Chapter 1

Introduction and Review of Literature
1.1. Parkinson’s Disease: An Overview

Parkinson’s disease (PD) is a neurological disorder named after Dr. James Parkinson, a London physician who reported the disease first time in 1817 in his report “Shaking Palsy”. PD is a neurodegenerative motor dysfunction caused due to loss of dopaminergic neurons in nigrostriatal pathways resulting in the reduction of striatal dopamine concentration. Generally, PD symptoms (e.g. tremor, rigidity, bradykinesia, and postural instability) arise after loss of 60% dopaminergic neurons in striatum. The annual prevalence rate of Parkinsonism has been estimated to be approximately 20.5 per 1,00,000 population in USA (Yokochi 1993), 80.6 per 1,00,000 population in Japan (Harada et al., 1983), and 57 per 1,00,000 population in China (Li et al., 1985). In India, no specific epidemiological data is available (Behari et al., 2002). Generally, the peak age of PD onset is between 55 and 65, however, the age of onset ranges from 20 to 80 years (Quinn et al., 1987; Yokochi 1993).

The specific etiology of the disease is yet not established, however, a number of factors such as environmental factors e.g., industrial chemicals, wood pulp mills, farming and exposure to pesticides, herbicides; exogenous toxins e.g., cyanide, lacquer thinner, organic solvents, carbon monoxide, and carbon disulfide; and endogenous toxins e.g., tetrahydroisoquinolines and β-carbolines, may increase the risk of developing PD (reviewed in Tanner & Langston 1990). However, no specific toxin has been found in the brain of PD patients. The most compelling evidence for an environmental factor in PD relates to the toxin 1,2,3,6-methyl-phenyl-tetrahydropyridine (MPTP), a byproduct of the illicit synthetic drug meperidine derivative, causing a syndrome markedly resembling to PD (Langston et al., 1983). MPTP induces toxicity in astrocytes through its conversion to the pyridinium ion (MPPC) by monooxidase type B (MAO-B) (Singer et al., 1987). MPPC causes a mitochondrial complex I defect in dopaminergic neurons similar to that found in PD (Nicklas et al., 1985), supporting the possibility that an environmental factor might cause PD.

Specific mutations in two different genes have been associated with a parkinsonian phenotype. The first of these “genetic parkinsonisms” was discovered as the result of
the identification of a large Italian family with a form of autosomal dominant Parkinsonism (Duvoisin and Golbe, 1995; Golbe et al., 1996). Linkage analysis in this family eventually led to the identification of a mutation in the encoding region for a protein known as α-synuclein (Polymeropoulos et al., 1997). A number of families of Greek origin are reported to have Ala53Thr mutation (Papadimitriou et al., 1999), however, other searches for the Ala53Thr mutation failed to identify additional cases, suggesting that Ala53Thr α-synuclein parkinsonism is extraordinarily rare (Chan et al., 1998a,b; Vaughan et al., 1998a,b; Farrer et al., 1998). A second missense mutation (Ala30Pro) in the same gene was identified in a German family with an autosomal dominant pattern of inheritance (Kru¨ger et al., 1988). Patients with “juvenile Parkinsonism” have disease onset before the age of 40 years, have a positive family history (Yamamura et al., 1973; Yokochi et al., 1984). Exon deletion mutations in a gene on chromosome 6, dubbed the”parkin”gene, have now been identified (Hattori et al., 1998; Kitada et al., 1998). Homozygous deletions and point mutations in the parkin gene have also been associated with early onset of Parkinsonism in European and North African families (Hattori et al., 1998). Another mutation has been associated in the form of missense mutation leu93Met in the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) gene located on chromosome 4p, observed in young-onset of PD, although their clinical characterization is limited (Leroy et al., 1998).

1.2. Pathophysiology of PD

The primary symptoms of Parkinson’s disease result from greatly reduced activity of dopamine-secreting cells caused by cell death in the pars compacta region of the substantia nigra (Obeso et al., 2008). Five major pathways in the brain connecting basal ganglia are motor, oculo-motor, associative, limbic and orbitofrontal and all are affected in PD exhibiting wide variety of malfunctions including movement, attention and learning (Obeso et al., 2008). The basal ganglia normally exert a constant inhibitory influence on the motor systems to generate high levels of dopamine, likely, to promote motor activity. Thus, the net effect of dopamine depletion is to produce hypokinesia, an overall reduction in motor output (Nagel et al., 2008; Obeso et al., 2008). Several
mechanisms of neurodegeneration have been reported (Davie et al., 2008; Obeso et al., 2010). The protein alpha-synuclein bound to ubiquitin in the damaged cells accumulates inside neurons forming inclusions called Lewy bodies (Schulz-Schaeffer et al., 2010). In patients with dementia, the Lewy bodies are formed in cortical areas. Proteosomal and lysosomal system dysfunction and reduced mitochondrial activity may cause neuronal cell death (Obeso et al., 2010). Iron accumulation in the substantia nigra in conjunction with the protein inclusions may be related to oxidative stress and protein aggregation causing neuronal death, however, the mechanisms are not fully understood (Hirsch et al., 2009).

1.3. Diagnosis and Treatment of Parkinson’s Disease

The characteristic features of PD are bradykinesia, rigidity and rest tremor. Early postural instability is suggestive of progressive supranuclear palsy (PSP) (Jankovic et al., 2008). Diffusion MRI technique, discriminates between typical and atypical Parkinsonism (Nicoletti et al., 2006, Paviour et al., 2007). Dopaminergic function in the basal ganglia can be measured with PET using radiotracer fluorodeoxyglucose ($^{18}$F) and SPECT using idoflupane ($^{123}$I) (DaTSCAN) and iometopane (Dopascan) radiotracers (David J. Brooks, 2009) to diagnose PD.

Treatment for PD can be divided into pharmacological, nonpharmacological, and surgical therapy for the management of PD (Waters et al., 1997). The pharmacological treatment of PD is symptomatic and do not reverse the course of the disease. The nonpharmacologic management includes education, support, exercise, and nutrition. The symptomatic medical therapy is determined by the degree of functional impairment (Rao et al., 2006). Levodopa (L-dopa) is the most effective drug for the symptomatic treatment of PD, which is combined with a peripheral decarboxylase inhibitor carbidopa or benserazide (Sinamet or Madopar) to block its conversion to dopamine in the systemic circulation and liver (before it crosses the blood-brain barrier) in order to prevent nausea, vomiting, and orthostatic hypotension. The common side effects of L-dopa therapy are nausea, somnolence, dizziness, and headache; however, serious
adverse reactions may include confusion, hallucinations, delusions, agitation, and psychosis. L-dopa treatment may induce moderate elevation in serum homocysteine levels, which may be associated with an increased risk of hip fractures in elderly patients. Selective monoamine oxidase (MAO) type B inhibitor (Selegiline, Eldepryl) also provides symptomatic relief in PD, and may have neuroprotective properties ([www.rxlist.com/eldepryl-drug/](http://www.rxlist.com/eldepryl-drug/)). Dopamine agonists (DAs) such as bromocriptine, pergolide, pramipexole, and ropinirole, are effective in patients with advanced PD complicated by motor fluctuations and dyskinesia. The pramipexole, ropinirole, transdermal rotigotine, and pergolide are effective as monotherapy in patients with early disease (Luthra et al., 2012). Adverse effects caused by dopamine agonists (DAs) are similar to those of levodopa. Peripheral edema with the chronic use of DAs is common, but rare in patients using levodopa alone. The catechol-O-methyl transferase (COMT) inhibitors tolcapone (Tasmar) and entacapone (Comtan) are useful as levodopa extenders (Rinne et al., 1998) and prolong and potentiate the levodopa effect. The most common side effects of tolcapone due to increased dopaminergic stimulation include dyskinesia, hallucinations, confusion, nausea, and orthostatic hypotension. The adverse effects are managed by lowering the dose of levodopa either before or after the addition of tolcapone. Centrally acting anticholinergic drugs such as trihexyphenidyl (Artane), benztropine (Cogentin) biperiden (Akineton), orphenadrine (Disipal), and procyclidine (Kemadrin) are used in PD (Poewe, 2002). Benztropine may increase the effect of dopamine by inhibiting its presynaptic reuptake.

Surgical therapies for the treatment of Parkinson’s disease (Mayers, 1951) were initiated in 1930’s. Thalamotomy is a useful treatment for tremor but has modest effect on bradykinesia or rigidity (Hassler and Riechert, 1954). Pallidotomy reduces rigidity, dyskinesia, and tremor (Wycis and Spiegel 1958), however, bilateral pallidotomy increases mortality. Furthermore, deep brain stimulation (DBS) procedure employed in the internal globus pallidus, subthalmic nucleus, and thalamus for the treatment of PD (Tasker, 1998) entails stereotactic implantation of electrodes into various subcortical structures (commonly the ventral intermediate nucleus of the thalamus, globus pallidus interna, and subthalmus nucleus) to persuade inhibition within these structures.
1.4. Role of Adenosine A\textsubscript{2A} Receptor in Parkinson Disease

Adenosine (an endogenous purine nucleoside) acts as versatile extracellular signal in variety of physiological and pathological conditions (Snyder, 1985) such as sleep and arousal, locomotion, nociception, seizure susceptibility, neuroprotection, drug addiction, and other vitally imperative processes (Ribeiro et al., 2002). Four mammalian adenosine receptor subtypes (A\textsubscript{1}R, A\textsubscript{2A}R, A\textsubscript{2B}R, and A\textsubscript{3}R) have been characterized (Fredholm et al., 2001). Adenosine A\textsubscript{2A} receptors (A\textsubscript{2A}R) co-localized with dopamine receptors (D\textsubscript{2}R) in striatum have emerged as promising non-dopaminergic target to control the motor impairment effect of PD (Chen and Schwarzschild, 2003).

The blockade of A\textsubscript{2A}Rs in striatopallidal neurons diminished postsynaptic effects of dopamine depletion and reduced the motor deficits of PD (Aoyama et al., 2000; Chen and Schwarzschild, 2003). A\textsubscript{2A}R antagonists inhibit basal ganglia pathway from striatum to thalamus via globus pallidus pars externa, subthalamic nucleus (STN), and internal pallidum and act through ‘indirect Pathway’ (Gerfen 1992). Selective A\textsubscript{2A}R-antagonists counteract depressant and cataleptic effects secondary to the genetic inactivation or pharmacological interruption of dopamine D\textsubscript{2} receptor (D\textsubscript{2}R) mediated neurotransmission (Ferre et al, 1993). The mechanism by which A\textsubscript{2A}R antagonist control motor impairment in Parkinson patient has been described in Figure 1. In the normal condition, inhibitory input from the striatonigral direct pathway and disinhibitory input along the striatopallidal indirect pathway are well balanced. Degeneration of nigrostriatal dopaminergic neurons depletes striatal dopamine in PD. The loss of striatal dopamine disinhibits striatal spiny projection neurons at the origin of the indirect pathway, which leads to a marked suppressed activity of the global pallidus leading to disinhibition of the subthalamic nucleus (STN). The resulting imbalance between the activity in the direct and indirect pathways leads to the alterations in the internal GP (GPi) and substantia nigra pars reticulata (SNr). Bradykinesia and akinesia in PD results from increased GABAergic inhibition of thalamic neurons, owing to excessive excitatory drive from the STN to the GPi and SNr. A\textsubscript{2A}R antagonists block the dual modulation of the striatopallidal medium spiny neurons by adenosine to restore the GP activity and relieves excessive excitatory drive from the STN to the GPi and
SNr, thereby normalizing the balance between the direct and indirect pathways (Schwarzschild, 2003).

![Fig. 1. Schematic presentation of the proposed mechanism of antiparkinsonian activity of adenosine A<sub>2A</sub> receptors (in figure as A<sub>2A</sub>) antagonists. (A) Normal condition, (B) Parkinson’s disease (PD), and (C) treatment with A<sub>2A</sub>R antagonists in PD.]

A<sub>2A</sub>R antagonists reversed Parkinsonian motor deficits in preclinical models without inducing or exacerbating dyskinesias in non-human primate models (Jenner et al., 2003) due to blockade of A<sub>2A</sub>R coexpressed with D<sub>2</sub>R in striatopallidal neurons, which inhibited the release of GABA in the globus pallidus, ultimately leading to enhanced motor function through the so-called indirect motor pathway of the basal ganglia (Mori et al., 1996; Hauser et al., 2003). Epidemiological data linking the consumption of caffeine (a non-specific adenosine antagonist) with a reduced risk of developing PD have demonstrated that caffeine and more specific A<sub>2A</sub>R antagonists protect against dopaminergic neuronal toxicity in vivo (Chen et al., 2001), suggesting that A<sub>2A</sub>R antagonists possess neuroprotective properties. A<sub>2A</sub>R form functional heteromeric receptor complexes with other G-protein-coupled receptors such as D<sub>2</sub>R and the mGlu5 receptor (Fuxe et al., 2003; Prakash and Luthra 2012). Action of A<sub>2A</sub>R and mGlu5 blockade synergistically reverses Parkinsonian deficits in rodents (Kachroo et al., 2005). Therefore, A<sub>2A</sub>R antagonists may be explored as promising treatments for Parkinson diseases in future.
1.5. Therapeutic Potential of Adenosine A$_{2A}$ Receptors in Parkinson’s Disease

Caffeine, a xanthine derivative and its metabolites such as theophylline, paraxanthine and theobromine, counteracted the locomotor depression persuaded by adenosine analogs, however, showed almost similar potency with AR-subtypes (A$_1$R, A$_{2A}$R, A$_{2B}$R, A$_3$R) (Fredholm et al., 1997; Snyder et al., 1981). At low concentrations, the effect of caffeine on locomotion is biphasic, however acts as a motor depressant at higher concentrations (Holtzman and Finn, 1988). Furthermore, the role of A$_{2A}$R in caffeine-mediated hyperlocomotion has been confirmed from transgenic mice lacking A$_{2A}$ receptors (Ledent et al., 1997). Endogenous adenosine acting at A$_{2A}$R is involved in neuroleptics such as haloperidol-induced catalepsy. Theophylline or caffeine alone reversed haloperidol-induced catalepsy (Hauber and Muenkle, 1996; Waldeck, 1973). Caffeine also accelerates the effect of bromocriptine-induced locomotion in reserpine-treated mice (Ferre et al., 1994). Furthermore, theophylline potentiates the anticonvulsant actions of N-methyl-D-aspartate (NMDA) receptor antagonists in haloperidol-treated animals, but does not potentiate the anticonvulsant effect of NMDA receptor antagonists on reserpine-induced catalepsy (Hauber and Muenkle, 1996). Due to non-selective nature of natural xanthines, xanthine derivatives were developed. Despite a low bioavailability (3.6%), oral administration of the A$_{2A}$R antagonist KF 17837(8-(3,4-dimethoxyphenyl)-7-methyl-1,3-dipropylpurine-2,6-dione) significantly decreases catalepsy induced by haloperidol in mice (Kanda et al., 1994). Xanthine derivative, KW-6002 was found to have 90 times more effective than KF 17837 to reverse the catalepsy induced by haloperidol or reserpine (Shimada et al., 1997). Intraperitoneal administration of CSC, a moderately A$_{2A}$R selective antagonist, also counteracted haloperidol and raclopride-induced catalepsy (Ward and Dorsa, 1999). The bilateral intrastratal injections of MSX-3(1-propargy-8-styrylxanthine), A$_{2A}$R antagonist prodrug, reversed the catalepsy induced by systemic administration of a D$_2$R as well as D$_1$R antagonist (Hauber et al., 1998) concluding that A$_{2A}$R antagonists potentiate both D$_1$ and D$_2$ receptor-induced contralateral rotation in animals with unilateral 6-OHDA lesions of the meso cortico frontal brain (MFB). In laboratory animals, A$_{2A}$R agonists with neuroleptics reduce spontaneous locomotion at lower concentrations than those needed to induce ataxia or catalepsy (Rimondini et al., 1997).
CV-1808 (2-Phenylaminoadenosine), an adenosine receptor agonist with selectivity for A$_{2A}$R showed that apomorphine-induced cage climbing was inhibited at doses that caused ataxia (Heffner et al., 1989). However, CGS 21680 was found to be more effective in counteracting apomorphine-induced climbing than in inducing catalepsy (Kafka and Corbett, 1996). Explicitly, A$_{2A}$ antagonists augment the effects of L-dopa (Fenu et al., 1997) and selective dopamine receptor agonists (Pinna et al., 1996) in models of PD. The studies have demonstrated that pretreatment, for longer periods of time, with adenosine receptor antagonists may protect against post-ischemic brain damage (Rudolphi et al., 1992). This protective effect was initially attributed to A$_1$R antagonism, however, administration of SCH 58261, a A$_{2A}$R antagonist showed neuroprotective effect, whereas the A$_1$R antagonist DPCPX was unsuccessful (Bona et al., 1997) indicating the imperative role of A$_{2A}$R in neuroprotection.

Indeed, the locomotor depressive effect of systemic, intracerebroventral (i.c.v.) or intrastriatal administration of the non-selective adenosine receptor agonist NECA was more effective than that for adenosine and selective A$_1$ receptor agonists (Durcan and Morgan, 1989). Thus, a potent locomotor depressant action of systemic, intrastriatal or intraaccumbal administration of CGS 21680(4-[2-[[6-Amino-9-(N-ethyl-β-D-ribofuranuronamidosyl)-9H-purin-2-yl]amino]ethyl]benzene propanoic acid ) has been exposed (Rimondini et al., 1997). This effect of CGS 21680 is completely abolished in mice pretreated with intrastraiatal injections with antisense oligonucleotides directed against A$_{2A}$R mRNA and in transgenic animals lacking functional A$_{2A}$R (Ledent et al., 1997). These studies evidence that A$_{2A}$R are involved in locomotor behavior. Several reports demonstrated that selective A$_{2A}$R antagonists provoke a mild hyperlocomotion in rodents (Popoli et al., 1998) and primates (Kanda et al., 1998).

1.6. Binding Site Cavity of Adenosine A$_{2A}$ Receptor Antagonist

The docking analysis as well as crystal structure of A$_{2A}$R co-crystalized with ZM241385 showed A$_{2A}$R active site extended longitudinal (14 Å) from TMs (TM3, 5, 6) to extracellular loops comprising two hydrophobic ends oriented towards TM2, TM3, TM5 and TM6 along with ECL2 (Jaakola et al., 2008). Lower hydrophobic
domain composed of residues A63, A81, V84, L85, T88, W246, L249 and H250 deeply embedded in TMs where as upper hydrophobic domain composed of residues I66, L167 (TM2) and F168 (ECL2) was oriented towards exposed membrane (Fig 2). The middle region of active site residues M177, N181, N253 were involved in polar interaction with antagonists. The polar interactions were also shown with E169 (ECL2) of upper domain and H250 (TM6) of lower domain (Prakash et al., 2012).

Fig. 2. (a) Backbone representation of the structure of human A2AAR-T4 lysozyme fusion protein with ZM241385 bound (PDB ID: 3EML). The missing part of extracellular loop 2 is modeled onto the structure (beginning and ending points are indicated by the dotted red line, (Jaakola et al., 2008) and “dummy” atoms (blue-colored dummy atoms in the cytoplasmic region and red-colored dummy atoms at the extracellular site). The receptor is colored blue at the amino terminus and changes gradually to red at the carboxyl terminus. Lipid, ligand, and sulfate ions are shown as stick models, and their polar interactions are shown as thin blue lines. Crystallographic waters in the binding cavity are shown as red balls. (b) ZM241385 docked with A2AAR (Cartoon view at Pymol). Important active site residues were labeled, polar interaction shown with yellow dots, water molecules present in the active site red dots shown with red dots (Luthra et al., 2009)

The predicted docking poses for A2AAR-selective antagonists suggest that the chemically diverse compounds bind similar to the A2AAR antagonist ZM241385 in the crystal structure (Jaakola et al., 2008). The core interaction for all ligands involved aromatic stacking with the conserved F168 of the receptor and additional hydrophobic interactions with the conserved I274 and L249 side chains. Strong polar interactions were formed with the side-chain of the conserved N253, and the role of the hydrogen bond donor was observed in high-affinity ligands possessing exocyclic amine group, with a notable exception of methylxanthine analogues (KW6002). The exocyclic amine group also forms a hydrogen bond to E169 side chain in the EL2 in subtypes A2AAR.
A$_{2B}$R and A$_1$R, but V169 in the A$_3$R. Most of the A$_2$A ligands have an acceptor for H-bonding with N253 amide donor (Luthra at al., 2009; Mishra et al., 2010). The aminoacid residues involved in interaction with A$_2$A receptor antagonist with their corresponding pharmacophoric features are shown in Table 1.

Table 1. Pharmacophoric features of A$_2$A active site residues

<table>
<thead>
<tr>
<th>HBD</th>
<th>HBAL</th>
<th>Hydrophobic aromatic</th>
<th>Ring aromatic</th>
<th>Posionizable</th>
</tr>
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</table>

A$_2$A active site residues categorized as pharmacophore features. HBD: Hydrogen Bond Donor, HBAL: Hydrogen Bond Acceptor Lipophilic.

1.7. Journey to the Development of Adenosine A$_{2A}$ Receptor Antagonist

In brief, the initial development of A$_2$A antagonist could be categorized in two groups:

(I) Xanthine derivatives
(II) Non-xanthine derivatives

(I) Xanthine Derivatives

The alkaloid caffeine (1), 1,3,7-trimethylxanthine, mediates its pharmacological actions by a blockade of A$_1$R, A$_2$A and A$_{2B}$R. The first “selective” A$_2$A antagonist was the caffeine analog, 7-dimethyl-1-propar-gylxanthine (DMPX, 2) (Seale et al., 1988). Like caffeine, the compound possesses low A$_{2A}$R affinity and moderate selectivity versus A$_1$R. The 8-phenyl-1,3-dipropylxanthine derivative (XAC 3) was the first highly potent A$_{2A}$R antagonist exhibiting low affinity, however, the compound is only moderately selective in humans (Jacobson et al., 1986). N7-methylation in 8-substituted xanthine derivatives was better tolerated by the A$_{2A}$R than the A$_1$R (Shamim et al., 1989) and that the 8-substituent had to be coplanar for achieving high A$_{2A}$R receptor affinity (Erickson et al., 1991; Müller et al., 1997), led to the first highly potent and selective A$_{2A}$ receptor antagonist, the xanthine derivatives CSC (4) followed KW-6002 (istradefylline, 5) (Jacobson et al., 1993).
Xanthine compounds showed low potency, nonselectivity for A1R and A2AR and nonspecific actions including phosphodiesterase inhibition and calcium mobilization (Daly, 1982; Fredholm and Persson, 1982; Rall, 1985; de Gubareff and Sleator, 1965). Therefore, the structural types with potent adenosine antagonist activity without phosphodiesterase inhibiting properties were explored. Some non-xanthine heterocycles like alloxazine, a benzo[g]pteridine, 9-methyladenine, pyrazolo [3,4-b]pyridinesetazolate, cartazolate, and tracazolate, pyrazolo[3,4-d]pyrimidines, particularly DJB-KK,15J6 and certain pyrazolo- [4,3-d]pyrimidin-7-ones showed mild A2AR receptor antagonist activity (Francis et al., 1988).

(i) Tricyclic Series

The discovery of CGS8216 (6) in John E. Francis laboratories as a potent benzodiazepine receptor led to the screening of other tricyclic heterocyclic structures. In 1979, Phillis and co-workers reported that theophylline, an adenosine antagonist,
antagonized the depressant action of flunitrazepam on cerebral cortical neurons in rats. Other investigators reported that theophylline and other xanthines block diazepam binding sites in brain tissue. Furthermore, inosine, an adenosine metabolite interacted with brain benzodiazepine receptor. These observations led to the suggestion that benzodiazepines and adenosine depress central neurons by acting at the same receptor. A comparison of CGS 8216 and theophylline in the adenosine-stimulated adenylate cyclase system present in guinea pig synaptoneurosom revealed that CGS 8216 indeed blocked adenosine activation more potently than theophylline. Subsequently, the triazoloquinazoline structure CGS 15943 (7) was discovered to be more potent than any adenosine antagonist reported at that time (January, 1983). It was approximately 500 times as active as theophylline and 250 times as potent as CGS 8216.

\[
\begin{align*}
\text{CGS8261} & \quad \text{CGS15943} \\
6 & \quad 7
\end{align*}
\]

\[
\begin{align*}
A_{2A}R & \text{Ki} = 1.2\text{nM} \\
A_1R & \text{Ki} = 6.4\text{nM}
\end{align*}
\]

(a) Pyrazolo-triazolo-pyrimidine Derivatives

Several modifications in CGS 15943 have been made by Gatta et.al in 1993, without any outcome (Gatta et.al in 1993). Baraldi et al replaced benzene ring of CGS15943 with substituted pyrazole ring which led to give pyrazolo-triazolo-pyrimidine derivatives. The compound with N7 phenyl ethyl side chain was found to be more potent and selective than CGS15943 and named as SCH58261 (8), a first potent and selective non-xanthine $A_{2A}$R antagonist (Baraldi et al., 1994). SCH-58261 showed efficacy in rodent models of PD after intraperitoneal (i.p.) administration, but suffered from poor solubility and oral inactivity.
Several SCH58261 derivatives have been reported and N7 position of SCH58261 substituted by 4-hydroxy phenyl propyl side chain (9) found to be more potent than parent compound. This compound showed Ki value 0.94 nM and 787 fold selectivity over A1R (Baraldi et al 1998). Neustadt et. al (2007) replaced phenyl ethyl side chain of SCH 58261 with an aryl-piperazine side chain, which led to discovery of more potent compound (10) SCH-412348 possessing high A2A R affinity and selectivity (>1600-fold) versus A1R.

Although, SCH-412348 reversed haloperidol-induced catalepsy in rats at 1 mg/kg dose, but compound showed poor aqueous solubility. The methoxy ethoxy analogue 11 (SCH-420814, preladenant), which showed high binding affinity for A2A R with good selectivity (1340-fold) over A1R with improved solubility. Preladenant (1 mg/kg) reversed haloperidol-induced catalepsy in rats and was characterized in multiple preclinical animal models of PD. Preladenant has completed phase II clinical trials for PD, and Merck (Schering-Plough, 2008) is processing several phase III trials for the compound (clinicaltrials.gov identifier: NCT01155479, NCT01227265, NCT01155466, NCT01294800, NCT01215227, NCT01323855).
A variety of fused heterocyclic derivatives of the SCH 58261 were prepared (Shah et al., 2008) with comparable $A_{2A}$ binding affinities to the SCH 58261 and preladen, found to be much less selective against the $A_1$R. The tetrahydrosulquinoline derivative 14 of this series showed excellent binding affinity and was 68-fold selective versus $A_1$R.

![Diagram of 14]

$A_{2A}$ $K_i = 2.5$ nM  
$A_1$ $K_i = 170$ nM

N8 substitution induced increase in the affinity with $A_{2A}$ but reduced selectivity over $A_1$R. Various N8 substituted derivatives of SCH58261 were synthesized, however, the compounds suffered with lower selectivity over $A_1$R (compounds 15 and 16) (Baraldi et al., 1994). Generally, the substitution at N7 position was more beneficial to achieve $A_{2A}$R antagonist selectivity.

![Diagrams of 15, 16, and 17]

$A_{2A}$ $K_i = 2.4$ nM  
$A_{2A}$ $K_i = 1.9$ nM  
$A_{2A}$ $K_i = 1.4$ nM  
$A_1$ $K_i = 30.4$ nM  
$A_1$ $K_i = 5.6$ nM  
$A_1$ $K_i = 7.4$ nM

The introduction of a substituent at the 9-position instead of a hydrogen leads to a loss of selectivity, but the receptor affinity is maintained. Like replacing hydrogen with methylthio or ethylamino at 9-position causes a significant loss of selectivity (Baraldi et al., 2003) (compounds 18, 19, 20).
A number of reports described that the amino group was necessary for receptor interaction with slight modifications (Baraldi et al., 2003; Francis et al., 1988; Gatta et al., 1993; Ongini et al., 2001). Compound 21 which has a free amino group at the 5-position, shows good affinity for the A\textsubscript{2A}R but, unfortunately, low selectivity. Transformation of the amino group into urea preserves A\textsubscript{2A}R affinity (compound 22), however, transformation of amino group into amide (Compound 23) decreases activity as well as selectivity. (Baraldi et al., 2002a).

In general, it has been observed that substitution of the furanyl moiety with phenyl/substituted phenyl ring causes a complete loss of affinity at the A\textsubscript{2A}R (compound 24, 25, 26) probably due to an increased steric hindrance by the aromatic rings (Francis et al., 1988; Gatta et al., 1993; Baraldi et al., 1999; Bolcato et al., 2008) and supported that the furanyl ring at the 2-position of the tricyclic structure is a necessary element for A\textsubscript{2A}R antagonists activity of the molecule, because oxygen atom produces a favorable electronic condition for the interaction with the adenosine receptor.
(b) Triazolo-triazolo-pyrimidine Derivatives

Pyrazolo ring of SCH58261 replaced by triazole ring led to decrease in affinity as well as selectivity towards $A_{2A}R$ compound 27, 28, 29 (Baraldi et al., 1994). The pyrazole ring was found to be more favorable than triazole ring for $A_{2A}R$ affinity as well as selectivity.

(c) Pyrolo-triazolo-pyrimidine Derivatives

Further modification of SCH 58261 has been carried by Baraldi et al., in 2012, and pyrazolo ring of SCH 58261 was replaced by pyrole ring. The compound 30 as a representative of the pyrolo-traizolo-pyrimidine derivatives showed low affinity as well as selectivity towards $A_{2A}R$ receptor as compared to SCH 58261 (Baraldi et al., 2012).
(d) Imidazolo-triazolo-pyrimidine Derivatives

Replacing the pyrazole ring of SCH58261 with an imidazole ring by Silverman et al in 2007 gave a series of 3H-[1,2,4]-triazolo[5,1-i]purin-5-amines. Compound 31 of this series contain aryl piperazine substituents which possessed good affinities for A\textsubscript{2A}R but reduced selectivity versus A\textsubscript{1}R (Silverman et al., 2007).

![Compound 31](image)

\begin{align*}
A_{2A}R \text{ Ki} &= 0.9nM \\
A_1 \text{ Ki} &= 602nM
\end{align*}

(e) Arylidenopyrimidines Derivatives

Methylene amine substituted arylidenopyrimidines were reported as dual A\textsubscript{2A}R/A\textsubscript{1}R antagonists (Shook et al., 2010). Compound 32 was the original lead compound that was potent in both A\textsubscript{2A}R and A\textsubscript{1}R functional assays and in the haloperidol-induced catalepsy model in mice, however, suffered from poor solubility, and was Ames positive. A variety of heterocyclic furan replacements were prepared that generally maintained good in vitro potency but lost in vivo activity. Replacing the furan with phenyl compound 33, showed good in vitro potency and comparable in vivo activity (ED50=8.0 mg/kg, p.o.) reversing catalepsy in mice. Compound 33 was negative in the Ames screen, but suffered from poor solubility. A variety of amines were incorporated at the 9-position of the scaffold to increase solubility and resulted in the synthesis of 34 that had good in vitro potency and had an ED50 of 3.8 mg/kg, p.o. in the mouse catalepsy model. Moving the pyrrolidine to the 8-position compound 35, showed equipotency with A\textsubscript{2A}R and was more potent for A\textsubscript{1}R, having significant increase in vivo (ED50= 0.2 mg/kg, p.o.) activity, in the mouse catalepsy model (Shook et al., 2010).
Further, evaluation revealed that metabolism of 35 resulted in the formation of reactive metabolites that were attributed to adverse events after the 28-day in toxicological studies in nonhuman primates (Shook et al., 2010). Oxidative metabolism was occurring on the pyrrolidine ring and at the benzylic methylene. Compound 36 is a representative amide that had good functional activity at both A2AR and A1R and reversed haloperidol-induced catalepsy in mice (ED50= 0.4 mg/kg, p.o.). The ether linked compound 37 was very potent in vivo with an ED50< 0.1 mg/kg, p.o. in the mouse catalepsy model (Shook et al., 2010).

(f) Pyrazolo-trizolo-pyrimidon-3-one Derivatives

Another tricyclic system pyrazolo-[4,3-e]-1,2,4-triazolo[4,3-c]pyrimidine-3-one have been developed by Joel et al. in 2011. The ethylene dimethyl amino analogue 38 showed good binding affinity for A2AR and is 269-fold selective versus A1R. In general, analogues of 38 exhibited good binding affinities but could not able to reverse haloperidol-induced catalepsy in rat.
However, tricyclic scaffold suffered from several drawbacks such as low water solubility, high molecular weight as well as low pharmacokinetic profile. These drawbacks led to develop bicyclic scaffold as a potential $A_2A$R receptor antagonist.

(ii) Bicyclic series

(a) Triazolo-triazin Derivatives

A novel $A_2A$R antagonist 4-(2-[7-amino-2-(2-furyl)-[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl) phenol, (ZM-241385) $39$ (Poucher et al., 1995) showed high affinity ($k_i$ value 0.8nM) and selectivity ($A_2A$R/$A_1$R = 280) for human $A_2A$R and exhibited protection against neuronal death produced by ischaemia or excitotoxicity. Currently, ZM241385 is used as tool in pharmacological studies.

![Chemical structures](image)

$A_2A$Ki= 0.8nM
$A_1$Ki= 225nM

$A_2A$Ki= 0.2nM
$A_1$Ki= 3300nM

A variety of compounds bearing ethanamine, ethylenediamine, pyrrolidine, piperazine and (S)-octahydro-1H-pyrido[1,2-a]pyrazine in the side chain have been synthesized based on ZM-241385. Peng et al. (2004) incorporated (S)-octahydro-1H-pyrido[1,2-a]pyrazine moiety between the triazolo-triazine nucleus and aromatic ring leading to the development of highly potent and selective $A_2A$R antagonists ($40-42$). The SAR studies revealed that substituted aryl fluorides are better $A_2A$R antagonists. In particular, 3-
fluorophenyl rendered the most potent $A_2\alpha$ antagonist, with greater than 16500-fold selectivity over the $A_1\beta$ (Peng et al., 2004). Furthermore, 6-quinolinyl moiety afforded potent $A_2\alpha$ binding with 5833-fold selectivity over $A_1\beta$, presumably due in part to the electron-withdrawing effect of the nitrogen atom in the 6-quinolinyl group, suggesting that the bulky groups are well-accommodated at the 7-cis-position. Similarly, 3-pyridyl group at the 7-cis-position exhibited better $A_2\alpha$ potency and 4666-fold selectivity over $A_1\beta$.

A number of [1,2,4]triazolo[1,5-a][1,3,5]triazine derivatives have been synthesized by Vu et al. (2004, 2005) as potent and selective $A_2\alpha$ antagonists incorporating a variety of diamines at C7 (compound 43). SAR of piperazine derivatives AR showed (a) Some form of capping group on piperazine nitrogen was needed for $A_2\alpha$ antagonistic activity; when a phenyl or heterocyclic group was installed as a capping group, the $A_2\alpha$ affinity was improved (compounds 44, 45), (b) the electron-withdrawing groups such as chloro and fluoro were more favorable than electron-donating groups such as OMe, on the phenyl ring (compound 46), (c) substituting fluoro for chloro was better for $A_2\alpha$ activity, and fluoro-trisubstitution on the phenyl ring gave potent and selective $A_2\alpha$ antagonist (compound 47, Vu et al., 2004). Similar trisubstitution on the phenyl ring of compound 48 also proved to be beneficial for $A_2\alpha$ affinity (Vu et al., 2005).
(b) Imidazolo-pyrimidine Derivatives

Cristalli, Klotz and colleagues in 1998 studied the structure activity relationships of series of adenine derivatives as adenosine receptor antagonists (Klotz et al., 2003). 8-ethoxy-9-ethyladenine (ANR-94, 49) with low molecular weight was found to exhibit good \( A_{2A} \) receptor affinity and selectivity (Pinna et al., 2005). 2-alkynyl-substituted adenine derivatives based on structures, initially introduced by Cristalli, were patented in Adenosine Therapeutics (Beauyan et al., 2005). The compound ATL-2 (50) of this series was the most potent \( A_{2A} \) receptor antagonist exhibiting high \( A_{2A} \) receptor affinity (\( K_i \) 0.95 nM)( Yan et al 2003). Adenine derivatives substituted at the 8-position by 1, 2, 3-triazole ring were developed as potent \( A_{2A} \) antagonists by Minetti et al in 2005. One of the most potent compounds was 9-methyl-2-phenethyl-8-[1, 2, 3] triazol-2-yl-adenine (51).

![Chemical structures of 49, 50, and 51](image)

(c) Pyrazolo-pyrimidine Derivatives

Chebib et al reported Pyrazolo pyrimidine derivatives in 2000 and SAR study of these derivatives suggested that substitutions at the 1 position, and aromatic ring attached to the nitrogen is essential for both affinity and selectivity at the \( A_{2A} \)Rs. The introduction of benzyl group led to 52, which proved to be a quite potent and selective \( A_{2A} \) antagonist who retained in vivo activity (Chebib et al., 2000; Gillespie et al., 2008)). Extension of the linker between the phenyl ring and pyrazole by one methylene group was detrimental to \( A_{2A} \)R potency, but further extension led to increase in \( A_{2A} \)R potency at the expense of \( A_1 \)R selectivity (compound 53).
(d) Naphthyridines Derivatives

1,8-Naphthyridines reported by Ferrarini et al. (2000, 2004) showed that a large part of the new 1,8-naphthyridine derivatives proved to be bovine $A_{1}$R selective, with a high affinity in the low nanomolar range. The affinity for the bovine and human $A_{2A}$R, 1,8-naphthyridine derivatives generally possess a moderate affinity. However, compound 54 synthesized by Manera et al. (2005) was found to possess better binding profile with $A_{2A}$R ($K_i = 35$ nM) and selectivity over $A_{1}$R in Naphthyridine series.

![Chemical structures](image)

A$_{2A}$ $K_i$ = 3 nM
A$_{1}$ $K_i$ = 468 nM

(e) Thieno-pyrimidine Derivatives

Thieno-pyrimidine derivatives developed by Gillespie et al. in 2001-2008 revealed that a reasonable affinity for $A_{2A}$R was exhibited by thieno [3,2-d]pyrimidine derivatives with small alkyl and alkylamino substituents at C-2 (Gillespie et al., 2001, 2004, 2008a). Furthermore, from SAR investigation of thienopyrimidines, it was observed that 2-thiazolyl group offered a significant advantage over other heteroaryl groups in the 4-position to give number of highly potent and selective analogues. Moreover, with a 2-thiazolyl substituent in place at the C-4 position, a wide range of C-2 substituents are tolerated and provide a series of highly potent and selective $A_{2A}$R antagonists. $A_{2A}$R affinity and selectivity over $A_{1}$R was better when the C-2 substituent was a small lipophilic group such as alkyl or dialkylamino as in compounds 55-58 (Gillespie et al., 2008b).
(f) Benzothiazole Derivatives

Novel series of 4-methoxy-substituted benzothiazole derivatives (compound 59, 60) as potent and selective $A_{2A}$R antagonists were developed (Alanine et al., 2001, 2006). 4-Morpholino-benzothiazole based series of compounds were patented by Hoffmann-La Roche. The imidazole derivatives 61 showed good binding affinities for $A_{2A}$, but the $A_{1}$R activity was not reported (Flohr et al., 2005, 2006). Further optimization of this scaffold led to the urea compound 62 which had good potency for $A_{2A}$R and was 270-fold and $>4000$-fold selective versus $A_{1}$R in binding and functional assays, respectively (Compound 62 had an ED50 of 0.5 mg/kg when dosed orally in the APEC-induced hypo-locomotion model in rats). In January 2007, Synosia acquired the rights from Roche to develop 62 (SYN-115) for the treatment of PD. SYN-115 successfully completed phase II clinical trial and is currently carrying phase II/III clinical trials for PD (clinicaltrials.gov identifier:NCT01283594) (Synosis Therapeutics. 2007).
(g) Triazolo-pyrazine Derivatives

Dowling et al reported [1,2,4]Triazolo[1,5-a]pyrazine derivatives in 2005 as A\textsubscript{2A}R antagonist. Substitution with N,N-diethylamide compound 63, could enhance the A\textsubscript{2A}R affinity but concomitantly conferred significant potency toward the A\textsubscript{1}R. Compound 64, which incorporates the amino-ethylphenol unit exhibited high A\textsubscript{2A}R affinity and impressive selectivity (300-fold) against the A\textsubscript{1}R (Dowling et al., 2005). SAR study was presented by Yao et al in same year (2005) for series of alkyne derivatives of triazolopyrazine as A\textsubscript{2A}R antagonist compound 65. The results indicated that presence of alkyl or aryl groups on the terminus of the acetylene side chain showed better affinity (compound 65, A\textsubscript{2A}R, 1.1 nM) and selectivity (A\textsubscript{1}R/A\textsubscript{2A}R=100) for the adenosine A\textsubscript{2A}R.

![Chemical structures of compounds 63, 64, and 65](image)

A\textsubscript{2A}Ki= 1nM  A\textsubscript{2A}Ki= 1 nM  A\textsubscript{2A}Ki= 1.1nM
A\textsubscript{1}Ki= 41nM  A\textsubscript{1}Ki= 320 nM  A\textsubscript{1}Ki= 100nM

(h) Triazolo-pyrimidine Derivatives

Another [1,2,4]triazolo[1,5-c]pyrimidine derivatives have been reported by Matasi et al. in 2005. SAR study of these derivatives point out that furan ring and free amino function at position 2 and 5 respectively are essential for A\textsubscript{2A}R antagonistic activity. Variety of aromatic and heteroaromatic substituents were placed at C-7 and the results were summarized as: (a) The tolyl analogs retained the affinity for A\textsubscript{2A}R but failed to improve desired selectivity; (b) the methoxy phenyl analogs provided compounds with high affinity for A\textsubscript{2A}R with moderate to good selectivity over A\textsubscript{1}R, Compound 66. (c) heteroaromatic analogs also retained single digit nanomolar potency for A\textsubscript{2A}R with no improvement in selectivity over the A\textsubscript{1}R (compound 67) (d) meta-substituted compounds retained single digit nanomolar A\textsubscript{2A}R affinity and acceptable selectivity over A\textsubscript{1}R; particularly compound 68, which was 135-fold selective.
A series of triazolo-9H-purines was reported as potent A2A R antagonists having modest selectivity versus A1R (Minetti et al., 2005). Compound with phenyl ethyl side chain 69 and having butyl side chain 70 (ST-1535) showed promising activity in the entire series developed by Sigma Tau (Minetti et al., 2005). ST-1535 characterized extensively in various animal models of PD showed minimum effective doses of 5.0 and 1.25 mg/kg in hypolocomotion and haloperidol-induced catalepsy models in mice, respectively (Stasi et al., 2006). Further studies show that ST-1535 potentiated L-dopa activity in 6-OHDA lesioned rats. In the MPTP-treated marmosets, ST-1535 at 40 mg/kg significantly increased locomotor activity but did not improve motor disability. ST-1535 is currently under active development in phase I clinical trials by Sigma Tau.

(i) Purines Derivatives

Holschbach et al. (2006) synthesized a series of oxazolo[5,4-d]pyrimidines, where compounds 71 and 72 were observed as the most A2AR-selective derivatives of the series. Furthermore, the compound 71 was synthesized in [3H]-labeled form to carry Position Emission Tomography (PET). Radioligand binding studies showed a high
degree of non-specific binding rendering the compound unsuitable as a potential ligand for PET after labeling with a neutron-deficient nuclide (Holschbach et al., 2006).

(k) Triazolo-pyrimidine Derivatives

Benzyl substituted triazolo[4,5-d]pyrimidines were synthesized by Gillespie et al. in 2009 to evaluate their A$_{2A}$ R antagonist potential. Compound 73 had potent A$_{2A}$R activity and exhibited moderate selectivity versus A$_1$R. A variety of substituted benzyl analogues were prepared which showed good A$_{2A}$R activity in vitro, however could not reverse haloperidol-induced hypo locomotion when dosed orally, which was attributed to low solubility and poor PK profiles and led to the synthesis of heterocyclic compounds such as the 2-pyridyl analogue 74. Despite good in vitro activity and increased solubility, 74 were not active in vivo at 30 mg/kg, p.o. Further optimization of this scaffold led to the discovery of vipadenant 75 (V2006/BIIB-014) which was evaluated in phase II clinical trials. Vipadenant showed excellent binding affinity for A$_{2A}$R and had modest selectivity against A$_1$R (Gillespie et al., 2009).
(iii) Monocyclic Derivatives

Developed bicyclic scaffold could not overcome the drawbacks present in tricyclic scaffold like low water solubility, low pharmacokinetic profile. Moreover tricyclic and bicyclic scaffold suffer from multi step synthetic strategy with low yield, which is major obstruction to bring a drug from laboratory bench to market (Luisi et al., 2011). These drawbacks illustrated to gaze for monocyclic scaffold as $A_{2A}\text{R}$ antagonists and attention for development of the compounds with simple synthetic process, high yield, better physicochemical and pharmacokinetic profile.

(a) Triazole Derivatives

Alanine et al. synthesized a series of monocyclic 1,2,4-triazole derivatives as $A_{2A}\text{R}$ antagonists in 2004. The initial SAR showed that the m-methoxy phenyl moiety is important for affinity with $A_{2A}\text{R}$ (compound 76, 77). The benzyl moiety afforded more scope for variation with hydrophobic substituents and introduction of a polar group dramatically abolished binding activity. The compounds 76 showed satisfactory aqueous solubility and compound exhibited good permeability tested with artificial membranes (Alanine et al., 2004).

\[
\begin{align*}
76 & \quad \text{A}_{2A} \text{Ki} = 20 \text{nM} \\
& \quad \text{A}_1 \text{Ki} = 1380 \text{nM}
\end{align*}
\]

\[
\begin{align*}
77 & \quad \text{A}_{2A} \text{Ki} = 10 \text{nM} \\
& \quad \text{A}_1 \text{Ki} = 70 \text{nM}
\end{align*}
\]

(b) Pyrazine Derivatives

Aminopyrazine based series was reported by Yonishi et al 2005 as dual $A_{2A}/A_1\text{R}$ antagonists. Compound 78 had potent affinity for both $A_{2A}\text{R}$ and $A_1\text{R}$ and significantly reversed haloperidol-induced catalepsy in mice. Further optimization of the pyrazine scaffold led to the identification of 79 (ASP-5854) that has been characterized extensively in numerous animal models of PD and cognition (Mihara et al., 2007). ASP-5854 reversed CGS21680-induced and haloperidol-induced catalepsy in mice.
SAR study of this series demonstrated that removal of cyano group from pyrazine core increased the affinity with A2AR receptor but reduced selectivity over A1R. However, all compounds of this series lacked selectivity towards A1R (Yonishi et al., 2005).

(c) Pyrimidine Derivatives

A series of trisubstituted pyrimidines was reported to have potent A2AR activity (Zhang et al., 2008). Compound 80 was a potent A2AR antagonist, but it lacked the desired selectivity versus A1R and had poor metabolic stability that was attributed to the unsubstituted furan moiety. SAR studies resulted in the synthesis of the thiazole substituted compounds 81 and 82 that maintained potency for A2AR with increase in selectivity over A1R. Further optimized by replacing the thiazole ring with the dimethylpyrazole ring to give compound 83 that was very potent for A2AR and had excellent selectivity versus A1R. Despite the increase in selectivity, compound 83 suffered from poor solubility, so basic amines were appended to the phenol ring to increase solubility and improve PK properties. A number of the amino alkyl compounds had good binding affinities for A2AR and good selectivity versus A1R, but had significant hERG liabilities. Compound 84 had the best hERG profile (patch clamp IC50 = 1.2µM) and showed oral activity in the haloperidol-induced catalepsy in rats (Zhang et al., 2008).
Compound 85 had a high affinity for A2AR and was 99-fold selective versus A1R (Slee et al., 2008). However, replacement of furyl group of compound 85 with methyl substituted furyl reduced 6 fold affinity towards A2AR with enhance selectivity over A1R (compound 86). Further modification of compound 85 was carried by replacing furyl ring with thiazole ring to give compound 87 (Ki = 9nM, A2AR/A1R = 1998). Analogues of pyrimidine-4-carboxamides have been reported to display good potency for A2AR and good selectivity over other AR-subtypes (Gillespie et al., 2009). Compound 88 having free amino group found to be more potent compared to other compound in this series.
(d) Thiazole Derivatives

A series of substituted 2-amino-5-benzoyl-4-(2-furyl) thiazoles reported by Cole et al., (2009), were designed by high through-put screening. Lead optimization led to compound 89 which showed good affinity for A<sub>2A</sub>R and excellent selectivity versus A<sub>1</sub>R. Interestingly, a simple methyl substitution at 2-amino substituent gave compound 90, which dramatically decreased the binding affinity for A<sub>2A</sub>R. Another carboxamides of 2-amino-4-phenyl-thiazoles were prepared by Sams et al in 2010 as adenosine A<sub>2A</sub>R antagonists. A lead optimization effort identified that the cyclopropyl amide was the optimal amide substituent. Further evaluation of the substitution at the 5-position of the thiazole led to the 5-methyl-[1, 2, 4]-oxadiazole compound 91 which had good affinity for A<sub>2A</sub>R and A<sub>1</sub>R. Compound 91 also showed good activity in the haloperidol-induced hypo locomotion model in mice with ED50 of 7 mg/kg.

![Chemical Structures](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>A&lt;sub&gt;2A&lt;/sub&gt; Ki</th>
<th>A&lt;sub&gt;1&lt;/sub&gt; Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>12 nM</td>
<td>1700 nM</td>
</tr>
<tr>
<td>90</td>
<td>8170 nM</td>
<td>10000 nM</td>
</tr>
<tr>
<td>91</td>
<td>28 nM</td>
<td>150 nM</td>
</tr>
</tbody>
</table>

(e) Triazine Derivatives

Congreve et al recently (2012) reported 1, 2, 4-triazine derivatives using structure based drug design approaches as A<sub>2A</sub>R receptor antagonists. Compound 92 of the series found to possess significant A<sub>2A</sub>R activity.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>A&lt;sub&gt;2A&lt;/sub&gt; Ki</th>
<th>A&lt;sub&gt;1&lt;/sub&gt; Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>7.5 nM</td>
<td>43 nM</td>
</tr>
</tbody>
</table>
1.8. Predicted Pharmacophoric Features for A2A R Antagonists

Pharmacophore model developed by Wei at al for selective A2A antagonists revealed that four features, one ring aromatic feature (R), one positively ionizable feature (P), one hydrogen bond acceptor lipid feature (L), and one hydrophobic feature (H) found to be essential for antagonists in terms of binding activity and A2AR selectivity (Wei et al., 2007). Pharmacophore model developed by Ye at al (Fig.3) suggested that two adjacent hydrophobic sites (one surrounded by residues, Ile92, Trp276, Ile66, and Ile244; the other by residues Ile92, Phe93, and Val186) accommodate the furan moiety along with a hydrophobic pocket which interacts with the side chains in the non-xanthine ligands. Electron rich core containing hydrogen bond donor /acceptor group is also essential for A2AR activity and selectivity (Ye et al., 2008).

Fig. 3. Extracted pharmacophore models based on binding mode from docking analysis representative of four featured pharmacophore model. (A) For xanthine type A2A antagonist. (B) For non-xanthine type A2A antagonist. Features are color-coded: green represents hydrogen bond acceptor; orange represents ring aromatic; light blue represents hydrophobic.

1.9. Physicochemical and Pharmacokinetic Properties

The assessment of physicochemical and pharmacokinetic properties is one of the critical stages in the drug development. Several compounds reported in literature as a potent A2AR antagonist suffered from low physicochemical and low pharmacokinetic profile and could not be developed as a drug. The computational models are used to demonstrate the molecular characteristics related to hydrogen bonding, lipophilicity, TPSA and molecular weight and predict the propensity for intestinal absorption or blood-brain barrier penetration (Pajouhesh and Lenz, 2005) for assessing the drug-like feature of new chemical entities. The computational tool Osiris property explorer, Mol
inspiration and ADMET Descriptors have been used to predict the physicochemical and pharmacokinetic profile of the compounds. ADMET includes descriptors for intestinal absorption, aqueous solubility, blood brain barrier penetration (BBB), plasma protein binding (PPB), cytochrome P450 2D6 inhibition (CYP2D6 Binding) and polar surface area (PSA).

1.10. Hypothesis and Workplan

In the present work, the rational development of novel A$_{2A}$R antagonists was carried to obtain a ligand with high potency and selectivity for A$_{2A}$R along with economically viable synthetic strategy (Figure 4). The synthesis of tricyclic thiazolo-triazolo-pyrimidines (Series 1; 27-39) was initiated based on SCH 25861 using 2-thioxo-N3-substituted-4-amino-5-nitrite-thiazoles. The same starting material was used to construct bicyclic, thiazolo-pyrimidine derivaties (Series 2; 40-135) tethered to urea (40-46), furonamide (47-51), and N3 as well as N6 substituted thiazolopyrimidines (52-135). Finally, monocyclic scaffold (Series 3; 136-147) 2-thixothiazole-5-carbonitrile substituted at N3 and N4 was developed with improved activity, selectivity and involved reduced synthetic steps. Moreover, the synthesis and characterization of tricyclic iso-oxaxolo-triazolo-pyrimidines (Series 4; 202-213) was initiated parallel to thiazolo-triazolo-pyrimidines for better pharmacokinetic profile based on SCH 25861. The interaction of the synthesized compounds with human A$_{2A}$R was studied by in silico docking analysis, in vitro, invivo and functional assay (Luthra et al., 2009, Mishra et al., 2010). Physicochemical as well as pharmacokinetic characteristics of synthesized compound were evaluated by free access software.

**Fig. 4. Series 1-4:** Series 1 (Thiazolo-triazolo-pyrimidines 27-39); Series 2 Thiazolo-pyrimidines (40-135); Series 3 Thioxothiazolederivatives (136-147); 4 Oxazolo-triazolo-pyrimidines (202-213).
1.11. Objective of Study

1. Synthesis and Structure Activity Relationship of tricyclic thiazolotriazolopyrimidine derivatives (Series 1)
2. Synthesis and Structure Activity Relationship of bicyclic thiazolopyrimidines (Series 2)
3. Synthesis and Structure Activity Relationship of monocyclic thiaoles (Series 3)
4. Synthesis and Structure Activity Relationship of tricyclic Isoxazoles (Series 4)
References


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Chapter 1
Introduction and Review of Literature


Prakash A, Luthra PM (2012) Insilico study of the A(2A)R-D (2)R kinetics and interfacial contact surface for heteromerization Amino Acids. 43:1451-64


Chapter 1

Introduction and Review of Literature


