Chapter 5
Adenosine A_{2A} receptor antagonism in Parkinson's disease

5.1 Introduction

As mentioned, PD is a neurodegenerative disorder, characterized by the loss of dopaminergic neurons in the SNpc (Dauer & Przedborski, 2003). The majority of the parkinsonian motor impairments are due to this progressive loss of dopamine producing neurons and the subsequent loss of dopamine input to the striatal motor structure (Shook & Jackson, 2012). The next logical step is to restore dopaminergic neurotransmission and currently the gold-standard treatment is provided by the dopamine precursor L-DOPA (LeWitt *et al.*, 2008; Shook & Jackson, 2012). Unfortunately, complications with long-term L-DOPA treatment develop, which include motor fluctuations and dyskinesia (Leung & Mok, 2005; Onofrj *et al.*, 2008). For this reason L-DOPA is often used in combination with various adjuvants to reduce its side-effects. Some of the adjuvants include COMT (Fung *et al.*, 2001) and MAO-B inhibitors (Fernandez & Chen, 2007). Unfortunately these treatments remain inadequate (LeWitt *et al.*, 2008).

The motor features of PD can be influenced by other pharmacological interventions that go beyond restoring of dopaminergic input to the striatal neurons (LeWitt *et al.*, 2008). Adenosine A_{2A} receptors are present in medium to high concentrations in several basal ganglia nuclei. These receptors may be able to influence motor activity by acting on the different basal ganglia levels (Morelli *et al.*, 2012). Dopamine D_2 receptors and adenosine A_{2A} receptors are co-localized at the indirect pathway of the basal ganglia (Cieślak *et al.*, 2008), the pathway which leads to inhibition of motor activity (Morelli *et al.*, 2012). These observations suggest that A_{2A} receptors may offer an attractive target to modulate dopamine receptor functions in a disease such as PD that is characterized by the progressive loss of dopaminergic neurons.

The rationale for the use of adenosine A_{2A} receptor antagonists, as a non-dopaminergic treatment for PD, will be outlined in this chapter. First, the role of A_{2A} receptor antagonists in motor control will be discussed with regards to the basal ganglia (section 5.2) and the ability of these receptors to form heteromeric complexes (section 5.3.3). A brief overview of the possible neuroprotective mechanisms exerted by adenosine A_{2A} receptor antagonists will be provided (section 5.3.4). Lastly, examples of known antiparkinsonian A_{2A} receptor antagonists are given to further support the rationale for designing adenosine A_{2A} receptor antagonists for the treatment of PD (section 5.4).

5.2 The basal ganglia and adenosine A_{2A} receptors

The basal ganglia plays an important role in movement and is comprised of the striatum, GPi, GPe, STN, SNpc, and the SNr (Bolam, 2000; Xu *et al.*, 2005; Ferraro *et al.*, 2012).

Normally striatal function in the basal ganglia is regulated by the dopamine D_1 and D_2 receptors. The signalling of these receptors is, in turn, modulated by the striatal adenosine A_1 and A_{2A} receptors (Fuxe *et al.,* 2007). As mentioned previously, motor function is dependent on the balance of two parallel pathways, namely the direct (striatonigral) and indirect (striatopallidal) pathways (Xu *et al.*, 2005). An imbalance between the direct and indirect pathways results in motor dysfunction (Ferraro *et al.*, 2012). Even though the above described basal ganglia model is an oversimplification of the direct and indirect pathways, it still embodies the basic concepts of movement via the basal ganglia. Figure 5.1 represents a basal ganglia model, demonstrating a possible mechanism of movement during normal state.

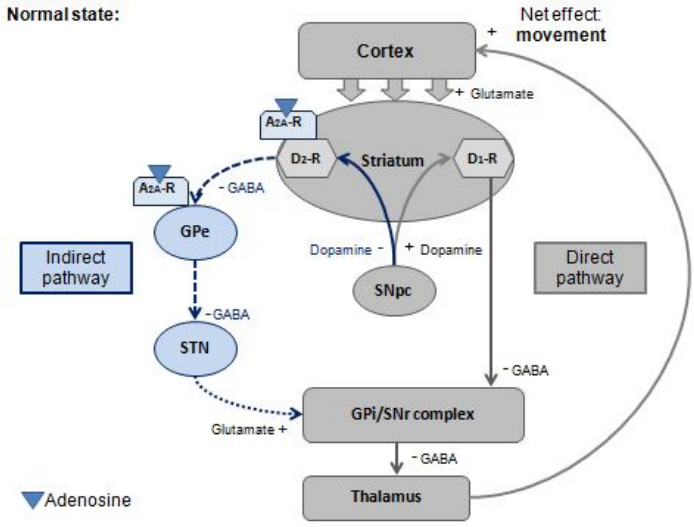


Figure 5.1: Schematic of the basal ganglia model in the normal state. Normal motor function requires the balance between the direct and indirect pathways. *Abbreviations: SNpc* = substantia nigra pars compacta*; SNr* = substantia nigra pars reticulata*; GPi* = globus pallidus internal*; GPe* = globus pallidus external*; STN* = subthalamic nucleus*;* D_1*-R* = D_1 receptors*;* D_2*-R* = D_2 receptors*; GABA* = gamma-aminobutyric acid.

In PD, the occurrence of motor symptoms can be attributed to the depletion of dopaminergic neurons in the SNpc, leading to an imbalance between the striatal output pathways (Cieślak *et al.*, 2008) and the subsequent range of functional modifications observed in the activity of the basal ganglia motor circuit (Ferraro *et al.,* 2012). In particular, the indirect (striatopallidal) output pathway plays a fundamental role in the motor fluctuations and dyskinesias observed in PD

(LeWitt *et al.,* 2008). The indirect pathway may be modulated by A_{2A} receptors. For example, overexpression of the A_{2A} receptor has been linked to parkinsonian associated motor symptoms (Shook *et al.*, 2012). The reason for this observation is A_{2A} receptors and D_2 receptors act in an antagonistic manner and it is believed that dopamine via D_2 receptor stimulation antagonizes A_{2A} receptor mediated signalling (Tanganelli *et al*., 2004; Vortherms & Watts, 2004). Thus, dopamine loss would lead to unopposed adenosine signalling, resulting in overactivity of the striatopallidal output pathways and consequently excess inhibition of movement (Fredholm & Svenningsson, 2003). The basal ganglia model may be used to demonstrate the mechanism involved with PD that results in motor impairment (Figure 5.2).

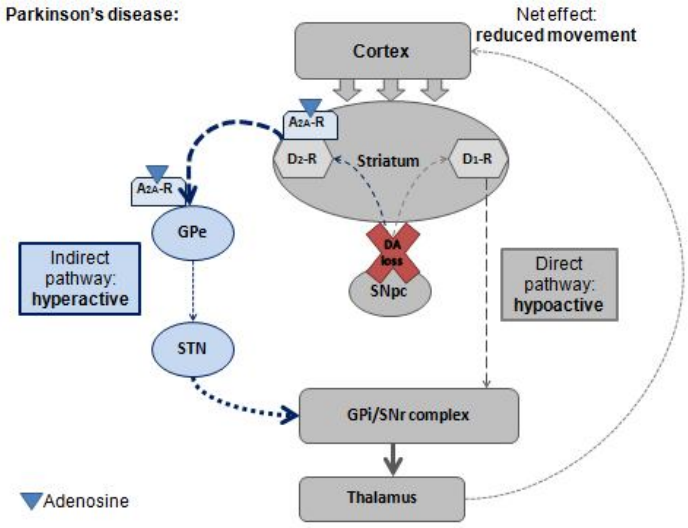


Figure 5.2: Schematic of the basal ganglia model, in PD. Degeneration of the SNpc leads to the loss of dopamine input to the striatum and consequently results in an unopposed overactivity of the indirect pathway (indicated in blue) that include the GPe and STN structures of the basal ganglia. In turn, depletion of dopamine in the direct pathway leads to a decrease in the activation of this pathway. The net effect is excessive inhibition of the thalamocortical neurons and as a result motor impairment. *Abbreviations: SNpc* = substantia nigra pars compacta*; SNr* = substantia nigra pars reticulata*; GPi* = globus pallidus internal*; GPe* = globus pallidus external*; STN* = subthalamic nucleus*; D_1-R* = D_1 receptors*; D_2-R* = D_2 receptors.

The rationale for the use of A_{2A} receptor antagonists in the therapy of PD is thus based on the role of A_{2A} receptors in the basal ganglia. Blockade of the A_{2A} receptor may result in an alternative or adjunctive therapeutic approach to the current dopamine restoring strategies used in PD, as depicted in Figure 5.3. In the early stages of PD it is thought that an inhibitory dopaminergic tonus compensatory mechanism (still undefined) is in place to delay motor

impairment (Gomes *et al.,* 2011). The latter justify the existence of a pre-symptomatic period in PD.

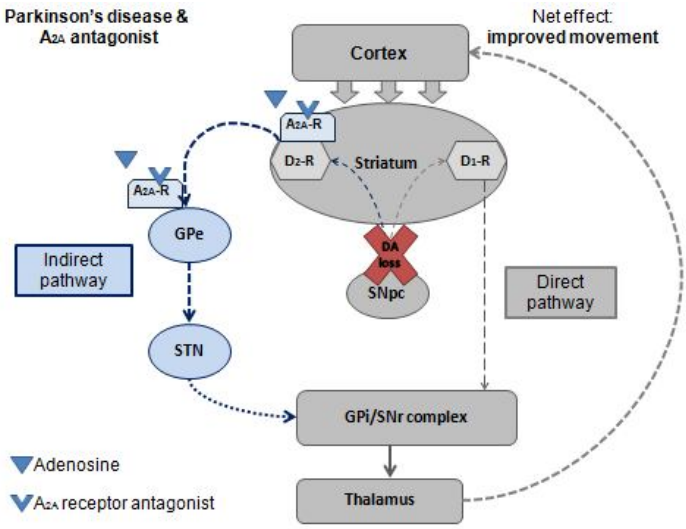


Figure 5.3: Schematic of the proposed anti-parkinsonian activity of adenosine A_{2A} receptor antagonists in a basal ganglia model of PD. In PD, degeneration of the SNpc is observed causing an imbalance of the indirect pathway (indicated in blue) and direct pathway. A_{2A} receptor blockade should result in recovery of the GPe activity and subsequent relief of the excessive inhibition of the GPi/SNr complex. The net effect is improvement of motor function as a consequence of the restoration of the balance between the direct and indirect pathway in PD. *Abbreviations: SNpc* = substantia nigra pars compacta*; SNr* = substantia nigra pars reticulata*; GPi* = globus pallidus internal*; GPe* = globus pallidus external*; STN* = subthalamic nucleus*;* D_1*-R* = D_1 receptors*;* D_2*-R* = D_2 receptors*;* A_{2A}*-R* = A_{2A} *receptors*.

Adenosine A_{2A} receptor antagonists may be used as adjuvants to L-DOPA treatment in PD. L-DOPA stimulates the direct pathway and inhibits the indirect pathway (Morelli *et al.*, 2012). PD animal studies have shown that a known A_{2A} receptor antagonist, KW-6002, when administed in combination with L-DOPA attenuates the outcome of motor impairments (LeWitt *et al.*, 2008). Chronic administration of L-DOPA is associated with complications such as dyskinesia (Leung & Mok, 2005; Onofrj *et al.*, 2008). This may be attributed to the overactivation of the direct pathway and particularly the enhanced indirect pathway leading to the overexpression of A_{2A} receptors (Morelli *et al.*, 2012). Even though an adenosine A_{2A} receptor antagonist does not counteract the excessive stimulation of the direct pathway, an A_{2A} antagonist co-administered with L-DOPA can stabilize the indirect pathway leading to motor activation possibly without aggravating dyskinesia (Morelli *et al.*, 2012).

5.3 The adenosine system

Adenosine is a purine nucleoside that consists of an adenine as the base and a ribose moiety as the sugar (Ongini *et al*., 2001). In the CNS adenosine is involved in numerous functions that include inhibitory neurotransmission and neuroprotective actions in pathological conditions (Latini & Pedata, 2001).

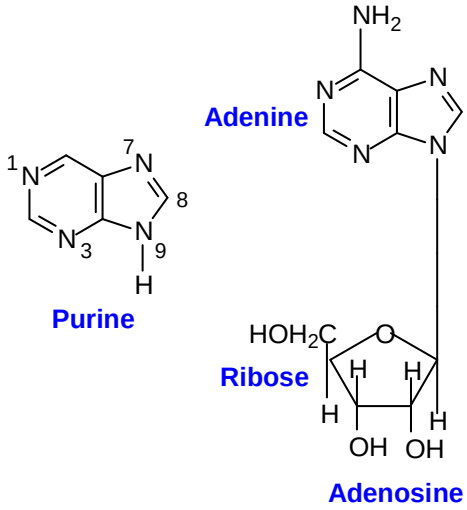


Figure 5.4: Chemical structures of purine and adenosine, an example of a purine nucleoside.

Adenosine is a neuromodulator which acts at adenosine receptors (Ongini *et al.*, 2001) and modulate the effects of dopamine and other neurotransmitters involved in motor function, mood, and learning and memory (Shook & Jackson, 2012).

Under normal conditions, adenosine is formed both intra- and extracellularly (Fredholm *et al.,* 2001) and equilibrative transporters keep the intra- and extracellular adenosine concentrations in equilibrium (Jenner *et al.,* 2009). In the CNS, adenosine is intracellularly formed via degradation of adenosine monophosphate (AMP) through 5'-nucleotidase, after which bi-directional nucleoside transporters keep the intracellular and extracellular concentrations of adenosine in equilibrium (Latini & Pedata, 2001; Pinna *et al*., 2005). The intracellular production of adenosine may also occur via hydrolysis of S-adenosyl-homocysteine. However, previous research revealed that the S-adenosyl-homocysteine pathway does not contribute significantly to adenosine concentrations in the brain (Latini *et al*., 1995; Latini & Pedata, 2001; Jenner *et al.,* 2009). Adenosine may also be formed extracellularly via the metabolism of released nucleotides by the action of the ecto-5'-nucleotidase enzyme (Latini & Pedata, 2001; Jenner *et al.,* 2009). Overall, the formation of adenosine depends upon the synthesis and breakdown of ATP (Pinna *et al.,* 2005).

5.3.1 The adenosine A_{2A} receptor

There are four subtypes of adenosine receptors that have been identified in the CNS and these are the A_1, A_{2A}, A_{2B} and A_3 subtypes (Ongini *et al.,* 2001; Cieślak*et al.*, 2008). These receptors are G-protein-coupled receptors (GPCRs) (Pinna *et al.,* 2005). The A_1 receptors couple with the G_i and G_o subfamilies of the G-proteins and inhibit adenylyl cyclase, while A_{2A} receptors couple with the G_s proteins and activate adenylyl cyclase (Ribeiro *et al.*, 2003; Cieślak *et al.*, 2008). The four identified subtypes of adenosine are all asparagine-linked glycoproteins. However, unlike the A_{2A} receptors, at the carboxyl terminal of the A_1, A_{2B} and A_3 receptors there are sites for palmitoylation (Ribeiro *et al.*, 2003).

The subtypes of adenosine receptors can be characterized as follows (Xu *et al.*, 2005; Cieślak *et al.*, 2008):

- A_1 and A_{2A} receptors with a high affinity for adenosine
- A_{2B} and A_3 receptors with a low affinity for adenosine

The A_{2A} receptors are mainly localized in the striatum and are expressed as follows (Cieślak*et al.*, 2008):

- 70% postsynaptically,
- 23% presynaptically,
- 3% on the neuron body and
- 3% on glial cells.

Dopamine D_2 receptors and adenosine A_{2A} receptors are co-localized at the indirect pathway of the basal ganglia (Cieślak *et al.*, 2008) and dopamine and adenosine have opposing effects in the brain (Ferré *et al.,* 2001). For example, a dopamine agonist and an adenosine antagonist or a dopamine antagonist and an adenosine agonist produce similar effects (Ferré *et al.,* 2001). An example of an antagonistic interaction between A_{2A} and D_2 receptors is found with haloperidol, a D_2 antagonist, which reduces dopaminergic neurotransmission. This effect can be countered by an adenosine A_{2A} antagonist (Ferré *et al.,* 2001).

5.3.2 The structure of the adenosine A_{2A} receptor

Computer modelling methods may be used as an aid for gaining insight into the possible binding modes of a compound within the binding site of the adenosine A_{2A} receptor. Unfortunately, no crystal structure of the human A_{2A} adenosine receptor exists in complex with a xanthine derivative, but there is a 2.6 Å crystal structure of the human adenosine A_{2A} receptor (PDB ID: 3EML) in complex with the non-xanthine adenosine A_{2A} receptor antagonist,

ZM241385 (4-(2-[7-amino-2-(2-furyl)1,2,4-triazolo[2,3-a]-[1,3,5]triazin-5-ylamino]ethylphenol) (Jaakola *et al.,* 2008).

As mentioned before, the adenosine A_{2A} receptor is part of the GPCRs (Pinna *et al.,* 2005). According to the crystal structure of the human adenosine A_{2A} receptor in complex with ZM241385 (Jaakola *et al.,* 2008), the overall three-dimensional structure consists of seven transmembrane α-helices, followed by one short membrane associated helix (helix VIII) that is stabilized by helix I (Jaakola *et al.,* 2008; Piirainen *et al.,* 2011). The residues of the transmembrane α-helices are summarized as follows (Jaakola *et al.,* 2008):

- Helix I: Gly-5 to Trp-32
- Helix II: Thr-41 to Ser-67
- Helix III: His-75 to Arg-107
- Helix IV: Thr-119 to Leu-140
- Helix V: Asn-175 to Ala-204
- Helix VI: Arg-222 to Phe-258
- Helix VII: Leu-269 to Arg-291
- Helix VIII: Arg-296 to Leu-308

The rest of the structure consists of an extracellular amino-terminus (N-terminus), a cytosolic carboxy-terminus (C-terminus), three extracellular loops (ECL1-3) and three intracellular loops (ICL1-3) (Jaakola *et al.,* 2008; Piirainen *et al.,* 2011).

The residues of the intra- and extracellular loops are as follows (Jaakola *et al.,* 2008):

- ICL1: Leu-33 to Val-40
- ICL2: Ile-108 to Gly-118
- ICL3: Leu-208 to Ala-221
- ECL1: Thr-68 to Cys-74
- ECL2: Leu-141 to Met-174
- ECL3: Cys-259 to Trp-268

In the crystal structure of Jaakola and co-workers (2008) the ICL3 is replaced by 160 residues from T4L lysozyme and the N-linked glycan associated Asn-154 has been removed enzymatically for improved crystallization. Figure 5.5 depicts the three-dimensional structure of human A_{2A} receptor in complex with ZM241385.

The adenosine A_{2A} receptor contains an ordered water cluster (HOH-501, HOH-514, HOH-522, HOH-528 and HOH-567) in the binding cavity. Speculation exists regarding the role of this water cluster and the possible interactions within the binding cavity. It is still unknown if this

water cluster interacts with residues, is important for agonist/antagonist selectivity and if these waters define the allosteric modulation site (Piirainen *et al.,* 2011).

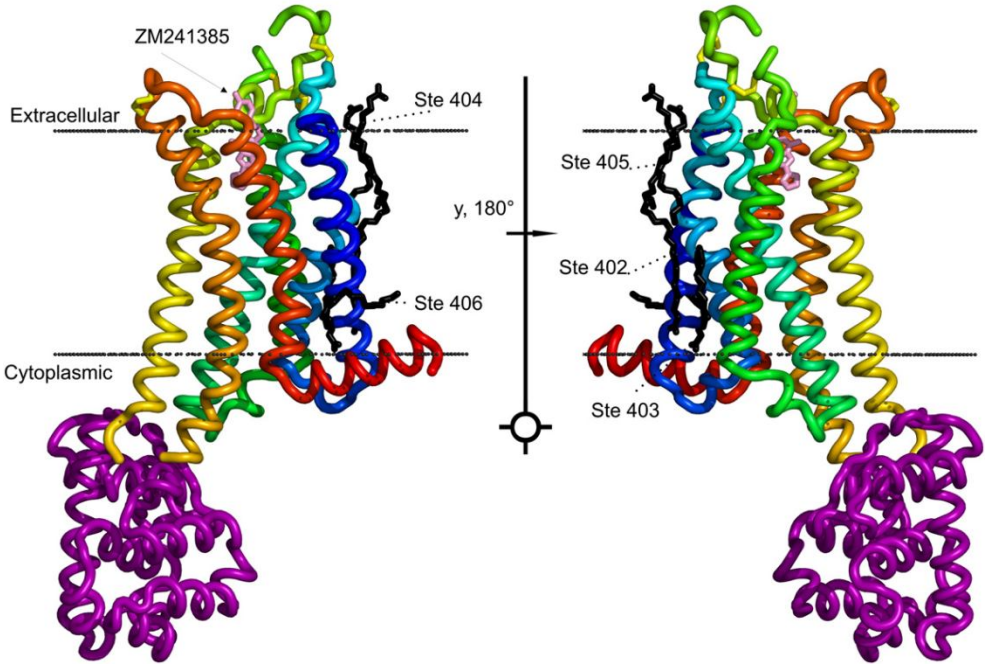


Figure 5.5: Crystal structure of the A_{2A} receptor in complex with ZM241385 (PDB ID 3EML). The cartoon diagram depicts the C-terminus in red and the N-terminus in blue. The T4L lysozyme is depicted in purple. The membrane boundary planes are indicated as grey coloured "dummy" atoms. Stick models are used to indicate ZM241385 in pink, with the black structures representing lipids Ste-402 and Ste-406 (Piirainen *et al.,* 2011).

ZM241385 binds perpendicular to the plane of the membrane bilayer. A hydrophobic π-stacking interaction with Phe-168 is observed as well as a hydrophobic interaction with Ile-274, and several hydrogen bond interactions (directly or indirectly) with water molecules (HOH-559, HOH-567, HOH-550, HOH-522 and HOH-519) (Piirainen *et al.,* 2011). The key regions and interactions of the human adenosine A_{2A} receptor in complex with ZM241385 are depicted in Figure 5.6.

A pharmacophore model was proposed by Müller and Ferré (2007) for the antagonism of A_{2A} receptors by xanthine derivatives. This pharmacophore model is depicted in Figure 5.7 and shows that the styryl side chain of KW-6002 fills the lipophilic pocket (indicated in pink). This pocket is also occupied by the ribose moiety of adenosine derivatives. The 2-oxo group and N1-substituent of the xanthines are required as electron-rich (indicated in green) while the 6-oxo group represent the hydrogen bond accepting group (indicated in blue). According to a

molecular docking study performed, KW-6002 and ZM241385 occupy the same cavity in the active site but differ in the orientation of the purine ring (Yuzlenko & Kiec-Kononowicz, 2009).

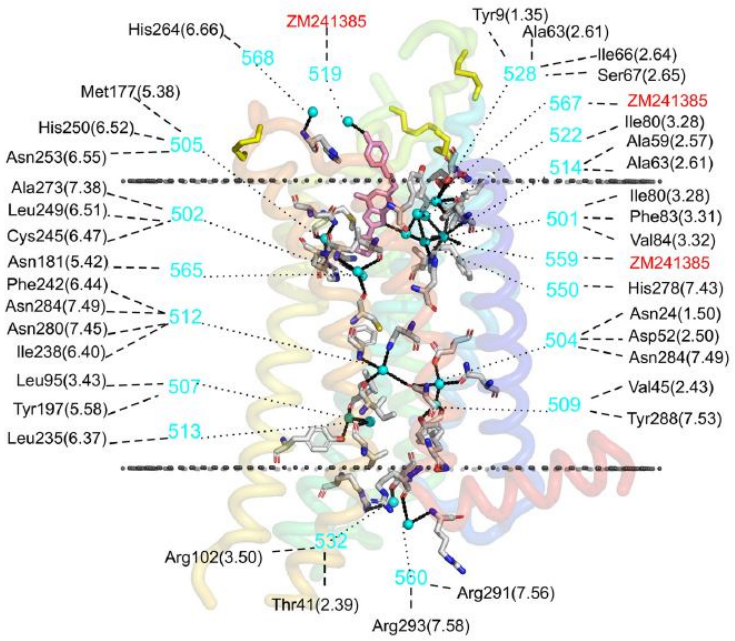


Figure 5.6: Key regions and interactions of the human adenosine A_{2A} receptor in complex with ZM241385 (PDB ID 3EML). Water molecules are indicated as blue dots. The interacting side chains are indicated as white sticks. Bound ZM241385 is shown as a pink stick model with the direct interactions with cluster water molecules HOH-519, HOH-559 and HOH-567 depicted. (Piirainen *et al.,* 2011).

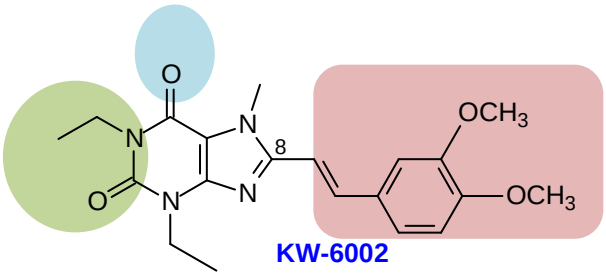


Figure 5.7: Pharmacophore model for the A_{2A} receptor-selective xanthine derivative, depicting KW-6002 in the binding cavity. The pink area indicates a lipophilic pocket, the green an electron-rich area and the blue indicates a hydrogen bond accepting region (Müller and Ferré, 2007).

It is important to take polar interactions into consideration when designing adenosine A_{2A} receptor antagonists, as it may play a role in adenosine A_3 receptor selectivity. It has been documented that a valine residue of the adenosine A_3 receptor is at the analogous position of Glu-169 in the A_{2A} receptor, thus removing the polar stabilizing interactions observed between ZM241385 and residues Asn-253 and Glu-169 (Piirainen *et al.,* 2011).

5.3.3 Molecular interaction of adenosine A_{2A} receptors with dopamine receptors

The discovery that adenosine A_{2A} receptors may form functional heteromeric receptor complexes further enhanced the advantages (both symptomatic and disease modifying) of A_{2A} receptor antagonists as anti-parkinsonian treatment. These heteromeric receptor complexes consist of the A_{2A} receptor and other G-protein-coupled receptors and may act either directly or indirectly (Schwarzschild *et al.,* 2006). Adenosine A_{2A} receptors may form a heteromeric complex with co-localized dopamine D_2 receptors of the striatopallidal pathway (Gomes *et al.,* 2011). The A_{2A}-D_2 receptor heteromers may be found in the dorsal and ventral striatopallidal pathway. Activation of the A_{2A} receptors results in a reduction of motor function due to a decreased D_2 receptor recognition, coupling and signalling (Fuxe *et al.,* 2007). In PD, A_{2A} receptor antagonists are used to amplify D_2 receptor signalling of the A_{2A}-D_2 receptor heteromers of the dorsal striatopallidal pathway (Fuxe *et al.,* 2007), suggesting potential benefits of administration of adenosine A_{2A} receptor antagonists, as monotherapy, in the early stages of PD (Schwarzschild *et al.,* 2006).

The fact that A_{2A} receptor antagonists may improve motor function in PD independently of the co-expressed D_2 receptors (Cieślak *et al.*, 2008; LeWitt *et al.*, 2008), leads to the speculation that another mechanism, other than the A_{2A}-D_2 heteromerization, may contribute to their anti-parkinsonian properties. Furthermore, three neurotransmitters have been identified to modulate dopamine-mediated neurotransmission and these include dopamine, adenosine and glutamate. Another heteromeric A_{2A} receptor complex is found in the ventral striatopallidal pathway. A_{2A} receptors also form complexes with the non-dopaminergic metabotropic glutamate $mGlu_5$ receptor, which has also been indicated as a possible therapeutic target in PD. The A_{2A}-$mGlu_5$ heteromers seem to exhibit a synergistic interaction of striatal plasticity. The interdependence of the A_{2A}, D_2 and $mGlu_5$ receptors is demonstrated by the finding that $mGlu_5$ antagonists induce motor activation only after the activation of A_{2A} and D_2 receptors (Schwarzschild *et al.,* 2006).

5.3.4 Neuroprotective properties of A_{2A} receptor antagonists in PD

It is a challenge to design neuroprotective therapy for PD as the underlying neurodegenerative processess of this disease remains unknown (Armentero *et. al.,* 2011). Since multiple mechanisms may be involved in PD, it is likely that these mechanisms act synergistically and form complex interactions to result in neurodegeneration (Yacoubain & Standaert, 2009). In PD, adenosine A_{2A} receptor antagonists may possess neuroprotective actions (Cieślak *et al.*, 2008).

Initially in PD, inflammation occurs to restore physiological tissue function. Unfortunately, the chronic stimuli of inflammatory reactions have emerged as a contributing factor in PD. This uncontrolled neuroinflammation is associated with toxic factors that enhance the underlying

disease states. In the basal ganglia, adenosine A_{2A} receptors are expressed by cells (such as astrocytes, microglia and oligodendrocytes) that are associated with the neuroinflammatory process. It is speculated that adenosine A_{2A} receptor antagonists may modify these neuroinflammatory processes (Armentero *et al.,* 2011). It is suggested that A_{2A} receptor antagonists potentially modulate astrocytes to reduce the inflammatory burden (Armentero *et al.,* 2011).

It is also documented that overstimulation of glutamate (in the basal ganglia) may lead to neurodegeneration (Gomes *et al.,* 2011; Armentero *et al.,* 2011). The chronic stimulation of glutamate in PD may act as a noxious stimulus over time and consequently results in neuronal death (Armentero *et al.,* 2011). It is known that A_{2A} receptor antagonists decrease glutamate release, pre- and postsynaptically, and indirectly may halt neural death.

5.4 The design of adenosine A_{2A} receptor antagonists

While numerous advances have been made with symptomatic treatment in PD, most of the antiparkinsonian agents fail to provide a neuroprotective effect. Modulation of adenosine A_{2A} receptors in the treatment of PD may proof valuable for their ability to control motor impairment (symptomatic treatment) and for the possibility of providing neuroprotection (disease modifying).

The functional interactions between dopamine and adenosine in the basal ganglia is of significance in the control of motor behaviour. The ability of A_{2A} receptors to control motor function may, in part, be attributed to the ability of A_{2A} receptors to modulate the function of the D_2 receptors, both at the level of intracellular signalling and via the formation of heteromers with D_2 receptors (Fuxe *et al.,* 2007; Gomes *et al.,* 2011) or possibly with the metabotropic glutamate mGlu5 receptor (Schwarzschild *et al.,* 2006).

In addition, various mechanisms have been proposed for adenosine A_{2A} receptor-mediated neuroprotection in PD. As mentioned, one of the neuroprotective mechanisms includes the modulation of neuroinflammation by adenosine A_{2A} receptors in the brain (Kalda *et al.,* 2006). Secondly, blockade of the adenosine A_{2A} receptors in PD decrease glutamate release. This is important as it is speculated that excessive glutamatergic stimulation may lead to neurodegeneration (Gomes *et al.,* 2011).

5.4.1 The adenosine A_{2A} receptor as drug target

It has been reported that motor functions may be influenced by adenosine A_{2A} receptor modulation in the basal ganglia (Harper *et al.,* 2006). In primate and rodent models of PD, adenosine A_{2A} receptor antagonists have been shown to exert motor activation, either alone or in combination with dopaminergic drugs such as L-DOPA and dopamine agonists (Harper *et al.,*

2006; Gomes *et al.,* 2011). It has been documented that dyskinesia associated with L-DOPA treatment is not enhanced with an adenosine A_{2A} receptor antagonist (Gomes *et al.,* 2011). Currently, the treatments of PD with dopamine restoring drugs are associated with at least two limitations: long-term side-effects (motor disability, including dyskinesia) and failure to prevent degeneration of the disease (Gomes *et al.,* 2011). Adenosine A_{2A} receptor antagonists may be divided into two classes: the xanthine and non-xanthine (the amino-substituted heterocyclic compounds) classes of compounds. These classes will be discussed in the following sections.

5.4.1.1 Xanthine class of adenosine A_{2A} receptor antagonists

To date some of the most effective adenosine A_{2A} receptor antagonists are substituted xanthines (Massip *et al.,* 2006; Bansal *et al.,* 2009) and their affinities and selectivities for adenosine receptors are well documented (Bansal *et al.,* 2009).

Caffeine (1,3,7-trimethylxanthine) is a known xanthine derivative. Another known methylxanthine is theophylline (1,3-dimethylxanthine). These natural occurring xanthine derivatives (Figure 5.8) were the first adenosine antagonists (Ongini & Fredholm, 1996) and possess low affinities for adenosine receptors. They may also be considered as non-selective towards A_1 and A_{2A} receptors (Erickson *et al.,* 1991; Ongini & Fredholm, 1996; Shimada *et al.,* 1997).

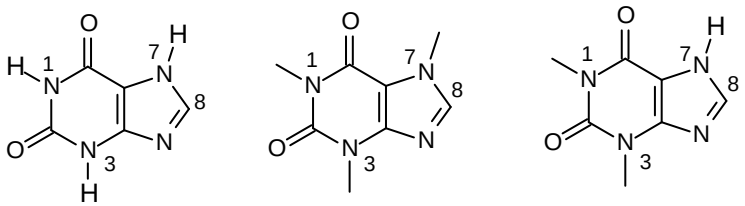
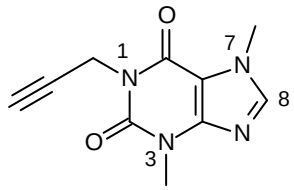


Figure 5.8: Chemical structures of xanthine, caffeine and theophylline.

Numerous xanthine derivatives have been synthesized in an attempt to develop more potent and selective antagonists for adenosine A_{2A} receptors (Erickson *et al.,* 1991). The first selective A_{2A} receptor antagonist described was 3,7-dimethyl-1-propargylxanthine (DMPX; Figure 5.9) (Müller *et al.,* 1998). It was documented that this compound has a low affinity for adenosine receptors although it was selective for A_{2A} receptors compared to A_1 receptors (Müller *et al.,* 1998).

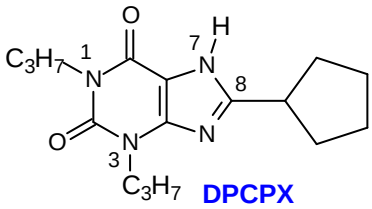


DMPX

Figure 5.9: Chemical structure of the adenosine A_{2A} receptor antagonist, 3,7-dimethyl-1-propargylxanthine.

It was found that substitution on the 8-position of the xanthine heterocyclic system has a significant effect on the potency of the xanthine derivative as an antagonist of adenosine receptors (Bansal *et al.,* 2009). Previous studies demonstrated that substitution at the 8-position of the caffeine ring with either a cycloalkyl or styryl group results in potent and selective A_1 and A_{2A} receptor antagonists, respectively (Ongini *et al.,* 2001).

A documented selective A_1 receptor antagonist bearing a cycloallkyl group at position 8 is 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; Figure 5.10). This compound is frequently used in biochemical and pharmacological studies as a reference A_1 receptor antagonist (Ongini *et al.,* 2001).



DPCPX

Figure 5.10: Chemical structure of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an adenosine A_1 receptor antagonist.

Very potent and selective derivatives of 8-styrylxanthine were developed during the past years as A_{2A} receptor antagonists (Müller *et al.,* 1998). In 1992, an 8-styrylxanthine A_{2A} receptor antagonist known as (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF17837; Figure 5.11), was documented with high affinity for A_{2A} receptors (Ongini & Fredholm, 1996; Kase *et al.*, 2004) and a good selectivity for A_{2A} receptors when compared to A_1 receptors (Ongini & Fredholm, 1996). Care must be taken, however, as exposure of a styryl-containing compound to daylight may convert the compound from the *E* isomer to an *E/Z* mixture. The *Z* isomer was found to be less active than the *E* isomer at A_{2A} receptors (Ongini & Fredholm, 1996).

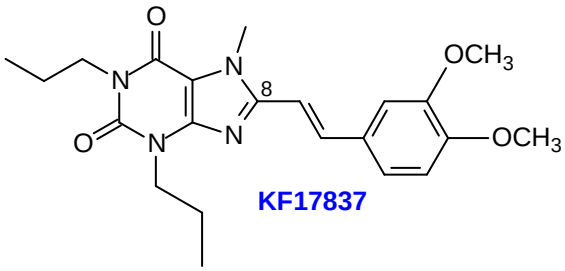


Figure 5.11: Chemical structure of the adenosine A_{2A} receptor antagonist, (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine.

During the 1990's (*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-xanthine (KW-6002; Figure 5.12) was developed (Harper *et al.,* 2006) as a derivative of KF17837 (Kase *et al.*, 2004). This compound is the first orally active A_{2A} adenosine receptor antagonist (Harper *et al.,* 2006) and is currently in Phase III clinical trials as PD therapy (Kalda *et al.,* 2006; Müller & Ferré, 2007). Today KW-6002 is seen as one of the most important xanthine derived A_{2A} receptor antagonists (Minetti *et al.,* 2005) and is reported to display high affinity for A_{2A} receptors with a K_i value of 2.2 nM and a 68-fold lower affinity for A_1 receptors (Harper *et al.,* 2006). It was also reported that KW-6002 exhibits K_i values of 841 nM and 12 nM for human A_1 and A_{2A} receptors, respectively (Müller & Jacobson, 2011). In numerous PD models, KW-6002 was shown to ameliorate motor dysfunction (Ongini *et al.,* 2001). The importance of selective A_{2A} receptor antagonists in PD therapy is demonstrated by the ability of KW-6002 to stimulate motor activity as either monotherapy or adjunct therapy with L-DOPA, as well as the ability of KW-6002 to reduce the tendency to develop dyskinesia with L-DOPA therapy (Kalda *et al.,* 2006; Schwarzchild *et al.,* 2006). Unfortunately, the development of dyskinesia is not prevented when KW-6002 is co-administered with the full treatment regime of L-DOPA (Blandini, 2003; Schwarzschild *et al.,* 2006). It has also been documented that KW-6002 is a moderate MAO-B inhibitor with a K_i value of 28 µM (Petzer *et al.,* 2003). The anti-cataleptic effect of KW-6002 was evaluated in a haloperidol-induced catalepsy model in mice and it was shown that this compound displays an EC_{50} value of 0.03 mg/kg for the reverse of haloperidol-induced catalepsy (Shimada *et al.,* 1997).

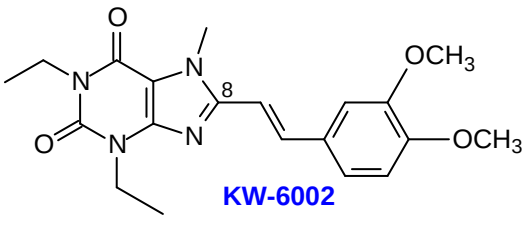


Figure 5.12: Chemical structure of the adenosine A_{2A} receptor antagonist, (*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine.

Another 8-styrylxanthine derived A_{2A} receptor antagonist, which also inhibits MAO-B, is (*E*)-8-(3-chlorostyryl)caffeine (CSC; Figure 5.13) (Vlok *et al.,* 2006). CSC is often used as a reference A_{2A} antagonist in *in vivo* pharmacological studies (Vlok *et al.,* 2006), and exhibits K_i values of 28,000 nM and 54 nM for A_1 and A_{2A} receptors, respectively (Müller & Jacobsen, 2011). A K_i value of 70 nM for the inhibition of MAO-B was also reported for CSC (Vlok *et al.,* 2006). It has previously been speculated that the neuroprotection by CSC may be a result of its inhibition effect on MAO-B (Gomes *et al.,* 2011).

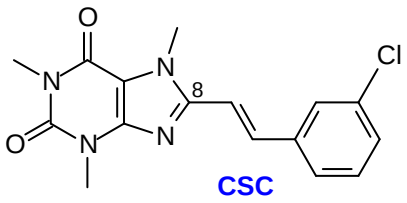


Figure 5.13: Chemical structure of the adenosine A_{2A} receptor antagonist, (*E*)-8-(3-chlorostyryl)caffeine.

5.4.1.2 Non-xanthine heterocyclic adenosine receptor antagonists

Another class of adenosine receptor antagonists is known as the non-xanthine A_{2A} receptor antagonists. These compounds are flat aromatic, 6:5-fused bicyclic, 6:6:5- or 6:5:5-fused tricyclic heterocycles that contain nitrogen with an exocyclic amino group (Ongini & Fredholm, 1996).

One of the first triazoloquinazolines with adenosine receptor antagonistic properties is CGS15943 (5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-*c*]quinazoline; Figure 5.14). This compound served as a lead compound for the design of potent and selective A_{2A} receptor antagonists (Ongini & Fredholm, 1996). However, it was found that this compound also potently blocks A_1 receptors and interacts with A_{2B} receptors (Ongini & Fredholm, 1996; Ongini *et al.,* 2001). In further studies the phenyl ring of CGS15943 was replaced with different heterocyclic rings, such as pyrazole or imidazole. Even though these compounds had little or no A_{2A} receptor selectivity compared to A_1 receptor selectivity, an improvement of antagonistic properties was found in functional assays (Ongini *et al.,* 2001).

Compounds were also developed with little or no interaction with A_1 or A_{2B} receptors. These include SCH58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3]-1,2,4-triazolo[1,5]-pyrimidine; Figure 5.15) with a K_i value of 2 nM at A_{2A} receptors and a selectivity of 50-100 over A_1 receptors (Ongini & Fredholm, 1996). In pharmacological studies SCH58261 is used as a reference A_{2A} receptor antagonist (Ongini *et al.,* 2001). The compound, ZM241385 (Figure 5.16), also displays high affinity and selectivity for A_{2A} receptors (Ongini & Fredholm, 1996;

Ongini *et al.,* 2001). However, this compound also interacts with A_{2B} receptors (Ongini *et al.,* 2001).

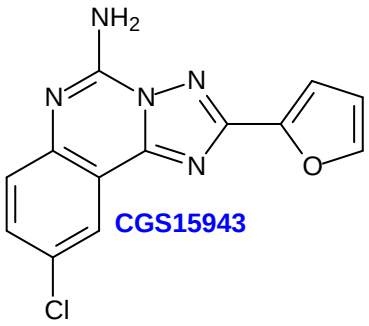


Figure 5.14: Chemical structure of the adenosine A_{2A} receptor antagonist, 5-amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-*c*]quinazoline.

Both SCH58261 and ZM241385 are derived from the prototype compound, CGS15943 (Minetti *et al.,* 2005). CGS15943 is also used as a reference A_{2A} antagonist (Müller *et al.,* 1997).

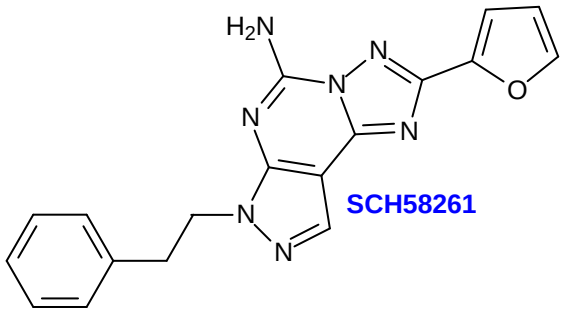


Figure 5.15: Chemical structure of the adenosine A_{2A} receptor antagonist, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3]-1,2,4-triazolo[1,5]pyrimidine.

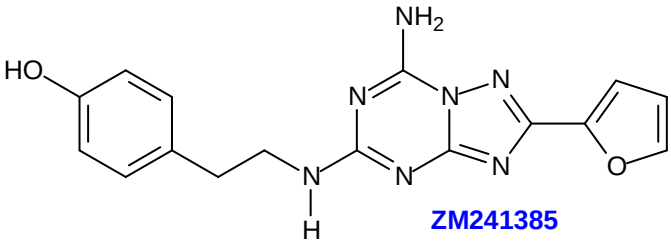


Figure 5.16: Chemical structure of the adenosine A_{2A} receptor antagonist, 4-(2-[7-amino-2-(2-furyl)1,2,4-triazolo[2,3-a]-[1,3,5]triazin-5-ylamino]ethylphenol.

5.5 Conclusion

Adenosine plays a very important role in the striatum and modulates the control of motor function. Four different adenosine subtypes can be identified, namely A_1, A_{2A}, A_{2B} and A_3 receptors. Previously, antagonistic interactions between A_{2A} and D_2 receptors have been

documented (Ferraro *et al.*, 2012). It is speculated that D_2 receptors are responsible for antagonizing A_{2A} receptor mediated signalling (Tanganelli *et al.*, 2004; Vortherms & Watts, 2004). According to the literature, A_{2A} receptors have an important role in the modulation of dopamine-mediated responses and the control of motor behaviour (Pinna *et. al.*, 2005). Also of significance, A_{2A} receptors are expressed almost exclusively in the striatum of the basal ganglia (Tanganelli *et. al.*, 2004; Pinna *et. al.*, 2005). The importance of adenosine is further emphasized by the co-localization of the dopamine D_2 receptors and adenosine A_{2A} receptors in the striatopallidal medium spiny neurons that constitute the indirect pathway of the basal ganglia (Ferré *et al.*, 2001; Schwarzschild *et al.,* 2006; Morelli *et al.*, 2012).

Adenosine A_{2A} receptor antagonists may address some of the current limitations associated with dopamine replacement therapy for PD. The limitations of dopamine replacement therapy include longterm adverse effects (including dyskinesia) (Mihara *et al.,* 2008; Gomes *et al.,* 2011) and the inability of therapy to stop the progressive degeneration process (Gomes *et al.,* 2011). These limitations of the current dopamine replacement therapies encourage the development of novel non-dopaminergic drugs, especially for the treatment of the middle and advanced stages of PD. The rationale for the use of adenosine A_{2A} receptor antagonists includes the improvement of motor function in PD, but also possible neuroprotective effects of these drugs.

According to Gomes and co-workers (2011), the role of the adenosine A_{2A} receptor in PD may be summarized as follow:

- the physiological role of A_{2A} receptors in control of motor function
- the ability of A_{2A} receptors to control glutamatergic transmission
- the unopposed A_{2A} receptor mediated activity in PD
- the eventual A_{2A} receptor involvement in neuroinflammation

A_{2A} receptor antagonists can be divided into two chemical classes, namely the xanthine and the non-xanthine derivatives. One of the important xanthine derived compounds is KW-6002, which is currently in Phase III clinical trails for the treatment of PD. This compound was found to reduce motor dysfunction and the risk of developing dyskinesia with L-DOPA treatment. Based on these observations, KW-6002 may be a clinically useful agent.

From the above, it is clear that the adenosine A_{2A} receptor is an important target to consider when developing novel treatments for PD. A_{2A} receptor antagonists may possibly be used as mono- or adjunct therapy to L-DOPA treatment and may reduce dyskinesia as well as motor dysfunctions in PD. In addition, A_{2A} receptor antagonists may also exert neuroprotective effects.

One of the aims of this thesis was to discover new adenosine A_{2A} receptor antagonists by synthesizing a series of xanthine analogues. The design, synthesis, results and discussion will be provided in Chapter 8 (article 3).

5.6 References

ARMENTERO, M.T., PINNA, A., FERRÉ, S., LANCIEGO, J.L., MÜLLER, C.E. & FRANCO, R. 2011. Past, present and future of A_{2A} adenosine receptor antagonists in the therapy of Parkinson's disease. *Pharmacology and therapeutics*, 132: 280–299.

BANSAL, R., KUMAR, G., GANDHI, D., YOUNG, L.C. & HARVEY, A.L. 2009. Synthesis of a series of 8-(substituted-phenyl)xanthines and a study on the effects of substitution pattern of phenyl substituents on affinity for adenosine A_1 and A_{2A} receptors. *European journal of medicinal chemistry*, 44: 2122–2127.

BLANDINI, F. 2003. Adenosine receptors and L-dopa-induced dyskinesia in Parkinson's disease: potential targets for a new therapeutic approach. *Experimental neurology*, 184, 556–560.

BOLAM, J.P., HANLEY, J.J., BOOTH, P.A.C. & BEVAN, M.D. 2000. Synaptic organization of the basal ganglia. *Journal of anatomy*, 196: 527–542.

CIEŚLAK, M., KOMOSZYŃSKI, M. & WOJTCZAK, A. 2008. Adenosine A_{2A} receptors in Parkinson's disease treatment. *Purinergic signalling*, 4: 305–312.

DAUER, W. & PRZEDBORSKI, S. 2003. Parkinson's disease: mechanisms and models. *Neuron*, 39: 889–909.

ERICKSON, R.H., HINER, R.N., FEENEY, S.W., BLAKE, P.R., RZESZOTARSKI, W.J. & HICKS, R.P. 1991. 1,3,8-Trisubstituted xanthines. Effects of substitution pattern upon adenosine receptor A_1/A_2 affinity. *Journal of medicinal chemistry*, 34: 1431–1435.

FERNANDEZ, H.H. & CHEN, J.J. 2007. Monoamine oxidase B inhibition in the treatment of Parkinson's disease. *Pharmacotherapy*, 27: 174S–185S.

FERRARO, L., BEGGIATO, S., TOMASINI, M.C., FUXE, K., ANTONELLI, T. & TANGANELLI, S. 2012. A_{2A}/D_2 receptor heteromerization in a model of Parkinson's disease. Focus on striatal aminoacidergic signalling. *Brain research,* 1476: 96–107.

FERRÉ, S., POPOLI, P., GIMENEZ-LLORT, L., RIMONDINI, R., MULLER, C.E., STROMBERG, I., ORGEN, S.O. & FUXE, K. 2001. Adenosine/dopamine interaction: implications for the treatment of Parkinson's disease. *Parkinsonism and related disorders*, 7: 253–241.

FREDHOLM, B.B., IJZERMAN, A.P., JACOBSON, K.A., KLOTZ, K.N. & LINDEN, J. 2001. International union of pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacological reviews*, 53: 527–552.

FREDHOLM, B.B. & SVENNINGSSON, P. 2003. Adenosine-dopamine interactions: development of a concept and some comments on therapeutic possibilities. Neurology, 61: S5–S9.

FUNG, V.S.C., HELY, M.A., DE MOORE, G. & MORRIS, J.G.L. 2001. Drugs for Parkinson's disease. *Australian prescriber*, 24: 92–95.

FUXE, K., FERRÉ, S., GENEDANI, S., FRANCO, R. & AGNATI, L.F. 2007. Adenosine receptor-dopamine receptor interactions in the basal ganglia and their relevance for brain function. *Physiology and behavior*, 92: 210–217.

GOMES, C.V., KASTER, M.P., TOMÉ, A.R., AGOSTINHO, P.M. & CHUNA, R.A. 2011. Adenosine receptors and brain disease: Neuroprotection and neurodegeneration. *Biochimica et biophysica acta*, 1808: 1380–1399.

HARPER, L.K., BECKETT, S.R., MARSDEN, C.A., MCCREARY, A.C. & ALEXANDER, S.P.H. 2006. Effects of the A_{2A} adenosine receptor antagonist KW-6002 in the nucleus accumbens *in vitro* and *in vivo*. *Pharmacology, biochemistry and behavior*, 83: 114–121.

JAAKOLA, V.-P., GRIFFITH, M.T., HANSON, M.A., CHEREZOV, V., CHIEN, E.Y.T., LANE, J.R., IJZERMAN, A.P. & STEVENS, R.C. 2008. The 2.6 Á crystal structure of a human A_{2A} adenosine receptor bound to an antagonist. *Science*, 322: 1211–1217.

JENNER, P., MORI, A., HAUSER, R., MORELLI, M., FREDHOLM, B.B. & CHEN, J.F. 2009. Adenosine, adenosine A_{2A} antagonists, and Parkinson's disease. *Parkinsonism and related disorders*, 15: 406–413.

KALDA, A., YU, L., OZTAS, E. & CHEN, J.F. 2006. Novel neuroprotection by caffeine and adenosine A_{2A} receptor antagonists in animal models of Parkinson's disease. *Journal of the neurological sciences*, 248: 9–15.

KASE, H., MORI, A. & JENNER, P. 2004. Adenosine A_{2A}-receptor antagonists: beyond dopaminergic therapies for Parkinson's disease. *Drug discovery today: therapeutic strategies*, 1: 51–57.

LATINI, S., CORSI, C., PEDATA, F. & PEPEU, G. 1995. The source of brain adenosine outflow during ischemia and electrical stimulation. *Neurochemistry international*, 28: 113–118.

LATINI, S. & PEDATA, F. 2001. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. Journal of neurochemistry, 79: 436–484.

LEUNG, H. & MOK, V. 2005. Parkinson's disease: aetiology, diagnosis, and management. *Hong Kong medical journal*, 11: 476–89.

LEWITT, P.A, GUTTMAN, M., TETRUD, J.W., TUITE, P.J., MORI, A., CHAIKIN, P. & SUSSMAN, N.M. 2008. Adenosine A_{2A} receptor antagonist istradefylline (KW-6002) reduces "Off" time in Parkinson's disease: a double-blind, randomized, multicenter clinical trial (6002-US-005). *Annals of neurology*, 63: 295–302.

MASSIP, S., GUILLON, J., BERTARELLI, D., BOSC, J.J., LEGER, J.M., LACHER, S., BONTEMPS, C., DUPONT, T., MÜLLER, C.E. & JARRY, C. 2006. Synthesis and preliminary evaluation of new 1- and 3-[1-(2-hydroxy-3-phenoxypropyl)]xanthines from 2-amino-2-oxazolines as potential A_1 and A_{2A} adenosine receptor antagonists. *Bioorganic & medicinal chemistry*, 14: 2697–2719.

MIHARA, T., IWASHITA, A. & MATSUOKA, N. 2008. A novel adenosine A_1 and A_{2A} receptor antagonist ASP5854 ameliorates motor impairment in MPTP-treated marmosets: comparison with existing anti-Parkinson's disease drugs. *Behavioural brain research*, 194: 152–161.

MINETTI, P., TINTI, M.O., CARMINATI, P., CASTORINA, M., DI CESARE, M.A., DI SERIO, S., GALLO, G., GHIRARDI, O., GIORGI, F., GIORGI, L., PIERSANTI, G., BARTOCCINI, F. & TARZIA, G. 2005. 2-*n*-Butyl-9-methyl-8-[1,2,3]triazol-2-yl-9*H*-purin-6-ylamine and analogues as A_{2A} adenosine receptor antagonists. Design, synthesis, and pharmacological characterization. *Journal of medicinal chemistry*, 48: 6887–6896.

MORELLI, M., BLANDINI, F., SIMOLA, N. & HAUSER, R.A. 2012. A_{2A} receptor antagonism and dyskinesia in Parkinson's disease. *Parkinson's Disease*, 2012: Article ID489853, 8 pages, doi:10.1155/2012/489853 (in press).

MÜLLER, C.E. & FERRÉ, S. 2007. Blocking striatal adenosine A_{2A} receptors: a new strategy for basal ganglia disorders. *Recent patents on CNS drug discovery*, 2: 1–21.

MÜLLER, C.E, GEIS, U., HIPP, J., SCHOBERT, U., FROBENIUS, W., PAWŁOWSKI, M., SUZUKI, F. & SANDOVAL-RAMIREZ, J. 1997. Synthesis and structure-activity relationships of 3,7-dimethyl-1-propargylxanthine derivatives, A_{2A}-selective adenosine receptor antagonists. *Journal of medicinal chemistry, 40:* 4396–4405.

MÜLLER, C.E. & JACOBSON, K.A. 2011. Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochimica et biophysica acta*, 1808: 1290–1308.

MÜLLER, C.E., SANDOVAL-RAMIREZ, J., SCHOBERT, U., GEIS, U., FROBENIUS, W. & KLOTZ, K.N. 1998. 8-(Sulfostyryl)xanthines: water-soluble A_{2A}-selective adenosine receptor antagonists. *Bioorganic & medicinal chemistry*, 6: 707–719.

ONGINI, E. & FREDHOLM, B.B. 1996. Pharmacology of adenosine A_{2A} receptors. *Trends in pharmacological sciences*, 17: 364–372.

ONGINI, E., MONOPOLI, A., CACCIARI, B. & BARALDI, P.G. 2001. Selective adenosine A_{2A} receptor antagonists. *Farmaco*, 56: 87–90.

ONOFRJ, M., BONANNI, L. & THOMAS, A. 2008. An expert opinion on safinamide in Parkinson's disease. *Expert opinion on investigational drugs*, 17: 1115–1125.

PETZER, J.P., STEYN, S., CASTAGNOLI, K.P., CHEN, J.-F., SCHWARZSCHILD, M.A., VAN DER SCHYF, C.J. & CASTAGNOLI, N. 2003. Inhibition of monoamine oxidase B by selective adenosine A_{2A} receptor antagonists. *Bioorganic & medicinal chemistry,* 11: 1299–1310.

PIIRAINEN, H., ASHOK, Y., NANEKAR, R.T. & JAAKOLA, V.-P. 2011. Structural features of adenosine receptors: from crystal to function. *Biochimica et biophysica acta*, 1808: 1233–1244.

PINNA, A., WARDAS, T.J., SIMOLA, N. & MORELLI, M. 2005. New therapies for the treatment of Parkinson's disease: adenosine A_{2A} receptor antagonists. *Life sciences*, 77: 3259–3267.

RIBEIRO, J.A., SEBASTIÃO, A.M. & DE MENDONÇA, A. 2003. Adenosine receptors in the nervous system: pathophysiological implications. *Progress in neurobiology*, 68: 377–392.

SCHWARZSCHILD, M.A., AGNATI, L., FUXE, K., CHEN, J.F. & MORELLI, M. 2006. Targeting adenosine A_{2A} receptors in Parkinson's disease. *Trends in neurosciences*, 29: 647–654

SHIMADA, J., KOIKE, N., NONAKA, H., SHIOZAKI, S., YANAGAWA, K., KANDA, T., KOBAYASHI, H., ICHIMURA, M., NAKAMURA, J., KASE, H. & SUZUKI, F. 1997. Adenosine A_{2A} antagonists with potent anti-cataleptic activity. *Bioorganic & medicinal chemistry letters*, 7: 2349–2352.

SHOOK, B.C. & JACKSON, P.F. 2012. Adenosine A_{2A} receptor antagonists and Parkinson's disease. *American chemical society chemical neuroscience*, 2: 555–567.

SHOOK, B.C., RASSNICK, S., WALLACE, N., CROOKE, J., AULT, M., CHAKRAVARTY, D., BARBAV, J.K., WANG, A., POWELL, M.T., LEONARD, K., ALFORD, V., SCANNEVIN, R.H., CARROLL, K., LAMPRON, L., WESTOVER, L., LIM, H.-K., RUSSELL, R., BRANUM, S., WELLS, K.M., DAMON, S., YOUELLS, S., LI, X., BEAUCHAMP, D.A., RHODES, K. & JACKSON, P.F. 2012. Design and characterization of optimized adenosine A_{2A}/A_1 receptor antagonists for the treatment of Parkinson's disease. *Journal of medicinal chemistry*, 55: 1402–1417.

TANGANELLI, S., SANDAGER NIELSEN, K., FERRARO, L., ANTONELLI, T., KEHR, J. & FRANCO, R. 2004. Striatal plasticity at the network level. Focus on adenosine A_{2A} and D_2 interactions in models of Parkinson's disease. *Parkinsonism and related disorders*, 10: 273–280.

VLOK, N., MALAN, S.F., CASTAGNOLI, N. jr., BERGH, J.J. & PETZER, J.J. 2006. Inhibition of monoamine oxidase B by analogues of the adenosine A2A receptor antagonist (*E*)-8-(3-chlorostyryl)caffeine (CSC). *Bioorganic & medicinal chemistry,* 14: 3512–3521.

VORTHERMS, T.A. & WATTS, V.J. 2004. Sensitization of neuronal A_{2A} adenosine receptors after persistent D_2 dopamine receptor activation. *Journal of pharmacology and experimental therapeutics*, 308: 221–227.

XU, K., BASTIA, E. & SCHWARZSCHILD, M. 2005. Therapeutic potential of adenosine A_{2A} receptor antagonists in Parkinson's disease. *Pharmacology and therapeutics*, 105: 267– 310.

YACOUBIAN, T.A. & STANDAERT, D.G. 2009. Targets for neuroprotection in Parkinson's disease. *Biochimica et biophysica acta,* 1792: 676–687.

YUZLENKO, O. & KIEC-KONONOWICZ, K. 2009. Molecular modeling of A_1 and A_{2A} adenosine receptors: comparison of rhodopsin- and β_2-adrenergic-based homology models through the docking studies. *Journal of computational chemistry*, 30: 14–32.