Novel antagonists for the human adenosine A2A and A3 receptor via purine nitration: synthesis and biological evaluation of C2-substituted 6-trifluoromethylpurines and 1-deazapurines
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1

Introduction
1.1 ADENOSINE AND ADENOSINE RECEPTORS
1.1.1 ADENOSINE

Adenosine belongs to the group of nucleosides, consisting of an adenine molecule connected with a ribose. Adenosine and its analogues play a vital role in a variety of biochemical processes, like energy transfer in the body via adenosine triphosphate (ATP), signal transduction via cyclic adenosine mono phosphate (cAMP) or as a neurotransmitter. Adenine is a building block in DNA, as it readily forms complexes via hydrogen bonding with thymine. Since DNA of living organisms can be compared with a blue print code for cell expression and protein formation, adenines and its analogues are highly important in biochemical processes.

![Chemical structures of adenine, adenosine, cAMP, and AMP](image)

Year over year adenosine and its derivatives have been associated with biological reactions. Since Drury and Szent-György reported the action of adenosine compounds on mammalian heart in the early 1920’s, it is well known that adenosine influences circulation, respiration and gastrointestinal motility. Later, important roles of adenosine were reported in brain tissue. Exposure to adenosine was found to increase accumulation of adenosine 3’,5’-cyclic monophosphate (cAMP) in brain slices. This effect was also observed after electrical stimulation. Both processes were blocked by methylxanthines. The presence of methylxanthine-sensitive adenosine receptors on nerve and glial cells from several species was widely established. Later it was found that adenosine was able to block the release of neurotransmitters like dopamine, acetylcholine, serotonin and noradrenalin, indicative for an important regulatory role for adenosine.
Under normal conditions there is continuous generation of adenosine both intracellularly and extracellularly. The intracellular production is mediated either by a 5'-nucleotidase, that dephosphorylates AMP, or by hydrolysis of S-adenosyl-homocysteine. Intracellular generated adenosine can be transported into the extracellular space via specific bidirectional transporters that efficiently control the intra- and extracellular levels of adenosine. In striatum, stimulation of dopamine D₁ receptors is found to enhance the NMDA (N-methyl d-aspartate) receptor-dependent increase of extracellular adenosine levels via ion channels. Adenosine can be converted into AMP through phosphorylation by adenosine kinase or degraded to inosine by adenosine deaminase. The normal levels of adenosine in rodent and cat brain have been determined by different techniques and have been estimated to be ca 30-300 nM.

1.1.2 ADENOSINE RECEPTORS

In 1980 it was found that different adenosine derivatives were able to either increase or decrease the concentration of intracellular cAMP, which indicated a mechanism via different receptors. The receptors that inhibited the cAMP generating adenylyl cyclase were initially classified as A₁ receptors and those that stimulated adenylyl cyclase as A₂ receptors. Up to this moment four distinct adenosine receptors have been identified: A₁, A₂A, A₂B, and A₃.

Adenosine receptors are present in a broad variety of tissues. The four subtypes however, do not have equal tissue distribution. In this way, adenosine is proposed as a homeostatic modulator with a global rather than a specific role. In general, each class of adenosine receptors demonstrates high overall homology for the same receptor subtype in different species (82-93%). Only the A₃ subtype has less homology between different species (rat-human 74%). Within species the different adenosine subtypes also share relatively high homology. As result of that, it is a challenge to design and develop subtype selective ligands. Since various therapeutic options have been linked to specific adenosine receptor subtype manipulations, research focuses on finding adenosine derivatives with high affinity and selectivity for only one of the receptor subtypes. Therapeutically A₁ and A₂A receptors are correlated with heart action and oxygen regulation, while in the brain these receptors play a pivotal role in neurotransmitter release like dopamine.³ Well known example of the wide application of adenosine receptors is the use of the endogenous agonist adenosine in hospitals as treatment for severe tachycardia⁴ (rapid heartbeat), directly slowing down the heart beat.
through action on all four adenosine receptors in heart tissue, as well as producing a sedative
effect through action on $A_1$ and $A_{2A}$ receptors in the brain. The $A_{2B}$, $A_3$ are located mainly
peripherally and are involved in processes such as inflammation and immune responses
(asthma) or cell signalling pathways (axon elongation, human melanoma cells).

1.2.1 ADENOSINE RECEPTOR (ANT-)AGONISTS
As described above, adenosine is the endogenous ligand for the adenosine receptors. It acts
as a general activator of adenosine receptors, which is by definition called an agonist. For
many years, derivatives have been synthesised to mimic the activating action of adenosine
with similar structures. This research produced a variety of compounds with different affinity,
agonistic potency and subtype selectivity, useful for various therapeutic applications.
Compounds that have a sub maximal response after complexing with the receptor are called
partial agonists. In special cases such a sub maximal response may be beneficial e.g. for a
subtle efficacy versus side effect balance.

Alternatively, blockade of adenosine receptors can be achieved with compounds that bind
to the receptor, but cannot activate it. These antagonists are important for regulating
adenosine mediated cell processes and can also have very promising therapeutic value.
It has been shown that three of four adenosine receptors can be blocked by naturally occurring
methylxanthines. From cohort studies with methylxanthines and caffeine it became clear that
caffeine is correlated to a lower risk of developing Parkinson’s disease. Biological
interactions with the $A_{2A}$ receptor may be responsible for these findings while dopamine
release is triggered. As a result of those studies, more evidence was found that subtype
selective antagonists need to be developed to decrease side effects during treatments.

1.2.2 LIGAND BINDING ON $A_{2A}$ RECEPTORS
Developing new selective molecular structures to complex with the receptor requires
knowledge about the receptor properties. Year over year, molecular models have been
proposed for rat and human $A_{2A}$ receptors to describe its properties and mechanism of action.
It is generally accepted that $A_{2A}$ receptors consist of seven trans membrane polypeptide
alpha-helices, forming a hydrophilic cavity in the cell and a hydrophobic receptor surface
(Figure 1.1). The region of ligand binding is focused around transmembrane domains TM5-
TM7. In rat and human A<sub>2A</sub> receptors two histidines were found to be crucial for ligand binding. The fact that one histidine (His<sub>278</sub>) at TM7 is located in the putative ribose-binding region suggests that this residue may also be involved in the retention of the receptor conformation. The hydroxy group of a serine (Ser<sub>277</sub>) was thought to be essential for high affinity binding of the agonist, but not for antagonists. A probably existing disulfide bridge between Cys<sub>166</sub> and a cysteine near the N-terminus of TM3 is important for attaching the E2-(extracellular)-loop in physical proximity to the ligand binding regions. Within these loop other residues may be contributing too for both agonist and antagonist binding.

Figure 1.1 Model of the A<sub>2A</sub> receptor. The critical regions and residues possibly involved in ligand binding are indicated.

For a long time, these model predictions were setup in analogy with the other adenosine receptors, using model compounds and model calculations on affinity data. Often the model predictions did not result in the anticipated A<sub>2A</sub> receptor affinity, as will follow from the biological evaluation of compounds in this thesis too. Recently, the exact structure of the adenosine A<sub>2A</sub> receptor was determined via crystallisation of the protein in complex with a
high affinity antagonist. Surprisingly, that revealed that the antagonist was twisted perpendicular in contrast to what was envisaged for years. The extracellular loops (four disulfide bridges in the extracellular domain) and the helical cores showed distinct changes from the other GPCR’s.

**Figure 1.2** Crystal structure of A2A receptor. A. Showing transmembrane helices I - VIII (brown), the four extracellular disulfide bonds (yellow), antagonist ZM241385 (light blue) and intracellular loops. B. Idem, rotated 180° around the x-axis.

It is suggested that the restriction of the movement of a tryptophan residue, important in the activation of these class A receptors is key in the efficacy of adenosine A2A receptor modulation. Revealing the change of (putative) position of the binding pockets, stimulated research towards generating new compounds, in depth study of (allosteric) modulators and other processes related to the receptor. The effect of this structural information resulted in
new insights and perspectives and had large implications for drug screening and structure-based drug design.\textsuperscript{12}

\subsection*{1.2.3 Therapeutic potential of $A_{2A}$ receptor antagonists}

$A_{2A}$ antagonists were studied for a wide set of possible therapeutic applications. For example: for neuroprotection, sleeping disorders, analgesia\textsuperscript{13}, anti inflammatory effects, anti depressant effects and cognitive effects. Most important application of adenosine receptor antagonists may be found in locomotor disorders such as Parkinson’s disease, and will be discussed in this thesis.

\textbf{Neuroprotective effects}

Endogenous adenosine plays an important role in protecting tissues from damage when subjected to increased metabolic demand and reduced energy levels\textsuperscript{14}. Adenosine concentration in brain rapidly increases when patients suffer from ischemia or other forms of hypoxia. Acute treatment of patients with $A_{2A}$ receptor antagonists resulted in significant protection of hippocampal neurons and can be of therapeutic value for the treatment of brain injuries.\textsuperscript{15} It was found that $A_1$ receptor activation in combination with $A_{2A}$ receptor antagonists presynaptically reduces the excessive amino acid release through inhibition of $Ca^{2+}$ influx and release of $K^+$ by opening $K^+$ ion channels. It is suggested that $A_{2A}$ receptor antagonists via such pathways can prevent neuronal damage and cell death in the central nervous system.\textsuperscript{16}

\textbf{Antidepressant effects}

In animal models, some adenosine analogues have been shown to produce depressant effects which may be related to human depressive disorders\textsuperscript{17}. Adenosine receptor antagonists have shown to reverse this effect.\textsuperscript{18} It was found that $A_{2A}$ receptor antagonists reduce the immobility time of mice in the tail suspension test and forced swim test. In the tail suspension test\textsuperscript{19} anti psychotics and anxiolytics increase immobility time, whereas $A_{2A}$ receptor antagonists decrease it.
**PSYCHIATRIC DISORDERS**

There is currently a major interest in the ability of A2A receptors to control synaptic plasticity at glutamatergic synapses. This makes A2A receptors a particularly attractive target to manage psychiatric disorders since adenosine may act as go-between glutamate and dopamine, two of the key players in mood processing.\(^\text{20}\)

**MOTOR DYSFUNCTION, PARKINSON’S DISEASE**

Many studies describe a decrease of locomotor activity, when adenosine and its agonistic derivatives, are targeted to A2 receptors. Actual reduction of motor activity is observed after stimulation of the A1 or the A2A receptor.

As A2A receptors are co-localised with dopamine D2 receptors, adenosine and dopamine tend to influence each other’s functions. Stimulation of the A2A receptor in the basal ganglia decreases affinity of D2 dopamine agonists for the D2 dopamine receptor. Therefore adenosine agonists have the same overall effect as dopamine antagonists. On the other hand, this implicates a potential role for adenosine antagonists reversing this effect. Indeed, while A2A receptor agonists dose dependently suppressed basal locomotor activity, A2A receptor blocking with selective antagonists increased locomotor activity. While many of the potential drugs for treatment of Parkinson's disease have shown benefit in the treatment of the resulting movement disorders, an advantage of adenosine A2A antagonist therapy was envisaged that its putative neuroprotective effects might beneficially influence the underlying neurodegenerative disorder.\(^\text{21}\)

In clinical trials, the (non-selective) adenosine antagonist theophylline (a dimethylxanthine, naturally occurring in tea and cocoa) showed significant improvements in Parkinson patients. In this respect also the most consumed, also non-selective, adenosine antagonist, caffeine should be mentioned.\(^6\) As for other methylxanthines most of its central effects, seem to be related to its adenosine receptor blocking effect\(^\text{22}\). A relation was described between coffee intake and genetic coding, via genetically scanning of over 4000 patients with Parkinson’s disease and mapping it with their caffeine intake. They identified a gene called GRIN2A that appeared to protect people who drink coffee from developing Parkinson's disease, which may help identifying and selection of patients for future therapy, as about 25 percent of the population seem to have the variant that boosts the protective effect of coffee.
1.3 PARKINSON’S DISEASE AND ADENOSINE ANTAGONISTS

A full description of the symptoms of Parkinson's disease was first published in 1817 by James Parkinson. By the end of the 19th century, the clinical features of Parkinson's disease had been defined, but its etiology or pathology was still not understood. Since 1919, it was recognised that the substantia nigra was the affected region of the brain. It was not until 1925 that it became widely accepted that the rigidity and tremor, characteristic of Parkinson's disease, were due to a loss of inhibition in the pathway that uses the axon cells of the substantia nigra which end primarily in the striatum (Figure 1.2). The loss of inhibition is caused by depletion of dopamine in the basal ganglia.

Figure 1.2 Brain regions affected by Parkinson's disease.

Degeneration of dopaminergic nigrostriatal neurons of the basal ganglia and progressive loss of dopamine producing cells cause a decrease in dopamine production. Absence of dopamine results in changes in the expression of mRNA encoding neuropeptides. This creates an increase in activity of the indirect output pathway and a decrease in activity of the direct
output pathway from the basal ganglia. The balance between these two output pathways is important for smooth and well-coordinated movement. It is thought that the imbalance in the activity of the two output pathways, as a result of dopamine depletion, is the neuronal cause of motor dysfunction in Parkinson’s disease symptoms (involuntary shaking, slow movement, stiffened muscle tone, and impaired balance). A controversy still exists about the reasons whether Parkinson’s disease is caused by genetic or environmental factors, a virus or otherwise.

The genetic claim is that there is a mutation on the alpha-synuclein protein which may play a role in the disease. Studies show that a mutation in PINK1 (PTEN-Induced Kinase 1) plays an important role in the hereditary early onset of Parkinson’s disease. Mutations in the PINK1 gene cause PARK6 familial Parkinsonism. Cell culture studies suggest that PINK1 is mitochondrially located and may exert a protective effect on the cell that is altered by the mutations, resulting in increased susceptibility to cellular stress. This study provides a direct molecular link between mitochondria and the pathogenesis of Parkinson’s disease. Other studies suggest that, in addition, PINK1 interacts with other signaling proteins implicated in Parkinson’s disease pathogenesis and mitochondrial dysfunction. Strong relations between PINK1 and parkin genes and mitochondrial damage are recently confirmed, leading to Parkinson’s disease over time.

Another theory is that exposure to environmental toxins may be the key player. The pesticides paraquat and rotenone can induce Parkinson-like symptoms in animal models, and are known to injure mitochondria. A chemical resemblance of some pesticides and herbicides with the proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been suggested. MPTP, which structure is shown in Scheme 1.1, is commonly used to induce dopamine depletion in mice models resulting in Parkinson’s disease. In the brain, MPTP is then converted to 1-methyl-4-phenylpyridine (MPP) by the enzyme monoamine oxidase B.
Scheme 1.1 Transformation of MPTP to neurotoxin MPP⁺ by the enzyme Mono Amino Oxidase B

MPP⁺ is produced by the catabolic machinery in substantia nigra cells. MPP⁺ can cause cellular death by disrupting mitochondrial metabolism. Moreover, MPP⁺ can induce lipid membrane destruction by the formation of free radicals. Free radicals are also formed by monoamine oxidase B during dopamine catabolism. Normally there are large concentrations of protective enzymes, such as superoxide dismutase and glutathione peroxidase, and free radical scavengers which neutralize these effects. In Parkinson's patients however, there is a reduced concentration of glutathione peroxidase, the enzyme responsible for cell protection against oxidative damage by reducing the levels of hydrogen peroxide and therefore decreasing the production of very reactive hydroxyl radicals. Other "protectors" are also reduced along with the aging process and consequently, more neurons die and the chance of acquiring the physical symptoms of Parkinson’s disease increases. Replication-defective lentiviruses have been shown to allow the sustained and long-lasting expression of transgenes in both rodent and primate central nervous system and to be powerful tools for gene delivery in the nervous system. Recently, this concept was used to demonstrate that a lentiviral vector carrying the human glutathione peroxidase GPX1 gene can exert neuroprotective effects in both in vitro and in vivo models of Parkinson’s disease.

Dopamine exerts regulatory control on pathways, mainly via the dopamine D₁ (direct pathway) and D₂ (indirect pathway) receptors. Progressive decrease of dopamine (due to the degeneration of nigrostriatal neurons), results in changes in the expression of the mRNA encoding for several neuropeptides, such as preproenkephalin increase and substance P.
decrease and a loss of D1 receptor-stimulated GABA release. This causes increasing activity of the indirect pathway and decreasing activity of the direct pathway. As mentioned, this imbalance in the activity of the two pathways is the eventual cause of motor dysfunction.

**Parkinson’s disease, L-dopa treatment**

The most effective and commonly used drug to substitute the decreasing level of dopamine, is L-dopa (3,4-dihydroxy-L-phenylalanine, Figure 1.3). L-dopa acts a zwitter ion and is neutral in physiological conditions, which assists in membrane passage. L-dopa, usually given in combination with a peripheral aromatic amino acid decarboxylase inhibitor to avoid total decarboxylation before reaching its target, is converted in the brain to dopamine. In Parkinson’s patients, L-dopa is initially effective in reducing the symptoms, but it does not treat the underlying problem of progressive cell loss. Therefore, steadily increasing doses become necessary to compensate for the degeneration of dopaminergic terminals.

![Dopamine and L-Dopa](image)

**Figure 1.3** Endogenous dopamine and the structurally related prodrug L-Dopa

The requirement to apply increasing doses during longer periods of time makes that the brain becomes supersensitive and may lead to other side effects like hallucinations, and even psychosis.

The loss of dopamine producing cells on the one hand, and the super sensitivity of the brain on the other, makes it more difficult to control motor movements. Tics, spasms, and muscle clenching may appear in addition to the usual Parkinsonian tremors and bradykinesia (unusually slow movements). This is termed L-Dopa-Induced Dyskinesia (LID) and is an almost inevitable result of chronic L-dopa treatment.

Currently, still large studies are carried out to find out the optimal conditions to use A2A antagonists in Parkinson’s disease treatment. Recent data in MitoPark mice with A2A antagonist MSX-3 prevented the reduction of spontaneous locomotor activity observed in
saline or L-Dopa treated animals. Unfortunately, the characteristic decline of dopamine levels was not reversed, indicating only effectiveness in mono therapy in early onset of Parkinson’s disease. One of the potential options for human treatment may be to use adenosine A$_{2A}$ receptor antagonists as new agents to improve the mode of action of L-dopa and increase susceptibility in neurons, alone or in combination with existing therapies to dopamine.

**MECHANISM OF ACTION OF ADENOSINE ANTAGONISTS IN PARKINSON DISEASE**

Adenosine A$_{2A}$ antagonists can stimulate the signal transduction by dopamine in striatum. In the GABAergic striatopallidal neurons, A$_{2A}$ receptors are colocalised with dopamine D$_2$ receptors on the same cell surface, where they can influence each other’s functions. A major mechanism for a direct interaction at the intramembrane level may involve formation of heterodimers between the two receptors.

**Figure 1.4** Signal transduction by dopamine receptors. The arrows indicate signal transduction by dopamine receptors. A: Depletion of dopamine causes low signal transduction, increased by the absence of A$_{2A}$ antagonists. B: Adenosine A$_{2A}$ receptor
deactivation by adenosine antagonists restores the functioning of the dopamine receptors and partly compensates for the lower amounts of dopamine

In Figure 1.4 scenarios for dopamine signalling output are described with a combination of dopamine, adenosine agonists (A) and with additional adenosine antagonists (B) in Parkinson patients. Stimulation of the A2A receptor may lead to allosteric changes, influencing the affinity as well as the G protein coupling thereby negatively influencing the efficacy of neurons. Blocking the A2A receptor (B) may change protein structure orientation and leads to higher success rate for signal transduction.

1.4 DEVELOPMENT OF A2A SELECTIVE ADENOSINE RECEPTOR ANTAGONISTS

Despite the fact that a large amount of ligands has been synthesised and evaluated for their adenosine A2A receptor affinity, the versatility of organic synthons creates many possibilities for designing novel heterocyclic compounds with potentially improved properties. Two major strategies are used in the search for new compounds.

The first one is to screen compounds for their receptor affinity and selectivity with high throughput screening techniques (HTS).

In the past, screening ligands for receptor affinity was an expensive and specialized process. With fast progress in technology (automation, miniaturisation, and computerized analysis) it has become a fast reliable technique. An advantage of high throughput screening of large and diverse libraries (e.g. 100,000 compounds) is the option to find new classes of compounds or lead compounds. These may be further explored by synthesizing analogues using follow up libraries or classical synthetic techniques. Whenever relevant information is available on potentially favourable structural features of the ligands pursued (endogenous ligand, synthetic ligands, receptor information), this may be used to bias (part of) the compounds to be screened. In that case millions of compounds in databases can be evaluated in silico for the required properties. The resulting selection may be further filtered for potentially adverse structural features (toxic or unfavourable ADME motives (absorption, distribution, metabolism, and excretion)) or their predicted (in-)capability to pass blood-brain barrier (if
medication into the brain is necessary). The final biased selection (e.g. ~10,000 compounds) may optionally be combined with a diverse set and screened experimentally.

The second strategy is to find possible new drugs by rational design and synthesis. Ligand based molecular modelling studies of active structures and extensive comparison of structural elements with putative receptor interaction; give detailed insight in the mechanism of action. By replacing moieties with new pharmacophoric groups new insight in biological activity may be found. Usually, analogues of endogenous ligands or prototype drugs which have already shown the desired biological activity are synthesized and modified. Alternatively, pharmacophoric groups of different drugs may be combined in a novel lead structure using rational design.

At Solvay Pharmaceuticals, since February 16, 2010 Abbot Healthcare Products B.V., an HTS campaign was performed to give interesting proprietary lead compounds. In parallel, based on modelling by Solvay, combined with synthetic expertise of our research group, an effort was made to rationally design new classes of adenosine antagonists.

**A2A ANTAGONISTS IN (PATENT-) LITERATURE**

Xanthine derivatives have been disclosed as adenosine A2 receptor antagonists, useful for treating various diseases caused by hyper functioning of adenosine A2 receptors, such as in Parkinson's disease. Theophylline (1,3-dimethylxanthine), as mentioned in paragraph 1.2.3, a bronchodilator drug which is a mixed antagonist of adenosine A1 and A2A receptors, has been studied clinically. To determine whether this adenosine receptor antagonist would be of value in Parkinson's disease an open trial was conducted on 15 Parkinsonian patients. They were treated for up to 12 weeks with a slow release oral theophylline preparation (150 mg/day), yielding serum theophylline levels of 4.44 mg/L after one week. The patients exhibited significant improvements in mean objective disability scores and 11 reported moderate or marked subjective improvement. Another prominent xanthine-derived adenosine A2A selective antagonist is CSC [8-(3-chlorostyryl)caffeine], see Figure 1.5. Its effect on the signalling pathway of levodopa induced motor fluctuation was reviewed recently.

KF17837 [(E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine] is a selective adenosine A2A receptor antagonist extensively used in preclinical studies, which on oral administration significantly ameliorated the cataleptic responses in rats induced by
intracerebroventricular administration of an adenosine A$_{2A}$ receptor agonist, CGS 21680.$^{38}$ The structure activity relationship of KF 17837 and analogues have been published.$^{39}$ Recent clinical data have also been provided on the A$_{2A}$ receptor antagonist KW-6002/Istradefylline [(E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine].$^{40}$ Phase II studies started in 1999 and continued to phase III studies in the end of 2004. Early 2007 an NDA (new drug application) was filed, however in 2008 it was not approved by the FDA (effectivity was not proven in the optimal way) and thus discontinued.$^{41}$ Xanthine derivatives continue to be developed.$^{42}$

![Figure 1.5 Some A$_{2A}$ receptor antagonists from (patent) literature](image)

New non-xanthine structures with pharmacological effect include SCH 58261 and its analogs.$^{43}$ SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-c]-1,2,4-

Chapter 1
triazolo[1,5-c]pyrimidine) is reported as being effective in the treatment of movement disorders\textsuperscript{44} and has been followed up later by several series of compounds\textsuperscript{45, 46}.

Another antagonist structurally related to SCH 58261 is ZM 241385 having even almost tenfold higher affinity for the A\textsubscript{2A} receptor. Cadus describes a series of 7-deazapurines with N-6 substitutions.\textsuperscript{47}

**Mefloquine**

The most intriguing compound with effect on adenosine receptors is Mefloquine (Lariam). Mefloquine (\(R,S\)-(±)-\(\alpha\)-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol) is an antimalarial drug, chemically related to quinine, that is effective against multidrug resistant strains of *Plasmodium falciparum*, the protozoan parasite causing malaria.

![Mefloquine / Lariam](image)

**Figure 1.6** Mefloquine, anti-malarial agent and A\textsubscript{2A} receptor antagonist

Although generally well tolerated, a number of clinical reports have emerged, suggesting that mefloquine is associated with infrequent but severe neuropsychiatric side effects, which include disturbed sleep, increased anxiety, panic attacks, depression, psychosis, and seizures. The mechanisms responsible for these effects were not known until recently.

Mefloquine is an asymmetric molecule that is marketed as a racemic mixture consisting of equal parts of (\(-\)-(\(R,S\))-mefloquine and (\(+\)-(\(S,R\))-mefloquine enantiomers. Both enantiomers are reported to possess antimalarial potency against *P. falciparum* in vitro and have been shown to penetrate the brain. Therefore, either one or both of the enantiomers might in principle account for the adverse CNS effects of the racemate. Preceding any literature reports, a potential relation between observed side effects and adenosine receptor affinity was
already considered in our research group. Mefloquine was included as a unique model compound in the search for novel adenosine antagonists, described in paragraph 1.5. Later, literature evidence was found. In an attempt to identify the mechanism responsible for the neuropsychiatric side effects of mefloquine, researchers from Vernalis company assessed both enantiomers in a series of receptor binding, enzyme, and functional assays. In addition, mefloquine enantiomers were assessed for in vivo behavioural effects in rats after short- and long-term dosing.

The results of these studies confirmed our initial hypothesis and showed that the (-)-(R,S)-enantiomer of mefloquine is a potent and moderately selective A2A antagonist. In an initial examination of the effects of mefloquine on 81 receptors and enzymes, the only significant interaction identified was displacement of high-affinity binding to bovine striatal adenosine receptors. In subsequent binding studies using membranes from cells expressing human adenosine receptor subtypes, (-)-(R,S)-mefloquine was found to possess high affinity for A1 and A2A receptors, with Ki values of 255 and 61 nM, respectively, and absence of relevant affinity for either the A2B or A3 receptor subtypes (Ki ~7 µM). The binding profile of racemic mefloquine was comparable with that of (-)-(R,S)-mefloquine, whereas (+)-(S,R)-mefloquine was found to have low affinity for adenosine receptors. (-)-(R,S)-mefloquine and racemic mefloquine were demonstrated to show competitive antagonism in functional assays. Schild analysis revealed pA2 values at the A2A receptor of 6.96 for (-)-(R,S)-mefloquine and 6.61 for racemic mefloquine, whereas at the A1 receptor, these compounds were found to possess pA2 values of 6.40 and 6.21, respectively.

<table>
<thead>
<tr>
<th>Table 1.1 Comparison of the binding affinities (nM) selected compounds from literature on human adenosine receptors</th>
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<td>Receptor</td>
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<tr>
<td>Compound</td>
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<td>KW-6002</td>
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<td>SCH-58261</td>
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<td>ZM-241385</td>
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<td>VER-6947</td>
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<td>racemic mefloquine</td>
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<td>(+)-(S,R)-mefloquine</td>
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<td>(-)-(R,S)-mefloquine</td>
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Radioligands A1: [3H]-DPCPX, A2A:[3H]-CGS21680, A2B:[3H]-ZM241385, A3:[125I]-ABMECA
1.5 **STRATEGY FOR NEW ADENOSINE RECEPTOR ANTAGONISTS**

Using adenosine A\textsubscript{2A} antagonist structures from literature, a ligand based design was conducted together with Solvay Pharmaceuticals to observe essential elements for binding affinity and selectivity. The special structure of mefloquine/Lariam with its trifluoromethyl groups was also evaluated in this study. Also 7-deazapurine ligands of Cadus were examined. An A\textsubscript{2A} receptor model was built from a large set of antagonists, followed by fitting the four structures in the model receptor.

![Chemical structures of adenosine receptor antagonists](image)

**Figure 1.7** Adenosine receptor antagonists fitted in a ligand based A\textsubscript{2A} receptor model
Figure 1.8 Overlay structures of the four compounds presented in Figure 1.7

Based on the overlay results of these studies we tried to formulate key structural features for future candidate ligands for the adenosine $A_{2A}$ receptor. As a starting point the compounds should have a flat core structure: an aromatic or pi-electron rich system is well tolerated. Like in the xanthine series, ring structures with heteroatoms are preferred. We focused on the development of 1-deazapurine substrates and trifluoromethylated purines. As Cadus and Vernalis already looked into (deaza-)purine substrates as a potential drug class, new synthetic pathways were necessary to realize new structures that are still patent free.

Given the basic starting points described above, we defined structural requirements as depicted in Figure 1.9. At C-6 an H bond donor/acceptor is good for affinity. At C-8 a lipophilic moiety is required. Substitution at N-9 with small alkyl groups [R] is tolerated. At C-2 alkoxy or amino substituents are well tolerated. A spacer is required linked to lipophilic or aromatic groups for effective interaction with a hydrophobic pocket. Finally, the indicated heteroatom [X] in the purine ring will optionally be replaced by a carbon atom and the potential change in interaction with the receptors studied.
Introduction

Given these requirements we selected and devised synthetic routes toward two series of potential new adenosine antagonists, as shown in Figure 1.10. In the first series, we introduce a trifluoromethyl group at the purine ring. This might give information on the intriguing activity of mefloquine. Moreover it is a new synthetic challenge to introduce the trifluoromethyl group on the purine skeleton in combination with substituents at C-2 and C-8.

In the second series, we focus on the synthesis and biological evaluation of a new class of 1-deazapurines. Since the synthesis of 2-substituted 1-deazapurines is unknown, this is also a clear synthetic challenge. The biological data might give further insight in the specific interaction of 1-deazapurines with adenosine receptors.

1.6 Outline of the thesis

In this thesis, the development of new synthetic methods is described for the synthesis of 6-trifluoromethyl substituted purines and 2-substituted 1-deazapurines to obtain new adenosine analogues as possible selective adenosine antagonists based on directed ligand based design. In chapter 2, attention is focused on the development of trifluoromethylated purines. It is discussed that via purine nitration the molecules are enhanced in reactivity, resulting in mild and efficient introduction of trifluoromethyl groups at C-6 (at 0°C and room temperature).
The effect of protective groups at N-9 is discussed and a new protective group, Bocom, for purine nitration is presented with excellent stability. Also the elucidation of the purine nitration mechanism using NMR techniques is presented. With $^{15}$N-NMR CIDNP experiments, the involvement of radicals was proven. The advantage of increased reactivity of nitrated purines towards C-2 substitution is presented in Chapter 3. Several series of amino substituted and alkoxy substituted 6-trifluoromethylpurines are reported. The effect and elegance of two electronegative substituents, i.e. the trifluoromethyl group in combination with the nitro group is shown via substitution at temperatures between -20°C and 0°C.

Chapter 4 deals with the introduction of substituents at C-8. Using standard amination procedures, amino groups are introduced at C-8. Using modern palladium coupling techniques (Suzuki, Stille and Sonogashira) a complete series of C-8 substituted 6-trifluoromethyl purines is constructed. In Chapter 5, the synthetic efforts towards the synthesis of 2-substituted 1-deazapurines are discussed. The synthesis of 1-deazapurines was highly optimised followed by efficient introduction of substituents using the electron withdrawing properties of the nitro group at C-2. This nitro group was converted to the highly reactive nitroso species, giving access to new series of compounds via condensation reactions with the nitroso group. The mechanism of 1-deazapurine nitration is studied and compared with pyridine nitration.

In Chapter 6 the compounds from the previous chapters are evaluated for their interaction with the adenosine receptors. An attempt is made to rationalize unexpected results with a further modelling study.
1.7 REFERENCES

47 Castelhano, A. L; McKibben, B.; Witter, D.J. WO 99/62518