Adenosine A2A Receptor Antagonists as Positron Emission Tomography (PET) Tracers


Abstract: The adenosine A2A receptor (A2AR) is highly concentrated in the striatum, and a therapeutic target for Parkinson’s disorder (PD) and Huntington’s disease. High affinity and selective radiolabeled A2AR antagonists can be important research and diagnostic tools for PD. Positron Emission Tomography (PET) can play an important role by measuring radiolabeled A2AR antagonists non-invasively in the brain. However, till date no complete review on A2AR PET ligands is available. The present article has been therefore focused on available PET tracers for A2AR and their detailed biological evaluation in rodents, nonhuman primates and humans. Drug design and development by molecular modeling including new lead structures that are potential candidates for radiolabeling and mapping of cerebral A2ARs is discussed in the present article. A brief overview of functions of adenosine in health and disease, including the relevance of A2AR for PD has also been presented.

Keywords: Adenosine A2A receptor, Parkinson’s disorder, positron emission tomography (PET), xanthine ligands, non-xanthine ligands, SCH442416, TMSX, 6-OHDA PD model.

1. INTRODUCTION

Adenosine, an endogenous ligand, functions as a cytoprotective and neuromodulator in response to stress to an organ or tissue under both physiological and pathophysiological conditions. It elicits intracellular signaling cascades through four subtypes of G-protein coupled adenosine receptors (ARs) namely A1, A2A, A2B and A3 (A1R, A2AR, A2BR and A3R, respectively) [1-4].

Cytoprotective mechanisms may be indicated by an increased blood supply (vasodilatation or angiogenesis), cerebral and cardiac preconditioning and / or suppression of inflammation [5]. Adenosine is believed to play an important role in promoting sleep and suppressing arousal, cognition and memory, neuronal damage and degeneration as well as neuronal maturation [5, 6]. Furthermore, adenosine is a local modulator for other neurotransmitters and counteracts glutamate excitatory effects. As a result, ARs are promising targets for investigation and treatment of cerebral and cardiac diseases, ischemic renal injury, endocrine, pain, and sleep disorders, immune and inflammatory disorders and cancers [6-10].

In the last two decades, the most extensively studied AR subtypes are high affinity adenosine A1 receptors (A1Rs) and adenosine A2A receptors (A2ARs), because adenosine activates these receptors in nanomolar concentrations. These subtypes are well-characterized biochemically and pharmacologically [11, 12]. The high affinity A2A subtype, when coupled with G-proteins, exhibits a lower affinity to adenosine, activation of A2AR assists neuronal function of neuronalotropic receptors like tropomyosin-related kinase B (TrkB) receptors and enhances neuronal communication [13]. A2ARs stimulate adenyl cyclase activity via Gs proteins [14]. They can also activate potassium channels but inactivate Ca2+ channels, modulate the activities of phospholipases C, D, and A2, and upregulate mitogen-activated protein kinases and inflammatory cytokines like IL-1β [14].

The regional distribution of A2AR within the human brain is more restricted than that of A1Rs. A2ARs are abundantly expressed in the basal ganglia and highest levels of expression occur in the substantia nigra (striatum Bmax 313 ± 10 fmol / mg protein) [15], nucleus accumbens and olfactory tubercle whereas A1Rs are highly expressed in the cerebral cortex, hippocampus, and dorsal horn of spinal cord [16]. Lower densities of A2ARs occur in the amygdala, cerebellum, brainstem and hypothalamus [17-19]. A2ARs are implied in several cerebral diseases such as Parkinson’s disease (PD), Huntington’s disease, Alzheimer’s disease, attention deficit hyperactivity and panic disorders, schizophrenia, pain, and sleep disorders. Also, A2ARs play an important role in cardiac diseases, immune and inflammatory disorders, and ischemic kidney injury [7-10, 20].
Symptomatic dopaminergic replacement strategy using L-DOPA and dopamine agonists is the current therapy for PD [21, 22]. However, with disease progression the therapy suffers from several limitations like negligible effects on non-motor symptoms, reduced effectiveness in reverting motor impairment, unwanted side effects like dyskinesia, motor fluctuations and neuropsychiatric complications and importantly, fails to delay disease progression [23-26]. A2ARs are mainly restricted to the indirect striatal output function [i.e., GABAergic neurons projecting to the globus pallidus (GP), pars externa] and are co-localized with dopamine D2 receptors (D2Rs) in the striatum. Along with D2Rs, blockade of A2ARs dampens the hyperactivity of the indirect dopamine pathway observed during PD, restores correct movement execution and suppresses the neurodegenerative process and hence has raised a lot of interest due to unmet medical needs of PD [26]. Co-localization and synergistic interaction between A2AR and metabotropic glutamate subtype 5 (mGlu5) receptor makes A2AR an important target for the therapy of PD [27, 28]. Heteromeric forms like A1/A2A, D3/A2A and cannabinoid CB1/A2A have all been observed [29, 30]. In addition, evidence for heterotrimers like CB1/A2A/D2, A2A/D2/ mGlu5 was also reported [29, 31].

Apart from its central location, A2ARs present in peripheral organs like heart, kidney, liver, muscle and lung [32]. In heart, adenosine is an important mediator in cardioprotective action [5, 32]. Myocardial protection action of adenosine is mediated mainly through A1R and A2AR. Activation of A2ARs causes coronary vasodilatation [33], increases myocardial contractibility [34], relaxes smooth muscle and inhibits cytokine production, increases coronary blood flow and inhibits platelet aggregation [5]. A2AR via the action of adenosine help in regulation of physiological functions of skeletal muscle like glucose uptake, blood flow and contractile force [35].

Positron emission tomography (PET) can contribute important information in drug development resulting in a more rapid evaluation of novel compounds. High affinity and selective radiolabeled A2AR antagonists can be used to assess changes of A2AR density during the progression of disease and the affect of therapy on such changes. Moreover, A2AR ligands can be employed to assess occupancy of the receptor population by therapeutic drugs in the human brain, which will allow correlation of receptor occupancy and therapeutic effects [36, 37]. PET is a non-invasive technique allowing studies of physiological processes in the brain of normal individuals and patients with neurologic illness [3]. Furthermore, PET can help to increase diagnostic specificity for dopamine-deficient parkinsonian syndromes and justify management decisions at initial stages of disease. Along with single photon emission computed tomography (SPECT) and proton magnetic resonance spectroscopy, 18F-DOPA PET is useful in discriminating atypical parkinsonian disorders (multiple system atrophy, progressive supranuclear palsy and corticobasal degeneration) from idiopathic PD with up to 80% specificity [38].

On the basis of these considerations, several A2AR antagonists (both xanthine and non-xanthine derivatives) have been produced and some of them are being tested as treatment for PD in several clinical trials as well as in preclinical studies [35, 39-45, 45-50]. Moreover, some of these chemical structures allow easy incorporation of radionuclides. Besides KF17837 and several related xanthine analogs, non-xanthine SCH442416 and its fluorinated derivative have been evaluated as PET ligands. In clinical studies, only one xanthine (11C]TMSX = [11C]KF18446) and a non-xanthine derivative ([11C]SCH442416) have been employed [3].

Adenosine antagonists and their PET tracers have been the topic of many reviews [2, 3, 21, 51-59]. These reviews have provided a discussion on adenosine functions in health and disease, PET tracers for mapping adenosine receptors (mainly A1R) and the development of potential novel radioligands. However, to date, no comprehensive review on PET ligands for A2AR is available. The major goals of the current article is three-fold: 1) to present an overview of A2AR antagonists used as PET tracers, 2) to summarize preclinical and clinical A2AR imaging data, and 3) to highlight the design and development of new lead compounds as potential tracers for mapping of A2ARs.

2. A2AR PET TRACERS

A2AR antagonist PET tracers (Fig. 1) can be mainly divided into two classes.

1. Xanthine PET tracers

2. Non-xanthine PET tracers

2.1. Xanthine Ligands

All xanthine type radioligands were synthesized either by N- or O-methylation of the corresponding desmethyl compounds using primarily [11C]CH3I with sufficient radiochemical yields suitable for routine use [60-64]. (Table 1). In one of the radioligand syntheses, the more reactive methylating agent [11C]CH3OTf has been used to achieve high radiochemical yield. However, reaction temperature, time and specific activity were not mentioned in the literature [65]. All xanthine analogues suffer from a serious photoisomerization (Fig. 1). The styryl group in the xanthine scaffold is isomerized to form a stable equilibrium mixture of E-isomer and Z-isomer in the presence of light. Therefore in experimental and clinical studies, all procedures should be carefully carried out under dim light [3].

2.1.1. In Vitro and Preclinical Studies

Researchers earlier emphasized the selectivity of KF17837 towards A2AR [67]. However, in a later study its specificity for A2AR was questioned because in dilute solution this styrylxanthine undergoes photoisomerisation to the less active Z-isomer (82 %) [68]. The Z-isomer has about 860 fold lower affinity (Kd value, 860 ± 120 mM) for the A2AR than the E-isomer (1.0 ± 0.1 mM) [60, 68].

Suzuki and co-workers developed the xanthine compound KF17837 [69, 70] and two other groups successfully labeled its desmethyl compound with carbon-11 by N-methylation reaction using [11C]methyl iodide (Table 1). Radiosynthesis was carried out under dim light in an amber glass vial, which preserved the E-isomer over the entire period of study [60, 62]. Biodistribution studies showed highest radioactivity uptake [15 % injected dose per gram (% ID/g)] in the heart at 5 min after injection of [11C]KF17837 in normal healthy mice but falling gradually thereafter. A high and saturable uptake of tracer by the mouse heart confirmed
Fig. (1). Current PET tracers for the adenosine A2A receptors.

Table 1. Radiochemical Synthesis of Xanthine Analogs

<table>
<thead>
<tr>
<th>Radiotracers</th>
<th>Methylating agent</th>
<th>Precursor (mg)</th>
<th>DMF (ml)</th>
<th>Base</th>
<th>Reaction temperature (°C)</th>
<th>Reaction time (min)</th>
<th>Radiochemical yield (%)</th>
<th>Specific activity (GBq/mol)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>[11C]KF17837</td>
<td>[11C]CH3I</td>
<td>0.5</td>
<td>0.25</td>
<td>5 mg Cs2CO3</td>
<td>120</td>
<td>1</td>
<td>19 - 48</td>
<td>37 - 64</td>
<td>[62]</td>
</tr>
<tr>
<td>[11C]CH3I</td>
<td></td>
<td>1 - 1.5</td>
<td>0.3</td>
<td>6 mg K2CO3</td>
<td>120 or Room temp</td>
<td>1 or 7</td>
<td>50 - 80</td>
<td>≥ 10</td>
<td>[64]</td>
</tr>
<tr>
<td>[11C]CSC</td>
<td>[11C]CH3I</td>
<td>1</td>
<td>0.4</td>
<td>10 mg K2CO3</td>
<td>60</td>
<td>10</td>
<td>44</td>
<td>1.85 - 5.55</td>
<td>[65]</td>
</tr>
<tr>
<td>[11C]KF21213</td>
<td>[11C]CH3I</td>
<td>0.5</td>
<td>0.25</td>
<td>5 mg Cs2CO3</td>
<td>120</td>
<td>3</td>
<td>31 - 62</td>
<td>43 ± 9</td>
<td>[68]</td>
</tr>
<tr>
<td>[11C]TMSX</td>
<td>[11C]CH3I</td>
<td>0.5</td>
<td>0.25</td>
<td>5 - 10 mg Cs2CO3</td>
<td>120</td>
<td>3</td>
<td>25 - 46</td>
<td>10 - 72</td>
<td>[63]</td>
</tr>
<tr>
<td>[11C]CH3OTf*</td>
<td></td>
<td>0.25</td>
<td>0.25</td>
<td>10 mg Cs2CO3</td>
<td>-</td>
<td>-</td>
<td>55 ± 5 (n = 3)</td>
<td>-</td>
<td>[67]</td>
</tr>
<tr>
<td>[11C]KW-6002</td>
<td>[11C]CH3I</td>
<td>0.5</td>
<td>0.25</td>
<td>5 mg Cs2CO3</td>
<td>120</td>
<td>1 - 5</td>
<td>25 - 46</td>
<td>10 - 72</td>
<td>[63]</td>
</tr>
<tr>
<td>[11C]KW-6002</td>
<td>[11C]CH3I</td>
<td>3</td>
<td>0.4</td>
<td>20 μl, 5.0 M NaOH</td>
<td>100</td>
<td>6</td>
<td>1.1 - 2.2*</td>
<td>-</td>
<td>[66]</td>
</tr>
</tbody>
</table>

* Radiochemical yield in GBq on each automated synthesis run and no specific activity data available.

its usefulness for mapping myocardial adenosine receptors [60]. Similar results were observed with a dynamic PET scanning of the heart in rabbits [71]. Regional brain distribution showed a higher uptake in striatum than in other regions (striatum / cerebellum ratio approximately 2.0 at 60 min). The compound’s affinity for A2AR-rich striatum was confirmed by carrier KF17837 co-injection and by sequential PET studies in the same rats using D2R ligand [11C]N-methylspiperone. Reduction by 68 % radioactivity in striatum after carrier injection at 30 min and also [11C]KF17837
accumulated in the same brain regions as observed using \([^{11}C]N\)-methylspiperone clearly indicating its specific uptake in the striatum [60] (Table 2).

Later studies were aimed at evaluation of \([^{11}C]KF17837\) as a central nervous system (CNS) tracer in rodents and monkey [72]. \textit{In vitro} autoradiography (ARG) experiments in rats showed 2.3 - 3.0 times higher striatal uptake than in other brain regions. On the contrary, results from a regional brain distribution study in mice, an \textit{ex vivo} ARG study in rats and a PET study in a monkey suggested only slightly higher uptake in the striatum than in other brain regions (1.1 - 1.5 times) (Table 2). Authors hypothesized that the \textit{in vivo} receptor binding sites of xanthine type antagonists may be different from those of non-xanthine type A2R ligands as \textit{in vivo} uptake of \([^{11}C]KF17837\) was not significantly decreased in a blocking study using subtype selective non-xanthine analogues such as SCH58621 or ZM241385 [73]. Their data indicated nonspecific binding and the presence of unknown but specific binding sites for \([^{11}C]KF17837\) in the cortex and cerebellum. In this study no clear conclusion was reached concerning its potential to bind adenosine A2B receptor (A2BR) because at the time of the study there was no subtype selective A2BR ligand. However, a weak A2B antagonist, alloxazine, did not reduce \([^{11}C]KF17837\) uptake in the brain [72].

In addition, another group evaluated \([^{11}C]KF17837\) in monkey by PET examination. PET biodistribution studies using 3-D mode of data acquisition was used to express radioactivity retention in the striatum, cerebellum, and cerebral cortex (1.1, 1.0 and 0.8, respectively). Radioactive accumulation was significantly different from the known relative A2AR densities in these regions [74-76] (Table 2). The ligand has limited usefulness for mapping the cerebral A2AR because of its limited diffusion through the blood brain barrier (BBB) and high non-specific binding. However, radiotracer uptake in the heart was rapid (maximum reached at 2 - 4 min post injection). In a saturation binding experiment, there was an indication of competition between the labeled and unlabeled drugs for the same receptor binding sites in heart. Hence, authors suggested further investigation to establish the specificity of the interaction of this tracer with myocardial A2ARs and other potent and selective A2AR antagonists (ZM241385 and SCH58261) could be considered as ligands for \textit{in vivo} PET studies [62]. In conclusion, both groups clearly demonstrated limited suitability of \([^{11}C]KF17837\) for A2AR quantification because of its low brain penetration and high degree of non-specific binding (Table 2). Investigated time is not an ideal point for comparing \textit{in vitro} and \textit{in vivo} studies as shown in (Table 2).

A comprehensive comparison of four xanthine PET tracers (carbon-11 labeled KF17837, KF19631, TMSX and CSC) was made in order to search for a selective A2AR ligand [61]. \([^{11}C]CSC\) had similar characteristics as \([^{11}C]KF17837\), but \([^{11}C]CSC\) showed higher uptake in the lung and small intestine and it was cleared more rapidly. Another study described in detail optimization of the radiochemical synthesis of \([^{11}C]CSC\) [63]. Also, significant \([^{11}C]CSC\) accumulation in the lung was detected whereas autoradiographic investigations indicated uptake in the striatum, consistent with observations by Ishiwata et al. [61]. Dynamic PET scans in rabbits showed rapid uptake of the radiotracer in the brain in less than 2 min after injection. An \textit{in vivo} competition study with cold CSC suggested that \([^{11}C]CSC\) binds specifically to A2AR in the rabbit brain [63]. However, the tracer’s rapid clearance, different distribution pattern from other xanthine PET tracers (i.e., high uptake in small intestine and lungs) and low affinity made it unsuitable as a PET tracer for A2AR.

Wang and co-workers continued their work in search for A2AR tracers with high affinity and selectivity and found KF21213 with higher selectivity for A2AR than KF17837 or KF18446 [66]. An \textit{in vitro} study showed that \(K_i\) values of KF21213 were 3.0 nM for A2AR and >10,000 nM for A1R whereas for KF18446 they were 5.9 nM for A2AR and 1600 nM for A1R (Table 3). In mice, regional brain distribution data of \([^{11}C]KF21213\) suggested a high striatal uptake for the first 15 min followed by a gradual decrease. A very low uptake was seen in the cortex and cerebellum. As a result of this, high uptake ratios of striatum-to-cortex (8.6 ± 1.6) and striatum-to-cerebellum (10.5 ± 2.1) were found at 60 min.

Table 2. Summary of Striatal Uptake of \([^{11}C]KF17837\)

<table>
<thead>
<tr>
<th>Species</th>
<th>Regional brain distribution (% ID / g)</th>
<th>In vitro ARG* (% ID / g)</th>
<th>Ex vivo ARG* (% ID / g)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Striatum-to- cerebellum</td>
<td>Striatum-to- cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>1.1 - 1.2</td>
<td>1.2 - 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(15 min)</td>
<td>(15 min)</td>
<td></td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(30 min)</td>
<td>(30 min)</td>
<td></td>
<td>[72]</td>
</tr>
<tr>
<td>Rat</td>
<td>-</td>
<td>2.3 - 3.0</td>
<td>1.2 - 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>(15 min)</td>
<td>(15 min)</td>
<td>[72]</td>
</tr>
<tr>
<td>Female Rhesus monkey (n = 1)</td>
<td>1.32</td>
<td>1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(30 min)</td>
<td>(30 min)</td>
<td></td>
<td>[72]</td>
</tr>
<tr>
<td>Male Cynomolgus monkey (n = 2)**</td>
<td>1.05 - 1.1</td>
<td>1.3 - 1.4</td>
<td></td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td>(20 min)</td>
<td>(20 min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Adenosine A2A Receptor Imaging

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5

post injection. On the other hand, the uptake of [11C]TMSX was higher in all three regions of brain but decreased more rapidly with time so that the striatum-to-cortex ratio was 2.8 ± 0.5 and striatum-to-cerebellum ratio was 2.7 ± 0.5 at 15 min. Co-injection of cold KF21213 and three other A2A antagonists (KF17837, KF18446 and SCH58261), but not the A1R antagonist KF15372, effectively blocked uptake of [11C]KF21213 especially in the striatum. However, no significant effect was seen in the cortex and cerebellum. Ex vivo ARG showed a high uptake in the caudate-putamen, GP and olfactory tubercle and good uptake ratios of striatum-to-cortex (4.0 ± 0.4) and striatum-to-cerebellum (3.7 ± 0.4) at 15 min post tracer injection. A PET study in rats indicated high striatal retention of [11C]KF21213 at 5 min followed by a gradual decrease whereas [11C]TMSX uptake cleared more rapidly [66]. However, its low BBB penetration, signal-to-noise ratio and poor water solubility made it not a very practical tracer for PET studies of the CNS.

In search for more pronounced A2AR-selectivity, 11C-labeled iodinated and brominated xanthine analogs were synthesized and evaluated for their capability of detecting A2AR changes in brain. In vitro binding assays showed that both IS-DMPX and BS-DMPX compounds had a high affinity and selectivity for A2AR (Table 3). However, because of their low in vivo uptake ratios of striatum to other brain regions and high nonspecific binding they were judged unsuitable for mapping cerebral A2ARs [79].

In another PET study, the xanthine-type radioligand [11C]KW6002 was reported. High striatal uptake [Standard Uptake Value (SUV) = 3.3] and low uptake in frontal cortex (SUV = 1.7) reflects specific binding [46, 64]. In a blocking study, specificity and selectivity of tracer was confirmed. However, extrastriatal regions like cerebellum and superior colliculi uptake can be seen where a low A2AR density was reported [46, 64]. Saturation binding study with A1R antagonist KF15372, non-xanthine type A2AR antagonist ZM241385 [64] and A2B-selective antagonist MRS1745 [46] failed to solve the nature of extrastriatal binding. In rats, specific in vivo binding of [11C]KW6002 to A2AR could not be observed and thus the compound does not appear to be a good PET tracer. Further study is warranted regarding its in vivo selectivity. In general, [11C]KW6002 has shown similar in vivo properties as [11C]KF17837 [64]. All xanthine A2A antagonists e.g., [11C]KF17837, [11C]TMSX, [11C]KF21213, [11C]KF19631 and [11C]KW6002 seem to interact with multiple binding sites and undefined binding sites are responsible for extrastriatal retention of radioactivity [46, 64, 66, 72, 78].

[11C]TMSX showed more desirable properties for mapping A2AR such as high retention in the rat brain and especially in the striatum (ratio of striatum uptake to other brain regions was up to 3.2). Additionally, [11C]TMSX- PET imaging of monkey brain showed 10 fold higher striatal uptake at 5-10 min than [11C]KF17837 but indicated a rapid washout pattern. However, uptake ratios of [11C]TMSX [striatum: cortex: cerebellum (1.0: 1.56: 1.46, respectively) at 60 min] in the monkey brain were slightly better than those of [11C]KF17837. An in vivo saturation binding experiment also suggested superiority of [11C]TMSX over other KF compounds [61].

Promising preliminary results stimulated further characterization of [11C]TMSX by in vitro ARG and in vivo biodistribution experiments [78]. The binding characteristics of [11C]TMSX (in vitro ARG) were slightly better than those of reference standard [3H]CGS21680. Non-specific binding of [11C]TMSX in the striatum was less than 10 % of the total uptake.

<table>
<thead>
<tr>
<th>A2A Antagonists</th>
<th>Affinity (Ki, nM)</th>
<th>Selectivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2A</td>
<td>A1 / A2A</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
<td>-----</td>
<td>---------</td>
</tr>
<tr>
<td>KF17837</td>
<td>62</td>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td>KF19631</td>
<td>&gt;10000</td>
<td>3.5</td>
<td>2857</td>
</tr>
<tr>
<td>KF18446 (= TMSX)</td>
<td>1600</td>
<td>5.9</td>
<td>270</td>
</tr>
<tr>
<td>CSC</td>
<td>28000</td>
<td>54</td>
<td>520</td>
</tr>
<tr>
<td>BS-DMPX</td>
<td>2300</td>
<td>7.7</td>
<td>300</td>
</tr>
<tr>
<td>IS-DMPX</td>
<td>&gt;10000</td>
<td>8.9</td>
<td>&gt;1100</td>
</tr>
<tr>
<td>KF21213</td>
<td>&gt;10000</td>
<td>3</td>
<td>&gt;3000</td>
</tr>
<tr>
<td>KW6002</td>
<td>150</td>
<td>2.2</td>
<td>68</td>
</tr>
<tr>
<td>SCH442416</td>
<td>1800</td>
<td>0.5</td>
<td>3600</td>
</tr>
<tr>
<td>SCH58261</td>
<td>1100 (h) **</td>
<td>0.048 (h) **</td>
<td>22916 (h) **</td>
</tr>
<tr>
<td>MRS5425</td>
<td>42.7 ± 0.6*</td>
<td>12.4 ± 0.8*</td>
<td>-</td>
</tr>
<tr>
<td>Fluoropropyl SCH442416</td>
<td>1000 ± 40</td>
<td>53.6 ± 25.5</td>
<td>19</td>
</tr>
</tbody>
</table>

* % inhibition at 10 μM ** h = humans.

Table 3. Binding Affinities of Adenosine A2A Receptor Antagonists
uptake and striatum-to-cortex ratio was 5.0 whereas related values for [3H]CGS21680 were estimated as 19 % and 4.6. TMSX had a very low affinity for various other 13 neuroreceptors like dopamine D_1 and D_2; histamine H_1 and H_2; nicotine acetylcholine in binding assays. In vitro blocking study with various A_2A-R and A_1-R antagonists indicated K_d values for [11C]TMSX of 9.6 nM in the striatum and 16.4 nM in the cerebral cortex. Also, all antagonists significantly reduced the binding of [11C]TMSX in the striatum and cortex but stronger effects were seen in the striatum. The in vitro ARG experiments suggested that xanthine ligands interact with unknown binding sites in the cortex and hippocampus that are different from known A_2A-R binding sites. Fredholm and co-workers also measured binding sites of an A_2AR radioare different from known A_2AR binding sites. The effect of age on the distribution of A_2A-R in the striatum and GP, suggesting the degeneration of A_2AR-expressing neurons and hence, specific uptake of [11C]TMSX [79].

On the basis of the previous promising results, extensive preclinical studies (stability test, internal dosimetry data for human organs and toxicological data) were carried out to establish [11C]TMSX as a radioligand for imaging human A_2A-R [80]. Theophylline challenge in mice resulted in a decrease of tracer uptake in the striatum, as theophylline is a non-subtype-selective adenosine antagonist. This suggested that [11C]TMSX-PET scan data should be interpreted with caution in patients who received theophylline. [11C]TMSX was metabolically stable as about 80 % and >98 % of radioactivity in plasma and striatum represented intact tracer at 30 min post injection. From the mouse data of tissue radioactivity distribution, absorbed doses of [11C]TMSX for human adults were estimated. The radiation absorbed doses in the brain (0.09 μGy / MBq) and heart (0.31 μGy / MBq) were very low. In rodents, neither mortality nor any other abnormality was found in an acute toxicity study, which was evaluated after single intraperitoneal administration of TMSX at a dose of 4.77 mg / kg and after intravenous injection of 3.3 - 3.9 μg / kg over a period of 15 days. An Ames test (with 4 strains of Salmonella typhimurium) suggested absence of mutagenic activity. All these findings encouraged the authors to use [11C]TMSX for the assessment of A_2A-R in the human brain [80].

2.1.2. Clinical Studies

A first human study with [11C]TMSX was reported concerning myocardial imaging. The levels of radioactivity in the left ventricular lateral wall, left ventricular anterior wall and interior ventricular septum increased during the first 2.5 min post injection and then gradually decreased with time. Time-activity curves in 3 heart regions and graphical analysis using Logan plot suggested that [11C]TMSX was taken up via a receptor-mediated mechanism. During the 60 min study period, [11C]TMSX was very stable in plasma (more than 90 % unchanged form). These preliminary findings suggested that [11C]TMSX-PET may be useful for myocardial imaging in the diagnosis of ischemia and other myocardial diseases [39]. This may be possible in combination with pharmacologic stress agent like regadenoson (an adenosine derivate) or with a flow tracer FDG.

An additional study evaluated [11C]TMSX for mapping A_2A-R of skeletal muscle and heart in humans using PET. In humans, the heart was clearly visualized at baseline. Radioactivity in three regions of the heart was in line with the previous result [39]. Theophylline (a non-subtype-selective adenosine antagonist, at a dose of 100 mg / kg) slightly decreased the distribution volume (DV) of [11C]TMSX in the heart (by 18 – 22 %) and muscle (by 10 %) suggesting some specific binding of the tracer [40].

Using PET, comparison of A_2A-R densities in cardiac muscle has been made in both endurance-trained subjects and untrained men at resting state [35]. In addition, a group from Japan evaluated receptor functions in the skeletal muscle using PET based on their previous result [40] whereas a Finnish group recorded the myocardial perfusion effect at rest and during adenosine-induced hyperemia [41]. Higher density of A_2A-Rs was found in cardiac muscle than in skeletal muscle. Also, higher levels of A_2A-R were recorded in cardiac and skeletal muscle (DV of [11C]TMSX in heart, 3.6 ± 0.3 vs 3.1 ± 0.4 ml g⁻¹, triceps brachii muscle 1.7 ± 0.3 vs. 1.2 ± 0.2 ml g⁻¹, respectively) of endurance-trained subjects than in untrained subjects [35]. As a follow-up the interrelation was studied between A_2A-R density and myocardial blood flow (MBF) in both endurance-trained men and untrained men. Neither difference in A_2A-R densities between groups nor affiliation of MBF with A_2A-R density & adenosine-induced hyperemia was found [41].

The effect of age on the distribution of A_2A-R in the striatum of healthy human subjects has been studied using
Kinetic modeling was carried out to investigate the behavior of $[^{11}C]$TMSX in the brain and to examine the usefulness of Logan plot [44]. In the study, estimation of binding potential (BP) using the Logan plot agreed to the three-compartmental model data with or without metabolite correction and arterial blood sampling. The estimated BP without metabolite correction was only 5 % lower than the true value acquired with or without arterial blood sampling [44].

In the report [43], the cerebral distribution of $[^{11}C]$TMSX was in agreement with the distribution of $A_2A$R known from post mortem studies in humans, rodents and primates [3, 20, 74, 75]. A two-tissue, three-compartment model was used to measure the distribution of $A_2A$R in the brain (n = 5) using metabolite corrected arterial input function. Specific binding was found to be 62 % in putamen. The BP was largest in the anterior putamen (1.25), posterior putamen (1.20), caudate nucleus (1.05) and thalamus (1.03) followed by the cerebellum, brainstem, posterior cingulate gyrus, occipital, temporal, parietal and frontal lobes [43]. $[^{11}C]$TMSX binding in human thalamus was relatively larger than in the thalamus of other mammals.

Furthermore, using $[^{11}C]$TMSX-PET, differences between $A_2A$R expression and the dopaminergic system in the striata of drug-naive PD patients, PD patients with dyskinesia and alterations of these receptor systems after antiparkinsonian therapy were studied [45]. In order to elucidate the relationship between changes in $A_2A$R density and dopaminergic system related tracers like $[^{11}C]$2β-carbomethoxy-3β-(4-fluorophenyl)tropane ($[^{11}C]$CFT), a marker for presynaptic dopamine transporter and $[^{11}C]$raclopride ($[^{11}C$RAC]), a marker for postsynaptic D₂R. In an early PD patient with right dominant PD symptoms, the left-side $[^{11}C]$CFT binding was more decreased than the right-side one, and the uptake of $[^{11}C]$RAC was increased bilaterally. In contrast, the $[^{11}C]$TMSX retention ($A_2A$R density) was decreased on the left side. These observations suggested that the changes in $A_2A$R binding measured with $[^{11}C]$TMSX were coupled with the asymmetry of the symptoms. The BP of $[^{11}C]$TMSX was increased in the putamen of PD patients with mild dyskinesia. The study also showed that $A_2A$R were significantly increased in human bilateral putamen of the drug-naive patients after antiparkinsonian therapy [45].

KW6002, a very potent, selective and orally active drug has completed clinical trials for the treatment of PD. In spite of extrastriatal in vivo binding result obtained in rats [64], $[^{11}C]$KW6002 uptake was well characterized in human study by a two-tissue compartmental model with a blood volume component and reversible kinetics were observed during the scan time [46]. The caudate (3.38) showed the highest BP, followed by putamen (2.90), nucleus accumbens (2.37), cerebellum (2.26), and thalamus (2.19). Oral dose of 20 - 40 mg daily produced >90 % of receptor occupancy in healthy volunteers [46]. Additional study is required to determine the dose-receptor binding relationship of KW6002 in PD patients.

The design and development of new $A_2A$R antagonist PET tracers is a hot research topic since there are still major problems, especially with xanthine PET tracers, including high non-specific binding, reduced tracer uptake, low signal to noise ratio and barely visible target areas in the brain [60, 62-64, 66]. Usefulness of all xanthine type PET tracers may be limited due to its photosomerisation problem, lower specific activity and selectivity towards $A_2A$R. On the basis of these considerations, non-xanthine compounds (Fig. 1) were developed and tested in many preclinical and clinical studies for the assessment of cerebral $A_2A$R. Below paragraphs are devoted to non-xanthine PET ligands.

### 2.2. Non-Xanthine Ligands

Non-xanthine $[^{11}C]$SCH442416 was synthesized by O-methylation of desmethyl compound using $[^{11}C]$CH₃I with radiochemical yield of 29 ± 7 % (decay corrected), radiochemical purity >96%, and specific activity of 1490 ± 978 mCi / μmol (n = 18). Overall synthesis time was about 40 min [81] (Fig. 2).

A two-pot radiosynthesis method was adopted using the fluorosynthon 2-[18F]-fluoroethyl- 3,4-dibromobenzensulfonate. The whole synthesis time including HPLC purification was about 130 min. The radiochemical yield was 15 % ± 4 % (n = 8) uncorrected for decay and the radiochemical purity was ≥98 % [48].

#### 2.2.1. In Vitro and Preclinical Studies

The development of the high affinity and selective novel non-xanthine compound SCH442416 as an in vivo probe for $A_2A$R using PET was reported Todde et al. [81]. In receptor binding studies, SCH442416 showed a very good selectivity for $A_2A$R as $K_i$ values were 0.048 nM for $A_2A$R, > 10,000 nM for $A_2B$ and $A_3$, and 1,111 nM for $A_1$R (Table 3). Biodistribution studies showed not only radioactive uptake in the adrenal glands and kidneys, where $A_2A$Rs are highly expressed, but also in highly perfused organs like lung and liver radio uptake reached maximum at 5 min post injection in normal healthy rats. $[^{11}C]$SCH442416 penetrated the BBB easily and was retained in the brain where the maximum uptake was reached at 5 to 15 min after injection. Regional brain distribution showed a high striatal uptake (0.46 % ID / g) whereas tracer levels in the cerebellum (0.10 % ID / g) and cortex (0.10 % ID / g) were lower. Striatum-to-cerebellum ratio was 4.6 ± 0.17 at the time of maximum uptake (at 15 min post injection). Two hydrophilic metabolites were found in plasma extracts (Retention time (tR) = 4.5 and 2.5 min) with about 40 % of an intact tracer at 60 min. The regional distribution of radioactivity, the good signal-to-noise ratio and low amounts of radioactive metabolites in the brain all suggested that $[^{11}C]$SCH442416 was the first non-xanthine ligand suitable for mapping of $A_2A$R using PET [81].

In a later study, $[^{11}C]$SCH442416 was tested as a new PET tracer for in vivo imaging of $A_2A$Rs in rat and monkey brain [85]. Preadministered $A_2A$R selective (SCH442416,
SCH58261 and KW6002) or non-subtype selective antagonists (caffeine) decreased tracer accumulation in the striatum at 15 min after injection. Caffeine and KW6002 significantly decreased tracer retention (71 % and 68 %, respectively) in the striatum. Interestingly, subtype selective, potent antagonists like SCH442416 and SCH58621 did not yield a good blocking effect. These compounds may pose formulation problems due to poor water solubility. Receptor-specific uptake was confirmed by intraatrial injection of quinolinic acid. Using PET, serial brain scans were made in a single monkey after injection of 30 MBq of [11C]SCH442416. Rapid uptake of the tracer was observed in 0 - 4 min. Higher uptake in the striatum was noticed during the second frame (2 - 4 min). Striatum-to-cerebellum ratios reached a maximum value of 2.2 at approximately 15 min. BP in the striatum was estimated as 0.74 with the cerebellum as an input function (reference tissue model). These findings suggested suitability of [11C]SCH442416 as an in vivo probe for A2AR [85].

Acute pharmacological interaction of adenosine receptor (A1-, A2A-, & A2B-R) antagonists with L-DOPA has been investigated [47]. Neither the A1R antagonist CPX (5 mg / kg) nor the A2BR antagonist alloxazine (3mg / kg) induced rotational behaviors in this PD model. In addition, neither SCH58261 (10 mg / kg) nor L-DOPA (2.5 mg / kg) nor the A2BR antagonist alloxazine (3mg / kg) induced rotational behaviors in this PD model. However, synergistically increased contralateral rotations were seen with the A2AR antagonist SCH58261. In contrast, when used separately, neither SCH58261 (10 mg / kg) nor L-DOPA (2.5 mg / kg) induced rotational behaviors in this PD model. In addition, levels of A2A-Rs in 6-OHDA-lesioned mice have been determined with the A2AR specific tracer [11C]SCH442416. No significant differences in striatal uptake were observed between the two groups indicating that 6-OHDA-induced lesions of the dopaminergic system do not affect the in vivo binding of the A2AR tracer [11C]SCH442416. This contrasts strongly with reported changes of A2AR m-RNA expression in human PD [86]. Overall, this study suggested a synergistic beneficial role of A2AR antagonists in the reduction of L-DOPA dosage in 6-OHDA-lesioned mouse model [47].

A2AR occupancy in Rhesus monkeys after administration of various doses of the A1R and A2AR antagonist ASP5854 has been assessed using [11C]SCH442416 and PET. The amount of receptor occupancy required to inhibit haloperidol-induced catalepsy (0.03 mg / kg, intramuscularly) was also determined [87]. Receptor occupancy was studied after a intravenous bolus injection of ASP5854 in animals (n = 3) and a dynamic PET scan was performed at 1, 4, and 8 hour after injection of approximately 740 MBq of [11C]SCH442416. Incidence and duration of catalepsy were monitored throughout the study. Catalepsy was scored on the basis of a forced posture test and all experiments were video-recorded. If animals maintained the cataleptic posture then the trial was scored as “onset-positive”; else it was scored “onset-negative”. The anticausal effect of ASP5854 was achieved at 85 - 90% occupancy of A2ARs. Dose-dependent receptor occupancy 9.0 % (0.001 mg / kg), 50.7 % (0.01 mg / kg), and 87.4 % (0.1 mg / kg) was seen in the caudate nucleus. Bound ASP5854 dissociated slowly from cerebral A2ARs and hence a single drug administration provides a long-lasting effect. ASP5854 might be useful for treatment of psychotic patients in combination with antipsychotics like haloperidol. [11C]SCH442416-PET might be a useful tool for estimating the effective doses of A2AR antagonists in humans.

It has been reported that [18F]-MRS5425 (a [18F]-fluoroethyl derivative of SCH442416) can map A2AR changes in the rat model of PD (6-OHDA model). Also, precursor synthesis for radiolabelling and a two-pot radiosynthesis of [18F]-MRS5425 were described [48]. In anesthetized rats, [18F]-MRS5425 was intravenously injected and PET data were collected. The % ID / g values in regions of interest were measured in the striatum of normal rats and in rats unilaterally lesioned with 6-OHDA after intravenous administration of saline (baseline), the D2R agonist quinpirole (1.0 mg / kg) or the D2R antagonist raclopride (6.0 mg / kg). In vitro and ex vivo brain ARG showed A2AR specific uptake in the striatum. In addition, in vivo biodistribution studies suggested radioactivity in the striatum reached a maximum at 30 and 60 min and then dropped slowly. Radioactivity levels in the striatum were two-fold higher than in blood, suggesting suitability of [18F]-MRS5425 as an in vivo probe for mapping A2ARs. Results of the PET study were comparable to the previous one using [11C]SCH442416 in anesthetized non-human primates [85]. Radioactivity uptake was higher (9 - 12 % in the % ID / g) in the lesioned striatum than in the contralateral intact side due to upregulation of A2ARs [48, 82]. Conflicting results (either upregulation or no change of A2ARs in animal models of PD) [47, 48] may be related to differences in the experimental setup and / or species differences (rat vs mice).

2.2.2. Clinical Studies

In an important study, A2AR availability in 12 PD patients with and without levodopa-induced dyskinesias (LIDs) and...
in age-matched healthy controls (n = 6) using PET and [\(^{11}\)C]SCH442416 was reported [49]. PD patients with LIDs have elevated striatal A\(_{2A}\)R availability (BPs up to 1.67 vs. 0.96 of patients without LIDs). However, no correlation was found between severity of dyskinesia and increased striatal A\(_{2A}\)Rs. Also, the increase was not correlated to age, disease duration, levodopa equivalent unit, or Hoehn and Yahr staging. Higher A\(_{2A}\)R binding was found in the caudate and putamen of PD patients with LIDs with respect to both control group and PD patients without LIDs. Hence, this study suggested A\(_{2A}\)R antagonists might be useful for the management of LIDs along with reductions in levodopa dosage. However, to prove the concept, further studies are required in larger groups of patients. [\(^{11}\)C]SCH442416-PET might provide a robust and reliable method for in vivo investigations of A\(_{2A}\)R availability [49].

[\(^{11}\)C]SCH442416 has been employed to assess A\(_{2A}\)R occupancy by a therapeutic drug, vipadenant, in the human brain in order to correlate receptor occupancy and therapeutic effect [50]. The drug crossed the BBB and displaced receptor-bound [\(^{11}\)C]SCH442416 in a dose-dependent fashion (2.5 - 100 mg /day for 10 or 11 days). The estimated receptor occupancy of the drug in the brain varied from 74 % to 94 % at the lowest daily dose (2.5 mg) [46]. Unfortunately, negative findings in preclinical toxicology studies led to discontinuation of this drug in July 2010 by Vernalis Plc [88]. Thus, [\(^{11}\)C]SCH442416 might be an excellent tool for measurement of the A\(_{2A}\)R occupancy of various compounds (both xanthine and non-xanthine antagonists).

3. TRENDS IN MEDICINAL CHEMISTRY INCLUDING MOLECULAR DOCKING OF A\(_{2A}\)R ANTAGONISTS AS POTENTIAL PET TRACERS:

Recent developments in medicinal chemistry (both in synthesis and biological evaluation) resulted in the identification of large number of ligands with high affinity and specific binding to the A\(_{2A}\)R. Potential candidates for radiolabeling and molecular imaging have been reviewed below.

In the last 20 years, a number of selective A\(_{2A}\)R antagonists have been developed and some of them are being evaluated for treatment of PD in several preclinical and clinical studies. As shown above, A\(_{2A}\)R antagonists can be divided in two main categories. i) xanthine-type compounds and ii) polyheterocyclic compounds.

The xanthine scaffold present in the most popular compound caffeine represented an important starting point for the development of antagonists for this class of receptors [89]. Extensive structure-activity relationship studies among the xanthine derivatives have already led to the clinical candidates KW6002 and TMSX, both of which were labeled with carbon-11 [39, 40, 64]. However, there is an increasing interest among researchers in this field (particularly A\(_{2A}\)R) to explore other class of compounds (polyheterocyclic compounds) as potential PET antagonists because xanthine-type antagonists seriously suffer from low subtype selectivity and poor physicochemical properties such as photosomisation and low water solubility [68, 90-92]. Attempts have been made to improve water solubility of styrylxanthines such as introduction of polar groups on phenyl ring and prodrug approach with the aim not to compromise on affinity and selectivity (for example, the introduction of the sulfonate group on the phenyl ring of DMXP and MSX-2, a phosphate prodrug of MSX-2) (Fig. 4) [93]. All these studies appeal strongly to reconsider the xanthine family for A\(_{2A}\)R. Later, tricyclic xanthines and aminopyridopyrimidinedione derivatives (xanthine adenine hybrid structures) were developed [92, 94-97]. However, lack of affinity and selectivity made them unsuitable for further development (Fig. 4).

Presence of adenine base, a partial adenosine structure, in CGS15943 was an important starting point for the investigation of non-xanthine compounds [93]. The 2-alkynyl-substituted adenine derivative and 1,2,3-triazole adenine derivative are the most potent compounds of this class exhibiting high A\(_{2A}\)R affinity (Ki, 0.95 nM and 4.7 nM, respectively) but moderate selectivity for A\(_{2A}\)R versus A\(_{1}\) receptors (Fig. 4) [92, 98]. Later, a biosensor of CGS15943 showing good affinity and enhanced selectivity towards A\(_{2A}\)R was reported [99]. Pyrazolo-triazolo-pyrimidine nucleus was introduced as lead tricyclic molecule to determine A\(_{2A}\)R affinity and selectivity. Selected compounds of this class are SCH58621 and SCH442416 (Fig. 4). These compounds proved to have very good affinity and selectivity for both rat and human A\(_{2A}\)Rs (Table 3). The phenylethyl group of SCH58621 was substituted with an arylpiperazin ethyl group to obtain a new series of non-xanthines with high potency and selectivity for A\(_{2A}\)R. Poor water solubility is also a limiting factor for these non-xanthines [93]. However, further introduction of ether substituents gave derivatives with high affinity, selectivity and improved water solubility (SCH420814). Synthetic routes to various pyrazolo[4,3-e] [1,2,4]triazolo[1,5-\text{c}]pyrimidin-5-amine analogs were described [82, 83, 99-102]. The methoxy group of SCH442416 was replaced with various conjugates like ester, carboxylic acid, amines, alkyne, fluoropropyl and fluorophore reporter groups. The fluoropropyl derivative of SCH442416 might be useful as a PET ligand after labeling with \(^{18}\)F because of its good affinity (Table 3) towards A\(_{2A}\)R [83]. Pyrazole of the tricyclic moiety was replaced by an imidazole ring resulting in enhanced potency and selectivity (isomer of SCH420814) [103]. Researchers from Jülich synthesized and tested oxazolopyrimidines, derived from the triazolotriazine derivative, ZM241385 and triazoloquinonoxaline series as potential PET tracers for imaging brain A\(_{2A}\)Rs. In vitro ARG experiments indicated high nonspecific binding which conceals specific binding to A\(_{2A}\)Rs. In addition, triazoloquinonoxaline derivatives lack good selectivity for A\(_{2A}\)R over A\(_{1}\)R and have poor water solubility [104, 105].

Many groups described several very potent and highly subtype-selective bicyclic and tricyclic non-xanthine analogs [84, 106-108]. Unfortunately, all the structures are not amenable to radiolabelling with \(^{11}\)C or \(^{18}\)F. However, more suitable candidates are: 2-(2-furanyl)-7-phenyl[1,2,4]triazolopyrimidineamine derivatives [109, 110], triazolopurinamine analogs [98], piperazine diamino and triamino derivatives of triazolotriazine, arylpiperazin derivatives of pyrazolotria zolopyrimidines [102] and triazolopyrimidine [92, 107, 110-114] which can be labeled both with \(^{11}\)C (potential radiolabelling position on -NCH\(_{3}\) or -OCH\(_{3}\) groups) or \(^{18}\)F (fluorine atom on aromatic ring). In addition to the above compounds, aminopyrimidine derivatives, benzothiazole, thiazolopyridine and 1,2,4-triazole derivatives have emerged as potential new lead molecules for the development of A\(_{2A}\)R antagonists [92] (Table 4).
To identify potent potential PET ligands, computer-aided drug design is of great value. Scientists made use of molecular docking software to predict the binding orientation of small molecules to their protein targets. Hence *in silico* docking is useful in rational drug design. Several A2AR antagonists were docked using different software packages and protein data bank (PDB) crystal structures by different groups. A co-crystal structure of the A2AR with the selective antagonist ZM241385 (PDB Identification number: 3EML) has been reported [115]. Important characteristic receptor-ligand interactions include H-bond interactions with Asn253 and Glu269 and hydrophobic interaction with Phe168.

Another study reported docking analysis of structurally diverse A2AR antagonists with the aim to characterize the binding sites of A2AR. They identified five transmembrane helices (TM2, TM3, TM5, TM6, and TM7), which surround the active binding site of all A2AR antagonists [116]. These results were consistent with site-directed mutagenesis studies [117]. The three most potent xanthine analogs (KW6002, KF17837 and BS-DMPX) were first subjected to a docking study to validate the residues involved in stable binding.
### Table 4. Development of Novel Medicinal Lead Compounds as Potential PET Tracers

<table>
<thead>
<tr>
<th>Compounds</th>
<th>A$_{2A}$R Binding affinity (K_i, nM)</th>
<th>Selectivity (A$<em>{1}$/A$</em>{2A}$)</th>
<th>Potential radiolabelling position for [11C] or [18F]</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-[2-furanyl]-7-phenyl[1,2,4]triazolopyrimidineamine derivatives</td>
<td>2.8, 2.7, 1.0</td>
<td>601, 642, 1059</td>
<td>Methoxy group Methoxy and fluorine atoms Methoxy group</td>
<td>[109, 110]</td>
</tr>
<tr>
<td>Bicyclic piperazine derivatives of Triazolotriazine</td>
<td>0.2</td>
<td>16500</td>
<td>Fluorine substituent in meta position on aromatic group</td>
<td>[113]</td>
</tr>
<tr>
<td>9H-purinylamine analogs</td>
<td>6.6, 3.3, 4.7</td>
<td>11.92, 7.84, 17.02</td>
<td>-NCH$_3$ group</td>
<td>[98]</td>
</tr>
<tr>
<td>Triazolopurinamine analogs</td>
<td>0.1, 0.9, 0.7</td>
<td>1695, 669, 519</td>
<td>Terminal Methoxy groups</td>
<td>[103]</td>
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<tr>
<td>Piperazine derivatives of Triazolotriazine</td>
<td>4.0, 5, 3</td>
<td>205, 100, 433</td>
<td>Fluorine atoms on aromatic ring Fluorine substituents on aromatic ring</td>
<td>[111]</td>
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</table>
(Table 4 contd....)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>A$_{2a}$R Binding affinity (K_i, nM)</th>
<th>Selectivity (A$<em>1$/A$</em>{2a}$)</th>
<th>Potential radiolabelling position for [${}^{11}$C] or [${}^{18}$F]</th>
<th>References</th>
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<tr>
<td><strong>Pyrazolopyrimidine</strong></td>
<td>2.0</td>
<td>-</td>
<td>Methoxy group</td>
<td>[92]</td>
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<td><strong>Diamino derivatives of triazolotriazines</strong></td>
<td>5, 8, 250</td>
<td>250</td>
<td>Fluorine atoms on benzene ring</td>
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<tr>
<td><strong>Thiazolopyridine</strong></td>
<td>3</td>
<td>450</td>
<td>Methoxy group</td>
<td>[92]</td>
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<tr>
<td><strong>Phenyl substituted triazole</strong></td>
<td>20</td>
<td>69</td>
<td>Methoxy group</td>
<td>[92, 93]</td>
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<td><img src="image" alt="" /></td>
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<td></td>
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<tr>
<td><strong>Arylpiperazine derivatives of pyrazolotriazolopyrimidines</strong></td>
<td>0.6, 0.6, 0.6, 1.1, 0.4, 0.6, 0.6, 1.1, 0.4, 0.6</td>
<td>894, 1600, 1498, 1340, 1736, 1158</td>
<td>Fluorine atoms on aromatic ring Methoxy group</td>
<td>[102, 118]</td>
</tr>
</tbody>
</table>
interactions between antagonists and the receptor. All three xanthine type antagonists have similar binding mode patterns. Binding energy scores of all non-xanthine analogs were in agreement with receptor affinity (Ki) of the molecules. Moreover, all compounds shared similar π-π interactions between receptor aromatic residues and antagonists [116]. Critical interaction for xanthine analogs was hydrogen-bonding formation between Ser277 of A2AR with the carbonyl group of ligands whereas for non-xanthine compounds the free NH₂ groups and the oxygen in the furan ring are important features for binding to the receptor [116].

Bidhan et al. studied in detail pyrazolotriazolopyrimidine (SCH442416) derivatives by induced fit docking methodology using GLIDE software and they reported the 2-fluoroethyl derivative of SCH442416 as a highly selective PET tracer for A2αR [82]. This fluorinated analogue showed a similar binding mode as ZM241385 in the crystal structure whereas key interactions with important amino acids in the active site cavity such as Asn253, Phe168 and Glu169 were maintained [82]. In the study, the in silico results were fully substantiated with in vivo results.

### 4. SUMMARY AND FUTURE PERSPECTIVES

All the xanthine analogs including [¹¹C]TMSX proved to be not very suitable for molecular imaging mainly because of low signal to noise ratio, roughly visible receptor density and high degree of non-specific binding. All xanthine A2α antagonists e.g., [¹¹C]KF17837, [¹¹C]TMSX, [¹¹C]KF21213,
sites of xanthine compounds are quite different from those of showed that multiple binding sites and undefined binding Through saturation binding experiment, previous studies [11C]SCH442416. However, recently, newly developed F-18 derivatives have emerged as potential new lead molecules for the development of A2AR antagonists and PET tracers for adenosine receptors. In this regard, [18F]fluoroethyl SCH442416 derivative was the first non-xanthine ligand being evaluated in 6-OHDA PD rat model [48]. Preliminary findings indicated that [11C]SCH442416 and its [18F]fluoroethyl SCH442416 are good tracers for mapping cerebral A2ARs [48, 81, 85].

Some studies performed by the Jülich group [104, 105] have indicated that A2AR antagonists can be very potent and have appropriate lipophilicity for crossing BBB, yet fail as radiopharmaceuticals because of high non-specific binding. Furthermore, receptor subtype selectivity plays an important role in PET. It may thus be a challenge to develop PET ligands with better in vivo properties than [11C]TMSX or [13C]SCH442416. However, recently, newly developed F-18 labeled SCH442416 derivatives are under consideration mainly because of longer physical half-life (109.8 vs 20.4 min) allowing tracer distribution to remote imaging centers without cyclotron facilities and can achieve longer biodistribution and scanning times [82, 83]. Aminopyrimidine derivatives, benzothiazole, thiazolopyridine and 1,2,4-triazole derivatives have emerged as potential new lead molecules for the development of A2AR antagonists and PET tracers for both therapeutic and diagnostic applications [92]. Further studies with these compounds and other fluorinated analogues of SCH442416 are warranted. Computer-aided drug design and rapid developments in medicinal chemistry may yield better radioligands for A2AR visualization and quantification in the nearest future.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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