

Review

# Pathophysiological Role and Medicinal Chemistry of A<sub>2A</sub> Adenosine Receptor Antagonists in Alzheimer's Disease

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**Abstract:** The A<sub>2A</sub> adenosine receptor is a protein belonging to a family of four GPCR adenosine receptors. It is involved in the regulation of several pathophysiological conditions in both the central nervous system and periphery. In the brain, its localization at pre- and postsynaptic level in striatum, cortex, hippocampus and its effects on glutamate release, microglia and astrocyte activation account for a crucial role in neurodegenerative diseases, including Alzheimer's disease (AD). This ailment is considered the main form of dementia and is expected to exponentially increase in coming years. The pathological tracts of AD include amyloid peptide-β extracellular accumulation and tau hyperphosphorylation, causing neuronal cell death, cognitive deficit, and memory loss. Interestingly, in vitro and in vivo studies have demonstrated that A<sub>2A</sub> adenosine receptor antagonists may counteract each of these clinical signs, representing an important new strategy to fight a disease for which unfortunately only symptomatic drugs are available. This review offers a brief overview of the biological effects mediated by A<sub>2A</sub> adenosine receptors in AD animal and human studies and reports the state of the art of A<sub>2A</sub> adenosine receptor antagonists currently in clinical trials. As an original approach, it focuses on the crucial role of pharmacokinetics and ability to pass the blood–brain barrier in the discovery of new agents for treating CNS disorders. Considering that A<sub>2A</sub> receptor antagonist istradefylline is already commercially available for Parkinson's disease treatment, if the proof of concept of these ligands in AD is confirmed and reinforced, it will be easier to offer a new hope for AD patients.

**Keywords:** A<sub>2A</sub> receptors; A<sub>2A</sub> antagonist discovery; Alzheimer's disease; memory loss; neurodegeneration

## 1. Introduction

Adenosine is a ubiquitous nucleoside derived largely from the hydrolysis of ATP, which acts as an extracellular autacoid to regulate a wide number of pathophysiological functions. The intracellular production of adenosine arises from the action of cytosolic 5'-nucleotidase on AMP or the action of SAH hydrolase on S-adenosyl-homocysteine (SAH) hydrolysis. Conversely, an extracellular pathway predominates when cells undergo increased metabolic activity or stress, e.g., in pathological conditions. In this case, the main source of adenosine is from the activity of specific enzymes known as ectonucleoside

triphosphate diphosphohydrolases (CD39s), which convert ATP into AMP, and ecto-5'-nucleotidase (CD73), which dephosphorylates AMP to produce adenosine [1]. Upon generation, the extracellular nucleoside enters cells through both concentrative and equilibrative nucleoside transporters, CNTs (SLC28 family) and ENTs (SLC29 family), respectively. These proteins are crucial to fine-tuning adenosine levels, with the ENTs playing the more important role. CNT proteins increase adenosine from the extracellular to intracellular pools driven by the sodium gradient, while ENTs allow bidirectional transport, following the nucleoside concentration gradient across the membrane. Normally, adenosine produced extracellularly enters into cells by these transporters, while during hypoxia this flux can be reversed [2].

The amount of extracellular adenosine is also regulated by its metabolism through the enzyme adenosine deaminase (ADA), which transforms it into inosine. This type of regulation occurs also in the intracellular pool, where adenosine may be either converted into inosine by intracellular ADA or phosphorylated to AMP by adenosine kinase (ADK). Due to the different  $K_m$  of these enzymes for adenosine, with ADK being more potent than ADA, under physiological conditions AMP generation is predominant, while in stressful environments its conversion to inosine principally occurs [3].

Adenosine effects are due to the stimulation of four transmembrane receptors, termed  $A_1$ ,  $A_{2A}$ ,  $A_{2B}$  and  $A_3$  adenosine receptors (ARs) [4]. These AR subtypes belong to the family of G protein-coupled receptors, either  $G_i$ -( $A_1$ ,  $A_3$ ) or  $G_s$ -( $A_{2A}$ ,  $A_{2B}$ ) coupled. Altogether, they are balanced in modulating adenylyl cyclase activity, with inhibitory or stimulatory effects depending on  $G_i$  or  $G_s$  coupling, respectively. In addition, the  $A_{2A}$  subtype in the striatum triggers recruitment of  $G_{olf}$  proteins [5,6]. Furthermore, the ARs modulate mitogen-activated protein kinases (MAPK) p38, ERK1/2, and JNK1/2 as well as Akt phosphorylation, thus regulating important intracellular signal transduction pathways, potentially affecting a wide number of pathophysiological functions [7–11].

Although all ARs are potential pharmaceutical targets for a variety of human diseases, the  $A_{2A}$  subtype is the one for which the understanding of biological relationships and the discovery of potent and selective ligands are further along a translational path. The first clinically approved selective  $A_{2A}$  AR antagonist istradefylline, a caffeine analogue, is now on the market for Parkinson's disease (PD) [12,13]. Therefore, this review will focus on a survey of recent literature on the  $A_{2A}$  adenosine receptor as a druggable target for another important neurodegenerative disorder, i.e., Alzheimer's disease (AD), considering the present technological state and directions for improving drug discovery and development from both biological and chemical perspectives.

## 2. $A_{2A}$ Receptors in AD

AD is a multifactorial neurodegenerative pathology that is experiencing exponential growth in the aging population, which is expected to triple by 2050 [14]. Its pathological indications include a progressive appearance of amnesic disabilities, associated with aphasia, apraxia, and agnosia. Within the scope of this disease are behavioral problems, consisting of problem solving and orientation disorders. Current pharmacological strategies to treat AD patients are to increase synaptic acetylcholine (ACh) concentrations, using the cholinesterase inhibitors donepezil, rivastigmine, galantamine, or to reduce glutamate excitotoxicity through memantine [15,16]. Unfortunately, these drugs offer merely symptomatic relief, and they do not impede disease progression, thus making the search for new therapeutic strategies urgent. Various novel pharmacological targets aim to accelerate the clearance of  $\beta$  amyloid ( $A\beta$ ) peptide and tau protein, or to impede neurodegenerative molecular pathways [17,18]. This is an ambitious challenge, but a new drug targeting  $A\beta$  protein accumulation, named aducanumab, recently entered the market, with still controversial clinical efficacy [18–20]. However, due to the failure of numerous clinical trials with drugs directed towards  $A\beta$  protein, it is questionable whether  $\beta$ -amyloid is certainly an effective target. Another strategy for the treatment of AD concerns tau neurofibrillary tangles that are strongly correlated with local neurodegeneration and cognitive impairment

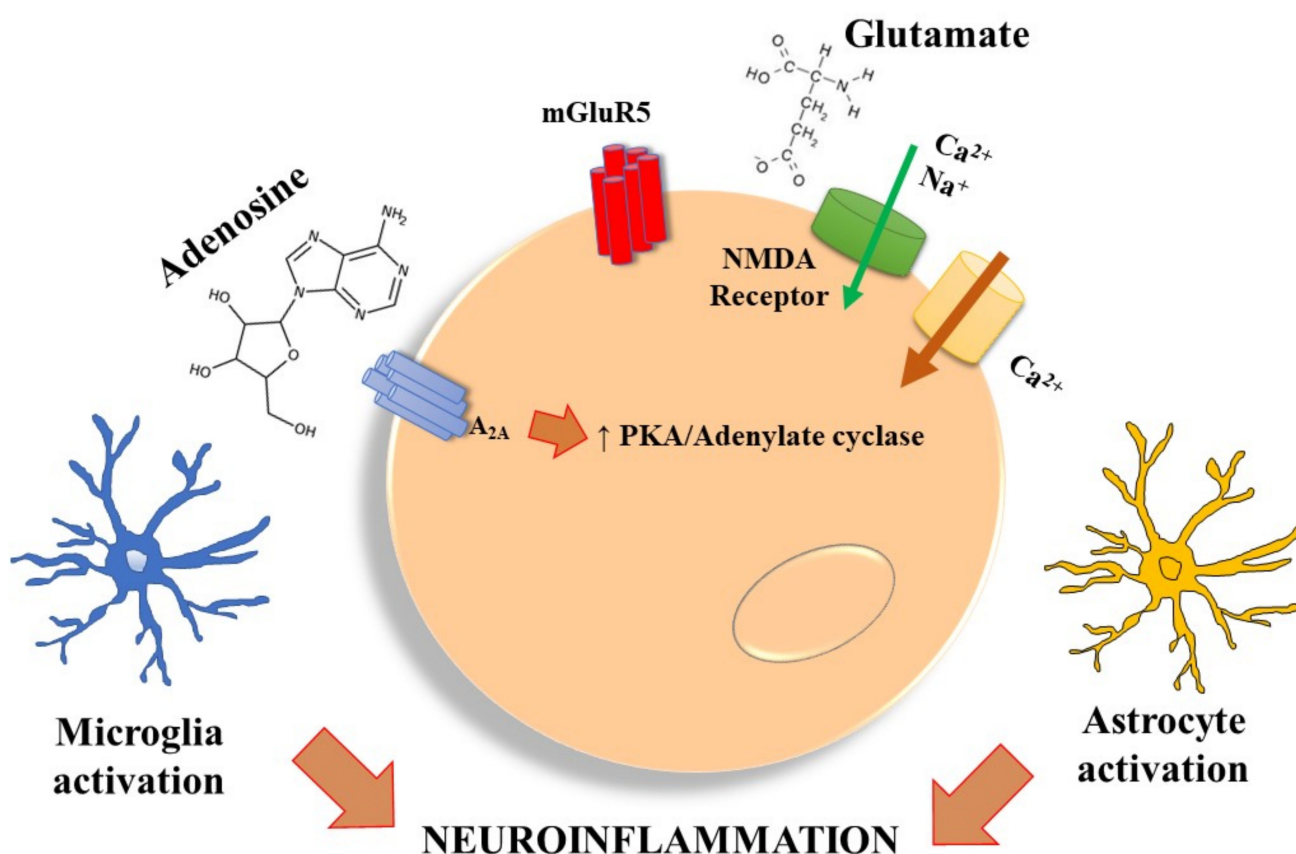
in AD. As a result, several approaches for lowering tau pathogenicity have been suggested, and tau-targeted active and passive immunotherapies are now being investigated for safety and effectiveness in patients with early-stage AD. However, because no clinical trials have been finished to date, no assumptions about the therapeutic effects of therapies for tau pathology can be established [16]. Interestingly, caffeine, the most commonly ingested  $A_{2A}$  receptor antagonist, as well as genetic elimination of  $A_{2A}$  receptors in mice, decreased hippocampus tau hyperphosphorylation, fought neuroinflammation, and restored the associated memory loss [13].

Indeed, the  $A_{2A}$  adenosine receptor may represent a novel and pleiotropic protein through which it is possible to alter the disease course. The  $A_{2A}$  adenosine receptor has been extensively characterized biochemically and structurally, with many X-ray and cryo-EM structures now available [12]. A lengthy intracellular carboxy-terminal tail, where phosphorylation and palmitoylation activities may cause receptor desensitization and internalization, is a structural feature of the  $A_{2A}$  subtype that other family members lack [21]. This subtype forms heteromers with other receptors, such as  $A_1$  adenosine and  $D_2$  dopamine receptors, that have different pharmacological properties compared to the receptor monomers [22]. In the central nervous system, the  $A_{2A}$  receptor is found at the highest levels in striatum, olfactory tubercle, and immune system, while lower amounts are present in the cerebral cortex, hippocampus and vasculature [23,24]. In any case, low expression does not imply lesser importance; indeed, cerebrocortical  $A_{2A}$  receptors account for most of the more relevant effects attributed to brain  $A_{2A}$  receptors (e.g., mood, memory). Likewise, proper recognition must be given to the sparse but highly significant cortical  $A_{2A}$  receptors.

$A_{2A}$  receptors in the brain are mostly detected in glutamatergic synapses, where the major consequence of their activation at the presynaptic level is reflected in increased glutamate release, which contributes to excitotoxicity. Indeed, under physiological conditions, hippocampal glutamate release is under the control of an inhibitory  $A_1$  receptor that, following adenosine increase, is counteracted by a presynaptic  $A_{2A}$  receptor resulting in the release of this excitatory neurotransmitter, possibly through induction of PKA-dependent calcium currents [25,26]. Postsynaptically, the  $A_{2A}$  receptor promotes long-term potentiation (LTP) by facilitating AMPA receptor-mediated currents, NMDA phosphorylation that is mGluR5-dependent and a  $Ca^{2+}$  increase [27–30]. The  $A_{2A}$  receptor reduces glutamate uptake in astrocytes, through modulating the expression of glutamate transporters, GLT-1 and GLAST, and induces astrocytic proliferation and activation. In microglia it plays an important role in the induction of proliferation, activation, process retraction and the production of molecules involved in inflammation, such as NO, PGE2, IL1 $\beta$  and cyclooxygenase [31] (Figure 1). In addition to affecting the inflammatory response,  $A_{2A}$  receptor promotes BDNF and GDNF secretion from microglia and astrocytes, respectively, in response to LPS [1].

It is widely recognized that glutamatergic synaptic failure and temporal lobe degeneration are the earliest signs of cognitive decline, preceding the production of A $\beta$  plaques and tau-derived neurofibrillary tangles [32]. Indeed, individuals with mild cognitive impairment (MCI) and early AD show synaptic loss in the hippocampus and posterior cingulate gyrus, indicating that this event is the starting point for memory loss [33–35]. Therefore,  $A_{2A}$  adenosine receptor engagement in glutamatergic synaptic physiology has suggested a relationship between this subtype and AD. The  $A_{2A}$  adenosine receptor expression in both hippocampal neurons and astrocytes is elevated in aging, AD animal models, and AD patients, elevating glutamate release, calcium influx, the LTP-to-long-term depression transition, and cognitive decline [26,36–46]. Overall, this evidence indicates that the important contribution of the  $A_{2A}$  adenosine receptor to synaptic and neuronal damage by increasing glutamate excitotoxicity and neuroinflammation, as evidenced by the neuroprotection provided by genetic or pharmacological  $A_{2A}$  adenosine receptor blockade. Indeed,  $A_{2A}$  receptor antagonism reduces hippocampus-dependent memory impairment and LTP changes in aged animals and AD models [36,47–51]. In addition,  $A_{2A}$  adenosine

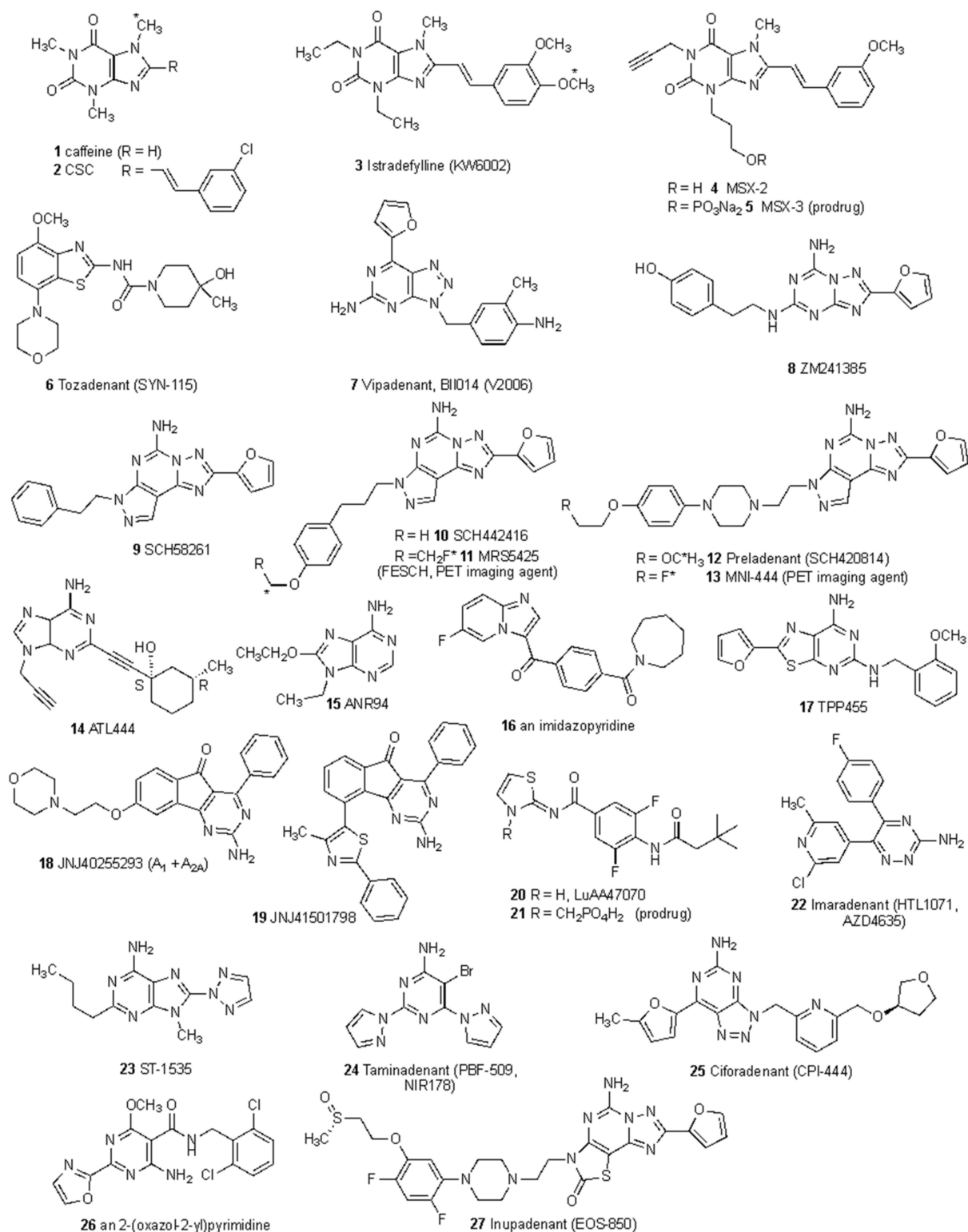
receptor genetic knockdown can ameliorate synaptic damage present in AD models, and numerous studies have found that  $A_{2A}$  adenosine receptor antagonists are effective at improving cognition after synaptic loss in AD animal models, providing a way to combat synaptic toxicity [40,52–54]. Adenosine levels in postmortem AD brains are higher in the parietal and temporal lobes than in the frontal cortex, implying enhanced  $A_{2A}$  receptor activation in overexpressing areas [55]. A study of  $A_{2A}$  receptor photoaffinity labeling in post-mortem striatal membranes provided the first hint that the  $A_{2A}$  receptor could be enhanced in the AD brain [56]. Indeed, in comparison to healthy, post-mortem subjects, brains from AD patients had a higher density of  $A_{2A}$  receptors. More interestingly, in comparison to both frontal grey matter and frontal white matter regions in AD but not in normal participants, the overexpression was observed primarily in the hippocampus and entorhinal areas, suggesting that the upregulation is likely connected to the existence of AD pathology. Indeed,  $A_{2A}$  expression appears to replicate the same AD scenario that extends in the brain from hippocampal/entorhinal structures to other cortical areas, with the hippocampal/entorhinal cortex having the highest density of pathology. As a result, the frontal white matter region, where the AD pathology is less prominent, has the lowest  $A_{2A}$  expression [24]. This leads to the idea that, once reliable PET ligands are validated, assessing  $A_{2A}$  receptor density in diseased brain circuits may become an essential biomarker of vulnerability to detect development of AD [57].



**Figure 1.** The picture represents the physiological effect of  $A_{2A}$  receptor activation on post-synaptic neurons, microglia, and astrocytes. In more detail, its post-synaptic stimulation leads to mGluR<sub>5</sub> activation and NMDA phosphorylation thus increasing calcium influx, while in microglia and astrocytes it raises activation and proliferation with an increase in neuroinflammation.

A further crucial component of  $A_{2A}$  adenosine receptor action in AD is its control of neuroinflammation via glial cells. The  $A_{2A}$  adenosine receptor was shown to be overexpressed in the astrocytes of AD patients, and its genetic silencing in both young and old mice improved long-term memory [40,58,59].  $A_{2A}$  receptors in astrocytes were required for

fine-tuning inhibitory and excitatory regulation of synaptic transmission through GABA and glutamate uptake.  $A_1$  and  $A_{2A}$  receptors, which can occur together as heterodimers, controlled GABA transport in different ways, with the  $A_1$  blocking and the  $A_{2A}$  boosting it [60]. Furthermore, glutamate uptake was hampered by  $A_{2A}$  receptor activation, which is required for the decrease of synaptic glutamate transporters GLAST and GLT-I that is produced by  $A\beta$  peptide [61]. An  $A_{2A}$  receptor antagonist or receptor knockout prevented the  $A\beta$ -induced decrease in glutamate transporters and astrogliosis.  $A_{2A}$  receptor overexpression causes significant transcriptional alterations, primarily impacting genes involved in immune responses, angiogenesis, and cell activation. Treatment with SCH58261 (Figure 2), a selective  $A_{2A}$  antagonist, restored the expression levels of numerous inflammatory and astrocytic activation-related genes. This reinforces the concept that blocking the  $A_{2A}$  receptor might reverse some astrocytic disorders caused by aberrant  $A_{2A}$  receptor expression, implying that receptor antagonists could be useful in  $A_{2A}$  receptor-induced transcriptional dysregulation, inflammation, and astrogliosis. Altogether, these findings shed light on the possible influence of  $A_{2A}$  receptor upregulation on astrocyte transcriptional dysregulation, paving the way for the development of  $A_{2A}$  receptor antagonists as a potential therapy for AD [62]. Activated microglia, in addition to astrocytes, are relevant cells for neuroinflammation and, interestingly, they overexpress the  $A_{2A}$  receptor. Microglia in AD also express the NMDA receptor, which is a crucial target in the fight against AD and might be counteracted by inhibiting the  $A_{2A}$  receptor through their crosstalk in heteromeric structures [63,64]. Furthermore, by inhibiting  $A_{2A}$  receptor-mediated cytokine production, this strategy may be able to reduce memory loss by attenuating neuroinflammation [65,66].



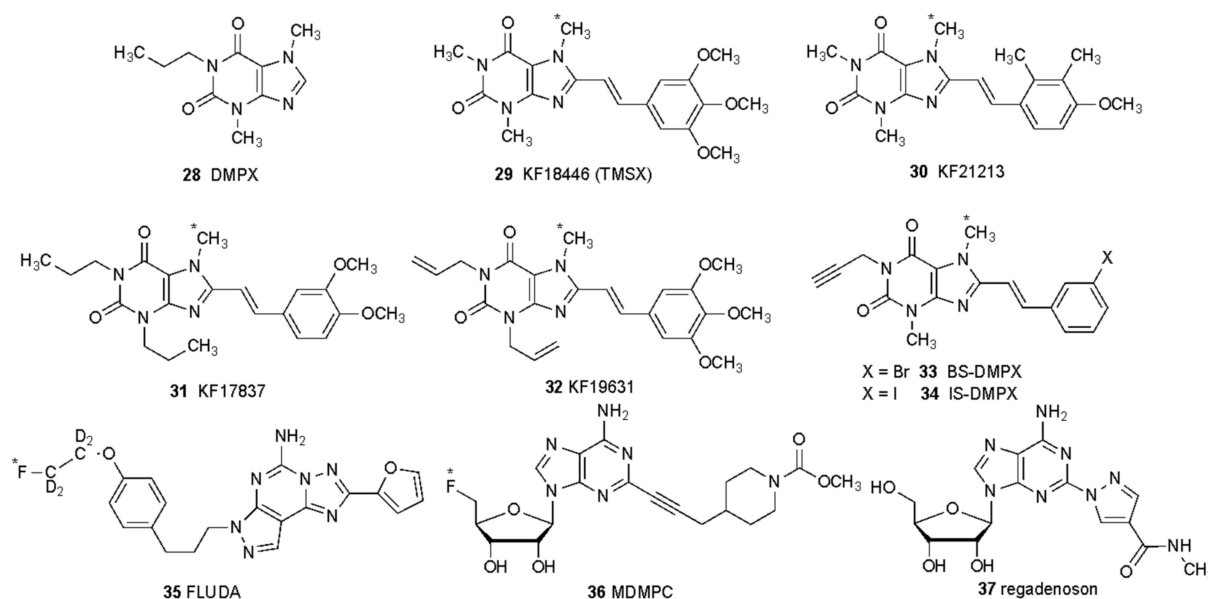
**Figure 2.** Structures of representative A<sub>2A</sub> receptor antagonists 1–27, including those listed in Table 1. Some of these pharmacological probes and clinical candidate compounds have been radiolabeled for PET. Other compounds not listed in Table 1 are described in the literature [67–69]. Additional dedicated PET ligands are shown in Figure 3. Compound 26 is one of a series of A<sub>2A</sub> antagonists discovered by AdoRx Therapeutics [68]. \* Indicates the location of a positron emitting isotope (<sup>11</sup>C or <sup>18</sup>F) when used as a PET tracer [70].

**Table 1.** A<sub>2A</sub> receptor antagonists that are or have been in clinical trials for neurodegenerative diseases and/or for cancer immunotherapy. This list does not include compounds that display mixed selectivity for A<sub>2A</sub> and A<sub>2B</sub> receptors.

Compound (hA <sub>2A</sub> R Affinity, pKi)	Company or Sponsor (Country)	Condition (Reference, Dose)	Clinicaltrials.Gov Number (Phase)
Caffeine (1), (5.0, non-selective)	University Hospital, Lille (France)	AD [50] 200 mg BID	NCT04570085 (3)
Istradefylline (3, KW-6002), (7.44)	Kyowa Hakko Bio (Japan)	PD [71,72] 20 or 40 mg BID	NCT00250393 (2) NCT00955526, 6002–009 (3) NCT01968031 (3)
Tozadenant (6, SYN-115), (8.30)	Hoffmann-La Roche (The Switzerland); Biotie Therapies (Finland)	PD [73,74] 120 or 180 mg BID	NCT01283594 (2) NCT03051607 (3)
Vipadenant (7, BIIB014), (8.89)	Vernalis (UK); Biogen Idec, RedoxTherapies (Juno Therapeutics) (US)	PD [67,75] 30 or 100 mg QD	NCT00438607 (2) NCT00442780 (2)
Preladenant (12, MK-3814, SCH 420814), (8.96)	Merck (US)	PD, antipsychotic drug side effects, cancer [67] 5 mg BID	NCT01155479, PARADYSE (3) NCT00686699, P04628 (2) NCT0309916 (1)
Imaradenant (22, AZD4635, HTL1071), (8.77)	Sosei Heptares (Japan, UK), AstraZeneca (UK)	cancer [76] 75 mg QD	NCT03980821 (1) NCT03381274 (1/2) NCT02740985 (1) NCT04089553 (2)
Taminadenant (24, NIR178, PBF-509), (7.92)	Palobiofarma SL (Spain), Novartis (The Switzerland)	PD, cancer [77] 80–640 mg BID	NCT02111330 (1) NCT02403193, AdenONCO (1/2) NCT03207867 (3) NCT04895748 (1)
Ciforadenant (25, CPI-444, V81444), (8.45)	Bristol-Myers Squibb (US); Corvus Pharmaceuticals (US); Vernalis (UK)	cancer [78] 100 or 200 mg BID	NCT03454451 (1) NCT03337698 (1/2) NCT03549000 (1)
Inupadenant (27, EOS100850, EOS-850)	iTeos (Belgium, US)	cancer [79] 20 mg QD–160 mg BID	NCT03873883 (1) NCT05117177 (1)
TT-10 (structure not disclosed)	Tarus Therapeutics (US)	cancer [80] 10–200 mg BID	NCT04969315 (1/2)
CS3005 (structure not disclosed)	CStone (China)	Cancer [81]	NCT04233060 (1)

Abbreviations: AD, Alzheimer’s disease; PD, Parkinson’s disease; PD-1, programmed cell death 1.

We already have proof that blocking A<sub>2A</sub> improves memory in humans. As a matter of fact, caffeine, the most commonly ingested A<sub>2A</sub> receptor antagonist, can ameliorate cognition providing the evidence for the A<sub>2A</sub> receptor’s relevance in AD through different strategies. Indeed, caffeine decreased hippocampal tau hyperphosphorylation, attenuated neuroinflammation and reversed the associated memory loss [12,50,53,82]. The Effect of CAFFeine on Cognition in Alzheimer’s Disease (CAFCA) is under investigation in the Phase 3 clinical trial NCT04570085. It is a multicenter, randomized, double-blind, placebo-controlled trial evaluating the effect of a 30-week caffeine treatment on cognition in AD at beginning to moderate stages (MMSE 16–24). The results of human research will help to clarify the function of caffeine in the prevention of AD. In addition, istradefylline, a potent and selective A<sub>2A</sub> antagonist, is already in use in the clinic for another neurodegenerative condition, PD. It was approved earlier in Japan and Korea, and in August 2019 in the United States after passing various clinical safety and efficacy tests (as Nourias) [73,83–85]. As for its role in AD, low istradefylline doses improved spatial memory and habituation in animal models of AD, providing important confirmation that A<sub>2A</sub> receptor blockade could be a novel target for countering cognitive deficits in AD patients [59,71]. This finding shows that A<sub>2A</sub> receptor-targeting drugs, such as istradefylline and hopefully novel ones, have a great deal of promise for treating dementia.



**Figure 3.** Structures of additional  $A_{2A}$  receptor ligands (antagonists 28–36) that have been radiolabeled for PET imaging, as described in Sun et al. [70] \* Indicates the location of a positron emitting isotope ( $^{11}\text{C}$  or  $^{18}\text{F}$ ). DMPX 28 is shown for comparison as an early, slightly selective  $A_{2A}$  antagonist that is too low in affinity to be used a PET tracer. In addition, the only synthetic  $A_{2A}$  agonist approved for human use (regadenoson 37, used in unlabeled form for myocardial perfusion imaging) is shown.

### 3. Chemistry of $A_{2A}$ Receptor Antagonists in Clinical Trials

Many selective  $A_{2A}$  antagonists have been reported and some have entered clinical trials (Table 1) [12,67,68,86] for either neurodegenerative diseases or cancer immunotherapy (including by co-administration with checkpoint inhibitors and other cancer immunotherapies). It is to be noted that  $A_{2B}$  antagonists and mixed  $A_{2A}/A_{2B}$  antagonists are also being evaluated in cancer immunotherapy [80]. Although not covered in this review,  $A_{2B}$  antagonists may also have neuroprotective properties [87]. The naturally occurring alkylxanthine caffeine, a weak non-selective adenosine antagonist, is in a Phase 3 clinical trial for AD in France [50]. Recently, the analogue of caffeine with full deuteration of the three methyl groups was shown to have a prolonged in vivo plasma half-life in the rat (from 1.9 h to 5 h) without losing adenosine receptor binding affinity [88].

Istradefylline 3 [72], a moderately potent ( $\text{pK}_i$  7.44) and selective  $A_{2A}$  antagonist that is structurally related to caffeine (Figure 2), is the only such ligand that is FDA-approved for clinical use, i.e., as a co-therapy with L-DOPA in PD treatment. A total of 23 clinical trials have been carried out to probe the safety and efficacy of istradefylline in PD and also to study the effects of hepatic impairment and antibiotic rifampicin on its metabolism.

Unlike some of the more recently introduced  $A_{2A}$  antagonists for cancer, tozadenant 6 (SYN115) was initially developed for treatment of PD and later for cocaine addiction [67,89–91]. Tozadenant has been studied both as a PD monotherapy and as a cotherapy with L-DOPA or dopamine agonists. Clinical efficacy in reducing off-time was observed without severe adverse effects in initial clinical trials, but a Phase 3 clinical trial turned disastrous with seven cases of sepsis out of 890 patients and six fatalities [86]. Therefore, its clinical development was terminated in 2018. Its potential use in treating cocaine addiction was previously studied [89], but there was insufficient clinical efficacy to proceed with this target. Tozadenant analogues were recently reported as  $A_{2A}$  antagonists, including  $^{18}\text{F}$ -labeled radiotracers for potential PET imaging [92]. Vipadenant 7 [67] is a potent  $A_{2A}$  antagonist ( $\text{pK}_i$  8.89) that was introduced for PD [74,93] and its development halted due to safety issues [68], but it later transitioned to use in cancer immunotherapy. Other  $^{18}\text{F}$ -labeled  $A_{2A}$  antagonists related to vipadenant were reported by Lai et al. [94]. Preladenant 12 followed a similar developmental path, first being introduced for PD and



later repurposed to cancer [68,95]. The  $^{11}\text{C}$  forms of a predecessor of preladenant, i.e., SCH442416 **10**, and preladenant itself, have been shown to be useful as  $A_{2A}$  receptor PET imaging agents [94]. The use of structure-based approaches has accelerated the discovery of  $A_{2A}$  antagonists. The earliest success of a structurally designed  $A_{2A}$  antagonist was imaradenant **22**. It was initially discovered using an inactive conformation-stabilized  $A_{2A}$  receptor, enabled by the ability to readily determine the three-dimensional structure of  $A_{2A}$  antagonist-bound receptors by strategic stabilizing mutations [96]. Early in its development it was positioned for use in treating attention deficit hyperactive disorder (ADHD), but it was repurposed for cancer immunotherapy. Taminadenant **24** and ciforadenant **25** are being developed for cancer immunotherapy [77,78], but ciforadenant has a long history of clinical development, initially for CNS application. An analogue of taminadenant (PBF-999, structure not shown) displayed dual  $A_{2A}$  receptor and phosphodiesterase (PDE)10 activity and is now in a clinical trial for cancer [68,94].

The role of pharmacokinetics and ability to pass the blood–brain barrier (BBB) are important factors in the discovery of new agents for treating CNS disorders. There are now protocols for predicting the likelihood that a new compound will cross the BBB with appropriate metabolic stability, such as the multi-parameter optimization (MPO) score [97–100]. The MPO score estimation for CNS drugs, along with binding/functional affinity measurement could be useful tool to predict and design radiotracers for PET. Use of such algorithms will aid in the discovery of future  $A_{2A}$  antagonists for the treatment of CNS disorders, such as PD and AD, or for treatment of peripheral conditions in which CNS penetration would cause side effects. Nevertheless, it must be kept in mind that multiple physicochemical and biochemical factors influence the suitability of a given compound for use as a CNS drug or imaging agent [100].

Table 2 shows the MPO scores calculated using standard commercial software of common  $A_{2A}$  antagonists, including those in clinical trials and those for use as PET imaging agents [98,99]. This is a means of predicting which antagonists might be suitable for use in treatment of neurodegenerative diseases. For CNS drug candidates expected to be readily brain penetrant, the high  $\text{clogBB}$  ( $\log([\text{brain}]:[\text{blood}])$ ) value ( $>-0.2$ ) and “no” substrate for the brain efflux pumps (e.g., P-gp), and a higher Intravenous CNS Profile Score [100] are preferable. A  $\log P$  value  $<4$  is one of many parameters predictive of effective oral dosing [100]. Thus, alkylxanthines caffeine and DMPX readily cross the BBB with  $\text{clogBB}$  values of  $-0.0323$  and  $-0.180$ . Most of the compounds shown in Table 1 have the ability to cross the BBB to varying degrees, but an anti-tumor agent, inupadenant ( $\text{clogBB} -1.25$ ), is not brain penetrant.

**Table 2.** Calculated MPO parameters of representative  $A_{2A}$  antagonists and one agonist **37** as shown in Figures 1 and 2, including compounds that are for use as PET tracers. The probabilistic scores indicative of CNS penetration were calculated for each small molecule with the StarDrop software package (Optibrium Inc., Cambridge, UK) [98,99]. Six physicochemical parameters that affect BBB passage are included in this calculation: molecular weight, most basic center’s  $pK_a$ , calculated  $\log P$  ( $\text{clogP}$ ), calculated  $\log D$  at pH 7.4 ( $\text{clogD}$ ), topological polar surface area (tPSA), and hydrogen bond donor number. The BBB  $\log([\text{brain}]:[\text{blood}])$  ( $\text{clogBB}$ ) parameter is a single indicator of probability of crossing the BBB at pharmacologically significant levels.

Number	Compound [Reference]	Intravenous CNS Scoring Profile Score	Predicted BBB $\log([\text{Brain}]:[\text{Blood}])$	P-gp Category	$\log P$
1	caffeine [12]	0.355	$-0.0323$	no	0.0231
2	CSC [10]	0.238	$-0.539$	no	2.24
3	istradefylline [73]	0.129	$-0.999$	yes	2.16
4	MSX-2 [10]	0.119	$-1.25$	no	1.12
6	tozadenant [74,92]	0.0786	$-0.891$	yes	2.47

Table 2. Cont.

Number	Compound [Reference]	Intravenous CNS Scoring Profile Score	Predicted BBB Log([Brain]:[Blood])	P-gp Category	logP
7	vipadenant [75]	0.103	−0.388	yes	2.12
8	ZM241,385 [70]	0.101	−1.10	yes	2.13
9	SCH58241 [70]	0.0783	−0.644	yes	2.60
10	SCH442416 [94]	0.0926	−0.779	yes	2.78
11	MRS5425 [70]	0.0747	−0.773	yes	3.08
12	preladenant [95]	0.105	−0.592	yes	2.41
13	MNI-444 [70]	0.0964	−0.495	yes	2.73
14	ATL444 [101]	0.163	−1.17	no	1.16
15	ANR94 [101]	0.147	−0.953	no	0.958
16	imidazopyridine [101]	0.113	0.0684	no	3.47
17	TPP455 [11]	0.0753	−0.876	no	3.14
18	JNJ40255293 [10]	0.121	−0.219	yes	2.72
19	JNJ41501798 [102]	0.0200	−0.600	no	5.14
20	LuAA47070 [101]	0.122	−0.672	no	2.87
21	imaradenant [76]	0.0833	−0.508	no	3.04
23	ST-1535 [10]	0.0767	−1.07	yes	1.46
24	taminadenant [77]	0.153	−1.01	no	1.02
25	ciforadenenant [78]	0.0507	−0.965	yes	2.21
26	2-(oxazol-2-yl)pyrimidine [68]	0.0723	−0.502	no	2.64
27	inupadenant [79]	0.0994	−1.25	yes	2.66
28	DMPX [70]	0.308	−0.180	no	0.629
29	KF18446 [70]	0.227	−0.947	no	1.34
30	KF21213 [70]	0.183	−0.826	no	2.34
31	KF17837 [70]	0.178	−1.02	yes	2.55
32	KF19631 [70]	0.183	−0.525	yes	1.74
33	BS-DMPX [70]	0.192	−0.603	no	2.21
34	IS-DMPX [70]	0.185	−0.616	no	2.26
35	FLUDA [103]	0.0674	−0.774	yes	3.08
36	MDMPC [70]	0.0934	−1.43	yes	0.678
37	regadenoson [101]	0.0551	−1.30	yes	−1.35

Furthermore, the application of in vivo imaging with PET ligands has become an important component in CNS drug development to determine target engagement and occupancy related to duration of drug action. Fortunately, there are numerous A<sub>2A</sub> antagonist PET ligands developed over decades, resulting in [<sup>18</sup>F]MNI-444 ([<sup>18</sup>F] **12**) and [<sup>11</sup>C]preladenant ([<sup>18</sup>F] **11**), which demonstrated high specific binding signal [82] as clinical imaging tools. The interaction of A<sub>2A</sub> antagonists and the D<sub>2</sub> dopamine receptor was also studied in humans using istradefylline (40 mg QD) and PET imaging of dopamine receptors with [<sup>11</sup>C]raclopride [104].

#### 4. Conclusions

This paper presents a brief overview of A<sub>2A</sub> receptor antagonist clinical use in the context of drug discovery for AD therapy. Many new A<sub>2A</sub> antagonists are being tested clinically, mostly for cancer, and some of these compounds are able to cross the BBB, which would enable their use in AD. Overexpression and functional upregulation of the A<sub>2A</sub> receptor is associated with early changes leading to progressive neurodegeneration. Thus,

PET ligands that are validated for A<sub>2A</sub> receptor imaging in the human brain could be useful for the early clinical diagnosis of AD or other neurodegenerative conditions, which is an unmet medical need. Given the generality of the involvement of this receptor in brain injury and other pathology, the clinical development of A<sub>2A</sub> receptor antagonists for treatment of these conditions promises to be on the verge of becoming a reality.

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