H, C1-*H*), 7.56-7.29 (m, 11H, Ph), 5.50 (s, 2H, CH_2). ^{13}C-NMR δ($CDCl_3$): 152.8, 148.0, 143.8, 140.3, 139.7, 136.2, 136.0, 132.0, 129.1, 128.8, 128.6, 128.1, 127.9, 127.1, 114.1, 47.0. MS (ES^+): 362.0 Da. Anal. ($C_{25}H_{19}N_3.0.2CH_3CN$) C, H, N.

2,6-diphenyl-1-deazapurine (6.4)



2,6-Diphenyl-9-benzyl-1-deazapurine (100 mg, 0.233 mmol), $Pd(OH)_2$/C (20% Pd, Pearlman's catalyst) (20 mg), ammonium formate (147 mg, 2.3 mmol, 10 eq.) and EtOH (3 mL) were heated in a sealed vessel in the microwave at 140 ºC for 5 mins (pressure reached 11 bar). Upon cooling the septum was pierced to release the remaining pressure trapped in the vessel. The TLC showed only a slight trace of a new product. Further amount of ammonium formate added (300 mg) and the vessel resealed and heated at 150 ºC for 15 mins (pressure reached 22 bar). The reaction mixture was pre-absorbed onto SiO_2 and chromatographed eluting with CH_2Cl_2 and MeOH (gradient elution 98:2 to 90:10). A white solid was obtained (41%). mp 259-261 ºC; ^{1}H NMR δ(DMSO): 8.52 (s, 1H, C8-*H*), 8.28-8.02 (m, 5H, Ph + C1-*H*), 7.65-7.41 (m, 6H, Ph). ^{13}C-NMR δ(DMSO): 163.0, 156.6, 156.3, 151.6, 139.4, 135.8, 29.0, 128.7, 126.9. MS (ES^+): 272.1 Da. Anal. ($C_{18}H_{13}N_3.0.2$DMF) C, H, N.

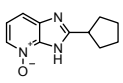
8-Cyclopentyl-1-deazapurine (6.1)



Polyphosphoric acid (~5 mL) was added to 2,3-diaminopyridine (0.43 g, 3.94 mmol), followed by cyclopentyl carboxylic acid (0.52 mL, 4.73 mmol, 1.2 eq.). This was then stirred at 100 ºC for 5 h, cooled in an ice-water bath to 0 ºC and neutralised carefully with cNH_4OH. The product was extracted with EtOAc (3 × 50 mL), the organic phases dried over $MgSO_4$ and then concentrated.

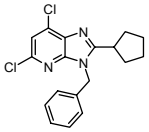
Chromatography on SiO_2, eluting with CH_2Cl_2 and MeOH (gradient elution 98:2 to 90:10) gave an off-white solid. Recrystallisation from MeOH provided the title product (10 %). mp 161 ºC; ^{1}H NMR δ($CDCl_3$): 8.18 (d, 1H, 4.4 Hz, C6-*H*), 7.90 (d, 1H, J = 7.32 Hz, C2-*H*), 7.11-7.06 (m, 1H, C1-*H*), 3.36 (pent, 1H, J = 8.0 Hz, C*H*), 2.17-1.63 (m, 8H, 4 × CH_2) ^{13}C-NMR δ(DMSO): 161.0, 149.2, 141.3, 135.0, 125.9, 117.3, 39.9, 31.8, 25.5. MS (ES^+): 188.2 Da. Anal. ($C_{11}H_{13}N_3.0.1H_2O$) C, H, N.

8-Cyclopentyl-1-deazapurine-N-oxide



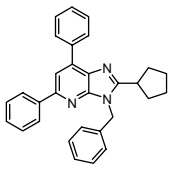
To a solution of 8-cyclopentyl-1-deazapurine (1.0 g, 5.51 mmol) in acetic acid (5 mL) was added H_2O_2 (35% in H_2O) (0.9 mL) and the mixture stirred at 70 ºC for 3 h. A further aliquot of H_2O_2 was then added (0.7 mL) and the reaction heated at 60 ºC for 16 h. The reaction mixture was allowed to cool to RT. The acetic acid was concentrated to approximately 1 mL, a few drops of H_2O were then added and the oil left to stand at room temperature, upon which precipitation occurred. The solid was collected and dried *in vacuo* to give a quantitative amount of the crude product. ^{1}H NMR δ(DMSO): 8.12-8.09 (d, 1H, J = 6.6 Hz, C6-*H*), 7.45-7.44 (m, 1H, C2-*H*), 7.17-7.11 (m, 1H, C1-*H*), 1.94-1.71 (m, 9H, 4 × CH_2).

9-Benzyl-8-cyclopentyl-2,6-dichloro-1-deazapurine



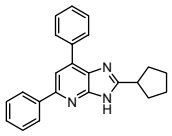
8-Cyclopentyl-1-deazapurine-N-oxide (1.1 g, 5.51 mmol) was dissolved in TFA at 0 ºC. To this was added fuming HNO_3 (3.5 mL) and the mixture was heated at 90 ºC for 3 h. This was allowed to cool to room temperature. Upon neutralisation with cNH_4OH no precipitation occurred (unlike for the unsubstituted analogue described earlier). The solution was then concentrated to half its volume whereby some solids appeared. This was probably inorganic matter (no UV chromophore) and thus the mixture was filtered, the filtrate collected and further concentrated. The resulting solids were taken on to the next step without further purification. These solids were dissolved in DMF (10 mL) at 0 ºC, careful addition of $POCl_3$ (25 mL) was followed by 30 mins reflux. The reaction mixture was then poured carefully onto ice (~300 mL) and brought to pH 6-7 with 2M NaOH. Organic material was extracted with EtOAc (3 × 150 mL), dried ($MgSO_4$) and solvents were evaporated *in vacuo*. The TLC at this point showed no clear product spot, thus the crude material was again taken on to the next step without further purification. Dissolution in DMF (10 mL) was followed by the addition of K_2CO_3 (3.4 g, 0.025 mol) and benzyl chloride (1.27 mL, 11 mmol). After stirring at RT for 48 h, the solids were filtered off. The filtrate was taken up in EtOAc (200 mL) and H_2O (200 mL) and separated. Further extraction of the aqueous layer with EtOAc (2 × 100 mL) followed. The combined organic phases were washed with H_2O (150 mL) and brine (150 mL), dried ($MgSO_4$) and concentrated to a brown oil. Chromatography on SiO_2 eluting with petroleum ether and EtOAc (gradient elution 5:1 to 3:1) resulted in a yellow oil which solidified upon standing, 140 mg. ^{1}H NMR δ($CDCl_3$): 7.37-7.2 (m, 6H, Ar), 5.80 (s, 2H, CH_2), 3.00-2.80 and 2.30-1.90 (m, 9H, cyclopentyl).

9-Benzyl-8-cyclopentyl-2,6-diphenyl-1-deazapurine



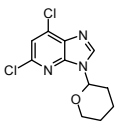
9-Benzyl-8-cyclopentyl-2,6-dichloro-1-deazapurine (140 mg, 0.39 mmol), $PhB(OH)_2$ (171 mg, 1.40 mmol, 3.5 eq.), $Pd(PPh_3)_4$ (27 mg, 0.02 mmol, 0.05 eq.), K_2CO_3 (97 mg, 0.7 mmol, 1.8 eq.) and toluene (5 mL) were heated in a sealed tube in the microwave at 150 ºC for 20 mins. The reaction mixture was then filtered, concentrated and chromatographed on SiO_2 eluting with petroleum ether and EtOAc mixtures (gradient elution 10:1 to 5:1). Yield 70%. ^{1}H NMR δ($CDCl_3$): 8.25-8.21 (m, 2H, Ph), 8.07-8.02 (m, 2H, Ph), 7.86 (s, 1H, C1-*H*), 7.59-7.40 (m, 11H, Ph), 5.89 (s, 2H, CH_2), 2.86-2.78, 2.47-2.04, 1.97-1.89 (m, 9H, cyclopentyl).

8-Cyclopentyl-2,6-diphenyl-1-deazapurine 6.13



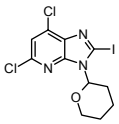
9-Benzyl-8-cyclopentyl-2,6-diphenyl-1-deazapurine (40 mg, 0.093 mmol), ammonium formate (120 mg, 0.47 mmol, 10 eq.), $Pd(OH)_2$/C (20% Pd, Pearlman's catalyst) (20 mg), and EtOH (3 mL) were heated in a sealed vessel in the microwave at 140 ºC for 5 min. The pressure reached 18 bar and upon cooling remained at 11 bar, this was released by piercing the septum carefully. Chromatography on SiO_2 eluting with petroleum ether and EtOAc mixtures (gradient elution 10:1 to 5:1) resulted in a white solid. Yield 32%. mp 236 ºC; ^{1}H NMR δ(MeOD + $CDCl_3$): 8.03-7.96 (m, 3H, Ph + N*H*), 7.89 (s, 1H, C1-*H*), 7.71-7.64 (m, 2H, Ph), 7.57- 7.40 (m, 6H, Ph), 3.41-3.35 (m, 1H, C*H*), 2.21-2.19 (m, 2H, CH_2), 1.99-1.95 (m, 2H, CH_2), 1.94-1.89 (m, 2H, CH_2), 1.77-1.76 (m, 2H, CH_2). ^{13}C-NMR δ(MeOD + $CDCl_3$): 157.1, 144.7, 142.6, 141.1, 133.6, 133.4, 133.2, 132.2, 131.9, 131.7, 131.3, 119.6, 44.9, 37.1, 30.3. MS (ES^+): 340.3 Da. Anal. ($C_{23}H_{21}N_3.1.2H_2O$) C, H, N.

2,6-Dichloro-9-THP-1-deazapurine[28]



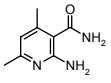
2,6-Dichloropurine (320 mg, 1.7 mmol) was stirred in EtOAc, and to this suspension was added pTSA (4.5 mg, 0.024 mmol) and the mixture stirred at 50 ºC. Dihydropyran (0.17 mL, 1.9 mmol, 1.1 eq.) was then added dropwise over 30 min. The mixture was stirred at 55 ºC for 1 h then allowed to cool to RT. cNH_4OH (2 mL) was then added and the product extracted with EtOAc (2 × 20 mL). The collected organics were then dried (Na_2SO_4) and concentrated to a clear oil that crystallised upon standing. Yield 90%. ^{1}H NMR δ($CDCl_3$): 8.28 (s, 1H, C8-*H*), 7.32 (s, 1H, C1-*H*), 5.82-5.76 (m, 1H, THP), 4.20-4.12, 3.84-3.71 (m, 2H, CH_2), 2.17-1.63 (m, 6H, 3 × CH_2).

2,6-Dichloro-8-iodo-9-THP-1-deazapurine



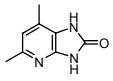
2,6-Dichloro-9-THP-1-deazapurine (135 mg, 0.5 mmol) was dissolved in dry THF (6 mL) and the solution cooled to -78 ºC. nBuLi (0.35 mL, 0.55 mmol, 1.1 eq.) was then added dropwise and the mixture stirred at -78 ºC for 1 h. N-Iodosuccinimide (124 mg, 0.55 mmol, 1.1 eq.) in THF (3 mL) was added and the reaction mixture stirred for a further hour at -78 ºC before allowing to warm to room temperature. Saturated aqueous NH_4Cl (10 mL) was then added and the mixture extracted with CH_2Cl_2 (3 × 10 mL). The collected organic layers were then washed with $NaHCO_3$, dried (Na_2SO_4) and concentrated. Chromatography on SiO_2 eluting with CH_2Cl_2 gave a white solid. Yield 40 %. ^{1}H NMR δ($CDCl_3$): 7.23 (s, 1H, C1-*H*), 5.73-5.67 (m, 1H, THP), 4.21-4.15, 3.77-3.67 (m, 2H, CH_2), 1.89-1.58 (m, 6H, 3 × CH_2).

2-Amino-4-6-dimethyl-nicotinamide[24]



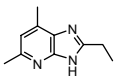
Malonamamidine hydrochloride (1.38 g, 10 mmol, 1 eq.) was added as a solid to a solution of potassium hydroxide (0.67 g, 12 mmol, 1.2 eq.) in methanol (35 mL). 2,4-Pentanedione (1.03 mL, 10 mmol) was then added to the solution and stirred at RT for 24 h. Solvents were then evaporated and the crude material chromatographed on SiO_2, eluting with CH_2Cl_2 and MeOH mixtures (gradient elution 95:5 to 80:20). Yield 34%. White solid. ^{1}H NMR δ(MeOD): 6.43 (s, 1H, py-*H*), 2.28 (s, 6H, 2 × *C*H_3).

2,6-Dimethyl-8-hydroxy-1-deazapurine[24]



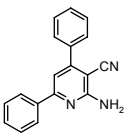
2-Amino-4-6-dimethyl-nicotinamide (0.56 g, 3.4 mmol) was dissolved in MeOH (12 mL). To this was added KOH (0.68 g, 12 mmol, 3.5 eq.) in MeOH (10 mL) and the mixture stirred for 30 mins before cooling to -5 ºC. Iodobenzenediacetate was then added (1.09 g, 3.4 mmol, 1 eq.) and the mixture allowed to warm to RT overnight. The crude solid was collected by filtration and purified by chromatography on SiO_2, eluting with CH_2Cl_2 and MeOH mixtures (gradient elution 95:5 to 80:20). Yield 48%. ^{1}H NMR δ(DMSO): 6.64 (s, 1H, py-*H*), 2.34 (s, 3H, C*H*$_3$), 2.23 (s, 3H, C*H*$_3$).

2,6-Dimethyl-8-ethyl-1-deazapurine (6.2)[24]



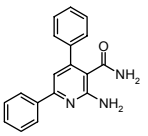
2,6-Dimethyl-8-hydroxy-1-deazapurine (0.27 g, 1.6 mmol) was added to a mixture of propionic acid (2.3 mL, 31 mmol, 19 eq.) and propionic anhydride (2.3 mL, 18 mmol, 11 eq.). $MgCl_2$ (0.16 mg, 1.6 mmol, 1 eq.) was added and the mixture heated at 120 ºC for 16 h. The reaction mixture was allowed to cool to approx. 60 ºC and MeOH (3 mL) was added. After stirring for 10 minutes the mixture was evaporated to near-dryness, followed by azeotropic distillation with H_2O to remove the excess acid/anhydride. Further co-distillation with toluene removed the last traces of water. The remaining solid was purified by chromatography on SiO_2, eluting with CH_2Cl_2 and MeOH mixtures (gradient elution 99:1 to 95:5). Yield 56%. mp 142-146 ºC (lit 143 ºC[5] and 147-148 ºC[24]) ; ^{1}H NMR δ(MeOD): 6.94 (s, 1H, C8-*H*), 2.92 (q, 2H, J = 7.3 Hz, C*H*$_2$), 1.40 (t, 3H, C*H*$_3$). ^{13}C-NMR δ(MeOD): 158.9, 153.1, 148.8, 145.2, 136.0, 119.7, 23.4, 23.3, 16.3, 12.7. MS (ES^+): 176.2 Da. Anal. ($C_{18}H_{13}N_3$.0.7 MeOH) C, H, N.

2-Amino-4,6-diphenyl-nicotinonitrile[25]



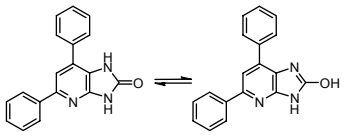
Chalcone (benzylideneacetophenone) (20.8 g, 100 mmol), malononitrile (6.6 g, 100 mmol, 1 eq.), ammonium acetate (61.6 g, 800 mmol, 8 eq.) were dissolved in EtOH (15 mL) and refluxed for 5 h, whereupon no starting material was evident by TLC. The reaction mixture was allowed to cool to RT and the solvents evaporated to leave a yellow solid. This was taken up in approx. 10 mL of hot EtOH and filtered. The remaining off-white solids were then washed with petroleum ether. This was recrystallised from hot ethanol to give white crystals. Yield 40% mp 170-174 ºC; ^{1}H NMR δ(DMSO): 8.17-8.14 (m, 2H, Ph), 7.73-7.50 (m, 8H, Ph), 7.30 (s, 1H, py-*H*), 7.05 (br s, 2H, N*H*$_2$). ^{13}C-NMR δ(DMSO): 160.8, 158.6, 154.9, 137.5, 137.0, 130.1, 129.5, 128.6, 128.3, 127.2, 117.0, 109.2. MS (ES^+): 272.0 Da. Anal. ($C_{18}H_{13}N_3$.0.16Hex) C, H, N.

2-Amino-4,6-diphenyl-nicotinamide[29]



2-Amino-diphenyl-nicotinonitrile (10.8 g, 39.7 mmol) was refluxed in 20% $KOH_{(aq.)}$ (30 g) and EtOH (150 mL) for 22 h. H_2O (100 mL) was then added and the reaction mixture allowed to stand, upon which crystallisation occurred. This yellow solid was collected and dried *in vacuo* at 40 ºC. Quantitative yield. ^{1}H NMR δ(DMSO): 8.03-8.00 (m, 2H, Ph), 7.62-7.58 (m, 2H, Ph), 7.47-7.31 (m, 6H, Ph), 6.98 (s, 1H, py-*H*).

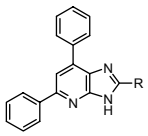
2,6-Diphenyl-8-hydroxy-1-deazapurine (6.3)[30]



2-Amino-4,6-diphenyl-nicotinamide (12.75 g, 44 mmol) was dissolved in a solution of KOH (6.43 g, 110 mmol, 2.5 eq.) in MeOH (300 mL) and stirred for 30 mins at RT. The reaction mixture was then cooled to -5 ºC, iodobenzenediacetate (14.2 g, 44 mmol, 1 eq.) added, allowed to warm to room temperature and left to stand for 40 h. The reaction mixture was then further dissolved/diluted with methanol (175 mL) and H_2O (100 mL) and the solution neutralised with 1M HCl, then stirred with cyclohexane to remove traces of the iodobenzenediacetate. The hexane layer was then separated and the remaining MeOH/H_2O layer concentrated to leave a yellow solid. Recrystallised from EtOH to give a white solid. Yield 27 %. mp 240-244 ºC; ^{1}H NMR δ(DMSO): 8.02-7.98 (m, 2H, Ph), 7.71-7.67 (m, 2H, Ph),

7.60-7.36 (m, 7H, C1-*H* + Ph). ^{13}C-NMR δ(DMSO): 155.2, 147.9, 145.8, 139.1, 135.2, 129.0, 128.6, 128.3, 128.0, 126.2, 120.3. MS (ES^+): 287.6 Da. Anal. ($C_{18}H_{13}N_3O.0.4EtOH$) C, H, N.

General procedure for the preparation of 8-alkyl-2,6-diphenyl-1-deazapurines (6.6-6.12, 6.14)



2,6-Diphenyl-8-hydroxy-1-deazapurine (200 mg, 0.7 mmol), isobutyric acid (1.5 mL, 16.2 mmol, 23 eq.), isobutyric anhydride (1.5 mL, 9.0 mmol, 13 eq.) and $MgCl_2$ (66 mg, 0.7 mmol, 1 eq.) were heated in a sealed vessel at 180 ºC for 10 h. The reaction mixture was then concentrated and co-distilled with water to remove the excess acid/anhydride. Co-distilling with toluene removed the last traces of water. The crude material was then purified by column chromatography on SiO_2, eluting with CH_2Cl_2 and MeOH (99:1), then recrystallised.

2,6-Diphenyl-8-methyl-1-deazapurine (6.6)

Yield 87 %. mp 188-192 ºC; ^{1}H NMR δ($CDCl_3$): 8.16-8.03 (m, 4H, Ph), 7.77 (s, 1H C1-*H*), 7.59-7.47 (m, 6H, Ph), 2.01 (s, 3H, C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 153.9, 151.8, 150.5, 140.3, 139.6, 136.3, 133.1, 129.0, 128.8, 128.6, 127.7, 114.6, 14.9. MS (ES^+): 286.0 Da. Anal. ($C_{19}H_{15}N_3.0.02CHCl_3$) C, H, N.

2,6-Diphenyl-8-ethyl-1-deazapurine (6.7)

Yield 36 %. mp 188-192 ºC; ^{1}H NMR δ($CDCl_3$ + MeOD): 7.93-7.87 (m, 4H, Ph), 7.62 (s, 1H C1-*H*), 7.42-7.36 (m, 6H, Ph), 2.72 (q, 2H, J = 7.3 Hz, C*H*$_2$), 1.22 (t, 3H, J = 7.3 Hz, C*H*$_3$). ^{13}C-NMR δ($CDCl_3$ + MeOD): 159.1, 152.1, 139.8, 136.0, 130.8, 128.7, 128.6, 128.5, 128.3, 127.1, 114.7, 22.5, 12.0. MS (ES^+): 300.0 Da. Anal. ($C_{20}H_{17}N_3.0.4\ H_2O$) C, H, N.

2,6-Diphenyl-8-propyl-1-deazapurine (6.8)

Yield 29 %. mp 156-158 ºC; ^{1}H NMR δ(MeOD): 8.05-8.04 (m, 2H, Ph), 8.00-7.89 (m, 2H, Ph), 7.70 (s, 1H C1-*H*), 7.54-7.35 (m, 6H, Ph), 2.88 (t, 2H, J = 7.3 Hz, C*H*$_2$), 1.88 (m, 2H, C*H*$_2$), 1.01 (t, 3H, J = 7.3 Hz, C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 153.4, 151.6, 150.0, 141.3, 137.4, 129.7, 129.4, 128.0, 115.3, 31.8, 22.6, 13.8. MS (ES^+): 314.0 Da. Anal. ($C_{21}H_{19}N_3.0.3EtOH$) C, H, N.

2,6-Diphenyl-8-isopropyl-1-deazapurine (6.9)

Yield 71 %. mp 232-234 ºC; ^{1}H NMR δ(MeOD + $CDCl_3$): 8.01-7.99 (m, 2H, Ph), 7.97-7.87 (m, 2H, Ph), 7.66 (s, 1H C1-*H*), 7.49-7.37 (m, 6H, Ph), 3.29-3.22 (m, 1H, C*H*), 1.43 (d, 6H, J = 7.3 Hz, 2 × C*H*$_3$). ^{13}C-NMR δ(DMSO): 162.1, 150.4, 150.2, 139.6, 137.1, 136.1, 131.6, 129.1, 128.7, 128.5, 128.3, 126.7, 112.2, 29.0, 21.1. MS (ES^+): 314.0 Da. Anal. ($C_{21}H_{19}N_3.0.1\ CHCl_3$) C, H, N.

2,6-Diphenyl-8-isobutyl-1-deazapurine (6.10)

Yield 65 %. mp 179-181 ºC; ^{1}H NMR δ($CDCl_3$): 8.22-8.19 (m, 2H, Ph), 8.10-8.06 (m, 2H, Ph), 7.80 (s, 1H C1-*H*), 7.55-7.44 (m, 6H, Ph), 2.24 (d, 2H, J = 7.3 Hz, C*H*$_2$), 1.86-1.80 (m, 1H, C*H*), 0.64 (d, 6H, J = 5.8 Hz, 2 × C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 157.2, 151.7, 150.4, 140.4, 139.5, 136.3, 133.1, 129.2, 128.7, 17.7, 114.6, 38.1, 22.1. MS (ES^+): 328.0 Da. Anal. ($C_{22}H_{21}N_3.0.6\ H_2O$) C, H, N.

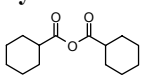
2,6-Diphenyl-8-(1-ethylpropyl)-1-deazapurine (6.11)

Yield 39 %. mp 196-197 ºC; ^{1}H NMR δ($CDCl_3$): 8.27-8.24 (m, 2H, Ph), 8.11-8.07 (m, 2H, Ph), 7.82 (s, 1H C1-*H*), 7.57-7.43 (m, 6H, Ph), 2.37-2.30 (m, 1H, C*H*), 1.53-1.43 (m, 4H, 2 × C*H*$_2$), 0.70-0.63 (m, 6H, 2 × C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 160.3, 151.8, 150.0, 140.4, 139.2, 136.4, 132.7, 129.3, 128.9, 128.8, 128.5, 127.5, 114.3, 43.5, 26.6, 11.7. MS (ES^+): 342.0 Da. Anal. ($C_{23}H_{23}N_3.0.05\ CHCl_3$) C, H, N.

2,6-Diphenyl-8-*t*Butyl-1-deazapurine (6.12)

Yield 76 %. mp 233-234 ºC; ^{1}H NMR δ($CDCl_3$): 8.33-8.29 (m, 2H, Ph), 8.01-7.98 (m, 2H, Ph), 7.82 (s, 1H C1-*H*), 7.55-7.41 (m, 6H, Ph), 1.44 (s, 9H, 3 × C*H*$_3$). ^{13}C-NMR δ($CDCl_3$): 163.8, 152.1, 150.1, 140.2, 139.0, 138.2, 136.2, 132.3, 129.3, 128.9, 128.8, 128.7, 128.4, 128.3, 127.4, 114.1, 33.6, 28.9. MS (ES^+): 328.0 Da. Anal. ($C_{22}H_{21}N_3.0.04\ CHCl_3$) C, H, N.

Cyclohexane carboxylic anhydride[26]



Cyclohexane carboxylic acid (1.24 mL, 10 mmol) was ground together with K_2CO_3 (6 g) for approx. 5 min. Tosyl chloride (0.95 g, 5 mmol, 0.5 eq.) was then added portionwise with a few drops of EtOAc. Grinding was continued for approx 40 min, whereupon the tosyl chloride was barely visible by TLC. The solids were then washed with CH_2Cl_2 (3 × 20 mL), filtered and the filtrate concentrated. Yield 26 %. ^{1}H NMR δ($CDCl_3$): 2.46-2.33 (m, 2H, C*H*), 1.99-1.23 (m, 20H, cHex).

2,6-Diphenyl-8-cyclohexyl-1-deazapurine (6.14)

Yield 49 %. mp 210-213 ºC; ^{1}H NMR δ($CDCl_3$): 8.32-8.22 (m, 2H, Ph), 8.11-8.08 (m, 2H, Ph), 7.83 (s, 1H C1-*H*), 7.55-7.45 (m, 6H, Ph), 2.51-2.44 (m, 1H, C*H*), 1.10-1.06 (m, 10 H, cHex). ^{13}C-NMR δ($CDCl_3$): 161.4, 151.8, 150.2, 140.2, 139.4, 136.4, 129.3, 129.0, 128.8, 128.6, 127.5, 114.3, 38.6, 31.3, 25.7. MS (ES^+): 354.0 Da. Anal. ($C_{24}H_{23}N_3.0.5$ EtOH) C, H, N.

6.4.2 Biology

Materials and Methods

[^{3}H]DPCPX and [^{125}I]AB-MECA were purchased from Amersham Biosciences (NL). [^{3}H]ZM 241385 was obtained from Tocris Cookson, Ltd. (UK). CHO cells expressing the human adenosine A_1 receptor were provided by Dr. Andrea Townsend-Nicholson, University College London, UK. HEK 293 cells stably expressing

the human adenosine A_{2A} and A_3 receptor were gifts from Dr. Wang (Biogen, USA) and Dr. K.-N. Klotz (University of Würzburg, Germany), respectively.
All compounds were tested in radioligand binding assays to determine their affinities at the human adenosine A_1, A_{2A} and the A_3 receptors as described previously in Chapter 3, with the exception of non-specific binding on the A_{2A} receptor was determined in the presence of 10 μM CGS21680 instead of 100 μM CPA. The human A_1 receptors were expressed in CHO cells, and [^{3}H]DPCPX used as the radioligand. The A_{2A} and A_3 receptors were expressed in HEK 293 cells, and [^{3}H]ZM 241385 and [^{125}I]AB-MECA were used as the respective radioligands.

Data Analysis

K_i values were calculated using a non-linear regression curve-fitting program (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA). K_D values of the radioligands were 1.6 nM, 1.0 nM and 5.0 nM for [^{3}H]DPCPX, [^{3}H]ZM 241385 and [^{125}I]AB-MECA, respectively.

6.5 References

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Chapter 7

A Series of Ligands Displaying a Remarkable Agonistic-Antagonistic Profile at the Adenosine A_1 Receptor

Adenosine receptor agonists are usually variations on the natural ligand, adenosine. The ribose moiety in the ligand has previously been shown to be of great importance for the agonistic effects of the compound. In this chapter, a series of non-adenosine ligands selective for the adenosine A_1 receptor with an extraordinary pharmacological profile is presented. 2-Amino-4-benzo[1,3]dioxol-5-yl-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile **7.70**, LUF 5853) shows full agonistic behaviour comparable with the reference compound CPA, whilst also displaying comparable receptor binding affinity (K_i = 11 nM). In contrast, compound **7.58** (2-amino-4-(3-trifluoromethyl-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile, LUF 5948) has a binding affinity of 14 nM and acts as an inverse agonist. Also present within this same series are compounds that show neutral antagonism of the adenosine A_1 receptor, for example compound **7.65** (2-amino-4-(4-difluoromethoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile, LUF 5826).

Based upon L. C. W. Chang, J. K. von Frijtag Drabbe Künzel, T. Mulder-Krieger, R. F. Spanjersberg, S. F. Roerink, G. van den Hout, M. W. Beukers, J. Brussee, A. P. IJzerman, J. Med. Chem. ***2005****, 48, 2045-2053.*

7.1 Introduction

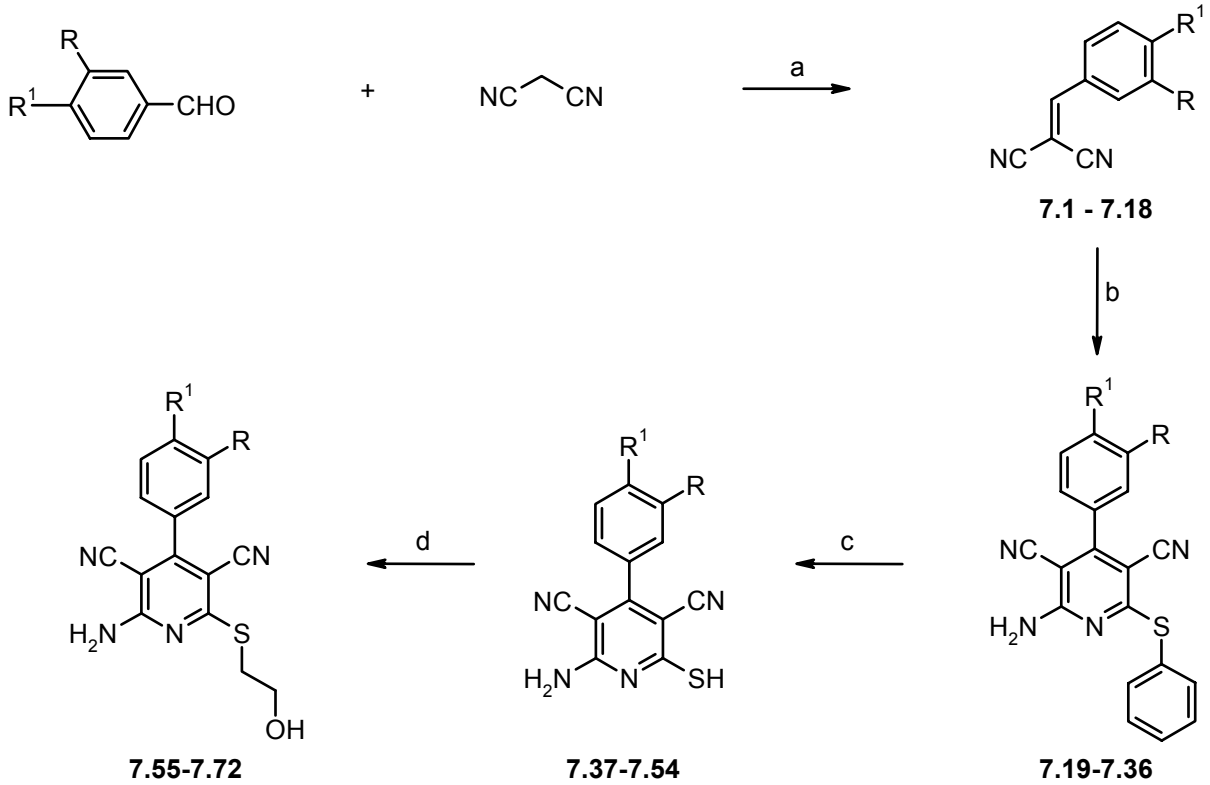
The adenosine A_1 receptor is the most extensively researched of the family of adenosine receptors, which also include the A_{2A}, A_{2B}, and the A_3 receptors. Many different classes of ligands have been designed, synthesised and tested at the A_1 receptor, and to date there are several recurring factors which almost define the affinity or efficacy of the compound. The antagonists can be generally split into two groups, those based on the xanthine core structure and those heterocycles which are non-xanthine compounds. This latter category encompasses all manner of different mono-, bi-, tri- and even quadri-cyclic mostly nitrogen containing aromatic compounds,[1-3] as detailed in Chapter 2. On the contrary, until very recently (partial) agonists were all derivatives of the natural ligand itself, adenosine. Research had shown that, in general, manipulation of the ribose moiety results in a reduction of the intrinsic activity towards the adenosine A_1 receptor,[4-7] and thus accordingly all new non-adenosine ligands were assumed to be antagonists. The main exception to this is modification at the 5' position, where replacement of the 5'-hydroxyl with 5'-N-carboxamides is particularly well tolerated, e.g., NECA and derivatives thereof, although these ligands tend not to be very selective.[1] Recently, based upon some data published in patent literature, it was shown that certain 2-amino-4-(substituted)phenyl-6-(1*H*-imidazol-2-yl-methylsulfanyl)-pyridine-3,5-dicarbonitriles had varying degrees of efficacy, ranging from full to partial agonists at the different adenosine receptors.[8] Most significantly, these compounds displayed a considerable affinity and efficacy at the A_{2B} receptor that had not been reported previously. However, these ligands were not particularly selective at any of the adenosine receptors (being only slightly more favourable towards the A_1 receptor). In this chapter a novel series based upon this same template is explored and the affinity and selectivity for the adenosine A_1 receptor is examined. The remarkable pharmacological profile of these compounds, in terms of the ability to block or activate the human A_1 receptor influencing the cAMP production in CHO cells stably expressing this receptor, is shown.

7.2 Results and Discussion

7.2.1 Chemistry

The compounds **7.55-7.72** were synthesised according to Scheme 7.1.[9,10] The aldehyde was reacted with malononitrile in a straightforward Knoevenagel condensation in the presence of a few drops of piperidine to give the intermediates (**7.1-7.18**) in moderate to good yields (30-93%). Pyridine formation occurred according to a preparation by Kambe *et al.*[11] to give the phenyl protected sulfide in the 6-position of the ring. The functionalised malononitrile was refluxed with another equivalent of malononitrile and an equivalent of thiophenol in ethanol and triethylamine, resulting in compounds **7.19-7.36** (generally between 20-50% yields). To obtain the free thiol in the 6-position of the pyridine ring, 3.3 equivalents of sodium sulfide in DMF at 80 °C for 2-3 hours resulted in quantitative yields of compounds **7.37-7.54**. Throughout the synthesis, purification of the crude product was not performed, nor were the reactions optimised. The yields stated correspond to the crude material, and it was this crude

substance that was used in subsequent reactions. The final step was the reaction of the free thiol with 2-bromoethanol in the presence of $NaHCO_3$ in DMF at room temperature to give compounds **7.55-7.72** in modest to good yields. The final products were purified by chromatography and subsequent recrystallisation gave clean, fully characterised compounds.



Scheme 7.1. Synthetic route to 2-amino-4-[(substituted)phenyl]-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitriles **7.55-7.72** (a) piperidine, EtOH, 1 h reflux; (b) malononitrile, thiophenol, triethylamine, EtOH, 4 h reflux; (c) (i) Na_2S, DMF, (ii) 1M HCl; (d) 2-bromoethanol, $NaHCO_3$, DMF.

7.2.2 Biology

All compounds were tested in radioligand binding assays to determine their affinities at the respective human adenosine receptors (Table 7.1). Compounds for which the affinities were determined (i.e., compounds which showed greater than 50 % displacement of the radioligand at 1 μM) were tested in functional assays for their ability to influence the levels of cAMP in CHO cells expressing the human adenosine A_1 receptor. The compounds were tested at concentrations of mostly 100 x K_i, and at least 20 x K_i , where the receptor sites should be almost fully occupied and the reference ligands CPA, DPCPX and N0840 were included in the assays. In [^{35}S]GTPγS binding assays on similar cell membranes from CHO cells these three particular ligands are respectively reported as a full agonist, an inverse agonist and a neutral antagonist.[12,13] In cAMP assays on whole cells expressing the human A_1 receptor, CPA has been shown to be a full agonist and DPCPX as an inverse agonist.[13] To exclude the possibility of effects caused by endogenous adenosine that may be present in the system, adenosine deaminase was added to the incubation medium.

7.2.3 Structure Activity Relationships

The results of the radioligand binding assays are presented in Table 7.1. The compounds are highly selective for the human A_1 adenosine receptor, with not one variation displaying more than 50% displacement of the radioligand at a concentration of 1 μM on the human A_{2A} or A_3 receptors. Fluorine substitution caused many different effects on both the binding and efficacy of the compounds compared to the non-fluorinated analogues. Compounds **7.56** and **7.57**, the 3- and 4-fluorophenyl derivatives, possessed a K_i value of 40 and 81 nM respectively, compared to the phenyl derivative (**7.55**), which had an affinity of 15 nM. In general, the highly electronegative fluorine atom was detrimental for binding affinity compared to its non-fluorinated analogue, but for two exceptions. One exception was compound **7.58** with a K_i value of 14 nM, showing more than 5 times more affinity to the A_1 receptor than the 3-tolyl derivative **7.60** (K_i = 81 nM). Compound **7.59** abides by the generalisation and possesses an affinity of 150 nM (cf., compound **7.61**, K_i = 49 nM).

The presence of the hydroxyl group (**7.62**, **7.63**) also increases the electronegativity of this region of the ligand and thus might also have been detrimental for binding affinity. However, the presence of the hydrogen atom probably induces hydrogen-bonding with the receptor, accounting for similarly high affinities (12 and 23 nM, respectively) to that of the non-substituted phenyl derivative (**7.55**, K_i = 15 nM). As mentioned earlier, the presence of the fluorine atom was generally detrimental for binding, and this was illustrated by the fluorine-substituted methoxyphenyl ligands (**7.64**, **7.66** and **7.67**) which showed less affinity for the receptor than the analogous methoxyphenyl ligands (**7.68**, **7.69**). The other exception to the detrimental effect of fluorine was the 4-difluoromethoxyphenyl ligand **7.65**, which displayed comparable affinity to the 4-methoxyphenyl derivative **7.69** in the 30-40 nM range. With an affinity of 4 nM the compound with the most encouraging potency at the A_1 receptor was the 3-methoxyphenyl ligand (**7.68**).

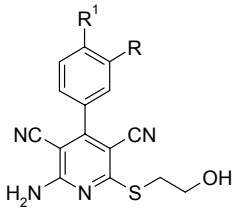
In general, substitution in the 3-position was more positive for binding than substitution in the 4-position of the phenyl ring. The only exceptions to this were the tolyl compounds (**7.60**, **7.61**). The spatial benefit of substitution in the 3- versus the 4-position is illustrated well by compounds **7.68**-**7.70**. The 3-methoxyphenyl derivative **7.68** displays almost 10-fold better affinity than the 4-methoxyphenyl ligand **7.69** at 4.3 nM and 41 nM, respectively.

Compound **7.70,** which can perhaps be seen as a compromise of these two moieties, holds an affinity consistent with this theory at 11 nM. The 3,4-dimethoxyphenyl derivative **7.71**, a combination of compounds **7.68** and **7.69**, has a much-reduced affinity at the A_1 receptor, with a displacement of the radioligand of only 25% at a concentration of 1 μM. It is obvious that the two methoxy groups create too much steric bulk for good binding in the pocket to occur. The 4-dimethylaminophenyl derivative **7.72**, like the methoxy-analogues, possesses a methyl group connected to a hydrogen-bond acceptor. However, the existence of a second methyl substituent on this hydrogen-bond acceptor (causing steric hindrance, both in terms of

optimal fit in the receptor and in perhaps preventing the occurrence of H-bonding) probably explains its relatively poor affinity at the A_1 adenosine receptor at 245 nM.

The behaviour of the compounds in efficacy assays is displayed in Figure 7.1. The ligands were tested at concentrations of mostly 100 × K_i, and at least 20 × K_i, where the receptor sites should be almost fully occupied. The actual percentage inhibition or stimulation relative to the full agonist CPA (100 % inhibition) and DPCPX (full inverse agonist, set at 100 % stimulation) is given in Table 7.1. As the A_1 receptor is coupled to an inhibitory G protein forskolin was used to induce the production of cAMP. N0840 (reported in [^{35}S]GTPγS assays to be a neutral antagonist)[12,13] was shown in this whole cell cAMP assay to have properties consistent with an inverse agonist, although on lower levels than DPCPX.

Table 7.1. Affinities of the 2-amino-4-[(substituted)phenyl]-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitriles (**7.55-7.72**) in radioligand binding assays at the human adenosine receptors and the effect in cAMP assays with the human A_1 adenosine receptor.



	R	R^1	K_i (nM) or % disp.[a]			% change in cAMP[e]
			hA_1[b]	hA_{2A}[c]	hA_3[d]	
CPA	-	-	10 ± 1.3	1652 ± 344	281 ± 56[14]	-100
DPCPX	-	-	6.1 ± 1.6	129[15]	1700 ± 170[12]	100
N0840	-	-	1081 ± 69	n.d.	15%[12]	72 ± 7
7.55	H	H	15 ± 4	23%	26%	-72 ± 6
7.56	F	H	40 ± 10	19%	8%	-102 ± 10
7.57	H	F	81 ± 5	4%	0%	-51 ± 10
7.58 (LUF 5948)	CF_3	H	14 ± 2	22%	49%	85 ± 12
7.59	H	CF_3	150 ± 38	13%	0%	48 ± 6
7.60	CH_3	H	81 ± 17	29%	5%	-78 ± 6
7.61	H	CH_3	49 ± 9	20%	0%	-16 ± 6
7.62	OH	H	12 ± 3	25%	16%	-105 ± 22
7.63	H	OH	23 ± 3	37%	0%	-93 ± 12
7.64	OCF_2H	H	25 ± 4	8%	12%	6 ± 5
7.65 (LUF 5826)	H	OCF_2H	34 ± 7	0%	23%	4 ± 6
7.66	OCF_3	H	30 ± 7	3%	11%	80 ± 13
7.67	H	OCF_3	156 ± 40	0%	10%	42 ± 12
7.68	OCH_3	H	4.3 ± 0.6	21%	18%	-58 ± 10
7.69	H	OCH_3	41 ± 8	8%	21%	-16 ± 19
7.70 (LUF 5853)	-OCH_2O-		11 ± 2	33%	13%	-111 ± 16
7.71	OCH_3	OCH_3	25%	25%	0%	-
7.72	H	$N(CH_3)_2$	245 ± 100	0%	7%	-65 ± 23

[a]K_i ± SEM (n = 3), % displacement (n = 2). [b]Displacement of specific [^{3}H]DPCPX binding in CHO cell membranes expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations. [c]Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cell membranes expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations. [d]Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cells expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations. [e]% change of cAMP ± SEM (n = 3) compared to CPA (full agonist, -100%) and DPCPX (full inverse agonist 100%). cAMP generation was stimulated with 10 μM forskolin and compounds were tested at mostly 100 × their K_i value (see text).

Almost full agonistic activity was found for four compounds (**7.56**, **7.62**, **7.63**, **7.70**), the 3-fluorophenyl, the hydroxyphenyl substitutents and the benzo[1,3]dioxol-5-yl derivative. Five further compounds (**7.55**, **7.57**, **7.60**, **7.68**, **7.72**) display high levels of agonism, with efficacy ranging from 50-80%, with respect to full inhibition by CPA. Lower levels of partial agonism were demonstrated by compound **7.61** (the 4-tolyl derivative) and **7.69** (the 4-methoxyphenyl substitutent). The compounds with trifluoro-derivatives (**7.58**, **7.59**, **7.66**, **7.67**) induce inverse agonism, resulting in an increase of cAMP levels. The two difluoromethoxyphenyl derivatives (**7.64** and **7.65**) show very little effect on the levels of cAMP even after stimulation by forskolin, indicating almost neutral antagonism. These compounds compare favourably with a previous exploration for neutral antagonists by De Ligt *et al.*[12] In that paper, an 8-substituted N^6-cyclopentyl-9-methyladenine, LUF 5674, possessed a binding affinity at the A_1 adenosine receptor of 75 nM whilst displaying neutral antagonism. In this study we have achieved neutral antagonism with K_i values in the region of 40 nM.

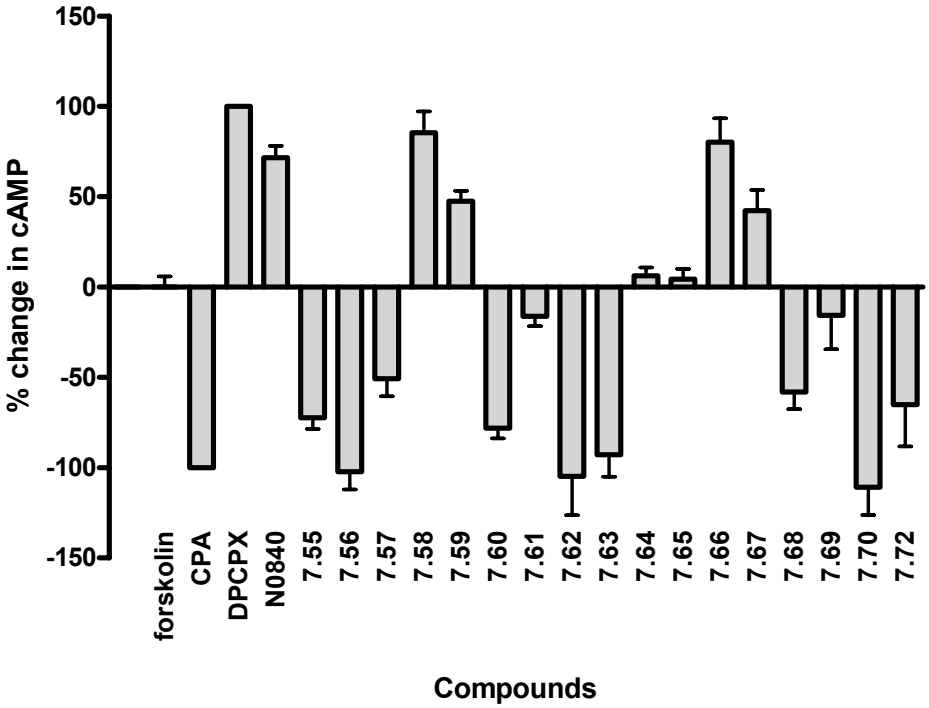


Figure 7.1 The effect of reference and synthesised ligands on forskolin-induced cAMP levels at hA_1 receptors compared to CPA (full agonist, 100% inhibition) and DPCPX (full inverse agonist, 100% stimulation).

For each pair of 3- and 4-substituted analogues the 3-substituent shows more acute behaviour than the 4-substituent. For example, the 3-fluorophenyl derivative **7.56** is a full agonist compared to partial agonism displayed by the 4-fluorophenyl derivative (**7.57**), and the 3-CF_3 derivative (**7.58**) displays a higher inverse agonistic behaviour than compound **7.59**, the 4-CF_3 analogue. Although binding affinity and efficacy are not necessarily correlated, these findings support the outcome of the binding studies. The results suggest that substitution in the 3-position fits the receptor pocket better than substitution in the 4-position to induce either activation of the receptor, or a stabilisation to return the receptor to the ground state.

The mode of activation of these ligands at the adenosine A_1 receptor is far from clear. The long held theory of the necessity of the ribose group to activate the receptor proposed the existence of a ribose-specific binding domain in the receptor in which the activation 'trigger' resides. This current series of compounds, all without a ribose moiety, possess possible hydrogen-bonding groups (OH or NH_2) that may bind in the ribose-specific domain and activate the receptor. However, the potential to reside in this specific domain alone may not explain the observations recorded. All the compounds in the series comprise of the same elements and only vary at the phenyl moiety, some distance away from the potential H-bonding hydroxyl and amino- functions, and yet it is these substitutions that cause the great differences in receptor activation. A review by Visiers *et al.*[16] highlights the complexities involved with receptor activation/inactivation and summarises our understanding of it. There are many proposed sites of importance in the activation of a G protein-coupled receptor (GPCR). The binding site itself for many endogenous ligands at rhodopsin-like receptors has been identified as being a spatially compact binding microdomain, most probably in transmembrane (TM) helices 3, 5 and 6, and there is evidence to suggest ligand orientation can be crucial for efficacy. Almaula *et al.*[17] described how not only the binding affinity, but also the activation of the serotonin 5-HT_{2A} receptor was affected by small differences in the mode of interaction of the ligand with the receptor pocket. Following this, Ebersole *et al.*[18] investigated partial agonism of the human serotonin 5-HT_{2A} receptor. Based on mutagenesis studies and computational simulation, they found that ligand orientation affected the H-bonding at serine residues in TM3 and TM 5 and caused a reduction in efficacy. In conclusion, it was hypothesised that the same structurally specific mechanism holds for other GPCRs. Translating the proposals to the current findings, we speculate that the strongly electron-withdrawing trifluoromethyl and trifluoromethoxy groups cause such a change in electron density about the molecule that the manner of binding (with respect to the non-substituted ligand **7.55**) at the receptor is distorted dramatically. This prevents not only activation of the receptor but induces the stabilisation of an already active receptor to adopt an inactive form. We can also hypothesise that the electron-withdrawing trifluoro-moieties (**7.58**, **7.59**, **7.66**, **7.68**) are capable of accepting hydrogen bonds from the protein in a manner stabilising the receptor, despite the potential from other parts of the molecule to activate the receptor. In comparison, the difluoromethoxy derivatives (**7.64**, **7.65**) possess less electronegativity than the trifluoro species, distorting the manner of binding less, and so resulting in neutral antagonists. In the same speculative vein, the singularly substituted fluorine moiety (**7.55**, **7.56**) is probably not sufficiently electronegative and not large enough to induce a different binding mode, thus agonism is conserved.

In an attempt to form a clearer basis to this speculation we looked at the electronic and spatial properties of these compounds in the molecular modelling package Spartan.[19] The agonists were modelled in comparison to the known agonist, CPA. For the clarity of the following discussion, CPA and its corresponding numbering system is shown in Figure 7.2. Assuming

that the conservation of the 3'OH is necessary for activation of the agonist (as reported by Taylor *et al.*[20]), we propose two alternative models of superimposition depicted in Figures 7.3 (CPA and compound **7.68**). The first proposition suggests that the -SCH_2CH_2OH region can be overlayed upon the 3'OH area (Figure 7.3**a**). Examining the electronic configuration of the compounds, the electronegative regions of the H-bond accepting nitrile groups seem to correspond to the electronegative N1 and N7 of the purine ring. The advantage of 3-substitution of the phenyl ring above 4-substitution can be seen clearly in this representation of compound **7.68** vs. CPA. The 3-methoxy group seems to be superimposable with the cyclopentyl moiety of CPA. In contrast 4-substitution would show much less overlap with the N^6 substituent of the adenosine derivative CPA.

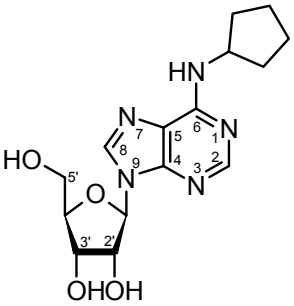


Figure 7.2 CPA and its numbering system.

An alternative superimposition is depicted in Figure 7.3**b**. This time the 2-amino group from the current series has been overlayed with the 3'OH of the ribose ring. The 3- and 5-nitrile groups of compound **7.68** now lie in the region of the 2'OH of the ribose group and the N7 of the purine ring, respectively. Again 3-substitution at the phenyl group confers overlap with the N^6-cyclopentyl domain of CPA, offering more spatial compatibility than 4-substitution.

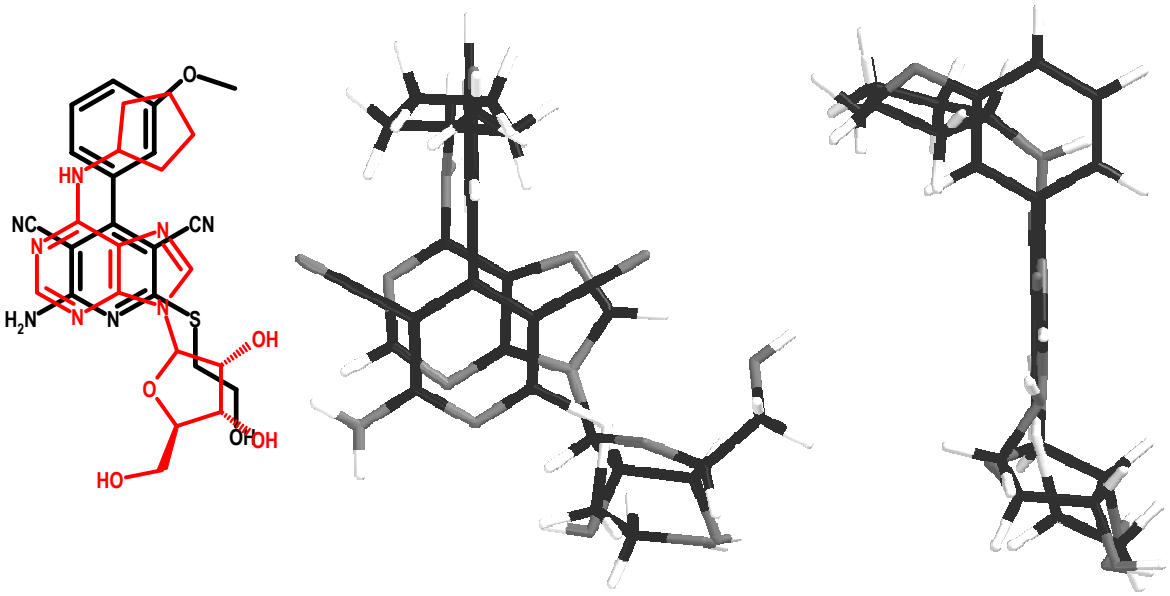
The rationale in this modelling procedure was to search for maximal overlap between the two structures. However, it should be kept in mind that the binding sites for the two compounds (CPA and **7.68**) may be (partially or even entirely) different, but converge in the subsequent activation of the receptor.

In Chapter 5, the original model proposed for 'good' adenosine A_1 receptor antagonists was refined and this refined model was subsequently exploited to achieve ligands with extremely low K_i values for the adenosine A_1 receptor (Chapter 6). Since members of this series of pyridine-3,5-dicarbonitriles also show antagonistic behaviour, it is appropriate to compare the properties of these molecules to those proposed by the model. As illustrated in Figure 7.4, the very vital hydrogen-bond interacting regions A, B and C are consistently represented in this new series of ligands. The L3 group is also well represented by the 4-phenyl substituent. The lowest K_i value reported for the antagonists/inverse agonists of this series was 14 nM, almost 50-times greater than compound **5.31** (0.29 nM). Thus the 'missing' elements of these pyridine-3,5-dicarbonitriles compounds (that may provide more compatibility to the proposed model) may account for its lower affinity for the A_1 receptor. Therefore, it is very likely that the antagonists in this series bind in the same position as the other antagonists detailed in previous chapters.

7.3 Conclusion

This chapter describes a series of ligands displaying very different activity at the A_1 adenosine receptor. Several compounds are full agonists with affinity and efficacy comparable to the reference ligand CPA. Most remarkably, they are not derivatives of adenosine. There are also

a



b

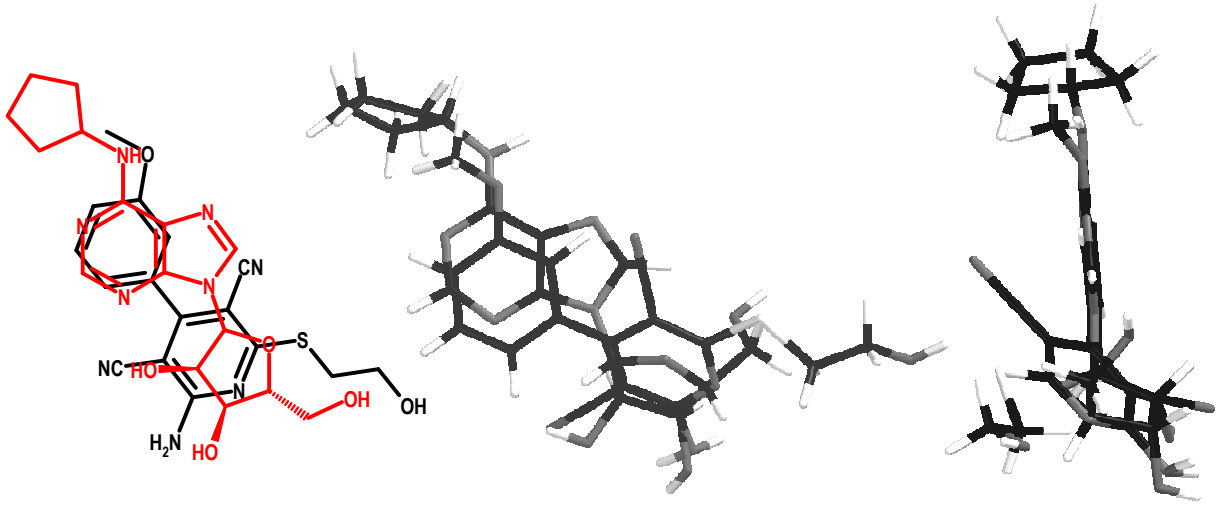


Figure 7.3. (**a**) 2-Amino-4-(3-methoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (**7.68**) superimposed upon CPA. The first two representations depict the proposed overlap of electron density between the two nitrile groups and the purine ring. The right-hand picture is the superimposition shown from a different angle and displays the steric overlap of the N^6 substituent of CPA and the 3-methoxy substituent of the phenyl group. Also seen more clearly in this illustration is the compatibility of the 3'OH group from the ribose ring and the hydroxyl moiety from compound **7.68**. (**b**) An alternative superimposition of compound **7.68** and CPA. Here the anchor point has been taken as the 2-amino group of **7.68** with the 3'OH of the ribose ring. The first two pictures suggest overlap of the ribose 2'OH group with the 3-nitrile moiety, and the purine N7 with the 5-nitrile group. The depiction on the right is the superimposition shown from a different angle and displays more clearly the overlap of the 2-amino group from **7.68** with the 3'OH of CPA.

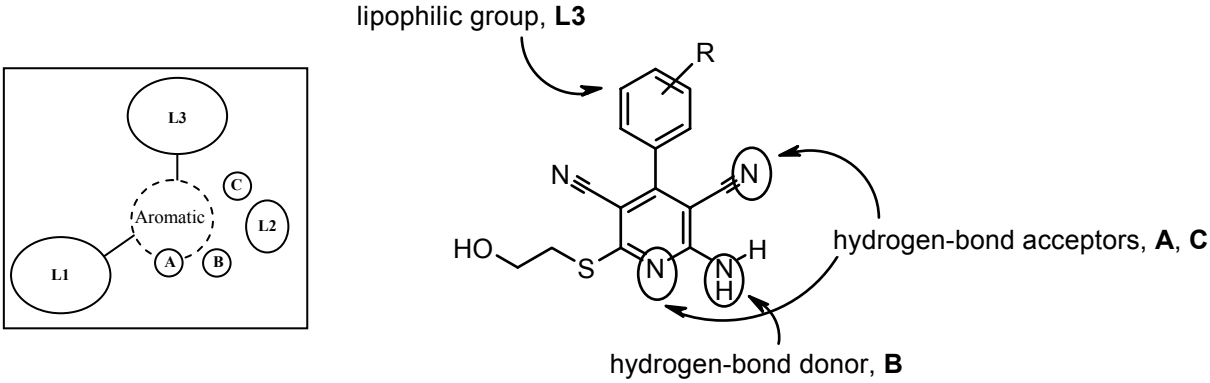


Figure 7.4 The model depicted on the left and manner in which the pyridine-3,5-dicarbonitriles may fit this model.

compounds in this series that display fully inverse agonistic behaviour, almost on levels comparable to the reference ligand DPCPX. Most strikingly, neutral antagonists have also been discovered which retain high affinity and selectivity for the adenosine A_1 receptor. It is perhaps particularly noteworthy that these effects vary with the presence or absence of fluorine atoms distant from the phenyl ring. This suggests the substantial influence of hydrogen-bond acceptors in this part of the molecule upon the activation of the adenosine A_1 receptor.

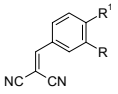
7.4 Experimental

7.4.1 Chemistry

Materials and Methods

All reagents used were obtained from commercial sources and all solvents were of an analytical grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ(ppm) and the following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Melting points were determined on a Büchi melting point apparatus, the lowest point of the melting point range is given and the values are uncorrected. Elemental analyses were performed by the Leiden Institute of Chemistry and are within 0.4 % of the theoretical values unless otherwise stated. Reactions were routinely monitored by TLC using Merck silica gel F_{254} plates.

General Procedure for functionalised malononitriles (7.1-7.18)



To malononitrile (1.32 mL, 20.8 mmol) dissolved in EtOH (14 mL) was added an aldehyde (20 mmol) followed by 3 drops of piperidine. This reaction mixture was then refluxed for an hour, and then allowed to cool to room temperature, upon which a precipitate formed. The crude product was collected by filtration and used without any further purification.

2-Benzylidene-malononitrile (7.1)

Off-white solid, 65% yield. ^{1}H NMR δ($CDCl_3$): 7.93-7.86 (m, 2H, phenyl-*H*), 7.79 (s, 1H, C*H*), 7.68-7.50 (m, 3H, phenyl-*H*).

2-(3-Fluoro-benzylidene)-malononitrile (7.2)

Off-white solid, 56% yield. ^{1}H NMR δ(DMSO): 8.59 (s, 1H, C*H*), 7.84-7.60 (m, 4H, phenyl-*H*).

2-(4- Fluoro-benzylidene)-malononitrile (7.3)

Off-white solid, 93% yield. ^{1}H NMR δ(DMSO): 8.55 (s, 1H, C*H*), 8.09-8.01 (m, 2H, phenyl-*H*), 7.55-7.47 (m, 2H, phenyl-*H*).

2-(3-Trifluoro-benzylidene)-malononitrile (7.4)

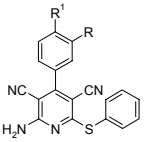
Off-white solid, 42% yield. ^{1}H NMR δ(DMSO): 8.67 (s, 1H, C*H*), 8.27-8.21 (m, 1H, phenyl-*H*), 8.09-8.05 (m, 1H, phenyl-*H*), 7.92-7.84 (m, 1H, phenyl-*H*).

2-(4-Trifluoro-benzylidene)-malononitrile (7.5)

Off-white solid, 88% yield. ^{1}H NMR δ(DMSO): 8.69 (s, 1H, C*H*), 8.14-7.99 (m, 4H, phenyl-*H*).

2-(3-Methyl-benzylidene)-malononitrile (7.6)
Off-white solid, 29% yield. ^{1}H NMR δ(DMSO): 8.51 (s, 1H, C*H*), 7.80-7.77 (m, 2H, phenyl-*H*), 7.54-7.51 (m, 2H, phenyl-*H*), 2.37 (s, 3H, CH_3).
2-(4-Methyl-benzylidene)-malononitrile (7.7)
Off-white solid, 46% yield. ^{1}H NMR δ(DMSO): 8.49 (s, 1H, C*H*), 7.87 (d, 2H, J = 8.0 Hz, phenyl-*H*), 7.45 (d, 2H, J = 8.0 Hz), 2.41 (s, 3H, CH_3).
2-(3-Hydroxy-benzylidene)-malononitrile (7.8)
Off-white solid, 40% yield. ^{1}H-NMR δ($CDCl_3$/MeOD): 7.92 (s, 1H, C*H*), 7.40-7.36 (m, 3H, phenyl-*H*), 7.13-7.10 (m, 1H, phenyl-*H*).
2-(4-Hydroxy-benzylidene)-malononitrile (7.9)
Off-white solid, 66% yield. ^{1}H-NMR δ($CDCl_3$/MeOD): 7.86 (d, 2H, J = 8.8 Hz, phenyl-*H*), 7.71 (s, 1H, C*H*), 6.94 (d, 2H, J = 8.8 Hz, phenyl-*H*).
2-(3-Difluoromethoxy-benzylidene)-malononitrile (7.10)
Off-white solid, 45% yield. ^{1}H-NMR δ($CDCl_3$): 7.79-7.75 (m, 2H, phenyl-*H*), 7.66 (s, 1H, C*H*), 7.56 (m, 1H, phenyl-*H*), 7.43-7.39 (m, 1H, phenyl-*H*), 6.58 (t, 1H, J = 72.3 Hz, OCF_2H).
2-(4- Difluoromethoxy-benzylidene)-malononitrile (7.11)
Off-white solid, 68% yield. ^{1}H-NMR δ($CDCl_3$): 7.96 (d, 2H, J = 8.76 Hz, phenyl-*H*), 7.75 (s, 1H, C*H*), 7.27 (d, 2H, J = 8.8 Hz, phenyl-*H*), 6.65 (t, 1H, J = 72.3 Hz, OCF_2H).
2-(3-Trifluoromethoxy-benzylidene)-malononitrile (7.12)
Off-white solid, 48% yield. ^{1}H-NMR δ($CDCl_3$): 7.89-7.86 (m, 1H, phenyl-*H*), 7.80 (s, 1H, C*H*), 7.74 (m, 1H, phenyl-*H*), 7.62 (m, 1H, phenyl-*H*), 7.51-7.47 (m, 1H, phenyl-*H*).
2-(4-Trifluoromethoxy-benzylidene)-malononitrile (7.13)
Off-white solid, 30% yield. ^{1}H-NMR δ($CDCl_3$): 7.99 (d, 2H, J = 8.8 Hz, phenyl-*H*), 7.78 (s, 1H, C*H*), 7.38 (d, 2H, J = 8.8 Hz, phenyl-*H*).
2-(3-Methoxy-benzylidene)-malononitrile (7.14)
Off-white solid, 82% yield. ^{1}H-NMR δ($CDCl_3$): 7.75 (s, 1H, C*H*), 7.45-7.39 (m, 3H, phenyl-*H*), 7.20-7.14 (m, 1H, phenyl-*H*), 3.87 (s, 3H, CH_3).
2-(4-Methoxy-benzylidene)-malononitrile (7.15)
Off-white solid, 68% yield. ^{1}H-NMR δ($CDCl_3$/MeOD): 7.96-7.91 (m, 3H, phenyl-*H* + C*H*), 7.04 (d, 2H, J = 8.8 Hz, phenyl-*H*), 3.90 (s, 3H, CH_3).
2-Benzo[1,3]dioxol-5-ylmethylene-malononitrile (7.16)
Off-white solid, 46% yield. ^{1}H-NMR δ(DMSO): 8.39 (s, 1H, C*H*), 7.57-7.54 (m, 2H, phenyl-*H*), 7.24-7.19 (m, 1H, phenyl-*H*), 6.25 (s, 2H, -OCH_2O-).
2-(3,4-Dimethoxy-benzylidene)-malononitrile (7.17)
Off-white solid, 53% yield. ^{1}H-NMR δ(DMSO): 8.39 (s, 1H, C*H*), 7.66-7.60 (m, 2H, phenyl-*H*), 7.27-7.22 (m, 1H, phenyl-*H*), 3.91 (s, 3H, CH_3), 3.83 (s, 3H, CH_3).
2-(4-Dimethylamino-benzylidene)-malononitrile (7.18)
Off-white solid, 45% yield. ^{1}H-NMR δ(DMSO): 8.08 (s, 1H, C*H*), 7.87 (d, 2H, J = 9.5 Hz phenyl-*H*), 6.89 (m, 2H, J = 9.5 Hz, phenyl-*H*), 3.13 (s, 6H, 2 × CH_3).

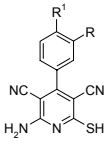
General Procedure for 2-Amino-4-(substituted)-phenyl-6-phenylsulfanyl-pyridine-3,5-dicarbonitriles (7.19-7.36) To a solution of the functionalised malononitrile (**7.1-7.18**) (10 mmol) in EtOH (10 mL), was added malononitrile (0.64 mL, 10 mmol), thiophenol (1.02 mL, 10 mmol) and triethylamine (50 μL), and the mixture heated at reflux for approximately 4 h. The reaction mixture was then allowed to cool to room temperature. The crude product precipitated upon cooling and was collected by filtration.



2-Amino-4-phenyl-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.19)
Yellow solid, 41% yield. ^{1}H-NMR δ(DMSO): 7.83 (br s, 2H, NH_2), 7.66-7.52 (m, 10H, phenyl-*H*).
2-Amino-4-(3-fluoro-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.20)
Yellow solid, 24% yield. ^{1}H-NMR δ(DMSO): 7.90 (br s, 2H, NH_2), 7.71-7.40 (m, 9H, phenyl-*H*).
2-Amino-4-(4-fluoro-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.21)
Yellow solid, 42% yield. ^{1}H-NMR δ(DMSO): 7.64 (br s, 2H, NH_2), 7.62-7.59 (m, 4H, phenyl-*H*), 7.52-7.40 (m, 5H, phenyl-*H*).
2-Amino-4-(3-trifluoromethyl-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.22)
Yellow solid, 34% yield. ^{1}H-NMR δ(DMSO): 7.85 (br s, 2H, NH_2), 7.83-7.70 (m, 4H, phenyl-*H*), 7.66-7.61 (m, 2H, phenyl-*H*), 7.56-7.51 (m, 3H, phenyl-*H*).
2-Amino-4-(4-trifluoromethyl-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.23)
Yellow solid, 50% yield. ^{1}H-NMR δ(DMSO): 8.01-7.92 (m + br s, 3H, NH_2 + CF_3-phenyl-*H*), 7.83-7.78 (m, 2H, CF_3-phenyl-*H*), 7.64-7.60 (m, 2H, phenyl-*H*), 7.53-7.43 (m, 3H, phenyl-*H*).

2-Amino-4-(3-methyl-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.24)
Yellow solid, 29% yield. ^{1}H-NMR δ(DMSO): 7.83 (br s, 2H, N*H*$_2$), 7.66-7.61 (m, 2H, phenyl-*H*), 7.56-7.49 (m, 3H, phenyl-*H*), 7.45-7.33 (m, 4H, tolyl-*H*), 2.41 (s, 3H, C*H*$_3$).
2-Amino-4-(4-methyl-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.25)
Yellow solid, 46% yield. ^{1}H-NMR δ(DMSO): 7.60 (br s, 2H, N*H*$_2$), 7.55-7.52 (m, 2H, phenyl-*H*), 7.50-7.38 (m, 7H, phenyl-*H* + tolyl-*H*), 2.43 (s, 3H, C*H*$_3$).
2-Amino-4-(3-hydroxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.26)
Yellow solid, 20% yield. ^{1}H-NMR δ(CDCl$_3$/MeOD): 7.60-7.32 (m, 6H, phenyl-*H*), 6.99-6.94 (m, 3H, phenyl-*H*).
2-Amino-4-(4-hydroxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.27)
Yellow solid, 25% yield. ^{1}H-NMR δ(CDCl$_3$/MeOD): 7.58-7.39 (m, 7H, phenyl-*H*), 6.95 (d, 2H, J = 8.8 Hz, phenyl-*H*).
2-Amino-4-(3-difluoromethoxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.28)
Yellow solid, 42% yield. ^{1}H-NMR δ(DMSO): 7.88 (br s, 2H, N*H*$_2$), 7.67-7.34 (m, 9H, phenyl-*H*), 6.95 (m, 1H, OCF$_2$*H*).
2-Amino-4-(4-difluoromethoxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.29)
Yellow solid, 32% yield. ^{1}H-NMR δ(DMSO): 7.85 (br s, 2H, N*H*$_2$), 7.69-7.38 (m, 9H, phenyl-*H*), 7.07 (m, 1H, OCF$_2$*H*).
2-Amino-4-(3-trifluoromethoxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.30)
Yellow solid, 16% yield. ^{1}H-NMR δ(DMSO): 7.89 (br s, 2H, N*H*$_2$), 7.75-7.51 (m, 9H, phenyl-*H*).
2-Amino-4-(4-trifluoromethoxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.31)
Yellow solid, 4% yield. ^{1}H-NMR δ(DMSO): 7.89 (br s, 2H, N*H*$_2$), 7.76 (d, 2H, J = 6.6 Hz, OCF$_3$-phenyl-*H*), 7.66 (m, 7H, OCF$_3$-phenyl-*H* + phenyl-*H*).
2-Amino-4-(3-methoxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.32)
Yellow solid, 32% yield. ^{1}H-NMR δ(CDCl$_3$/DMSO): 7.57-7.42 (m, 6H, phenyl-*H*), 7.10-6.95 (m, 3H, phenyl-*H*), 3.87 (s, 3H, C*H*$_3$).
2-Amino-4-(4-methoxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.33)
Yellow solid, 32% yield. ^{1}H-NMR δ(DMSO): 7.77 (br s, 2H, N*H*$_2$), 7.65-7.50 (m, 7H, phenyl-*H*), 7.15 (d, 2H, J = 8.8 Hz, phenyl-*H*), 3.87 (s, 3H, C*H*$_3$).
2-Amino-4-benzo[1,3]dioxol-5-yl-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.34)
Yellow solid, 34% yield. ^{1}H-NMR δ(DMSO): 7.80 (br s, 2H, N*H*$_2$), 7.64-7.60 (m, 2H, S-phenyl-*H*), 7.53-7.50 (m, 3H, S-phenyl-*H*), 7.20-7.04 (m, 3H, phenyl-*H*), 6.18 (s, 2H, -OC*H*$_2$O-).
2-Amino-4-(3,4-dimethoxy-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.35)
Yellow solid, 28% yield. ^{1}H-NMR δ(DMSO): 7.77 (br s, 2H, N*H*$_2$), 7.65-7.50 (m, 7H, phenyl-*H*), 7.15 (d, 2H, J = 8.8 Hz, phenyl-*H*), 3.67 (s, 3H, C*H*$_3$), 3.63 (s, 3H, C*H*$_3$).
2-Amino-4-(4-dimethylamino-phenyl)-6-phenylsulfanyl-pyridine-3,5-dicarbonitrile (7.36)
Yellow solid, 32% yield. ^{1}H-NMR δ(DMSO): 7.68 (br s, 2H, N*H*$_2$), 7.64-7.60 (m, 2H, phenyl-*H*), 7.54-7.48 (m, 2H, phenyl-*H*), 7.44 (d, 2H, J = 8.8 Hz, 4-NMe$_2$-phenyl-*H*), 6.86 (d, 2H, J = 8.8 Hz, 4-NMe$_2$-phenyl-*H*), 3.03 (s, 6H, 2 × C*H*$_3$).

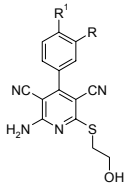
General Procedure for 2-Amino-4-(substituted)-phenyl-6-mercapto-pyridine-3,5-dicarbonitriles (7.37-7.54)



The pyridine (**7.19-7.36**) (3 mmol) was dissolved in DMF (10 mL) and to this was added sodium sulfide (0.78 g, 10 mmol) and the mixture stirred at 80 °C for 2 h. Upon cooling to room temperature, 1 M HCl (20 mL) was added, resulting in the formation of a yellow precipitate. The crude product was collected by filtration.

2-Amino-4-phenyl-6-mercapto-pyridine-3,5-dicarbonitrile (7.37)
Yellow solid, quantitative yield. ^{1}H-NMR δ(CDCl$_3$/MeOD): 7.51-7.20 (m, 5H, phenyl-*H*).
2-Amino-4-(3-fluoro-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.38)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.65-7.59 (m, 1H, phenyl-*H*), 7.48-7.35 (m, 3H, phenyl-*H*).
2-Amino-4-(4-fluoro-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.39)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.58-7.52 (m, 2H, phenyl-*H*), 7.44-7.39 (m, 2H, phenyl-*H*).
2-Amino-4-(3-trifluoromethyl-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.40)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.91-7.78 (m, 4H, phenyl-*H*).
2-Amino-4-(4-trifluoromethyl-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.41)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.96 (d, 2H, J = 8.0 Hz, phenyl-*H*), 7.76 (d, 2H, J = 8.0 Hz, phenyl-*H*).

2-Amino-4-(3-methyl-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.42)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.57-7.30 (m, 4H, phenyl-*H*), 2.40 (s, 3H, C*H*$_3$).
2-Amino-4-(4-methyl-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.43)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.53-7.35 (m, 4H, phenyl-*H*), 2.41 (s, 3H, C*H*$_3$).
2-Amino-4-(3-hydroxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.44)
Yellow solid, quantitative yield. ^{1}H-NMR δ(CDCl$_3$/MeOD): 7.46-7.34 (m, 1H, phenyl-*H*), 6.95-6.93 (m, 3H, phenyl-*H*).
2-Amino-4-(4-hydroxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.45)
Yellow solid, quantitative yield. ^{1}H-NMR δ(CDCl$_3$/MeOD): 7.41 (d, 2H, J = 8.8 Hz, phenyl-*H*), 6.94 (d, 2H, J = 8.8 Hz, phenyl-*H*).
2-Amino-4-(3-difluoromethoxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.46)
Yellow solid, quantitative yield. ^{1}H-NMR δ(MeOH): 7.46-7.33 (m, 4H, phenyl-*H*), 6.89 (t, 1H, J = 73.4 Hz, OCF$_2$*H*).
2-Amino-4-(4-difluoromethoxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.47)
Yellow solid, quantitative yield. ^{1}H-NMR δ(MeOD): 7.59 (m, 2H, phenyl-*H*), 7.30 (m, 2H, phenyl-*H*), 6.97 (t, 1H, J = 73.1 Hz, OCF$_2$*H*).
2-Amino-4-(3-trifluoromethoxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.48)
Yellow solid, quantitative yield. ^{1}H-NMR δ(MeOD): 7.67-7.62 (m, 1H, phenyl-*H*), 7.54-7.47 (m, 3H, phenyl-*H*).
2-Amino-4-(4-trifluoromethoxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.49)
Yellow solid, quantitative yield. ^{1}H-NMR δ(MeOD): 7.67-7.63 (m, 2H, phenyl-*H*), 7.48-7.44 (m, 2H, phenyl-*H*).
2-Amino-4-(3-methoxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.50)
Yellow solid, quantitative yield. ^{1}H-NMR δ(CDCl$_3$/MeOD): 7.47-7.39 (m, 1H, phenyl-*H*), 7.09-7.01 (m, 3H, phenyl-*H*), 3.89 (s, 3H, C*H*$_3$).
2-Amino-4-(4-methoxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.51)
Yellow solid, quantitative yield. ^{1}H-NMR δ(CDCl$_3$/MeOD): 7.49 (m, 2H, phenyl-*H*), 7.03 (m, 2H, phenyl-*H*) 3.88 (s, 3H, C*H*$_3$).
2-Amino-4-benzo[1,3]dioxol-5-yl-6-mercapto-pyridine-3,5-dicarbonitrile (7.52)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.13-7.00 (m, 3H, phenyl-*H*), 6.17 (s, 2H, -OC*H*$_2$O-).
2-Amino-4-(3,4-dimethoxy-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.53)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.17-7.13 (m, 3H, phenyl-*H*), 3.85 (s, 3H, OC*H*$_3$), 3.81 (s, 3H, OC*H*$_3$).
2-Amino-4-(4-dimethylamino-phenyl)-6-mercapto-pyridine-3,5-dicarbonitrile (7.54)
Yellow solid, quantitative yield. ^{1}H-NMR δ(DMSO): 7.41 (d, 2H, J = 8.8 Hz, phenyl-*H*), 6.83 (d, 2H, J = 8.8 Hz, phenyl-*H*), 3.03 (s, 6H, 2 × C*H*$_3$).



General Procedure for 2-Amino-4-[(substituted)phenyl]-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitriles (7.55-7.72) The free thiol (**7.37-7.54**) (1 mmol) was stirred with 2-bromoethanol (84 µL, 1 mmol), NaHCO$_3$ (0.34 g, 1 mmol) in DMF (2 mL) at room temperature for 2 h. Water (10 mL) was added to precipitate the crude product, which was collected by filtration. Purification by column chromatography on SiO$_2$, eluting with ethylacetate and/or ethylacetate-methanol mixtures, followed by recrystallisation gave the desired products.

2-Amino-4-phenyl-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.55)
White Solid, 54% yield. mp: 211 ºC. ^{1}H-NMR δ(MeOD): 7.56-7.48 (m, 5H, phenyl-*H*), 3.80 (t, 2H, J = 6.6 Hz, OC*H*$_2$), 3.40 (t, 2H, J = 6.6 Hz, C*H*$_2$S). ^{13}C-NMR δ(MeOD): 167.1, 159.6, 158.3, 134.0, 130.3, 128.7, 128.4, 115.4, 115.3, 96.1, 85.7, 59.5, 32.6. MS (ESI): 296.9. Anal. (C$_{15}$H$_{12}$N$_4$OS·0.34H$_2$O.0.3DMF) C, H, N, S.
2-Amino-4-(3-fluoro-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.56)
White Solid, 38% yield. mp: 189 ºC. ^{1}H-NMR δ(DMSO): 8.09 (br s, 2H, N*H*$_2$) 7.70-7.59 (m, 1H, phenyl-*H*), 7.52-7.37 (m, 3H, phenyl-*H*), 5.05 (t, 1H, J= 5.5 Hz, O*H*), 3.72-3.64 (m, 2H, OC*H*$_2$), 3.40-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.8, 159.5, 130.3, 130.2, 123.7, 117.1, 116.7, 115.2, 114.8, 114.3, 105.6, 83.3, 59.8, 31.5. MS (ESI): 314.8 Anal. (C$_{15}$H$_{11}$FN$_4$OS·0.3H$_2$O) C, H, N, S.
2-Amino-4-(4-fluoro-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.57)
White Solid, 54% yield. mp: 133 ºC. ^{1}H-NMR δ(DMSO): 8.06 (br s, 2H, N*H*$_2$) 7.68-7.60 (m, 2H, phenyl-*H*), 7.49-7.40 (m, 2H, phenyl-*H*), 5.04 (t, 1H, J= 5.5 Hz, O*H*), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.45-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.0, 166.0, 160.0, 157.4, 131.1, 131.0, 130.4, 116.0, 115.6, 115.4, 115.2, 85.8, 83.9, 59.4, 32.6. MS (ESI): 315.0. Anal. (C$_{15}$H$_{11}$FN$_4$OS·0.3MeOH) C, H, N, S.
2-Amino-4-(3-trifluoromethyl-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.58)
White Solid, 60% yield. mp: 164 ºC. ^{1}H-NMR δ(DMSO): 8.10 (br s, 2H, N*H*$_2$), 8.00-7.83 (m, 4H, phenyl-*H*), 5.05 (t, 1H, J= 5.5 Hz, O*H*), 3.75-3.66 (m, 2H, OC*H*$_2$), 3.42-3.36 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.2,

159.6, 156.8, 135.1, 132.7, 130.0, 129.8, 129.2, 127.0, 126.6, 125.6, 115.3, 115.1, 93.7, 85.9, 59.5, 32.7. MS (ESI): 364.9. Anal. ($C_{16}H_{11}F_3N_4OS \cdot 0.07CH_2Cl_2$) C, H, N, S.

2-Amino-4-(4-trifluoromethyl-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.59)

White Solid, 69% yield. mp: 158 ºC. ^{1}H-NMR δ(DMSO): 8.14 (br s, 2H, N*H*$_2$), 7.99 (d, 2H, J = 8.0 Hz, phenyl-*H*), 7.81 (d, 2H, J = 8.0 Hz, phenyl-*H*), 5.05 (t, 1H, J= 5.5 Hz, O*H*), 3.74-3.65 (m, 2H, OC*H*$_2$), 3.45-3.36 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 178.9, 167.2, 159.5, 157.0, 138.2, 130.8, 130.1, 129.6, 126.6, 125.7, 115.2, 115.0, 93.4, 85.7, 59.4, 32.7. MS (ESI): 364.6. Anal. ($C_{16}H_{11}F_3N_4OS \cdot 0.2CH_2Cl_2$) C, H, N, S.

2-Amino-4-(3-methyl-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.60)

White Solid, 74% yield. mp: 178 ºC. ^{1}H-NMR δ(DMSO): 8.02 (br s, 2H, N*H*$_2$), 7.47-7.30 (m, 4H, phenyl-*H*), 5.04 (t, 1H, J= 5.5 Hz, O*H*), 3.71-3.65 (m, 2H, OC*H*$_2$), 3.40-3.34 (m, 2H, C*H*$_2$S), 2.40 (s, 3H, C*H*$_3$). ^{13}C-NMR δ(DMSO): 167.1, 159.6, 158.4, 138.1, 134.0, 131.0, 128.7, 125.5, 115.5, 115.3, 93.6, 85.9, 59.5, 32.7, 20.9. MS (ESI): 311.2. Anal. ($C_{16}H_{14}N_4OS$) C, H, N, S.

2-Amino-4-(4-methyl-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.61)

White Solid, 81% yield. mp: 206 ºC. ^{1}H-NMR δ(DMSO): 8.00 (br s, 2H, N*H*$_2$), 7.46-7.36 (m, 4H, phenyl-*H*), 5.03 (t, 1H, J = 5.5 Hz, O*H*), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.39-3.33 (m, 2H, C*H*$_2$S), 2.41 (s, 3H, C*H*$_3$). ^{13}C-NMR δ(DMSO): 167.0, 159.6, 158.3, 140.2, 131.1, 129.2, 128.4, 115.5, 115.3, 93.6, 85.7, 59.5, 32.6, 20.9. MS (ESI): 310.8. Anal. ($C_{16}H_{14}N_4OS$) C, H, N, S.

2-Amino-4-(3-hydroxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.62)

White Solid, 70% yield. mp: 227-229 ºC dec. ^{1}H-NMR δ(DMSO): 8.01 (br s, 2H, N*H*$_2$) 7.41-7.33 (m, 1H, phenyl-*H*), 6.98-6.89 (m, 3H, phenyl-*H*), 5.05 (t, 1H, J = 5.5 Hz, O*H*), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.42-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.1, 135.2, 130.0, 118.9, 117.3, 115.4, 115.3, 115.1, 93.6, 86.0, 59.5, 32.7. MS (ESI): 312.8. Anal. ($C_{15}H_{12}N_4O_2S \cdot 0.6H_2O$) C, H, N, S.

2-Amino-4-(4-hydroxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.63)

White Solid, 40% yield. mp: 224 ºC dec. ^{1}H-NMR δ(DMSO): 7.95 (br s, 2H, N*H*$_2$) 7.39 (m, 2H, phenyl-*H*), 6.93 (m, 2H, phenyl-*H*), 5.04 (br s, 1H, O*H*), 3.70-3.64 (m, 2H, OC*H*$_2$), 3.32-3.19 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.1, 130.3, 124.3, 115.7, 115.4, 59.5, 32.7. MS (ESI): 312.7. Anal. ($C_{16}H_{14}N_4O_2S \cdot 0.1$MeOH), C, H, N, S.

2-Amino-4-(3-difluoromethoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.64)

White Solid, 36% yield. mp: 140 ºC. ^{1}H-NMR δ(MeOD): 7.60-7.52 (m, 1H, phenyl-*H*), 7.38-7.31 (m, 3H, phenyl-*H*), 6.86 (t, 1H, 73.4Hz, CF$_2$*H*), 3.79 (t, 2H, J = 6.6 Hz, OC*H*$_2$), 3.39 (t, 2H, J = 6.6 Hz, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 169.4, 161.4, 158.5, 152.8, 137.0, 131.7, 126.5, 122.0, 120.4, 117.6, 116.1, 116.0, 95.5, 87.2, 61.4, 33.5. MS (ESI): 363. Anal. ($C_{16}H_{12}F_2N_4O_2S$) C, H, N, S.

2-Amino-4-(4-difluoromethoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.65)

White Solid, 44% yield. mp: 156 ºC. ^{1}H-NMR δ(DMSO): 8.04 (br s, 2H, N*H*$_2$) 7.65 (m, 2H, phenyl-*H*), 7.38 (m, 2H, phenyl-*H*), 7.43 (s, 1H, CF$_2$*H*), 5.04 (t, 1H, J = 5.5 Hz, O*H*), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.38-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.1, 159.6, 157.4, 152.4, 130.7, 118.4, 115.5, 115.3, 93.4, 85.8, 59.5, 32.7. MS (ESI): 363.0. Anal. ($C_{16}H_{12}F_2N_4O_2S$), C, H, N, S.

2-Amino-4-(3-trifluoromethoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.66)

White Solid, 69% yield. mp: 125 ºC. ^{1}H-NMR δ(DMSO): 8.08 (br s, 2H, N*H*$_2$) 7.73-7.59 (m, 4H, phenyl-*H*), 5.04 (t, 1H, J = 5.5 Hz, O*H*), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.38-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.1, 159.5, 156.5, 148.2, 136.1, 131.0, 127.7, 123.3, 122.9, 121.5, 115.2, 115.0, 85.8, 59.5, 32.6. MS (ESI): 380.7. Anal. calc. for $C_{16}H_{11}F_3N_4O_2S$ (C 50.53; H 2.92; N 14.73; S 8.43) found (C 50.46; H 2.39; N 15.01; S 8.16) %.

2-Amino-4-(4-trifluoromethoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.67)

White Solid, 75% yield. mp: 144 ºC. ^{1}H-NMR δ(DMSO): 8.08 (br s, 2H, N*H*$_2$) 7.74-7.58 (m, 4H, phenyl-*H*), 5.04 (t, 1H, J = 5.5 Hz, O*H*), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.37-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.2, 159.6, 157.1, 149.6, 133.2, 131.0, 121.2, 115.4, 115.2, 93.6, 85.8, 59.5, 32.7. MS (ESI): 380.8. Anal. ($C_{16}H_{11}F_3N_4O_2S$) C, H, N, S.

2-Amino-4-(3-methoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.68)

White Solid, 63% yield. mp: 188 ºC. ^{1}H-NMR δ(DMSO): 8.01 (br s, 2H, N*H*$_2$) 7.53-7.45 (m, 1H, phenyl-*H*), 7.17-7.06 (m, 3H, phenyl-*H*), 5.06 (t, 1H, J = 5.5 Hz, O*H*), 3.82 (s, 3H, C*H*$_3$), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.39-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.1, 159.7, 159.1, 158.1, 135.3, 130.1, 120.6, 115.9, 115.5, 115.3, 114.1, 93.7, 85.8, 59.5, 55.4, 32.7. MS (ESI): 326.9. Anal. ($C_{16}H_{14}N_4O_2S \cdot 0.2$MeOH), C, H, N, S.

2-Amino-4-(4-methoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.69)

White Solid, 54% yield. mp: 188 ºC. ^{1}H-NMR δ(DMSO): 7.96 (br s, 2H, N*H*$_2$) 7.51 (d, 2H, J = 8.8 Hz, phenyl-*H*), 7.13 (d, 2H, J = 8.8 Hz, phenyl-*H*), 5.03 (t, 1H, J = 5.5 Hz, O*H*), 3.86 (s, 3H, C*H*$_3$), 3.73-3.64 (m, 2H, OC*H*$_2$), 3.37-3.33 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.1, 160.8, 159.8, 158.0, 130.2, 125.9, 115.7, 115.6, 114.1, 85.7, 59.7, 59.5, 55.4, 32.6. MS (ESI): 327.0. Anal. ($C_{16}H_{14}N_4O_2S \cdot 0.2H_2O$) C, H, N, S.

2-Amino-4-benzo[1,3]dioxol-5-yl-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.70)
White Solid, 42% yield. mp: 192 ºC. ^{1}H-NMR δ(DMSO): 7.99 (br s, 2H, N*H*$_2$) 7.16-7.01 (m, 3H, phenyl-*H*), 6.17 (s, 2H, -OC*H*$_2$O-), 5.02 (t, 1H, J = 5.5 Hz, O*H*), 3.67 (m, 2H, OC*H*$_2$), 3.38-3.32 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 197.7, 167.0, 159.7, 157.9, 148.9, 147.4, 127.4, 123.0, 115.6, 115.4, 108.9, 108.5, 101.8, 93.8, 59.5, 32.6. MS (ESI): 341.1. Anal. calc. for $C_{16}H_{12}N_4O_3S$ (C 56.46; H 3.55; N 16.46; S 9.42) found (C 56.31; H 3.11; N 16.38; S 9.46) %.
2-Amino-4-(3,4-dimethoxy-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.71)
White Solid, 35% yield. mp: 209 ºC. ^{1}H-NMR δ(DMSO): 7.96 (br s, 2H, N*H*$_2$) 7.19-7.13 (m, 3H, phenyl-*H*), 5.02 (m, 1H, O*H*), 3.86 (s, 3H, OC*H*$_3$), 3.81 (s, 3H, OC*H*$_3$), 3.69-3.66 (m, 2H, OC*H*$_2$), 3.32-3.19 (m, 2H, C*H*$_2$S). ^{13}C-NMR δ(DMSO): 167.0, 159.7, 158.0, 150.4, 148.3, 126.0, 121.5, 115.7, 115.6, 112.3, 111.5, 92.7, 85.9, 59.5, 55.7, 55.6, 32.8. MS (ESI): 356.9. Anal. ($C_{17}H_{16}N_4O_3S$), C, H, N, S.
2-Amino-4-(4-dimethylamino-phenyl)-6-(2-hydroxy-ethylsulfanyl)-pyridine-3,5-dicarbonitrile (7.72)
White Solid, 43% yield. mp: 268 ºC dec. ^{1}H-NMR δ(DMSO): 7.87 (br s, 2H, N*H*$_2$) 7.39 (d, 2H, J = 8.8 Hz, phenyl-*H*), 6.82 (d, 2H, J = 8.8 Hz, phenyl-*H*), 4.99 (t, 1H, J = 5.5 Hz, O*H*), 3.69-3.60 (m, 2H, OC*H*$_2$), 3.38-3.32 (m, 2H, C*H*$_2$S), 3.00 (s, 6H, 2 × C*H*$_3$). ^{13}C-NMR δ(DMSO): 167.1, 160.0, 158.3, 151.5, 129.9, 120.0, 116.1, 116.0, 111.3, 93.2, 85.0, 59.6, 39.5, 32.6. MS (ESI): 339.8. Anal. ($C_{17}H_{17}N_5OS \cdot 0.2CH_2Cl_2$) C, H, N, S.

7.4.2 Biology

Materials and Methods
[^{3}H]DPCPX and [^{125}I]AB-MECA were purchased from Amersham Biosciences (NL). [^{3}H]ZM 241385 was obtained from Tocris Cookson, Ltd. (UK). CHO cells expressing the human adenosine A_1 receptor were provided by Dr. Andrea Townsend-Nicholson, University College London, UK. HEK 293 cells stably expressing the human adenosine A_{2A} and A_3 receptor were gifts from Dr. Wang (Biogen, USA) and Dr. K.-N. Klotz (University of Würzburg, Germany), respectively.
All compounds were tested in radioligand binding assays to determine their affinities at the human adenosine A_1, A_{2A} and the A_3 receptors as described previously in Chapter 3, with the exception of non-specific binding on the A_{2A} receptor was determined in the presence of 10 μM CGS21680 instead of 100 μM CPA. The human A_1 receptors were expressed in CHO cells, and [^{3}H]DPCPX used as the radioligand. The A_{2A} and A_3 receptors were expressed in HEK 293 cells, and [^{3}H]ZM 241385 and [^{125}I]AB-MECA were used as the respective radioligands. Compounds for which the affinities were determined (i.e., compounds which showed greater than 50% displacement of the radioligand at 1 μM) were tested in functional assays for their ability to influence the levels of cAMP in the test system. The compounds were tested at concentrations of mostly 100 × K_i, and at least 20 × K_i , where the receptor sites should be almost fully occupied. The behaviour of the compounds was observed with reference to known adenosine receptor ligands; CPA (a full agonist), DPCPX (an inverse agonist) and N0840 (reported as a neutral antagonist).
CHO cells expressing the human adenosine A_1 receptor were grown overnight as a monolayer in 24 well tissue culture plates (400 μL/well; 2 × 10^5 cells/well). cAMP generation was performed in Dulbecco's Modified Eagles Medium (DMEM)/ N-2-hydroxyethylpiperazin-N'-2-ethansulfonic acid (HEPES) buffer (0.60 g HEPES/ 50 mL DMEM pH 7.4). Each well was washed twice with HEPES/DMEM buffer (250 μL), and the following added, adenosine deaminase (0.8 IU/mL), rolipram (50 μM), cilostamide (50 μM). This was then incubated for 30 minutes at 37 °C, followed by the introduction of the compound of interest. After a further 10 minutes of incubation, forskolin was added (10 μM). After a subsequent 15 minutes, incubation was stopped by aspirating the assay medium and by adding 200 μL of ice-cold 0.1 M HCl. The amount of cAMP was determined by competition with [^{3}H]cAMP for protein kinase A (PKA). Briefly, the sample, approximately 1.8 nM [^{3}H]cAMP, and 100 μL PKA solution were incubated on ice for 2.5 hr. The incubations were stopped by rapid dilution with 2 mL of ice-cold Tris HCl buffer (pH 7.4) and bound radioactive material was then recovered by filtration through Whatman GF/C filters. Filters were additionally rinsed with 2 × 2 mL Tris HCl buffer and then the radioactivity counted in Packard Emulsifier Safe scintillation fluid (3.5 mL). All data reflect three independent experiments performed in duplicate.

Data Analysis
K_i values were calculated using a non-linear regression curve-fitting program (GraphPad Prism 4, GraphPad Software Inc., San Diego, CA, USA). K_D values of the radioligands were 1.6 nM, 1.0 nM, and 5.0 nM for [^{3}H]DPCPX, [^{3}H]ZM 241385, and [^{125}I]AB-MECA, respectively. The data from the functional assays were also analysed with GraphPad Prism. Figure 7.1 and the results shown in Table 7.1 were generated by evaluating the data to relate to the known ligands CPA and DPCPX. The first step involved normalising the raw data with respect to CPA and DPCPX. CPA was chosen as the baseline value as it has previously been shown to be a full agonist, and DPCPX as the highest value, assuming full inversely agonistic behaviour. After normalisation, the Y-axis was shifted to present the data as being above or below that of the forskolin-generated levels of cAMP.

Finally, the data were again normalised to assume 100% inhibition of cAMP by the agonist CPA and 100% full inverse agonism by DPCPX.

7.4.3 Molecular Modelling

Molecular modelling work was performed with the SPARTAN molecular modelling package Spartan Pro 1.0.2. Default values in the Merck Force Field were used in Molecular Mechanics minimisations. The molecular electrostatic potential was calculated using the semi-empirical AM1 Hamiltonian. The electrostatic potential was sampled over the entire accessible surface of the molecules (roughly to a Van der Waals contact surface).

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General Conclusions and Perspectives

The research recorded in this thesis furthers our understanding of the adenosine A_1 receptor. By analysing a series of adenosine A_1 receptor antagonists with molecular modelling techniques, reoccurring electronic and spatial properties were identified and clustered into a new model. This model provided a new perspective from which to design A_1 receptor antagonists. Despite (or because of) the simplicity of the model, four series of ligands were designed and synthesised with extremely promising affinity for the adenosine A_1 receptor. This illustrates that there are few, though specific, essential features necessary for good interaction with the receptor.

It is vital that the compounds developed in this period of research are further examined and investigated, especially in a physiological system. Only in this way can their full potential be discovered. It is a step in the right direction that the PSA of a compound is factored into the design stage; however this is only an indication of the potential blood-brain barrier (BBB) penetration (or otherwise). The confirmation of this will only be certain after more research. However, the scope and the resources available to an academic research group may render such high aspirations unfeasible. The BBB is a complete area of research in itself and there are numerous groups devoted entirely to this subject, be it aspects of the barrier itself,[1,2] the transporters,[3-6] or the penetration and effects of various different pharmaceutical preparations.[7] *In vitro* investigations and animal models may shed light on some of the questions, but it may be only after *in vivo* studies in humans (after, of course, the numerous lengthy and time-consuming safety and toxicity questions are answered) that the real effects of a particular 'drug' in the brain are revealed.

The next step to take in this line of research is perhaps to undertake the same procedure for the A_{2A} and A_3 receptors, the lack of existing high affinity ligands for the A_{2B} receptor may limit this method of development for this particular receptor. Since some affinity (albeit much lower than that attained for the A_1 receptor) is achieved at the A_{2A} and A_3 receptors with some of the compounds detailed in this thesis, it may be possible that these heterocyclic cores can also be manipulated to enhance the affinity and selectivity for these two receptors. On the contrary, it may also be the case that it is only incidental that some affinity is shown at the A_{2A} and A_3 receptors and that their binding site requirements may be only partially satisfied by the heterocycles presented here. The vast expansion in the development of A_{2A}[8] and A_3[9] ligands over the past few years holds much promise in a methodical analysis and development of a ligand-based pharmacophore.

One discrepancy of adenosine receptor research continues to be the A_{2B} receptor. The low affinity nature of this receptor accounts for a lack of good selective ligands and thus appropriate radioligands (e.g., [^{3}H]MRS1754 and [^{3}H]MRE2029-F20)[10,11] have only just recently become available. The inclusion of the A_{2B} receptor as part of the standard assessment procedure is still not the norm and although expectations are low for compounds designed specifically for another receptor, it is a routine that should be striven to be practised. In an attempt to redress this somewhat and to substantiate our general observations that these series are (better) A_1 receptor ligands, a selection of compounds from each series described in this thesis have also been assessed at the A_{2B} receptor. Although the data are not shown in this

thesis (due to only the partial evaluation of each series), the indications are that these ligands, like many of their predecessors, perform poorly at the adenosine A_{2B} receptor.

As in many areas of science, no matter how well-trodden the path, there are quite often unexpected surprises still waiting to be uncovered. After so many years of adenosine research, the notion that the ribose group was necessary for activation of the receptor was a deeply embedded belief. The discovery of the pyridine dicarbonitrile series was perhaps by the use of screening methods or by chance, nevertheless, this now opens up the potential of exploring the bare necessities to achieve activation of the adenosine receptors. That the activation of the receptor with the pyridine dicarbonitriles described in Chapter 7 was so dependent upon the substituent present (or the lack of) is an extraordinarily interesting area to research further. The answer may hold clues to the secrets of activation of GPCRs in general.

In light of these investigations, cAMP assays have subsequently been performed with a selection of the compounds detailed in Chapters 3-6 to determine their effects at the adenosine A_1 receptor. These experiments have displayed the antagonistic-inverse agonistic behaviour typical of most non-ribose compounds. For example, from the compounds in Chapter 3, LUF 5767 (**3.14**) was tested in a whole cell cAMP assays in the presence of forskolin and CPA to determine its ability to antagonise the A_1 receptor. Due to the adenosine A_1 receptor's coupling to an inhibitory G protein, which leads to a decrease in the levels of cAMP upon stimulation, forskolin was added to generate a measureable amount of cAMP in the system. The amount of cAMP generated by the sole addition of forskolin was thus set at 100%. CPA, a well-known full agonist of the A_1 receptor, reduced the levels of cAMP (set thus at 0%). At a concentration of 1 μM, **3.14** displayed antagonistic behaviour in the test system, generating cAMP levels at approximately 60% of the forskolin-induced levels. At a higher concentration of the compound (10 μM), the effects of CPA were reversed completely to such an extent that inverse agonistic behaviour was recorded.

At the end of Chapter 7, the properties of the pyridine dicarbonitriles were matched to the model defined and refined earlier in this thesis. It can clearly be seen that the pyridine dicarbonitriles do possess some of the requirements defined by the model, and thus although not optimal for A_1 binding according to the set criteria, the affinity was in a range to match. An interesting line of investigation would be the modification of these pyridine dicarbonitriles to be fully compatible with the pharmacophore, e.g., the exchange of the thioethanol group for a phenyl ring. The resulting ligand(s) may reveal clues to the activating components in the receptor. A systematic dissection of the pyridine dicarbonitriles, examining each section (substituent or functional group) methodically may also gauge the importance and influence upon activation of the receptor. In combining this with the pharmacophoric model defined earlier, more information may be gathered on the orientation of the ligand in the A_1 receptor. Ensuing mutagenesis studies, in combination with computational work, may further determine the exact amino acids involved in receptor-ligand interactions and elicit clues towards the actual structure of the adenosine receptor(s). Medicinal chemistry remains a multi-disciplinary field.

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Summary

The adenosine receptors have been a well-explored field of research over the years. The therapeutic potential that is on offer by being able to manipulate these receptors is immense because the distribution of the receptors is so wide-spread in the physiological system. Chapter 1 begins with the introduction of the adenosine receptors and covers some of the investigations into potential therapeutic uses. Caffeine, as the archetypical adenosine antagonist and its wide consumption, is also given some attention in this introductory chapter. The scope and the general outline of this thesis are then detailed.

The A_1 receptor in particular has been investigated thoroughly and many different categories of ligands have been specifically synthesised and tested at this receptor. These encompass both the variety of different structures and the particular role that the ligand may fulfil, as an antagonist, an agonist or even a modulator in an allosteric binding position. Caffeine, as detailed in Chapter 1, is a well-known stimulatory 'drug' and most of its effects are a result of interactions with the adenosine A_1 receptor. It is a member of the xanthine family of compounds and this class has become the prototypic example of adenosine A_1 receptor antagonists. There are, however, issues arising from the development of xanthines, regarding such problems as solubility, bioavailability and specificity for the adenosine receptors, and thus the design of non-xanthine antagonists evolved.

The resulting vast assortment of non-xanthine antagonists is detailed in Chapter 2. Despite the large numbers of compounds that have been made, the eventual goal, adenosine A_1 receptor ligands with the potential to be developed as an eventual medicine, is still a distant target. The scope therefore, to create compounds that possess 'better' characteristics, in terms of affinity, selectivity, activity, solubility, absorption or indeed novelty, to name but a few desirable properties, is tremendous. The general restriction to G protein-coupled receptor (GPCR) research (i.e., the lack of a fully disclosed binding site) means that notwithstanding the many years of investigations, there are still many unknowns in the discipline.

Of the many approaches to understanding the adenosine A_1 receptor, computer modelling has become one of the mainstays of recent years and has contributed immensely to the knowledge-base. These techniques include 3D modelling of the protein itself, incorporating the crystal structure of rhodopsin (a very different GPCR) and results from site-directed mutagenesis studies. Another methodology is the so-called ligand-based approach, where the characteristics of the various different ligands that have shown good affinity for the receptor are compared and contrasted. The different computational practices have numerous advantages and disadvantages. It was the latter method that was chosen as the foundation of this thesis. Although the ligand-based approach has its limitations, it has generated pharmacophores and subsequent new ligand classes with positive effects at the receptors in question. Chapter 3 describes the use of such an approach. After a bout of intense research in the early 1990s, a model was revealed upon which to design adenosine A_1 antagonists, further

investigations did not lead to any particular advances in the design and planning processes. The general rules for A_1 adenosine antagonist design were that the structure should be (i) planar, (ii) aromatic and (iii) a nitrogen-containing 6:5 fused heterocycle. This did indeed lead to new compounds which displayed good affinity and selectivity for the A_1 receptor, but new insight into the reasons were not so forthcoming. In the year prior to starting the research detailed in this thesis, two new series of compounds were revealed which did not altogether abide by these rules. One was the 6:6 naphthyridine series and the other was a series of thia(dia)zoles. It was thus a more than appropriate time to update the model and the accompanying ingrained mind-set. Molecular modelling of a number of highly active compounds revealed a surprisingly simple configuration of hydrogen-bonding aspects and lipophilic groups. A series of 4-amido-2,6-diphenyl-pyrimidines were then designed and synthesised based on this pharmacophore. The biological results were very encouraging. The most effective ligands possessed an affinity of 2 nM at the adenosine A_1 receptor with a promising selectivity ratio.

The most well-known effects of caffeine are primarily due to the blockade of receptors in the brain. For a compound to be active at the brain, it has to be able to penetrate the blood-brain barrier (BBB). The BBB has been thoroughly investigated in recent years and computational/screening methods to predict its passage have become well-developed. The outcome of one such prediction method is to limit the calculated polar surface area (PSA) of a compound. Chapter 4 reports on the verification of a method to calculate the PSA and the application of it as a first parameter to predict the degree of penetration of adenosine A_1 receptor antagonists to the brain. A series of new ligands incorporating this new criterion were made. These 2-amido-4,6-diphenyl-pyrimidines were both within the preset PSA limits and were very effective at the adenosine A_1 receptor. The best compound in terms of affinity, at less than 4 nM, was comparable to that in Chapter 3. However, the selectivity of this ligand (and in general, of the whole series) over the A_{2A} and A_3 receptors was better than that obtained for the 4-amido-2,6-diphenyl-pyrimidines.

Fixing the hydrogen-bond accepting group corresponding to the oxygen of the amido-functionality of the aforementioned 4-amido-pyrimidines (Chapter 3) to the central aromatic ring gives rise to purine derivatives. Purines have been thoroughly explored as adenosine receptor antagonists, but some features have become almost institutional; namely the presence of the N^6-amino moiety and the requirement of an N9-substituent. With the aid of the model proposed in Chapter 3, a fresh take on the purine ring was explored in Chapter 5. The resulting ligands produced some of the lowest K_i values for the A_1 receptor in our group to-date. In particular compound **5.31**, LUF 5962, 8-cyclopentyl-2,6-diphenylpurine, at 0.29 nM was outstanding. The observations drawn from both this series of purines and the two preceding series of pyrimidines led to the refinement of the model described in Chapter 3.

Analysing the 2-amido-pyrimidines in a similar manner as described in the preceding paragraph produces a set of triazolopyrimidines. However, triazolopyrimidines are not fully

compliant with the criteria of the (refined) model; the extra nitrogen in the central core changes the planarity of the ring and is also superfluous to the hydrogen-bonding requirements. Exchanging this for a carbon atom generates the 1-deazapurine heterocycle. To confirm our observations from Chapter 5 and the subsequently adopted refinements to the model, a series of 1-deazapurines were made and tested. These investigations are recounted in Chapter 6 and the new refined model substantiated with the observation that five of the compounds displayed sub-nanomolar affinity.

In a slightly different twist to this thesis, Chapter 7 describes the development of a series of compounds with a surprisingly different activity at the adenosine A_1 receptor. The pyridine-3,5-dicarbonitriles were very selective for the A_1 receptor and depending on the substituent at the 4-position of the pyridine ring, the activity ranged from being fully agonistic, comparable to the reference compound CPA, to displaying full inverse agonism in our test system.

Finally, general conclusions about the research described in this thesis are drawn. This is also supplemented by an outlook on some potential aspects of research to be pursued, based upon the potent and interesting results obtained from this work.

Samenvatting

Adenosinereceptoren zijn al jarenlang het onderwerp van intensief wetenschappelijk onderzoek. Het bleek al snel dat deze receptoren wijdverspreid voorkomen in diverse fysiologische systemen van het menselijk lichaam en dus een belangrijke therapeutische potentie bezitten. Hoofdstuk 1 geeft een introductie over adenosinereceptoren (A_1, A_{2A}, A_{2B} en A_3) en behandelt enkele potentiële therapeutische toepassingen. Verder wordt hier aandacht besteed aan cafeïne, het schoolvoorbeeld van een adenosine-antagonist die op zeer grote schaal geconsumeerd wordt. Tenslotte wordt de doelstelling en de indeling van dit proefschrift aangegeven.

Met name aan de adenosine A_1 receptor is de afgelopen jaren veel onderzoek verricht en diverse categorieën liganden zijn specifiek gesynthetiseerd voor, en getest op, deze receptor. Deze grote aantallen van verbindingen staan beschreven in Hoofdstuk 2. Desondanks is het uiteindelijke doel om adenosine A_1 receptorliganden die een bepaalde rol vervullen, zoals die van antagonist, agonist of zelfs modulator/regulator op een allostere bindingsplaats, met de potentie om uiteindelijk als geneesmiddel ontwikkeld te kunnen worden, nog ver weg. De motivatie om verbindingen te synthetiseren met 'betere' eigenschappen wat betreft affiniteit, selectiviteit, activiteit, oplosbaarheid en absorptie, om maar een aantal wenselijke eigenschappen te noemen, is enorm sterk om deze redenen. De voornaamste beperking van het adenosine-onderzoek is het gebrek aan een volledig opgehelderde receptorstructuur, hetgeen betekent dat er nog steeds veel onbekende factoren zijn in dit onderzoek.

Van de vele mogelijke benaderingen om de adenosine A_1 receptor te begrijpen is computermodellering één van de belangrijkste en het heeft de laatste jaren substantieel bijgedragen aan de hoeveelheid vergaarde kennis. De verschillende computertechnieken hebben zowel voor- als nadelen. De technieken behelzen 3D modellering van het receptoreiwit zelf, gebruikmakend van de kristalstructuur van rhodopsine (een ander GPCR eiwit) en de resultaten van sitegerichte mutagenesestudies. Een andere methodologie is de benadering die uitgaat van het ligand. Daartoe worden de structuren van verschillende liganden, met een bewezen goede affiniteit voor de receptor, driedimensionaal met elkaar vergeleken. De laatstgenoemde methode werd gekozen als uitgangspunt voor dit proefschrift. Hoewel de benadering uitgaande van het ligand zijn beperkingen kent, heeft deze methode modellen opgeleverd van waaruit nieuwe groepen liganden zijn ontwikkeld met de gewenste effecten op de bestudeerde receptoren. In Hoofdstuk 3 wordt de toepassing van een dergelijke benadering beschreven. Na een intensieve onderzoeksfase in de vroege jaren '90, die een model opleverde voor het ontwerpen van adenosine A_1 antagonisten, heeft vervolgonderzoek niet geleid tot daadwerkelijke vooruitgang van het ontwerp- en planningsproces. De algemene voorwaarden aan het ontwerp van adenosine A_1 antagonisten waren dat de structuur (i) vlak, (ii) aromatisch en (iii) een stikstofhoudende 6:5 heterobicyclische verbinding diende te zijn. Dit leidde inderdaad tot nieuwe verbindingen met goede affiniteit en selectiviteit voor de A_1

receptor, maar ging niet gepaard met nieuwe inzichten en mogelijke verklaringen hiervoor. In het jaar voorafgaand aan het in dit proefschrift beschreven onderzoek werden twee nieuwe series verbindingen gepresenteerd die niet voldeden aan deze regels. De ene was de 6:6 nafthiridine serie en de andere was een serie thia(dia)zolen. Het was daarom meer dan gerechtvaardigd het model en de bijbehorende denkwijze te herzien. Moleculaire modellering van een aantal zeer actieve verbindingen liet een verrassend simpele configuratie van waterstofbruggen en lipofiele groepen zien. Een serie 4-amido-2,6-difenylpyrimidines, gebaseerd op deze farmacofoor, werd vervolgens ontworpen en gesynthetiseerd. De biologische resultaten waren zeer bemoedigend. De effectiefste liganden hadden een affiniteit van 2 nM voor de adenosine A_1 receptor met een veelbelovende selectiviteit.

Cafeïne is een welbekend stimulerend middel en het belangrijkste deel van het effect is het gevolg van interacties met de adenosine A_1 receptor. Cafeïne behoort tot de familie der xanthines, die het schoolvoorbeeld zijn van adenosine A_1 antagonisten. De effecten die aan deze liganden worden toegeschreven zijn voornamelijk het gevolg van een blokkering van receptoren in de hersenen. Echter, een verbinding die effecten veroorzaakt in de hersenen moet in staat zijn om de bloed-hersen-barrière (BHB) te passeren. In de afgelopen jaren is aan deze BHB uitgebreid onderzoek verricht en de computationele en screeningsmethoden voor het voorspellen van de doordringbaarheid zijn sterk verbeterd. De uitkomst van een dergelijke methode is het stellen van een limiet aan de grootte van het berekende polaire oppervlak (polar surface area, PSA) van een verbinding. In Hoofdstuk 4 wordt verslag gedaan van de verificatie van een berekeningsmethode voor de PSA-berekening en de toepassing hiervan als de eerste parameter voor het voorspellen van de penetratiegraad van adenosine A_1 antagonisten naar de hersenen. Een serie nieuwe liganden die voldoen aan dit nieuwe criterium werd vervolgens bereid. Deze 2-amido-4,6-difenylpyrimidines voldeden aan de gestelde PSA-limiet en waren zeer effectief als liganden voor de adenosine A_1 receptor. De beste verbinding wat betreft affiniteit, met een waarde van minder dan 4 nM, was vergelijkbaar met het beste ligand van Hoofdstuk 3. De selectiviteit van dit ligand (en van de serie als geheel) ten opzichte van de A_{2A} en A_3 receptor was beter dan die van de 4-amido-2,6-difenylpyrimidines.

Het fixeren van de waterstofbrugacceptorgroep overeenkomend met het zuurstofatoom van de amidogroep van de eerdergenoemde 4-amidopyrimidines (Hoofdstuk 3) aan de centrale aromaatring resulteert in purinederivaten. Purines zijn uitgebreid onderzocht als adenosinereceptor antagonisten en enkele eigenschappen worden schier onmisbaar geacht, namelijk de aanwezigheid van een N^6-aminogroep en de voorwaarde van een N9-substituent. In Hoofdstuk 5 werden met behulp van het model, zoals voorgesteld in Hoofdstuk 3, nieuwe purinederivaten ontwikkeld en gesynthetiseerd. De resulterende liganden leverden de hoogste affiniteit op die tot dusver in onze researchgroep gemeten is voor de A_1 receptor. In het bijzonder verbinding **5.31**, LUF 5962, 8-cyclopentyl-2,6-difenylpurine, met een K_i-waarde van 0.29 nM, is uitmuntend. De verkregen informatie van zowel deze serie purines als die van

de voorgaande twee series pyrimidines leidde tot een verfijning van het model uit Hoofdstuk 3.

Analyse van de 2-amidopyrimidines op eenzelfde manier als beschreven in de voorgaande paragraaf resulteerde in een serie triazolopyrimidines. Echter, triazolopyrimidines voldoen niet volledig aan de criteria van het (verfijnde) model en bezitten overbodige elementen. Het extra stikstofatoom in de centrale ring verandert wellicht de aromaticiteit van de ring en is tevens overbodig voor de waterstofbrugeisen. Door uitwisseling van dit stikstofatoom voor een koolstofatoom ontstaat 1-deazapurine. Een serie 1-deazapurines werd vervolgens gemaakt en getest om de waarnemingen uit Hoofdstuk 5 en de daaropvolgende verfijning van het model te bevestigen. Deze resultaten staan in Hoofdstuk 6 beschreven en de juistheid van het nieuwe verfijnde model werd bevestigd met de waarneming dat vijf van de verbindingen subnanomolaire affiniteit vertoonden.

Als een soort zijstap van dit proefschrift staat in Hoofdstuk 7 de ontwikkeling van een serie verbindingen met verrassend afwijkende activiteiten ten opzichte van de adenosine A_1 receptor beschreven. De 3,5-dinitrilpyridines blijken zeer selectief te zijn voor de A_1 receptor en, afhankelijk van de substituent op de 4-positie van de pyridinering, blijkt de activiteit te variëren van volledig agonistisch, vergelijkbaar met de referentieverbinding CPA, tot volledig invers agonistisch.

Tenslotte worden algemene conclusies uit het onderzoek, beschreven in dit proefschrift, getrokken. Dit wordt gecompleteerd met enkele toekomstperspectieven inzake het onderzoek aan adenosine A_1 receptorliganden.

Summary for Non-Scientists/ Samenvatting voor Leken/ 中文

The morning coffee, the afternoon tea, the mid-day (sugar-free!) cola drink, these are all everyday rituals that many people habitually partake in, in the name of ‘refreshment’, but what exactly are the ‘refreshing’ properties common to these beverages? Most people would hazard a guess at caffeine and they’d be correct. Caffeine is the major stimulant in some of our most popular drinks, with the ‘average’ cup of filtered coffee containing approximately 100 mg, (UK brewed) tea approximately 50 mg and standard cola drinks between 40-50 mg.

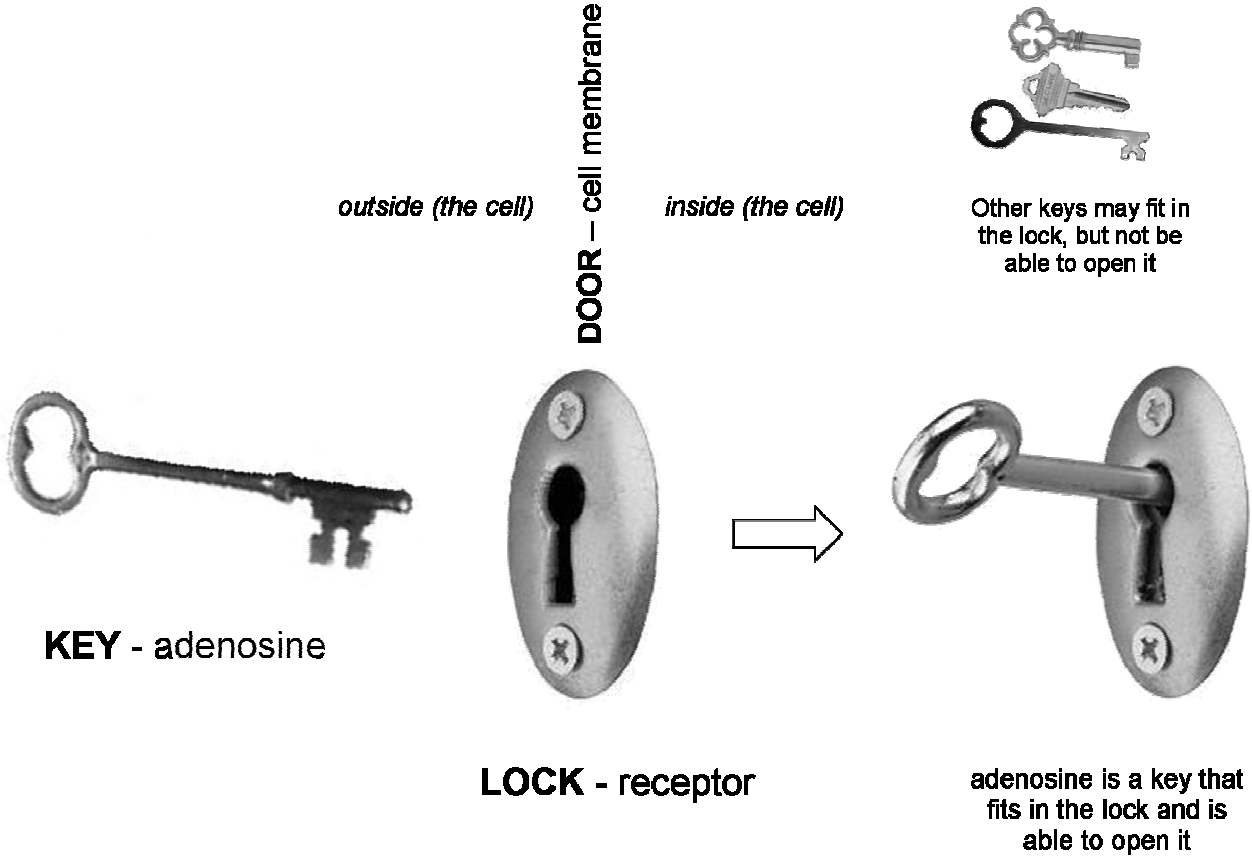
How and why does caffeine work? Caffeine acts at receptors in the body. These receptors you can imagine as small locks sitting in the surface of a cell. The body produces a substance that activates these receptors, acting much like a key where only the correct teeth or grooves fit to open the lock. The natural compound that activates these receptors is called adenosine and these receptors are called adenosine receptors. Caffeine works by blocking the receptor, like a key of the right size and shape, but with no teeth. A substance, like caffeine, which is able to block a receptor and not activate it, is called an antagonist. The adenosine receptors are found in many different areas of the

De kop koffie vroeg in de morgen, de thee in de middagpauze en cola (light!) in de middag, het zijn voor veel mensen dagelijks terugkerende rituelen wanneer we een opkikker nodig hebben. Maar wat geeft deze versnaperingen eigenlijk precies hun opwekkende eigenschappen? De meeste mensen zouden waarschijnlijk 'cafeïne' gokken, en dat is inderdaad juist. Cafeïne is het voornaamste stimulerende middel in onze favoriete drankjes, met een gehalte van rond de 100 mg per kop filterkoffie, 50 mg per kop (Engelse) thee en tussen 40 en 50 mg per glas cola.

Hoe en waarom werkt cafeïne? Cafeïne grijpt aan op receptoren in het lichaam. Deze receptoren kan men opvatten als kleine openingen ('sleutelgaten') aan de buitenkant van een lichaamscel. Het lichaam produceert een stofje dat deze receptoren activeert, op dezelfde manier als waarop een sleutel met de juiste groeven en tanden een slot kan openen. De stof in het menselijk lichaam die deze receptoren activeert is adenosine en de receptoren worden adenosinereceptoren genoemd. Cafeïne blokkeert de adenosinereceptor op dezelfde manier als een sleutel met de juiste afmetingen, maar zonder groeven en tanden. Een stofje als cafeïne, dat in staat is een receptor te blokkeren in plaats van te activeren, heet een antagonist. Adenosinereceptoren bevinden zich op zeer uiteenlopende plaatsen in

喝咖啡，茶或无糖的可乐饮料是人们用来提神的一种习惯方式。为什么这些饮料都能让人精神振奋呢？答案在于咖啡因。它是这些大众饮料有提神醒脑作用的主要原因。一般来说，一杯普通的咖啡含咖啡因100毫克，（英国）茶含咖啡因50毫克，而可乐则含40到50毫克。

咖啡因是如何起作用的呢？它的功能通过作用于我们身体内的腺苷酸受体而实现。这些受体可以被想象成为细胞表面的许多小锁，他们可以被我们身体分泌的与其形状匹配的物质打开（这种状况我们称之为激活）。腺苷酸受体得名于其天然底物——腺苷酸，腺苷酸与腺苷酸受体就像锁与钥匙一样形状完全吻合，所以能象开锁一样的将其激活。而咖啡因缺乏必要的激活腺苷酸受体的结构，我们可以将它想象为钥匙上缺了一个齿，所以它不能激活腺苷酸受体。但是



Above – The lock and key analogy to adenosine and its receptor.

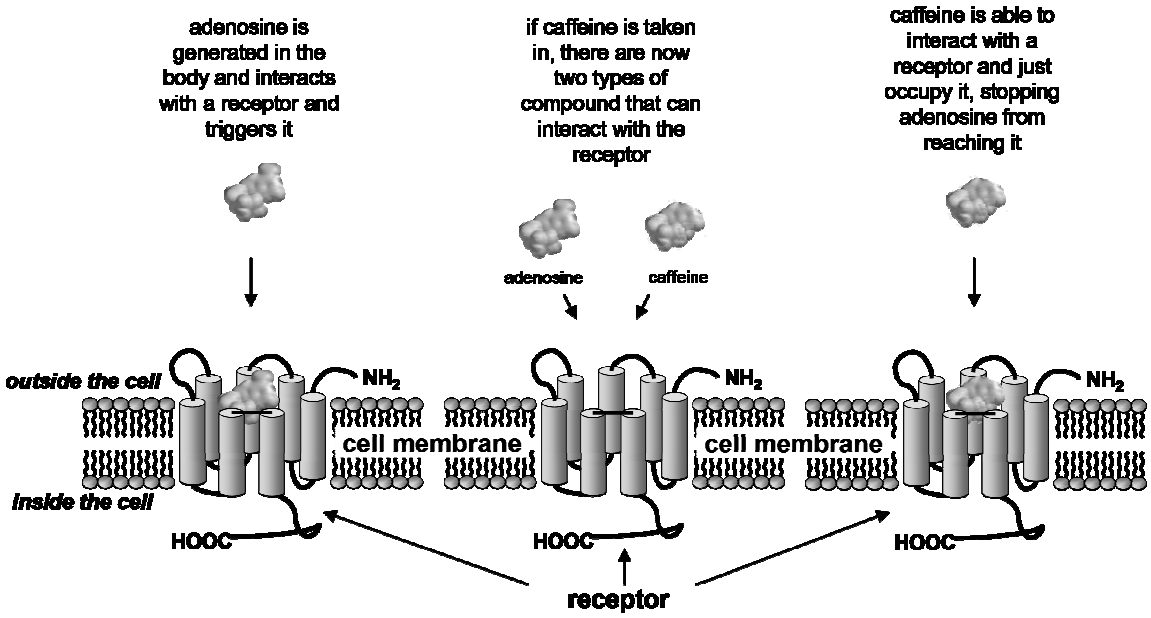
Boven – De sleutel-slot analogie van adenosine en zijn receptor.

上图是腺苷酸和腺苷酸受体的锁钥关系图示.

Below – How adenosine works and how caffeine is able to block the receptor.

Onder – De werking van adenosine en de blokkering van de receptor door cafeïne.

下图是腺苷酸激活受体的作用原理和咖啡因阻遏原理



body and there are four different types of adenosine receptor. As levels of adenosine rise in the brain we become sleepier (that mid-afternoon dip). We then drink a cup of tea or coffee and the caffeine that we take in is able to sit in two of the four different types of adenosine receptors. It has been found that the blockade of the so-called A_1 adenosine receptors in the brain makes you feel more awake. The blockade of the A_1 receptors has also been found to be potentially useful in the treatment of a range of diverse conditions such as asthma, Alzheimer's disease and kidney failure. An illustration of how adenosine and caffeine work can be found on the previous page.

As a short aside for those who may be baffled by some of the chemistry terms in this passage, 'Compounds' and 'substances' are used here interchangeably and refer to a chemical material. These compounds are built up of a number of different atoms, which is the smallest part into which an element can be reduced to. In the medicinal world, the most common and important atoms to know are C (carbon), H (hydrogen), N (nitrogen) and O (oxygen). An example is paracetamol, the structure of which is shown in the figure below.

As mentioned earlier, caf-

het lichaam en er bestaan vier verschillende typen van. Als de adenosineconcentratie in de hersenen stijgt voelen we ons slaperig worden (de bekende 'after-lunch dip'). Wanneer we dan een kop koffie of thee drinken kan de cafeïne die we binnenkrijgen twee van de vier adenosinereceptoren blokkeren. Uit onderzoek is gebleken dat blokkering van de zogenaamde A_1 adenosinereceptor in de hersenen ons wakker en alert houdt. Daarnaast is ook bekend dat blokkering van de A_1 receptor potentie heeft in de behandeling van aandoeningen als astma, de ziekte van Alzheimer en nierafwijkingen. De werking van adenosine en cafeïne is geïllustreerd op de vorige pagina.

Een korte toelichting voor diegenen die niet vertrouwd zijn met de chemische terminologie in deze samenvatting: 'stofje' en 'verbinding' worden hier door elkaar gebruikt en verwijzen naar een chemisch product. Deze verbindingen zijn opgebouwd uit een reeks verschillende atomen, de kleinste ondeelbare onderdelen waaruit een element is opgebouwd. In de geneesmiddelenwereld zijn de meest voorkomende en belangrijkste atomen om te onthouden C (koolstof), H (waterstof), N (stikstof) en O (zuurstof). Ter illustratie is hieronder de chemische structuur van paracetamol weergegeven.

Zoals eerder gezegd: cafeïne blokkeert meer dan één van de vier adenosinereceptoren en is dus niet selectief. Een ander probleem van cafeïne is dat nogal

咖啡因和腺苷酸的结构又是那么相近，所以它能堵着“锁孔”，从而不让受体与腺苷酸结合。象咖啡因这样只占据受体结合部位而又不能激活受体的物质我们称为拮抗剂。

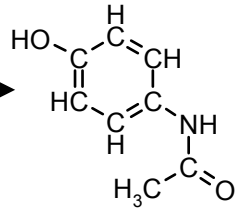
腺苷酸受体一共有4种，我们身体内的许多地方都有它们的分布。当脑内的腺苷酸浓度升高时，人们就会觉得困倦（人们午饭后常觉得犯困就是腺苷酸在作怪）。而这时如果喝上一杯茶或咖啡就能阻止其中2种腺苷酸受体的激活，从而使得人们感觉更加清醒。A_1型腺苷酸受体就是其中之一。此外，阻断A_1型腺苷酸受体同时还被应用在在治疗多种疾病，如哮喘，阿尔海茨默氏症和肾衰竭等上。

在这篇文章里化合物和物质同时用于指某些化学物质。化合物由许多不同的原子组成。在化学界，最常见的原子是C (碳), H (氢), N (氮) 和 O (氧)。举个例子，化合物

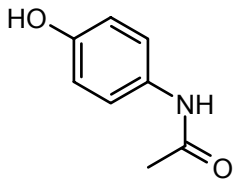
feine is able to sit at more than one of the four adenosine receptors; another problem with caffeine is that it takes a fair amount of it to have an effect. If we would like to use such a substance as a medicine then we would like to use a much smaller amount of it and make sure it acts predominantly at one of four different types of receptor. Thus this project was based on designing and making new substances that act in very small amounts as antagonists at the adenosine A_1 receptor.

This is not a new idea, adenosine receptors have been well researched a target in medicinal chemistry for more than twenty years, and the A_1 receptor in particular has had a lot of attention. However, although many compounds have been made aimed at the adenosine A_1 receptor, an actual medicine is still lacking despite its great potential. These compounds are summarised in detail in Chapter 2, and most notable is the depth and variety of different structures. This collection of many years work was the starting point for this thesis. The initial work was to build up a model from which compounds could

the chemical structure of paracetamol,showing all the carbon, hydrogen, nitrogen and oxygen atoms



paracetamol drawn in the conventional way used by scientists



grote hoeveelheden nodig zijn om een effect te genereren. Als we een verbinding als cafeïne als geneesmiddel zouden willen gebruiken zou dat in een veel lagere dosering, en met een effect op slechts één van de vier verschillende typen receptoren moeten zijn. Het doel van dit project was dan ook het ontwerpen en het maken van nieuwe verbindingen die zich in kleine doseringen gedragen als selectieve antagonisten voor de A_1 receptor.

Dit idee is zeker niet nieuw, aangezien adenosinereceptoren, met name de A_1 receptor, al meer dan twintig jaar uitgebreid onderzocht zijn als aangrijpingspunt voor geneesmiddelen. Hoewel vele verbindingen gericht op de adenosine A_1 receptor zijn gemaakt is er nog steeds geen geneesmiddel, de grote potentie van deze verbindingen ten spijt. Een overzicht van al deze verbindingen wordt gegeven in Hoofdstuk 2, waarbij vooral de grote variëteit aan structuren in het oog springt. Dit overzicht van jarenlange inspanningen vormde het uitgangspunt voor het in dit proefschrift beschreven onderzoek. De eerste taak was het maken van een model met behulp waarvan nieuwe verbindingen ontworpen konden worden. Dit doel werd bereikt met behulp van een

paracetamol 就是由这些原子组成，它的结构如下图所示。

如前所述，咖啡因作用于不止一个腺苷酸受体，而且产生效应需要量较多，因此难免会带来一些副作用。如果将其用于医药，则需对其进行改造，使其用量愈少，药效愈专（只针对其中某一种受体）愈好。本项研究的目的就是设计和合成新的针对于腺苷酸A_1受体的物质，低浓度时即可抑制受体，因而可以作药。

这并不是新的想法，在化学领域里，腺苷酸受体已经被深入研究了近20年，而A_1受体则是其中备受关注的一种。然而，尽管针对它的的化合物合成了不少，但是仍然没有具有良好疗效的化合物可以作为临床药物。对这些化合物的总结见第2章。前

be designed. This was achieved by using a computing package to analyse the properties of compounds that have been made before, in terms of their shape and size and actual atom distribution.

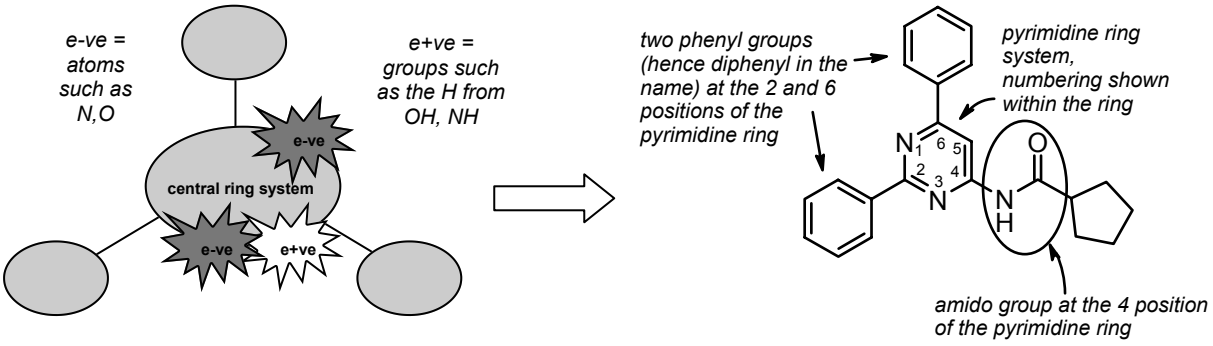
Analysing these compounds in this way led to a 'model' being built. This model stipulates a number of different conditions that a compound should meet to be good at the A_1 receptor (summarised in the illustration below). Having obtained a theoretical model, proof comes with the design, synthesis and testing of new structures. In this thesis, four series of substances have been made

computerprogramma dat de eigenschappen (vorm, afmeting en rangschikking der atomen) van eerder gemaakte verbindingen kon analyseren.

De analyse van deze eerder gemaakte verbindingen resulteerde in een model dat een aantal randvoorwaarden aangeeft waaraan een verbinding die op de A_1 receptor aangrijpt dient te voldoen (zie de Figuur hieronder). Na het definiëren van het theoretische model volgt de fase van het bewijzen van de juistheid ervan door middel van het ontwerpen, maken en testen van nieuwe verbindingen. In dit proefschrift staan vier series verbindingen beschreven die gebaseerd zijn op

人多年的工作是本项研究的起点。

我们最初使用分析化合物性质（如化合物的形状，大小和原子的分布）的软件包建立了一个模型，此模型规定了被设计的化合物结构必须和A_1受体吻合（见图示），因此可以用来引导化合物的设计。模型的好坏则经过了合成新的化合物的检验。在本项研究中，我们基于此模型合成了4个系列的



model derived from analysing many different compounds

one compound in the series of 4-amido-2,6-diphenyl pyrimidines

based on this model. The first one (Chapter 3) is a series called 4-amido-2,6-diphenyl pyrimidine (its naming scheme can be seen in the figure above). This was a very successful series with some particularly potent compounds.

Since we would like to copy some of the actions of caffeine, in particular the stimulatory ones (to use in perhaps condi-

dit model. In Hoofdstuk 3 staat de eerste serie, de 4-amido-2,6-difenyl pyrimidines (de naamgeving is nader toegelicht in de Figuur). De serie bleek zeer succesvol en bevat enkele zeer actieve verbindingen.

Aangezien we de eigenschappen van cafeïne willen nabootsen, met name de stimulerende effecten (voor toepassingen zoals tegen de ziekte van Alzheimer), dienen we er zeker van te zijn dat de verbindingen die we maken de

化合物。第一系列化合物（见第3章）叫做4-amido-2,6-diphenyl pyrimidine（其命名原则如下图所示），是一系列高效的化合物。

我们合成的物质可以模仿咖啡因的某些性质，尤

tions such as Alzheimer's), we need to know that the compounds we make reach the brain. In the body there is a physical barrier between the brain and the rest of the circulatory system. This is called the Blood-Brain Barrier (BBB) and is there to protect the brain from an assortment of different substances. It has been found that a compound has to hold certain assets to be able to cross the BBB. One of the ways of predicting this was effectively incorporated into the design of the second series in Chapter 4.

Chapters 5 and 6 describe two more series of compounds that were created as adenosine A_1 receptor antagonists. The series in Chapter 5 was made based upon a slight modification of the series recounted in Chapter 3; this led to the most potent compound ever discovered in our laboratories. The combination of the discoveries made in these three series led to the re-analysis and the refinement of the model. The last series (Chapter 6) was thus synthesised as a result of this, proving that the new model is a very good summary of the properties a compound should possess to be effective at the adenosine A_1 receptor.

The very last chapter of this thesis comes from a slightly different angle. Although we gain benefit from caffeine blocking the effects of adenosine, we need to know that the compounds we make reach the brain. In the body there is a physical barrier between the brain and the rest of the circulatory system. This is called the Blood-Brain Barrier (BBB) and is there to protect the brain from an assortment of different substances. It has been found that a compound has to hold certain assets to be able to cross the BBB. One of the ways of predicting this was effectively incorporated into the design of the second series in Chapter 4.

In de Hoofdstukken 5 en 6 staan tot slot twee series verbindingen beschreven die als adenosine A_1 antagonist zijn ontworpen. De verbindingen van Hoofdstuk 5 zijn het resultaat van een kleine modificatie van de serie uit Hoofdstuk 3; dit heeft geresulteerd in de actiefste verbinding ooit gemaakt in ons laboratorium. De ontdekkingen die volgden uit deze eerste drie series verbindingen hebben geleid tot een verdere verfijning van het model. De laatste serie verbindingen is hierop gebaseerd en het bewijs werd geleverd dat het verfijnde model een nauwkeurige beschrijving is van de essentiële eigenschappen van een effectieve verbinding voor de A_1 receptor.

In het laatste Hoofdstuk van dit proefschrift is voor een iets andere invalshoek gekozen. Hoewel we gebaat zijn bij het blokkerende effect van cafeïne op de werking van adenosine in de hersenen kan het in andere delen van het lichaam verbindingen die we maken de hersenen kunnen bereiken. Het lichaam heeft een fysieke barrière tussen de hersenen en de rest van de bloedsomloop. Deze zogenaamde bloed-hersen-barrière (BHB) beschermt de hersenen tegen een groot aantal schadelijke stoffen. Het is gebleken dat een verbinding over bepaalde eigenschappen dient te beschikken om de BHB te kunnen passeren. Eén van de manieren om dit te kunnen voorspellen is toegepast om een tweede serie verbindingen te ontwerpen, dit staat beschreven in Hoofdstuk 4.

In de Hoofdstukken 5 en 6 staan tot slot twee series verbindingen beschreven die als adenosine A_1 antagonist zijn ontworpen. De verbindingen van Hoofdstuk 5 zijn het resultaat van een kleine modificatie van de serie uit Hoofdstuk 3; dit heeft geresulteerd in de actiefste verbinding ooit gemaakt in ons laboratorium. De ontdekkingen die volgden uit deze eerste drie series verbindingen hebben geleid tot een verdere verfijning van het model. De laatste serie verbindingen is hierop gebaseerd en het bewijs werd geleverd dat het verfijnde model een nauwkeurige beschrijving is van de essentiële eigenschappen van een effectieve verbinding voor de A_1 receptor.

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其是刺激大脑的作用（可以用于治疗阿尔海茨默氏症），但是为了检测其是否有用作药物的可能性我们还需要了解这些物质是否可以到达大脑。人体内有一层天然的血脑屏障使得血液中携带的物质不容易自由进入大脑，从而对大脑起到一定的保护作用。只有具备一定特性化合物才能穿过血脑屏障最终到达大脑。预测化合物是否能穿过需脑屏障详见第4章。第4章同时还阐述了第2系列化合物的设计过程。

第5章和第6章描述了另外两个我们称作腺苷酸A_1受体拮抗剂的系列化合物。第5章中的化合物是基于第3章中的化合物作了轻微修改，因此而得到了现有的最强的化合物。根据前3个系列化合物的检测结果我们对原始模型进行了优化。第4系列化合物则基于新的模型而设计，其检测结果表明改良模型很好地归纳了能高效作用于腺苷酸A_1受体的化

sine in the brain, it can be therapeutically beneficial to replicate the effects of adenosine in other parts of the body. For example, the activation (as opposed to the blockade) of adenosine A_1 receptors has been shown to be able to return an irregularly high heartbeat to normal sinus rhythm. Adenosine can only exist in the body for about 1 second, that is to say it is very quickly broken down by the body. So the use of adenosine itself is very limited – it is used as a direct injection into the heart in emergency situations when patients show an irregularly high heart rate. Compounds that can mimic the effect of adenosine are called agonists. Chapter 7 describes a new series of compounds, which although are very similar in size and shape have very different effects at the adenosine receptor. Some of these substances act as agonists whilst others as antagonists. The most noteworthy factor of this chapter is the fact that these compounds look nothing like adenosine; to date all other substances that did not resemble adenosine could not activate the receptor.

Having conducted four years of research in this topic, devised a model and proved its worth with a number of different series of compounds, does this mean that 'the end' is in sight? I'm afraid the answer is

therapeutisch wenselijk zijn de effecten van adenosine te copiëren. Verbindingen die het effect van adenosine kunnen nabootsen worden agonisten genoemd. De activering (in plaats van blokkering) van adenosine A_1 receptoren, bijvoorbeeld, blijkt een ongewoon hoge hartslag terug te brengen naar een normaal sinusoïdaal ritme. Adenosine kan slechts ongeveer één seconde bestaan in het lichaam omdat het zeer snel wordt afgebroken. De toepasbaarheid van adenosine is daarom beperkt – het wordt wel gebruikt voor directe injectie in het hart in noodsituaties bij patiënten met een ongewoon hoge hartslag. In Hoofdstuk 7 wordt een nieuwe serie verbindingen besproken die, hoewel erg op elkaar gelijkend wat betreft vorm en afmeting, zeer uiteenlopende effecten op de adenosine A_1 receptor hebben. Sommige van deze verbindingen gedragen zich als agonist en andere als antagonist. Het opvallendste aspect in dit Hoofdstuk is het feit dat deze verbindingen in het geheel niet lijken op adenosine; tot dusver zijn alle verbindingen die niet op adenosine lijken niet in staat gebleken om de adenosinereceptor te activeren.

Na vier jaar onderzoek gedaan te hebben aan dit onderwerp, een model ontworpen te hebben en de waarde hiervan bewezen te hebben met een groot aantal verbindingen, kan nu de vraag gesteld worden of 'het einde' in zicht is. Ik ben bang dat het antwoord hierop 'nee' is, nog niet. Een medicijn tegen de ziekte van Alzheimer is nog steeds

合物的特性。

论文最后一章则从另一个角度来看待问题。尽管我们得益于用咖啡因抑制大脑内的腺苷酸受体而使我们保持清醒，我们也可以考虑模仿腺苷酸的功能去治疗大脑以外身体其它部分的疾病。比如说，激活（和抑制相对）A_1受体可以减慢心率。腺苷酸在体内的寿命只有1秒，时间如此之短因此不能直接用它作为药物。而被我们称为激活剂的模仿腺苷酸的的物质则有可能解决这个问题。第7章中描述了一系列具有极相似形状和大小但是对腺苷酸受体有不同作用的化合物，其中有些是激活剂，另一些是拮抗剂。最奇特之处在于这些化合物的结构与腺苷酸完全不同，而在此之前从来没有人发现具有与腺苷酸不同结构的物质可以激活腺苷酸受体。

4年光阴专注于这一个主题，建立了一个模型并用一系列化合物验证其可靠性。这是否意味着项目

no, not yet. That cure for Alzheimer's is still a long way off. This is just one step towards such a heady target. We know that these compounds have an effect at the adenosine A_1 receptor (and not at the other adenosine receptors), but we do not know the (other) effects it may have in a real live system. These compounds have to be tested further; amongst which, the way that it is taken up and spread about a bodily system has to be investigated, the toxicity of the compounds has to be assessed, and the speed with which this all happens is also a matter of research. In all, I hope I can summarise my achievements by saying that I have made a good, solid contribution to the understanding and advancement of adenosine receptor research.

ver weg. Dit proefschrift is een stapje in de goede richting van zo'n groot doel. We weten nu dat de gemaakte verbindingen een effect hebben op de adenosine A_1 receptor (en niet op de andere adenosinereceptoren), maar we weten nog niet welke andere effecten er zullen zijn in een levend organisme. Deze verbindingen zullen dus verder getest moeten worden; de manier van opname en verspreiding door het lichaam dient onderzocht te worden, de giftigheid van de verbindingen moet onderzocht worden en de snelheid waarmee de verschillende processen plaatsvinden is van belang.

Tenslotte, mijn resultaten samenvattend, hoop ik te kunnen zeggen dat ik een duidelijke bijdrage heb kunnen leveren aan het begrip en de voortgang van het adenosinereceptoronderzoek.

的完结？回答是否定的。找到合适的药物治疗阿尔海茨默氏症还有很长一段路要走，这个项目只是其中一步。我们已知合成的这些化合物专一作用于腺苷酸A_1受体而不影响其它受体，但我们不知道它们进入人体内是否会带来其它的副作用。因此这些化合物将接受进一步的检验，比如说它们在人体内的吸收与分布，是否有毒副作用以及它们的反应速度都将被检测与评估。最后，我希望我能用一句话总结我这几年的成就，那就是我对腺苷酸受体的研究作出了优秀和坚实的贡献。

For further information about caffeine, the following websites may be of interest:
http://www.teahealth.co.uk
http://www.cosic.org
http://www.bbc.co.uk/health/healthy_living/nutrition/drinks_caff.shtml
http://en.wikipedia.org/wiki/Caffeine
http://www.koffiethee.nl/koffie/htm/cafeine.asp
http://www.koffieengezondheid.nl/

List of Abbreviations

Asp	Aspartic acid
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
Bn	Benzyl
Bu	Butyl
cAMP	Cyclic adenosine monophosphate
CHO	Chinese hamster ovary
CNS	Central nervous system
CPA	N^{6}-Cyclopentyladenosine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DPCPX	1,3-Dipropyl-8-cyclopentylxanthine
Et	Ethyl
GDP	Guanosine diphosphate
GPCR(s)	G(uanylyl-nucleotide-binding) protein-coupled receptor(s)
GTP	Guanosine triphosphate
HEK	Human embryonic kidney
Hex	Hexyl
His	Histidine
IC_{50}	Inhibitory concentration (50%)
K_i	Equilibrium inhibition constant
Me	Methyl
NMR	Nuclear magnetic resonance
Pent	Pentyl
Ph	Phenyl
Pr	Propyl
PSA	Polar surface area
RT	Room temperature
SAR	Structure activity relationship
Ser	Serine
TCM	Traditional Chinese medicine
THF	Tetrahydrofuran
THP	Tetrahydropyran
TM	Transmembrane

Curriculum Vitae

Lisa Chang was born on the 19th of February 1976 in Salford (UK). Her secondary education was completed with the attainment of 'A' levels in Chemistry, Mathematics, Physics and General Studies at Loreto College, Manchester. She then went on to read Chemistry at Imperial College, University of London in October 1994. In the third year of her studies, she partook in the Socrates/Erasmus student exchange programme, where she spent a nine-month research period in the laboratories of Prof. J. Reedijk at Leiden University. After graduating in 1998, she was then employed at the Pfizer Global Research Laboratories in Sandwich, Kent. From February 2001 until February 2005 she performed the research described in this thesis at the Division of Medicinal Chemistry in the Leiden/Amsterdam Center for Drug Research.

Lisa Chang werd geboren op 19 februari 1976 in Salford (Verenigd Koninkrijk). Haar middelbare schoolopleiding werd afgesloten met 'A-levels' in Scheikunde, Wiskunde, Natuurkunde en Algemene Studies aan het Loreto College in Manchester. In oktober 1994 begon zij aan de studie Scheikunde aan het Imperial College, Universiteit London. In het derde jaar werd in het kader van een Socrates/Erasmus studentenuitwisselingsprogramma een stage van negen maanden uitgevoerd bij de vakgroep Coördinatie en Bio-Anorganische Chemie onder begeleiding van Prof. dr. J. Reedijk aan de Universiteit Leiden. Na haar afstuderen in 1998 was zij werkzaam bij Pfizer Global Research Laboratories in Sandwich, Kent. Van februari 2001 tot februari 2005 werd het in dit proefschrift beschreven onderzoek uitgevoerd in de vakgroep Farmacochemie van het Leiden/Amsterdam Center for Drug Research.

List of Publications

L.C.W. Chang, R.F. Spanjersberg, J.K. von Frijtag Drabbe Künzel, T. Mulder-Krieger, J. Brussee, A.P. IJzerman. 2,6-Disubstituted and 2,6,8-Trisubstituted Purines as Adenosine Receptor Antagonists. *Manuscript in preparation.*

L.C.W. Chang, J.K. von Frijtag Drabbe Künzel, T. Mulder-Krieger, J. Westerhout, T. Spangenberg, J. Brussee, A.P. IJzerman. 2,6,8-Trisubstituted-1-Deazapurines as Adenosine Receptor Antagonists. *Manuscript in preparation.*

L.C.W. Chang, J.K. von Drabbe Frijtag Künzel, T. Mulder-Krieger, R.F. Spanjersberg, S.F. Roerink, G. van den Hout, M.W. Beukers, J. Brussee, A.P. IJzerman. A Series of Ligands Displaying a Remarkable Agonistic-Antagonistic Profile at the Adenosine A_1 Receptor. *Journal of Medicinal Chemistry* **2005**, *48*, 2045-2053.

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W.L. Driessen, L. Chang, C. Finazzo, S. Gorter, D. Rehorst, J. Reedijk, M. Lutz, A.L. Spek. Two Pyrazolato-Bridged, Linear Trinuclear Cu(II) Complexes. Crystal Structures and Magnetic Properties. *Inorganica Chimica Acta* **2003**, *350*, 25-31.

D. Rehorst, L. Chang, C. Finazzo, M. Lutz, A.L. Spek, S. Gorter, W.L. Driessen, J. Reedijk. Two New Linear Trinuclear Cu(II) Complexes with Bridging Pyrazolato Ligands. *Journal of Inorganic Biochemistry* **1999**, *74*, 274.

Acknowledgements

It is perhaps easy to be too excited at this stage and thank literally everyone and anyone that you have been in contact with over your lifetime, let alone all those that have in some way made some contribution to this thesis or the work contained herein. However, with a degree of pragmatism I will limit myself to those people who have had the most influence and have made the completion of 'the book' itself possible. I should rightly begin with those who have had the greatest practical contributions – Jacobien and Thea, without these two, the bioassays and thus the actual results, or perhaps more aptly 'the proof of the pudding', would have been much longer in coming and the four years probably extended to at least five. As my first ever supervisee, Ron made a major contribution to the pyrimidines and I am very glad that he returned after graduating to make his mark on other series. Other people who have been involved experimentally with the work presented in this thesis are Joost, Thomas, Gijs and Sophie. For the latter two, I should also pay tribute to the guidance of their supervisors, and in addition I should mention that I should have included Elisabeth in the pyridines paper. My introduction to the lab, my orientation with the facilities available, finding the right people and generally finding my feet was achieved with the help of Rian. As a long-term resident of the Gorlaeus, Reynier too was a great source of knowledge, especially with all the tricks to reboot the NMR machine after hours. Other practical assistance I was afforded comes in the guise of analytical support; the prompt maintenance and technical assistance from the NMR team, namely Fons and Kees, the efficient and jovial elemental analysis service by Jos and last but by no means least, the numerous timely MS measurements by Jopie.

The dynamic environment of a university means that there are many people who have made a contribution to the atmosphere and thus the enjoyment of the many coffee breaks, lunches and extra-curricular activities. I would like therefore in one fell swoop to include here all those that have been (and those that still are) a member of the Medicinal Chemistry group over the past few years. The support from the group too has been immaculate.

I'm indebted to Qilan and Kai for the translation of the summary into Chinese, now my mother can finally understand that there's more than 'cooking' to my work! One of the very last things to do was probably the most important, as far as most people are concerned, and so my thanks go to Arjen for his top-quality work (worthy of a job in the profession!) in getting the cover together.

Family and friends have, of course, played their part in the support network and it is also good to know that distance and water doesn't seem to get too much in the way. I only hope that a bit more distance and a little more water (and a vast amount of land and a few hours' more time difference) doesn't change that.

To two people who have already been mentioned, but deserve a second dazzle in the limelight, Jacobien and Ron. They've have had a huge impact on the progress of the research, were my 'kamergenoten' (and everything that encompasses) for the majority of my time here and have, much to my delight, agreed to be my 'paranimfen'.

However, there has to be one concluding statement to this piece of work and there is only one person it can be dedicated to. This last paragraph must be reserved for the one who has lived through it all, the highs, the lows and all the accompanying mood swings! Whatever I write here is an understatement, so I'll keep it short and sweet. Leon, you're supercalifragilisticexpialidocious!!