History and perspectives of A_{2A} adenosine receptor antagonists as potential therapeutic agents

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Abstract/Synopsis: Growing evidence emphasizes that the purine nucleoside adenosine plays an active role as a local regulator in different pathologies. Adenosine is an ubiquitous nucleoside involved in various physiological and pathological functions by stimulating A_1, A_{2A}, A_{2B} and A_3 adenosine receptors (ARs). At the present time, the role of A_{2A}ARs is well known in physiological conditions and in a variety of pathologies, including inflammatory tissue damage and neurodegenerative disorders. In particular, the use of selective A_{2A} antagonists has been reported to be potentially useful in the treatment of Parkinson's disease (PD). In this review, A_{2A}AR signal transduction pathways, together with an analysis of the structure-activity relationships of A_{2A} antagonists, and their corresponding pharmacological roles and therapeutic potential has been presented. The initial results from an emerging polypharmacological approach are also analyzed. This approach is based on the optimization of the affinity and/or functional activity of the examined compounds toward multiple targets, such as A_1/A_{2A}ARs and monoamine oxidase-B (MAO-B), both closely implicated in the pathogenesis of PD.

Key words: human A_{2A} adenosine receptors; A_{2A} antagonists, Parkinson's disease, polypharmacology.

1. Introduction

Adenosine, a well known purine nucleoside, acts as an endogenous modulator in the human body in both the central and peripheral nervous systems by interacting with four G protein coupled receptors (GPCRs) identified as A_1, A_{2A}, A_{2B} and A_3 adenosine receptors (ARs).[1,2] The A_1 and A_3ARs are negatively coupled to adenylyl cyclase and exert an inhibitory effect on cyclic adenosine monophosphate (cAMP) production.[2] The A_{2A} and A_{2B}ARs stimulate the activity of the adenylyl cyclase, inducing an increase of cAMP levels.[3] A_{2A}ARs have a distinct tissue localization, different biochemical pathways, and specific pharmacological profiles (Section 2). It has been reported that A_{2A}ARs form homo- and heterodimers that could be very interesting in new drug design and development approaches (Section 3). Important progress has been made toward understanding the role of A_{2A} antagonists in physiological conditions and in a variety of pathologies such as the neurodegenerative disorders as well as other known diseases of the central nervous system (CNS, Section 4).[3,4] Over the past three decades the search for novel A_{2A} antagonists has been greatly expanded and a large number of ligands have been synthesized and tested (Section 5).[5-7] Many potent A_{2A} antagonists have been designed as promising candidates for their beneficial effects on motor deficits, suggesting that they could be used positively in neurological conditions related to dopaminergic dysfunction such as Parkinson's disease (PD).[8,9] Moreover, the blockade of A_{2A}ARs may be useful in brain disorders as ischemia, epilepsy, Huntington's disease (HD) or Alzheimer's disease (AD) and affords benefits in some psychiatric diseases (Section 6).[10,11]

In this review, the structure, distribution, and signal transduction pathways of A_{2A}ARs, together with the structure-activity relationships (SAR) of some of the most interesting A_{2A} antagonists, have been discussed, combined with the corresponding pharmacological roles

and therapeutic potential of the compounds. The bibliographic material employed to prepare the different topics of this review has been collected from the databases PubMed (mainly for the pharmacology sections) and SciFinder Scholar (for the medicinal chemistry part) setting as keywords: "A_{2A} adenosine receptor", "A_{2A} adenosine receptor antagonists" and "Parkinson's disease". Results were further refined with the terms "polypharmacology", "multi-target approach" or "dual acting molecules". The retrieved documents were individually scrutinized and selected for their relevance to the field. Since our aim was to give a comprehensive overview of both the history and perspectives of A_{2A} antagonists, we did not apply severe criteria with regard to the publication date. Nevertheless, particular attention has been given to describing the most recent achievements of this intriguing research field (since 2005 to date). Finally, we organized the chemistry papers by subdividing the described chemical scaffolds into mono- bi- and tricyclic systems, then analyzing similarities within each subclass.

2. Structure, distribution and biochemical pathways of A_{2A}ARs

The gene for the A_{2A}AR has been cloned from several species, including dog, rat, guinea pig, mouse and human, showing an high degree of homology between human, mouse, and rat.[12] The A_{2A}AR gene is composed of multiple exons which encode alternative transcripts initiated from at least four independent promoters.[13] Due to a long carboxy terminal domain, A_{2A}ARs show a greater molecular weight (45 kDa) in comparison to the other adenosine subtypes, even though their structure is similar to those of typical GPCRs. A_{2A}ARs are composed of a central core, consisting of seven transmembrane helices (TM1-7) each having 20-27 amino acids. The TM domains, that are mainly α-helices, are linked by three intracellular (IL1-3) and three extracellular (EL1-3) loops where TM3 and EL2 contain cysteine residues forming a disulfide bond. An additional short helix (TM8) runs parallel to

the membrane cytoplasmic surface.[12] The human A_{2A}AR is characterized by 49% amino acid sequence identity with the human A_1AR, 58% with human A_{2B}AR, and 41% with human A_3AR.[14] In the TM1-7 domains, the residues critical for interaction with the ligand are near the extracellular part of the receptor and are highly conserved. All transcripts identified contain the same coding region for the A_{2A}AR, a common 3′ untranslated region (UTR) and a distinct 5′UTR that mediates a strong homology.[13] Transcriptional and translational regulation of the A_{2A}AR appears to be critical in controlling the response of adenosine under pathophysiological conditions.[3] The A_{2A}AR C-terminus has been defined as a specific region where different accessory proteins may interact, such as D_2 dopamine receptors (D_2DRs), α-actinin, adenosine diphosphate (ADP)-ribosylation factor nucleotide site opener (ARNO), ubiquitin-specific protease (USP4) and translin-associated protein X (TRAX), the presence of which may explain conflicting results linked to A_{2A}AR activation. In addition, the C-terminus of A_{2A}ARs is highly conserved among species and mediates different G protein-independent actions. The interaction between ARNO and A_{2A}ARs modulates the stimulation of mitogen activated protein kinases (MAPK) closely associated with these receptors.[15] Another A_{2A}AR-interacting protein, TRAX, is involved in the ability of A_{2A}ARs to suppress cell proliferation.

Several mutagenesis studies have been performed on A_{2A}ARs to explore which amino acid residues are important for binding to different ligands.[16] This data can be combined with novel structural information and SAR of the ligands providing an ideal situation for characterizing ligand binding through computational modeling.[17] Extensive site-directed mutagenesis data on agonist and antagonist binding to A_{2A}ARs is also available.[18] The A_{2A}AR has also been examined by using a free energy calculation scheme to model alanine scanning mutagenesis and compute ligand binding free energies for receptor mutants.[19] In a previous molecular modeling study it was suggested that a glutamic acid in the first transmembrane

domain and a histidine in the seventh transmembrane domain are involved in the agonist binding to A_{2A}ARs.[20] It also has been reported that the overall loss of ligand-water interactions for mutations is correlated with losses in ligand binding affinity, suggesting the primary role of water-mediated interactions in ligand design projects.[16] The recent X-ray analysis of the crystal structure of both agonists and antagonists bound to the hA_{2A}AR furnished important insights on receptor topology and mechanisms of ligand-induced GPCR activation.[21-25] These investigations provided noteworthy information about receptor conformational changes that are triggered by ligand binding at the extracellular side and lead to the intracellular signaling cascade. In addition, the analyzed structures reveal precious details with respect to receptor-ligand interactions in the ligand-binding cavity, thereby validating receptor-based approaches for the identification of new A_{2A} antagonists.[17,26] For example Katritch et al. performed a virtual screening of more than 4 million compounds, succeeding in predicting unexplored potential ligands.[27] The structures of the antagonists ZM241385 (Figure 2), XAC (N-(2-aminoethyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)phenoxy]-acetamide hydrochloride) and caffeine (Figure 2) bound to the hA_{2A}AR have been to date reported.[21-23] As far as agonist ligands, the endogenous adenosine, NECA (5'-*N*-Ethylcarboxamidoadenosine) and UK432097 (6-(2,2-diphenylethylamino)-9-((2*R*,3*R*,4*S*,5*S*)-5-(ethylcarbamoyl)-3,4-dihydroxytetrahydrofuran-2-yl)-*N*-(2-(3-(1-(pyridin-2-yl)piperidin-4-yl)ureido)ethyl)-9*H*-purine-2-carboxamide) have been co-crystallized with the receptor.[22,24,25] A recent paper provides an excellent review of the key findings on the binding mode of agonists and antagonists.[28]

Several studies report that A_{2A}ARs exist in a wide variety of organs including heart, liver, lung, spleen, and thymus.[3] In the heart, the presence of A_{2A}ARs in atrial and ventricular tissues and in coronary vessels is consistent with their ability to mediate vasodilation.[29] In the

rat brain, A_{2A}ARs are found in various areas such as the striatum, nucleus accumbens, olfactory tubercle, cortex, and hippocampus, implying a role of adenosine in neuronal development, in neuroprotection, and in different homeostatic functions.[30] High expression of A_{2A}ARs has been found in platelets, leukocytes, neutrophils, vascular smooth muscle, and endothelial cells, with important implications in the regulation of inflammatory responses.[9,31,32] The signaling pathways used by A_{2A}ARs depend on the type of cells and tissues where the receptor is localized, by the specific G protein involved, and from the kinases present in the cells.[33] In the peripheral nervous system, a G protein α subunit is involved, while in the striatum A_{2A}ARs primarily stimulate Golf, another member of the Gs proteins coupled to adenylyl cyclase.[34] A_{2A}AR activation causes the exchange of guanosine diphosphate (GDP) for the guanosine triphosphate (GTP) bound to the G protein α subunit and the dissociation of βγ heterodimer. A_{2A}ARs stimulate adenylyl cyclase activity through coupling with Gs proteins, leading to cAMP production that activates protein kinase A (PKA)[12] (Figure 1). PKA phosphorylates and activates various receptors, ion channels, phosphodiesterases, cAMP responsive element binding protein (CREB), and dopamine- and cAMP-regulated phosphoprotein (DARPP32).[35] In brain, A_{2A}AR modulation is involved with MAPK pathways and with extracellular signal regulated kinases (ERK).[4] Kinase phosphorylation mediates specific cellular responses, such as the phosphorylated CREB that determines A_{2A}AR dependent anti-inflammatory effects and inhibition of nuclear factor kB (NF-kB) activation, suppressing cytokine expression.[36] The regulation of DARPP32 phosphorylation appears to be linked to A_{2A} antagonists providing a positive feedback amplification mechanism for shutting down the PKA signaling pathways. In various cell lines, the activation of A_{2A}ARs stimulates the formation of phospholipase C, which modulates inositol phosphates, raises intracellular calcium, and activates protein kinase C (PKC).[37]

3. A_{2A}AR homo- and hetero-dimers

For several years, it has been reported that a large number of receptors, especially GPCRs, are able to form multimers and/or dimers.[38] To fully appreciate the contribution of these heteromers to normal physiology of the brain and use them for selective drug targeting in specific pathologies, it is necessary to investigate the functional interrelationships between the receptors in a heteromer.[39] Technologies based on the use of fluorescent-fused proteins and different adaptations of resonance energy transfer (RET) or bioluminescence (BRET) techniques have been very useful.[39] In particular, bioluminescence assays have demonstrated the existence of an A_{2A}AR-A_{2A}AR interaction that represents the functional form of the receptor on the plasma membrane.[40] Immunoprecipitation studies suggest that in crude cell lysates, the ratio of homodimers to monomers is approximately 60:40, whilst on the cell surface the ratio rises to 90:10.[40]A deletion mutant version of the A_{2A}ARs, lacking the C-terminal domain, is able to form both monomeric and dimeric species, suggesting that this domain does not participate in the dimerization.[40] However, the C-terminal tail of A_{2A}ARs is known to be involved in heteromers formed by these ARs and D_2DRs.[41] While A_{2A}AR homodimers are the functional species at the cell surface, they coexist with A_{2A}AR-D_2DR heterodimers that are expressed to a lesser extent.[42]

The A_{2A}AR-A_{2B}AR heteromers appear to mediate an hypoxia-induced immunosuppression and to modulate anti-inflammatory pathways, preventing chronic inflammation associated with different pathologies.[43] Recently, A_{2A}AR-A_1AR heteromers have been identified in presynaptic membranes and in astrocytes *via* co-immunoprecipitation experiments. These heteromeric receptors are able to modulate glutamate release and gamma-aminobutyric acid (GABA) transport.[44,45] Direct evidence shows that A_1AR-A_{2A}AR heteromers in astrocytes are expressed as heteromers of homomeric multimers, with a

minimal structure consisting of an A_1AR-A_1AR-A_{2A}AR-A_{2A}AR complex leading to GABA uptake facilitation.[44] The presence of A_{2A}AR-A_1AR heterodimers in the CNS could increase the complexity by which these two receptors regulate neuronal excitability.[46] A_{2A}AR-A_1AR heteromers couple to two different G proteins such as Gs and Gi/o both regulating GABA transport in an opposite way, with the A_1AR promoter mediating inhibition of GABA transport and the A_{2A}AR promoter mediating facilitation of GABA transport. This suggested the role of a potential dual amplifier to control ambient GABA levels at neuron-glia synapsis.[44] Since GABA is an inhibitory neurotransmitter, a dual antagonist could conceivably have the desired inhibitory effect at elevated concentrations and actually increase GABA transport, and therefore neuronal firing, at low concentrations.

Alternatively, the co-administration of an A_{2A} antagonist and a cannabinoid CB_1 antagonist in an animal model of PD has shown neuroprotective potentials where the treatments counteract dopaminergic cell death and neuroinflammation.[47] Another approach, related to A_{2A}AR-D_2DR heteromers, shows that adenosine acting at A_{2A}AR counteracts the action of dopamine on D_2DR, suggesting the potential use of bivalent drugs able to activate homo- and heterodimers in neurodegenerative disorders.[48] The activation of A_{2A}AR leading to the increase of cAMP required concurrent activation of CB_1 receptors controlling how each of these receptors responds to their specific agonists.[49] A combination of drugs, or a single compound, selectively acting on A_{2A}AR-CB_1-D_2DR heteromers could represent a novel approach in PD pharmacological treatment.[50] It is well known that A_{2A}ARs can couple with metabotropic glutamate receptor type 5 (mGlu5) and form functional heteromeric complexes by the involvement of PKA-dependent or independent PKC signaling, suggesting that these synergistic interactions could have important implications for striatal neuronal function and dysfunction.[51,52]

It is known that inhibitors of monoamine oxidase-B (MAO-B) are considered useful in PD, showing marked neuroprotective properties.[53] It is also known that targeting of a single pathway may not be sufficient to modulate the specific pathology of neurodegenerative diseases, requiring instead a multifaceted approach.[54] However, the toxicities associated with drug combinations may be higher compared to multitarget compounds.[55] In addition, the use of drug cocktails can negatively impact patient compliance and increase the risks of drug-drug interactions. For these reasons, the development of multitarget drugs could offer significant advantages, particularly with regard to the relevant cost-effective aspects.[56] Considering that the brain is characterized by an age-related increase in MAO-B activity and an A_{2A}AR up-regulation,[9,53] compounds that are able to both inhibit MAO-B and antagonize A_{2A}ARs could represent novel anti-PD agents with dual mechanisms of action.

Therapies that act at multiple targets provide both symptomatic and neuroprotective benefits and may be more effective in treating complex neurodegenerative diseases. A_{2A} antagonists and MAO-B inhibitors potentiate the motor restorative effects of L-3,4-dihydroxyphenylalanine (L-DOPA), by acting at different targets and the combination of these two activities in a single drug may be advantageous, enhancing the neuroprotective properties.

In accordance with this view, it is recognized that a multitarget drug could be very useful by modulating multiple targets characterizing complex diseases such as PD or AD.[55] The polypharmacology approach, in contrast to combination drugs, is based on the use of more effective drugs that could be developed modulating multiple target. As a consequence, a single molecule with dual activity could have a better pharmacodynamic profile compared to different drugs administered in combination.

4. Pharmacological role and preclinical studies of A_{2A} antagonists

The co-expression of A_{2A}ARs with D_2DRs has been reported in the GABAergic striatopallidal neurons where adenosine and dopamine exert opposite effects in the regulation of locomotor activity.[7] Biochemical studies have demonstrated the existence of an antagonistic A_{2A}AR-D_2DR interaction by which the stimulation of A_{2A}AR decreases the affinity of D_2DR and affects its signal transduction to the G protein.[57-58] The negative interaction between these receptors is at the basis for a non-dopaminergic therapeutic approach of using A_{2A} antagonists in the treatment of PD.[59] Prospective epidemiological studies have strongly associated caffeine consumption with a reduced risk of developing PD.[8] Moreover, it has been observed that A_{2A} antagonist treatment could extend the duration of action of L-DOPA, a dopamine precursor, and reduce various motor symptoms such as gait and tremor or dyskinesia.[60] As a consequence, the development of bivalent ligands able to activate D_2DRs and block A_{2A}ARs may be a promising strategy for the treatment of neurodegenerative diseases with positive clinical implications.[5,61]

It is known that chronic inflammation impedes microglial motility induced by the injury and that the neuroprotection observed with an A_{2A} antagonist, such as preladenant, involves the ability of activated microglia to respond to tissue damage, restoring microglial responses under pro-inflammatory conditions.[62]

Recent studies demonstrated that both caffeine and A_{2A} antagonists prevent the accumulation of amyloid-β-peptide (Aβ) in and around cerebral blood vessels, which, if untreated, could result in cognitive deficits.[63,64] In an *in vivo* model of AD in mice, chronic caffeine consumption reverses cognitive impairment and decreases brain Aβ levels.[65-67] Moreover, caffeine promotes neuronal survival and reduces the process of neurodegeneration in the striatum and/or cortex, which may contribute to its beneficial effects against AD.[68]

Several lines of evidence supported a possible pathophysiologic role of A_{2A}ARs in HD. Alterations in their presence and functionality may represent an early vulnerability of medium spinal neurons that selectively express A_{2A}ARs.[69-70] The prominent role of glutamatergic neurotransmission in HD and the beneficial effects exerted by A_{2A} antagonists such as SCH58261 (Figure 3) or ZM241385 (Figure 2) in animal models of HD are well known.[71-72] Moreover, *in vivo* A_{2A}AR blockade influences NMDA receptor expression and functions in HD.[73] On the other hand, the involvement of A_{2A}ARs in both protective and pro-toxic pathways at pre and post-synaptic locations, makes their role rather unpredictable and their therapeutic application highly difficult.[74]

The potential neuroprotective effect of A_{2A}ARs in status epilectus is based on the effect of ZM241385, which had no influence on the progression of amygdala-kindled seizures but had a potent anti-convulsant profile with few adverse effects, suggesting good efficacy against the amygdala-kindled seizures.[75] One of the possible mechanisms involved in the genesis of the injury process triggered by status epilecticus is the imbalance between cerebral glucose consumption and blood flow that occurs during severe epileptic seizures where A_{2A}ARs could play a role. These receptors are present in cerebral blood vessels and can promote vasodilation and self-regulation of cerebral blood flow, suggesting that A_{2A}ARs might be directly contributing to the neurodegenerative process. Inhibiting A_{2A}ARs might alter blood flow homeostasis in the brain, worsening the imbalance between the metabolic demand and the energy supply during epilepsy.[76]

Pharmacological blockade of A_{2A}ARs mediates a significant protection in the CNS after a spinal cord injury (SCI) by reducing the excessive release of neurotransmitters caused by high levels of intracellular calcium ions, which can lead to neuronal death through increased excitotoxicity.[11] For example, enhanced release of adenosine has been related to the

development of many known functional motor and sensory deficits. Thus, the blockade of both A_1 and A_{2A}ARs may provide a protective role against SCI-induced pain, inflammation and cell death caused by excessive neuronal activity.[77]

Substantial evidence shows a protective role for A_{2A} antagonists in striatal and nigral neurons through prevention of glutamate-dependent neuronal death, thereby reducing cortical damage in a variety of ischemic stroke models. In A_{2A}AR knockout (KO) mice, transient focal ischemia causes less neuronal damage compared to wild-type (WT) mice. The apparent paradoxical finding that an A_{2A} agonist reduces ischemic reperfusion injury, mediating protection in the kidney, liver, and spinal cord by suppression of cytokine or chemokine production, has been previously discussed.[78] The selective A_{2A} antagonist SCH58261 (Figure 3) reduced ischemic brain damage in an adult rat model of focal cerebral ischemia.[79] The same antagonist, subchronically administered, was protective against both brain damage and neurological deficits.[80,81] Evidence suggests that A_{2A} antagonists provide early protection via centrally mediated control of excessive excitotoxicity, while A_{2A} agonists provide protracted protection by controlling massive blood cell infiltration in the hours and days after ischemia. As a consequence, the A_{2A}AR has a dual role: in a first phase of ischemia, it potentiates excitotoxicity, while hours and days after ischemia, A_{2A}AR on immune blood cells potentiates cell adhesion mechanisms and infiltration in the ischemic parenchyma.[82] A novel therapeutic strategy could involve, when possible, early treatment with A_{2A} antagonists to reduce excitotoxicity followed by A_{2A} agonist treatment for the control of later secondary injury.[83]

The expression and/or activation of more than one AR is needed for optimal cardioprotection from ischemic reperfusion injury, as indicated by the failure of an A_1 agonist to exert a cardioprotective effect in either A_{2A}AR or A_{2B}AR knockout mice.[84] Increased A_{2A}AR expression and calcium release has been found in atrial fibrillation patients and A_{2A}

antagonists are able to significantly reduce these elevated calcium levels.[85] In addition, A_{2A}ARs contribute to coronary vasodilation in response to cardiac ischemia *via* activation of voltage dependent potassium channels. Consequently, A_{2A} antagonists effectively inhibit A_{2A}AR-mediated coronary vasodilation.[86]

It is now well established that stimulation of A_{2A}ARs in immune cells induces anti-inflammatory effects, primarily due to their ability to increase cAMP levels, which affords marked immunosuppressive effects.[87] Activation of A_{2A}ARs inhibits neutrophil adherence to the endothelium, degranulation of activated neutrophils and monocytes, and superoxide anion generation. Thus, A_{2A}ARs play a key role in inflammation and in the regulation of immune cells responsible for the physiological control of inflammatory status.[88] In the peripheral system, it has been reported that the potential use of an A_1 agonist and an A_{2A} antagonist mix could be considered a new strategy for ischemic damage prevention in the retina, enhancing the recovery of retinal function.[89]

A_{2A}AR activation has been also demonstrated to promote wound healing and participate in dermal tissue protection and repair through increased collagen production.[90] On the other hand, the application of a selective A_{2A} antagonist, ZM241385, has been shown to decrease scar size and enhance the tensile strength of scar tissue *via* improved collagen alignment and modified collagen composition.[91] Additionally, treatment with ZM241385 was shown to reduce the number of myofibroblasts and angiogenesis in the scar, but did not affect infiltration of the scar by macrophages.[91] Adenosine has been shown to be involved in the pathogenesis of dermal fibrosis, and in the development of fibrosis in murine models of scleroderma and cirrhosis, suggesting a potential role for A_{2A} antagonists as a novel therapeutic approach to the treatment and prevention of dermal fibrosis.[92] A_{2A}ARs are increased in scleroderma fibroblasts and produce significant fibrogenic effects, further

suggesting that A_{2A} antagonists may be useful in the treatment of dermal fibrosis. Mice treated with ZM241385 are protected from developing bleomycin-induced dermal fibrosis through an A_{2A}AR regulation of fibrocyte recruitment to the dermis.[93]

Experimental studies have demonstrated that the activation of A_{2A}ARs significantly enhances the proliferation of breast cancer cell lines and promotes tumor angiogenesis, due to the high level of receptor expression associated with endothelial cells.[94,95] Preclinical studies indicate that adenosine within the tumor microenvironment markedly impairs anti-melanoma T cell responses, and that ZM241385 could enhance the antitumor effect of these cells.[96] Therefore, blocking adenosine-induced immune suppression by inhibiting A_{2A}ARs with ZM241385 may improve cancer immunotherapy, including tumor vaccination.[97]

5. Medicinal chemistry of A_{2A} antagonists

A_{2A} antagonists have typically been divided into xanthine-based and non-xanthine derivatives.[7] The most representative compounds of the xanthine family have a caffeine core (**1**, Figure 2), commonly with a styryl moiety substitution at the 8-position. Styryl xanthines have been widely investigated as a potential source of potent A_{2A} selective antagonists, but their development has been notoriously hampered by limited water solubility and photo-instability due to photo-isomerization (see the section "Xanthine-based derivatives" for details). To overcome these intrinsic limitations, a wide number of non-purine heterocycles with remarkable structural diversity have been identified.[98]

In 1987, Ciba-Geigy laboratories reported the triazoloquinazoline derivative CGS15943 (**2**, Figure 2) as a potent A_{2A} antagonist.[99] About 10 years later, Zeneca Pharmaceuticals described the triazolo[2,3-*a*][1,3,5]triazine antagonist labeled ZM241385 (**3**, Figure 2).[100] Both ligands are known as prototypical tools for the pharmacological

characterization of A_{2A}AR-mediated pathophysiological responses. Based on these structures, several optimized molecules have been identified.[6]

The medicinal chemistry and clinical advancements associated with small-molecule modulators of the A_{2A}AR as a drug discovery target have been recently reviewed.[3,6,7,28,98,101,102] In this section, we furnished a comprehensive analysis and an updated overview of the SAR profile of the most representative A_{2A} antagonists, characterized by tricyclic, bicyclic and monocyclic chemotypes. In addition, we review the initial results from the emerging polypharmacological approach,[54,103] based on optimization of the affinity and/or functional activity of small molecules toward multiple targets implicated in the pathogenesis of PD (specifically A_1AR/A_{2A}AR and MAO-B). On this subject, we have extended the description to novel non-xanthine templates never before reviewed, to the best of our knowledge.[53]

Pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidines (PTPs). The [1,2,4]triazolo[1,5-*c*]quinazoline derivative CGS15943[99,104] (**2**, Figure 2, Table I) can be considered the parent compound of the PTPs. Displaying subnanomolar affinity for the A_{2A}AR, but low selectivity toward the remaining AR subtypes, the compound was considered an interesting starting point worthy of further optimization. Gatta and co-workers reported the first example of a PTP (see compound 8FBPTP, **4**, Figure 3) derived by replacement of the CGS phenyl ring with a substituted pyrazole.[105] Since then, a large library of PTPs has been prepared and evaluated, derived from the systematic substitution of the C^2-, C^5-, C^9-, N^7-, and N^8-positions.[5,106-109] As emerged from the binding profiles of SCH58261 (**5**)[110,111] and SCH63390 (**6**, Figure 3, Table I),[112] developed in our laboratories, the selectivity for the hA_{2A}AR subtype was promoted by the introduction of an appropriate arylalkyl chain (i.e. phenylethyl and phenylpropyl) at the N^7-position. The radioligand [^{3}H]SCH58261[113] has been widely employed for the

pharmacological characterization of the A_{2A}AR. In addition, the compounds [^{11}C]SCH442416[114] (**8**, Figure 3) and the fluoroethoxy ^{18}F-labeled **9**[115,116]have been investigated as positron emission tomography (PET) ligands for the *in vivo* imaging of the receptor.

Showing strong *in vivo* activity when dosed intraperitoneally in animal models of PD, SCH58261 was devoid of efficacy if administered *per os* and was characterized by poor A_{2A} versus A_1AR selectivity in addition to low water solubility. Our first attempt to improve aqueous solubility was by introduction of an hydrophilic or salifiable function on the phenyl ring of the N^7-side chain (see compounds **10**-**13**, Figure 3), which also led to outstanding potency and selectivity (Table I).[117,118] The introduction of an aryl-piperazine moiety was particularly effective in enhancing water solubility, due to the ability to form hydrochloride salts. This strategy furnished valuable tools, such as the sulfonamide **14,** that were employed in *in vivo* animal models of PD,[119,120] but also the clinical candidate known as preladenant (SCH420814, **15**, Figure 4), selected by Schering-Plough for evaluation in Phase I-III trials.[101,121-124] Even though some of these studies would indicate significant efficacy of the molecule (mainly if co-administered with L-DOPA or dopamine agonists), Merck/Schering-Plough discontinued further development. The reason for this decision may be due to the lack of effectiveness when preladenant was used as monotherapy (see section 6 for details).

The SAR optimization work that led to the identification of preladenant has been recently reported in multiple papers, and focused on the manipulation of the N^7-side chain, where substitutions of the distal phenyl ring were primarily evaluated.[121,125,126] Among the resulting compounds, the 2,4-difluoro derivative SCH412348 (**16**, Figure 4, Table I) showed good A_{2A} versus A_1AR selectivity (K_i A_1AR/K_i A_{2A}AR > 1600) and relatively long lasting anti-cataleptic activity at a dose of 1.0 mg/kg in rat (80% inhibition 4 h after oral

administration).[121] The replacement of the aryl-piperazino moiety with biaryl functions (see compound **17**) and fused heteroaryl bicycles (see the quinoline derivative **18**) generally resulted in an improved *in vitro* and/or *in vivo* profile when compared to SCH58261, but poor water solubility.[125] In order to enhance hydrophilicity, an additional basic nitrogen was introduced, such as in the isoindole **19**, the tetrahydroisoquinoline **20**, the benzazepine **21,** and the tetrahydronaphthyridine **22**. In particular, the latter compound showed good oral bioavailability (F = 71%), a long half-life (11.3 h), and low plasma clearance (4.7 mL/min/kg) in a rat catalepsy model (80% and 65% inhibition after oral administration of 3 and 1 mg/kg, respectively).[126]

The pyrazole ring of PTP was also the subject of bioisosteric manipulation. For example, the replacement of the pyrazole ring of preladenant with an imidazole ring led to a significant loss of selectivity versus the A_1AR subtype as shown by compound **23** (Figure 5, Table I), although the compound showed a good *in vivo* pharmacokinetic profile.[127] In a competitive radioligand binding assay at the hA_{2A}AR, the closely correlated imidazolone derivative **24** has been claimed to have a K_i value between 0.1 and 10 nM.[128] King Pharmaceuticals described a series of pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amines as A_{2A} antagonists (i.e. **25** and **26**).[129] Compound **25** exhibited a significant increase in the number of controlateral rotations at 30 mg/kg when tested in the 6-hydroxydopamine lesioned rats challenged with a threshold dose of apomorphine. The *in vitro* potency of compounds **23-26** would suggest that the nitrogen at the 8-position of the PTP nucleus is not involved in determinant interactions with the A_{2A}AR. Nevertheless, we recently demonstrated the importance of the pyrazole portion of the PTP nucleus in modulating selectivity.[130] Other examples of PTP-related A_{2A} antagonists resulted from the substitution of the pyrazole ring with a thiazole,[131] such as in the thiazolo[5,4-e][1,2,4]triazolo[1,5-c]pyrimidine derivative

labeled as PTTP (**27**, Figure 5). This compound seems to combine a good binding profile (K_i hA_{2A} = 6.3 nM, K_i hA_1 = 29 μM) with high potency in a cAMP functional assay and efficacy in an *in vivo* animal model of PD without significant neurotoxicity.[132]

Schering-Plough has described several attempts to replace the 2-furanyl ring of SCH58261 analogs with other aryl groups, more stable from a metabolic point of view.[127,133] Nevertheless 2-phenyl/heteroaryl derivatives have been found to be generally less potent and/or selective. We have found a similar result in the pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine series reported from King Pharmaceuticals.[129] Benzyl substitution was also investigated in a recent series of pyrazolo[4,3-e][1,2,4]triazolo[4,3-c]pyrimidin-3-one derivatives structurally related to PTPs (see compound **28**, Figure 5).[134] Although the substitution led to good A_{2A} affinity, removal of the furan moiety determined a dramatic loss of *in vivo* efficacy.[134] Finally, the [1,2,4]triazolo[4,3-*a*]quinoxalinone **29**, displaying a K_i value of 0.4 nM in hA_{2A}AR competition binding assays, combines a benzyltriazolone isomer of **28** with the phenyl portion of CGS15943.[135] Unfortunately, the selectivity profile of this molecule was not reported.

Indenopyrimidines. A library of indeno[1,2-*d*]pyrimidin-5-one derivatives (see compounds **30**-**33**, Figure 6, Table I) has been screened by the Janssen group that detected a promising binding profile for the new tricyclic chemotype.[136] Hit-optimization furnished very potent A_1/A_{2A} dual antagonists with remarkable *in vivo* potency.[137-139] The co-localization of the A_1ARs and A_{2A}ARs in the striatum suggested a complementary role in the modulation of dopamine signaling. In particular, A_1 antagonists stimulate dopamine release at the pre-synaptic level, while A_{2A} antagonists enhance post-synaptic responsiveness to dopamine, revealing a rationale for the development of dual antagonists in PD treatment. In addition, A_1AR blockade is known to have positive effects on PD-related cognitive disorders.[136]

The SAR of indenopyrimidines designated the free amino group at the 2-position, the 4-phenyl ring, and the cycloalkylamino-methylene substitution of the 8-position as important structural elements to assure both *in vitro* and *in vivo* potency. Pyrrolidine derivative **31** was a very potent bivalent ligand (IC_{50} hA_1AR = 17 nM, IC_{50} $hA_{2A}AR$ = 4.1 nM) showing outstanding *in vivo* potency (ED_{50} = 0.2 mg/kg in a mouse catalepsy model, p.o.) across a number of animal models of PD.[140] Nevertheless, it displayed genotoxicity due to metabolic oxidation of the pyrrolidine nucleus, providing a reactive iminium species.[136, 141] Manipulation of this side of the molecule resulted in the ether (**32**, JNJ40255293) and amide (**33**) derivatives, in which the 8-methylene spacer of **31** was masked to avoid biotransformation.[136,142] Morpholine **32** showed improved *in vivo* potency (ED_{50} < 0.1 mg/kg in the mouse catalepsy model, p.o.), excellent brain exposure, was devoid of the metabolic liability seen with **31**, and exhibited a promising pharmacokinetic profile in mouse, rat and monkey.[136] Preclinical studies performed in rat with JNJ40255293 have shown beneficial effects on cognitive functions due to the interaction with A_1ARs, whilst the increase of motor activity is mediated by A_{2A}AR blockade. This suggests the importance of novel dual A_{2A}/A_1 antagonists as possible non-dopaminergic treatment for PD.[143]

Tricyclic xanthines. The tetrahydropyrimidino[1,2-f]purindione **34**[144] and the tetrahydropyrazino[2,1-f]purindiones **35**-**36**[145] (Figure 6, Table I) are very recent examples of tricyclic xanthines discovered during a research program for the identification of multitarget drugs for the treatment of neurodegenerative diseases. Although displaying moderate dual affinity for the A_1/A_{2A}ARs, their concomitant activity as MAO-B inhibitors (see Table IV) makes the scaffold interesting for the future development of triple-target compounds. MAO-B inhibitors are known to increase dopamine levels in the central nervous system (CNS), thereby enhancing the efficacy of L-DOPA when administered in combination therapy.[146]

Moreover, MAO-B inhibitors are able to counteract oxidative stress mechanisms that contribute to neurodegeneration.[147] Thus, modulation of MAO-B activity could have an additive or synergistic effect with A_1/A_{2A}AR blockade. The basic nitrogen of the N-benzyl derivatives **35** and **36** was expected to be protonated at the physiologic pH of the stomach, affording oral bioavailability through improved water solubility over the 8-styryl-xanthines. The dichlorobenzyl derivative **35**, was shown to be equipotent at the rat A_1AR, A_{2A}AR and MAO-B, suggesting that it could be considered as a potential tool for the preclinical evaluation of the multi-target approach. Compound **36** was identified as the most interesting multi-target ligand of the series against the human targets.

Xanthine-based Derivatives. Caffeine and theophylline are among the first compounds recognized as AR antagonists, characterized by a relatively low affinity (K_i values in the high micromolar range) and selectivity (equally potent toward A_1, A_{2A}, A_{2B}ARs). Efforts to develop more potent and selective compounds have focused on systematic functionalization of the 1-,3-,7- and 8- positions of the xanthine core. As mentioned before, significant A_{2A}AR selectivity and potency was achieved by researchers at Kyowa Hakko in 1992 with the introduction of an 8-styryl substitution. In particular, introduction of two or three methoxy groups on the 8-styryl phenyl ring of (*E*)-1,3-dipropyl-7-methyl-derivatives was shown to enhance A_{2A}AR affinity and selectivity, resulting in compounds like KF17837 (**37**, Figure 7, Table II), one of the first A_{2A} selective antagonists ever reported.[147,148] Structure-activity relationships of 8-styrylxanthines were further explored by workers in the laboratories of the NIH, resulting in the identification of 8-(*m*-chlorostyryl)caffeine (CSC, **39**, Figure 7).[149] Substitutions at the 1- and 3-positions have been occasionally exploited to introduce polar groups that improve aqueous solubility (see MSX2,[150] **40**, Figure 7) or have been functionalized to produce prodrugs with improved "drug-likeness" (see **41**[150] and **42**[151]).

Istradefylline (KW6002, **38**, Figure 7) is the only compound of this class that entered clinical trials and the only A_{2A} antagonist that has been licensed for use as an anti-parkinsonian drug in Japan (see section 6 for details).[101] The advancement of 8-styrylxanthines as drugs undoubtedly has been hampered by physico-chemical liabilities, such as poor water solubility and light sensitivity.[152] Representative 8-stryrylxanthines in the (*E*)-configuration have been shown to isomerize in dilute solution to the corresponding (*Z*)-isomers possessing little or no affinity for the A_{2A}AR. The photosensitivity seems to persist in the solid state following exposure to daylight or UV light that catalyzes dimerization *via* a [2+2]cycloaddition of the styryl double bond. The resulting dimerization products exhibited lower A_{2A}AR affinity than their parent compounds.[153] Some PEGylated analogues of KW6002 with improved water solubility and photostability have been synthesised recently.[154] CV Therapeutics also developed (*E*)-6-styrylthieno[2,3-*d*]pyrimidine-2,4-diones as KW6002 analogs in which the imidazole ring was replaced by a thiophene.[155] A novel series of 8-(substituted)phenyl/benzyl-xanthines has been investigated recently, with the chloropropoxy derivative **43** showing good affinity and selectivity for the A_{2A}AR subtype, as well as potent bronchospasmolytic effects in guinea pigs.[156] This molecule represents the only member of this class lacking an 8-styryl substitution.

The dual-activity behaviour of styryl-xanthines as A_{2A} antagonists and MAO-B inhibitors has been evaluated from the perspective of a multi-target approach for the treatment of PD.[53,54,157-159] CSC displayed good A_{2A}AR affinity (K_i = 54 nM) and potency toward monoamine oxidase B (IC_{50} = 70 nM, see Table IV).[158] Considering that caffeine is essentially devoid of MAO-B inhibitory activity,[145] the 8-styryl function is thought to establish important interactions with the enzyme. Indeed saturation of the styryl double bond (whose *E* geometry was found to be important for MAO-B activity, as well) led to reduced

inhibitory potency.[158] However, istradefylline is a weak inhibitor of MAO-B (IC_{50} = 28 μM), indicating that substitutions on the caffeinyl core can be discriminative. Specifically, an electron withdrawing group at C^3 (i.e. an halogen atom) of the styryl-ring was beneficial for the dual-activity profile, while an electron donating group at C^3 and/or C^4 (i.e. a methoxy group) was detrimental.[159] Similarly, N^7-methylated compounds were more potent MAO-B enzyme inhibitors and A_{2A} antagonists than N^7-unsubstituted compounds. In contrast, 1,3-diethyl substitution was beneficial for A_{2A}AR affinity but detrimental for MAO-B inhibitory activity.[158]

Recently, 9-deazaxanthines have been identified as a promising scaffold to develop dual-acting agents, among which **44** (ST3564, Figure 7) showed high selectivity versus hMAO-A (K_i A_{2A} = 260 nM, IC_{50} MAO-B = 200 nM, and IC_{50} MAO-A = 10 μM). Moreover, the compound showed good selectivity over a panel of more than 50 receptors, ion channels, and transporters, with the exception of A_1AR and A_3AR (see Table IV).[160] Under the same screening conditions, CSC was significantly less potent as an MAO-B inhibitor (IC_{50} = 588 nM). 8-Styryl-9-deazaxanthines of this series exhibited an SAR profile quite different from that of their nitrogenous analogues, as *m*-chlorine substitution of the styryl-ring was not strictly required, while *p*-substitution with electron withdrawing groups (such as CF_3, F or Cl) afforded the most potent dual-activity ligands. Less conservative modification of the styryl group (i.e. chlorinated benzyloxy groups) occasionally resulted in higher potency toward MAO-B, but compromised A_{2A}AR affinity. ST3564 significantly reversed haloperidol-induced catalepsy in mice when administered by the oral route at doses of 30 and 100 mg/kg, while it was inactive at a dose of 10 mg/kg.[160]

Replacement of the styryl group of CSC-related compounds with an 8-(*E*,*E*)-phenylbutadiene moiety resulted in polyvalent (A_{2A}/MAO-B) ligands (see compounds **45** and

46, Figure 7, Table IV).[54,157] Derivatives with an 8-butadiene moiety showed slightly improved MAO-B inhibitory activity, but somewhat reduced A_{2A}AR affinity, when compared to the corresponding 8-styryl analogs evaluated under the same experimental conditions.

9*H*-Purine Derivatives. A series of purine derivatives has been designed at Sigma-Tau through the application of a molecular modeling analysis of known AR antagonists with different selectivity profiles for the hA_1 and A_{2A}AR subtypes.[161,162] Starting with an adenine scaffold, which clearly mimics the endogenous ligand lacking the intact ribose moiety responsible for receptor agonist activity,[22] the 8-triazolyl moiety of **47** (ST1535) and **48** (Figure 8, Table II) was introduced with the aim of improving water solubility and, at the same time, providing additional H-bonds. The A_{2A}AR affinity of these analogs was enhanced by long/bulky 2-alkyl substitutions, such as the 2-*n*-butyl derivative in ST1535, the most representative lead compound of the series, or the 2-phenylethyl in **48**, which exhibited significantly higher selectivity versus the A_{2B}AR subtype. ST1535 showed increased spontaneous locomotor activity in a dose-dependent manner when administered orally to rats and has been extensively studied for the treatment of PD, reaching Phase I clinical trials.[163-165] Good tolerability was observed following single ascending oral doses of the molecule. Further important information about the safety and pharmacokinetics of ST1535 will be provided from the ongoing trials (see section 6 for details). Five metabolites of ST1535 have been recently identified, arising from the enzymatic oxidation of the 2-butyl group. Their A_1/A_{2A}ARs binding profiles have been investigated,[166] and all metabolites showed antagonistic properties *in vitro* and *in vivo* at the human A_{2A}AR, with affinities and intrinsic activities comparable to those of ST1535, though slightly lower selectivity versus the A_1AR subtype.[162] This could possibly explain the long-lasting pharmacological activity of the parent compound.

The 2-amino-6-furyl-9-benzyl purines **49** and **50**[167] (Schering-Plough) and the 9-carboxamide analogs **51** (VER6947) and **52** (VER7835, Vernalis, Figure 8)[168,169] are further examples of purine-based A_{2A} antagonists.[170] Binding data in table II suggests that the 9-benzyl substitution of **49** and **50** may be preferred for A_{2A}/A_1AR selectivity, compared to the urea moiety of **51** and **52** in the same position. The 9-benzyl group was maintained in a series of 8-azapurine analogs described by Gillespie *et al*. in 2009.[171] This led to the identification of Vipadenant (BIIB014/V2006, **53**, Figure 8),[172-174] which is clinically the most advanced member of the triazolo[4,5-*d*]pyrimidine class, showing clinical efficacy in Phase I/II trials both alone and in combination with L-DOPA. Nevertheless, further advancement was interrupted by Vernalis and Biogen in favour of compound V81444 (structure not disclosed), possibly due to toxicity. The second-generation lead gave evidence for a promising safety and pharmacokinetic profile in healthy volunteers during a Phase I trial (see section 6).[101]

Some C^2-, N^7- and N^9- trisubstituted-purin-8-ones (i.e. **54** K_i hA_{2A}AR = 9 nM, K_i hA_1AR = 1967, IC_{50} rA_{2A}AR = 18 nM),[175] 2-alkynyl-N^9-propargyl adenine derivatives (i.e. **55** K_i hA_{2A}AR = 0.56 nM, K_i hA_{2B}/K_i hA_{2A}ARs = 1.300, K_i hA_1/K_i hA_{2A}ARs = 10)[176,177] and 7-amino-2-arylpyrazolo[4,3-d]pyrimidine derivatives (i.e. **56**, K_i hA_{2A}AR = 55 nM, K_i hA_1AR = 5.3 nM)[178] have been reported as examples of purine-related A_{2A} antagonists or A_1/A_{2A} dual antagonists.

Triazolo-triazines/pyrimidines. The Biogen group have extensively investigated different biaryl cores structurally related to ZM241385 (see Figure 2) with the aim of improving oral bioavailability, metabolic stability, brain penetration, and *in vivo* efficacy.[179] ZM241385 can be considered a bicyclic analog of the PTPs resulting from the removal of the pyrazole ring. In view of the beneficial effects obtained with the introduction of piperazine moieties into the tricyclic A_{2A} antagonists (see Figures 4 and 5), the company first developed

[1,2,4]triazolo[1,5-*a*][1,3,5]triazines, typified by structures **57** and **58** (Figure 9, Table II).[180] The SAR around the 5-position indicated that a single methylene spacer between the distal (hetero)aryl ring and piperazine was preferred to direct substitution or longer chains. Moreover 2,6-disubstitution of the (hetero)aryl nucleus with electron-withdrawing groups, especially fluorine, was found to enhance both affinity and selectivity at the A_{2A}AR. However, poor oral bioavailability was initially observed. Replacement of the furan ring with substituted-phenyl groups or aza-heterocycles, although well tolerated in terms of *in vitro* binding potency and selectivity, resulted in lower *in vivo* activity in most cases.

Introduction of a flexible alkylamino spacer between the piperazine and triazolo-triazine core led to compound **59**,[181] with improved *in vivo* potency. In addition, replacement of the piperazine with alternative diamines led to the selection of an (*R*)-2-(aminomethyl)pyrrolidine sidechain.[182] In this series, the 2,6-disubstitution of the *N*-benzyl moiety with fluorine atoms promoted *in vitro* affinity, with the (*R*)-isomer (**60**),being slightly preferred over the (*S*)-isomer. Although **60** was a potent and selective A_{2A} antagonist *in vitro*, this derivative was significantly less effective *in vivo* than the related series with a piperazine ring, presumably due to a lack of oral bioavailability with the parent compound.

Further optimization efforts were undertaken to address the ADME (absorption, distribution, metabolism, and excretion) liabilities of the piperazine/pyrrolidine side chains, where metabolic *N*-dealkylation was thought to produce active metabolites responsible for the *in vivo* activity occasionally observed, despite poor oral bioavailability.[183] A strategy that proved to be somewhat helpful was to constrain the piperazine into a rigid bicyclic scaffold.[183] In particular, the octahydropyridopyrazine (**61**) or octahydropyrrolopyrazine (**62**) were considered in the place of the *N*-substituted-piperazine or *N*-substituted-pyrrolidine. Some compounds with subnanomolar potency and outstanding A_{2A}AR vs A_1AR selectivity

were identified. Despite this, a discrepancy between affinity, *in vivo* potency, and metabolic stability was still observed.[183]

The 5-substituted derivative **63**[184] (K_i = 16.9 nM) is a potent and water soluble antagonist of the triazolo[1,5-*a*]-1,3,5-triazine family whose $hA_{2A}AR$ selectivity was assessed against the full panel of the remaining hAR subtypes (see Table II). Docking studies highlighted an interaction pattern for these derivatives that would fully mimic that of ZM241385 bound to $hA_{2A}AR$ as defined through crystallographic data. Interestingly, compound **63** has been recently conjugated to a molecule of dopamine via a succinic spacer in order to obtain a potential antiparkinsonian prodrug.[185] The conjugated molecule was designed with the aim to explore beneficial effects against PD of a combined D_2 activation (mediated by dopamine) and $A_{2A}AR$ blockade (promoted by **63**) focused on striatal A_{2A}-D_2 heteromers. Both the prodrug and related hydrolysis products demonstrated the ability to increase dopamine affinity toward D_2 receptors by counteracting the activity of A_{2A} agonists. Additional examples of heterobivalent ligands, containing a D_2 agonist and an A_{2A} antagonist were recently investigated as probes for central A_{2A}-D_2 heteromers.[186] These findings clearly suggest a novel D_2/A_{2A} multi-target approach that deserves to be explored as possible polypharmacological treatments of PD.

Several triazolo[1,5-a]pyrimidines,[179,183] 1,2,4-triazolo[1,5-a]pyrazines,[187] pyrazolo[3,4-*d*]pyrimidines,[169,188] pyrazolo[4,3-*d*]pyrimidines[178] and pyrrolo[2,3-d]pyrimidines[169] have been considered as possible bioisosters of the triazolotriazine template, but a general decrease in $A_{2A}AR$ binding affinity and/or selectivity was found, with the exception of **64** developed by Schering-Plough (Figure 9). The compound showed an acceptable *in vitro* profile combined with elevated plasma levels after oral administration and protracted *in vivo* activity in a rat haloperidol-induced catalepsy model.[133] Compound **65** is

representative of a very recent series of triazolopyrimidines exerting AR antagonist activity with a different selectivity profile (mainly A_{2A} or A_3) in relation to the kind of substitutions at the 5- and 8-positions.[189] A free 5-amino group combined with the 8-ethoxycarbonyl function led to good hA_{2A} binding affinity (K_i hA_{2A} AR = 3.32 nM) and reasonable selectivity over the other ARs (see Table II).

Thienopyrimidines. Through a *de novo* approach, J&J laboratories identified and developed a series of thieno[2,3-*d*]pyrimidines with A_{2A} antagonist activity.[190-192] Interestingly, the early examples of this series (**66** and **67**, Figure 10, Table II) were functionalized with a free amino group and a furan ring at the 4- and 2-positions, respectively, sharing structural similarities with some purines/triazolotriazines described above. At the 6-position, bulky cycloalkyl amines with relatively low basicity were associated with a greater capability to cross the blood brain barrier. In particular, 6-cycloalkylamino-methyl substitution was found to promote *in vitro* activity, as well as good efficacy in reversing haloperidol-induced catalepsy in mice (ED_{50} = 1.3 and < 0.1 mg/kg for compounds **66** and **67**, respectively). Substitutions other than methyl at the 5′-position of the furan ring or replacement of the furan ring with alternative 5-membered heterocycles were found to be detrimental for *in vitro/in vivo* potency. Further attempts to overcome the typical metabolic instability of mono-substituted furans led to the identification of 6-phenyl derivatives, such as **68,** in which the cyano-group at the 3-position was found to be essential for maintaining *in vitro* A_{2A} antagonistic activity and *in vivo* potency. More recently, the effect of alternative 6-arylalkyl substitutions has been explored, such as in **69**.[193,194] Even though active *in vivo* after a single oral dose of 3 mg/kg (ED_{50} < 1 mg/kg), this benzyl derivative suffered from poor selectivity versus the A_1AR subtype and a short duration of action.

The Vernalis group also explored the potential of the thieno[3,2-*d*]pyrimidine isomer, as represented by **70** (Figure 10).[195,196] The subsequent optimization resulted in the identification of **71** (VER6623), characterized by notable A_{2A}AR binding affinity, but only moderate selectivity over A_1AR,[197] and poor oral bioavailability.[198] Hit compounds with similar structures also have been identified via a virtual screening approach by Katritch et *al.* in 2010.[27]

Benzofurans. A high-throughput screening performed by the Kyowa Hakko group resulted in the identification of the benzofuran hit **72** (Figure 11), displaying micromolar affinity for the A_{2A}AR (58 and 86% binding inhibition at 10^{-7} and 10^{-6} mol/L, respectively) and about 50% inhibition of CGS21680-mediated catalepsy *in vivo* at 10 mg/kg po.[199] The introduction of a 4-phenyl group to replace the methoxycarbonyl functionality, such as in compound **73,** enhanced binding affinity (83 and 100% binding inhibition at 10 nM and 100 nM, respectively), as well as *in vivo* potency (74% catalepsy inhibition at 10 mg/kg, p.o.). Inversion of the amide moiety at C^2 with arylcarbamate or arylurea functions led to a general decrease of *in vitro* potency. Replacement of the 4-phenyl group of **73** with a morpholine ring, as with compound **74**, resulted in a compound with enhanced water solubility and metabolic stability that exhibited reasonable A_{2A}AR affinity (30 and 73% binding inhibition at 10^{-8} and 10^{-7} mol/L, respectively) and selectivity (4% inhibition for A_1 and 21% inhibition for A_{2B} at 1 μmol/L, respectively). Good potency *in vivo* (76% CGS catalepsy inhibition, 10 mg/kg, po) with a long lasting positive effect on motor disability and locomotor activity was also observed.[200] The patent literature on this compound class would suggest that an additional amide function at the 3-position could be beneficial in terms of A_{2A}AR affinity, as in compounds **75**[201] with subnanomolar potency. Compound **76** is representative of a related series of furo[2,3-b]pyridines claimed by Kissei Pharmaceutical in the same field.[202]

Benzothiazoles. The benzo[*d*]thiazole skeleton characterized potent A_{2A} antagonists claimed by Roche in multiple patents.[203-206] The combination of the 4-methoxy, 7-phenyl or 7-morpholino and 2-phenylcarboxamide or 2-urea substitutions of **77**, **78** and **79** (SYN115, see Figure 12),[204] clearly shows analogy to the structure of benzofurans **73** and **74** (Figure 11). Benzoxazole analogs of this series have also been investigated.[207]

The morpholine derivative SYN115, also known as Tozadenant,[206] is the most intensively characterized member of this class.[205,208] The *in vitro*/*in vivo* pharmacological profile, as well the pharmacokinetic properties and efficacy on parkinsonian syndrome in animals and humans, have been recently reviewed.[28,101,209] Tozadenant has been evaluated in two Phase II trials and initial results suggest that twice daily oral administration of SYN115 improved PD symptoms versus placebo, either alone or in combination with sub-therapeutic doses of L-DOPA. In addition, the safety of the molecule seems promising, as indicated by the limited scenario of side effects (see section 6 for details).[101]

As in the case of benzofurans, the effect of the bioisosteric replacement of the phenyl ring with 6-membered heterocycles has been evaluated, such as the thiazolo[5,4-*c*]pyridine **80**[162] and the thiazolo[4,5-*d*]pyrimidine **81,** with picomolar A_{2A}AR affinity (see Table II) and functional potency (IC_{50} of 0.14 pmol/mL).[210]

Another example of 6,5-bicyclic structures are the imidazo[1,2-*a*]pyridine derivatives claimed by Domain Therapeutics in 2010.[211] Although the compounds are reported to have remarkable A_{2A}AR affinity of these molecules (K_i in the low nanomolar range), no information about selectivity is available thus far.

6,6-Bicyclics. As an alternative to the existing chemotypes, the 2-(acyl)amino-3,1-benzothiazin-4-one ring system was initially described as a versatile scaffold for the

identification of AR antagonists with a mixed selectivity profile.[212] For example, the *N*-benzylpiperazine derivative **82** (Figure 13) showed dual hA_{2A}/hA_{2B}AR affinity. Further efforts led to the identification of the thiazinone **83**, exhibiting nanomolar affinity and optimized selectivity for the hA_{2A}AR subtype together with the interesting capability to reversibly inhibit hMAO-B in the same concentration range (Table IV).[213] The compound proved to be devoid of the intrinsic instability of styrylxanthine-based A_{2A}AR/MAO-B dual-activity molecules. In this series, substitution at the 6- and/or 7-positions of the benzothiazinone core led to a marked decrease of A_{2A}AR affinity. An unsubstituted phenylpropionyl moiety in the place of the phenylbutyryl function of **83** maintained the MAO-B inhibitory activity, but led to a loss of selectivity, especially over the A_{2B} and A_3AR subtypes. In the same way, some 2-acylaminothienothiazinones bioisosterically related to **83** exhibited good A_{2A}AR/MAO-B potency, but reduced selectivity over the remaining ARs.[213]

As in other therapeutic areas, the virtual screening approach has been profitably employed in the search for innovative 6,6-bicyclic templates for the development of AR ligands. One of these studies[214] resulted in the identification of chromone-based compounds whose SAR optimization led to potent and quite selective A_{2A} antagonists, such as derivative **84** (Figure 13). Despite the presence of a thiazole ring that has been suggested as a possible source of reactive metabolites, chromone ligands have been recently investigated with structure-based molecular modeling-driven techniques for enhancing binding potency, selectivity and ligand efficiency.[215] This work led to the identification of **85** (Figure 13) that exhibited good selectivity for the A_{2A}AR against all the other ARs (see Table II). Further characterization of ADME properties and *in vivo* potency will assess potential of such chemotype.

Finally, aminoquinoxalines and aminoquinolines (see the general structure **86**) with high affinity (K_i values in the low nanomolar range) for the A_{2A}AR have been claimed by Schering Corporation in 2009.[216] Unfortunately, selectivity data over the other AR subtypes are unavailable for these molecules.

Pyridines and Pyrimidines. A series of 2-amino-6-(furan-2-yl)-4-substituted nicotinonitriles has been developed and reported in 2008 by the group of professor IJzerman from Leiden University (see for example compounds **87** and **88** in Figure 14).[217] These were designed thanks to a pharmacophore model based on molecular superimposition of previously known A_{2A} non-xanthine antagonists. The substitutions at the 4- and 6- position of the central monocyclic core were optimized with various combinations of (hetero)aromatic ring systems and several compounds with low nanomolar affinity for the A_{2A}AR were identified. SAR studies indicated that a five-membered heterocycle with an H-bond accepting heteroatom (i.e. furan) is favored over a phenyl ring at the 6-position. Compound **87**, with two furan rings, exhibited the highest affinity for the A_{2A} subtype (K_i = 1.0 nM) with 12- and 34-fold selectivity over the A_1AR and A_{2B}AR, respectively. In the functional assay, **87** was found to be a very potent antagonist with a pA_2 value of 9.87. Replacement of the metabolically reactive furan ring with more stable moieties led in most cases to reduced A_{2A} affinity. Interestingly, some derivatives with higher affinity toward A_1AR than A_{2A}AR have been identified, as well (see for example compound **88**). These molecules could be considered promising dual acting agents whose potential in view of the multi-target approach should be further investigated.

Researchers from Almirall and Neurocrine Biosciences reported in 2008 a series of water soluble pyrimidine-acetamide derivatives with interesting affinity and antagonist activity for the A_{2A}AR.[218-220] The original substitution pattern of the pyrimidine nucleus is

represented by compound **89** (Figure 14, Table III), in which acylation of the amino group at the pyrimidine 4-position was shown to enhance A_{2A}AR *vs* A_1AR selectivity and provided the opportunity to introduce salifiable moieties, such as a piperazine, that could improve water solubility. Dimethylation of the pyrazole ring (compound **90**, Figure 14) significantly contributed to an enhanced selectivity profile. Both molecules showed promising efficacy in a haloperidol-induced catalepsy model in rat at a dose of 10 mg/kg p.o., being about 10-fold less potent in binding to the rA_{2A}AR, rather than the hA_{2A}AR. Due to concern for poor metabolic stability, the mono-substituted furyl moiety was replaced in an initial stage with alternative heterocycles (pyridine, thiazole, oxazole, 5-methyl furan).[221] The 5-methyl-furan (**91**, **92**) and 2-thiazolyl moiety (**93**, **94**) improved the pharmacokinetic profile and when combined with a dimethylpyrazole at the 6-position (compound **94**), provided a satisfactory level of affinity and selectivity for the hA_{2A}AR. Further investigation of **92** highlighted potential liabilities of the compound, such as weak inhibition of the hERG channel (IC_{50} = 950 nM) and an atypical, species-specific binding to the human and rat A_{2A}AR isoforms (K_i hA_{2A}AR = 12 nM, K_i rA_{2A}AR = 131 nM).[222] While activity on the hERG channel may suggest the possibility of cardiac side effects, the lower affinity for the receptor in rodent species could compromise the *in vivo* efficacy. For these reasons, the piperazine side chain was the object of optimization efforts that led to the pyrrolidine derivative **95**, showing high affinity for the hA_{2A}AR (K_i = 2.4 nM), reasonable selectivity vs A_1AR (262-fold) and over the hERG channel (IC_{50} = 2280 nM), but a relatively low *in vivo* efficacy up to 30 mg/kg p.o. This was attributed to the lower rA_{2A}AR affinity (K_i = 41 nM) combined with low exposure in the rat brain. For this reason, non-basic side chains were also scrutinized at the 4-position, such as substituted-phenyl/phenoxyacetamides[223,224] or a simple acetylamino moiety.[225,226] In particular, the 4-acetylamino group was combined with several (hetero)aryl substitutions at the 2- and 6-positions. In the methoxypiperidine derivative **96** (Figure 14), the typical 3,5-

dimethylpyrazole substitution was shifted from the 6- to the 2-position and a basic pyridine was introduced at the 6-position, somewhat restoring the loss of water solubility resulting from the removal of the 4-piperazine tail. The compound was found to be equipotent at the human and rat A_{2A}ARs and displayed good efficacy in different animal models of PD.[226] Further attempts to improve drug-like properties included the incorporation of aliphatic (cyclo)alkylamines in place of pyridine, such as in the pyrrolidine **94** (Figure 14, Table III)[227] that was characterized by satisfactory pharmacokinetic properties, good *in vitro*/*in vivo* potency, but low selectivity vs hA_3AR. Very recently, the same authors published results from the bioisosteric substitution of the 4-acetamide function, assumed to be a potential site of chemical and metabolic instability.[228,229] Among the evaluated bioisosters (namely pyridine, pyrimidine, pyrazine, thiazole), pyridine was the most effective in enhancing hA_{2A}AR affinity, as shown by compound **98** that displayed a subnanomolar K_i value. The preclinical investigation of **98** revealed efficacy in a haloperidol-induced catalepsy model at 5, 10 and 30 mg/kg i.p., and a good ability to cross the blood brain barrier. Nevertheless, the molecule still suffered a metabolic liability that prompted further optimization work.[229]

A parallel research activity performed by the Vernalis group confirmed the pyrimidine nucleus as a possible source of A_{2A} antagonists.[230,231] Interestingly, these compounds, typified by **99**[231] and **100**,[230] also bear a carboxamide function at the 4-position. In addition, the presence of a 2-furyl ring (**99**) or the more stable 5-methyl-2-furyl (**100**) moieties characterize these AR ligands with different templates. The free amino group at the 2-position improved the physicochemical and pharmacokinetic properties of carboxamides **99** and **100**, resulting in very promising *in vivo* oral activity (minimum effective dose of 0.1 and 1 mg/kg, respectively).

Finally, Palobiofarma has disclosed 5-bromo-2,6-di(thiazol-2-yl)pyrimidin-4-amine **101**, with a K_i of 1 nM in the A_{2A}AR binding assay and a K_i of 12 nM in the cAMP assay.[232] The company is also reported to be developing the molecule labeled as PBF509 (structure not disclosed) currently in a Phase I clinical trial for the treatment of PD (see section 6).[101]

Triazines. A virtual screening program at Heptares Therapeutics identified a series of hits suitable for optimization into A_{2A} antagonists, characterized by an atypical triazine scaffold.[214] Initial studies focused attention on the 1,3,5-isomers **102** and **103** (Figure 15, Table III), endowed with high affinity for the target, but improvable selectivity over the A_1AR subtype. An independent, receptor-based approach at the Scripps Research Institute confirmed the potential of this cluster.[233] A proposed binding mode guided the optimization to the 1,2,4-triazine isomers as potentially capable of being better accommodated by the receptor domain usually involved in interaction with the ribose moiety of the endogenous ligand. Compound **104** resulted from an innovative X-ray structure-directed optimization based on co-crystallization of representative molecules of this class in the receptor.[234] The SAR profile, also described in the related patent,[235] indicates that an un-substituted 5-phenyl ring would be preferred to increase A_{2A}AR affinity. Introduction of a fluorine atom on the 5-phenyl ring and/or 3,5-disubstitution of the 6-phenyl ring contributed to a slight improvement of A_{2A}AR selectivity over A_1AR selectivity (see compound **105**). Replacement of the 6-aryl nucleus with a substituted-morpholine (**106**) or a phenoxy group (**107**) determined a significant improvement of selectivity, with a concomitant decrease of A_{2A}AR affinity. Following its promising *in vitro* profile, the pharmacokinetics of compound **104** were evaluated, revealing good ADME properties, low plasma protein binding, and a lack of inhibition of cytochrome P450 and the hERG channel. In addition, compound **104** showed acceptable clearance (42

mL/min/kg), rapid oral absorption, excellent brain penetration, and a remarkable *in vivo* efficacy, reversing haloperidol-induced catalepsy in rats with an ED_{50} = 0.2 mg/kg, p.o.

Pyrazines. Astellas Pharma claimed a series of pyrazine derivatives as adenosine $A_1AR/A_{2A}AR$ dual ligands, potentially useful for the treatment of PD if administered alone or in combination with L-dopa.[236] Compounds **108** and **109** (Figure 16) display low/sub nanomolar binding potency against the two targets with a slight preference for the $A_{2A}AR$ subtype. Of this class, the 4-F-phenyl compound **110** (ASP5854) was selected for preclinical evaluation in animal models of PD and cognition.[237-239] Interestingly, in the rat passive avoidance test, ASP5854 significantly reversed scopolamine-induced memory deficits, whereas istradefylline (a selective A_{2A} antagonist) did not. This may support the potential beneficial effects of a multi-target approach.

5-Membered heterocycles. A virtual screen of 1.4 million compounds toward the crystal structure of the $hA_{2A}AR$ suggested the triazoles **111** and **112** (Figure 17) as an unusual chemotype for the development of selective antagonists with high ligand efficacy.[233] In addition, thiazole[240] and oxazole[241] derivatives with affinity for the $A_{2A}AR$ have been claimed in recent patent literature. In particular, the *in vitro*/*in vivo* profile of a series of thiazole-based amides has been recently described.[242]Of these compounds, **113** (Figure 17) displays a K_i value of 5.9 nM from the binding assay at the $hA_{2A}AR$ and good to excellent selectivity versus the remaining ARs as well as over a panel of 60 GPCRs, ion channels, and transporters. As for the functional assay, **113** is a competitive antagonist with a K_b of 1.3 nM and the molecule exhibited promising drug-like properties following *in vitro*/*in vivo* ADME characterization. Oral administration of **113** in mice revealed dose-dependent efficacy in reversing haloperidol-induced hypolocomotion with an ED_{50} value of 0.5 mg/kg. Nevertheless, an intrinsic limitation of **113** is its poor water solubility that negatively affects

oral bioavailability of solid dose formulations. To overcome this problem, the water soluble phosphonooxymethylene prodrug **114** (LuAA47070) was designed and synthesised. This is fully converted to **113** *in vivo*, with no detectable prodrug in systemic circulation. Due to the improved properties and ADME profile, LuAA47070 was advanced to preclinical characterization and exhibited *in vivo* efficacy (3.75–30 mg/kg i.p.) in reversing the motor and motivational effects of the D2 receptor antagonists pimozide and haloperidol in rodent model of PD. LuAA47070 has been suggested as potentially useful for the treatment of PD and for some of the motivational symptoms of depression.[243]

6. Therapeutic applications of A_{2A} antagonists in clinical trials

The therapeutic value of A_{2A} antagonists has been investigated for several years and medicinal chemistry research has indicated a principal role of these compounds in PD pharmacology.[6] From a clinical point of view, PD is second most common neurodegenerative disorder, currently affecting more than 4 million people worldwide, that is expected to increase to approximately 8.7 million by 2030.[244] Estimates of the incidence of PD range from 8-18 per 100,000 person years, with a significant age-related increase after age 60.[245] PD is a progressive, neurodegenerative disease characterized by bradykinesia, resting tremor, rigidity, postural instability, and a variety of non-motor symptoms such as sleep disturbances and depression.[246] Current PD therapy is primarily based on dopamine replacement therapy, using agents such as L-DOPA, dopamine D_2 agonists, or dopamine reuptake inhibitors.[59] L-DOPA represents the gold standard in PD treatment, although several adverse effects are associated with its use, such as a very short effect, wearing-off, less predictable responses, and involuntary muscle movements.[247] The pharmacological treatment of motor fluctuations and dyskinesia involves L-DOPA with catechol-O-methyltransferase (COMT) or MAO-B inhibitors, or the use of long-acting dopamine agonists.[248] Among the various strategies to

ameliorate the side-effects of PD drugs, A_{2A} antagonists are considered one potential approach to treatment of the disease.[246] At the present time, several pharmaceutical companies have progressed A_{2A} antagonists to clinical trials, including KW6002 or istradefylline (Figure 7) from Kyowa Hakko Kirin Co; ST1535 from Sigma-Tau (Figure 8); Tozadenant (SYN115, Figure 12) from Biotie Therapies & UCB Pharma; V81444 (structure not disclosed) and Vipadenant (V2006, Figure 8) from Vernalis-Biogen; PBF509 (structure not disclosed) from Palobiofarma; and Preladenant (SCH420814, Figure 4) from Merck & Co.

Istradefylline (KW6002) has completed different clinical trials in Phase II and Phase III for the oral treatment of PD as monotherapy and combination therapy with L-DOPA or dopamine agonists. It shows good pharmacokinetic properties and improves the symptoms of the disease without increasing the incidence or severity of side effects.[249] Different studies have been conducted in North America or in Europe, in PD patients with "wearing-off" phenomenon on treatment with L-DOPA alone or L-DOPA administered concomitantly with other PD medications. Based on the results from these trials, the reduction in the percentage of awake time, which served as an indicator of the improvement in the "wearing-off" phenomenon, KW6002 is able to increase the half-life of the L-DOPA dose mediating the reduction of off-time (a period in the day when medications for the treatment of PD are not working well, characterized by decreased mobility) in PD patients with motor fluctuations.[250] Two Phase II trials conducted in PD patients with "wearing-off" in the absence or in the presence of dyskinesia have demonstrated that KW6002 significantly increased on-time with non-troublesome dyskinesia and decreased off-time period.[251] The use of KW6002 in clinical trials for advanced PD patients has revealed a dose response in reducing the off-time and in improving the Unified Parkinson's Disease Rating Scale (UPDRS) motor scores.[252] In PD patients, a full efficacy of KW6002 in reducing off-time has been shown, suggesting that 20

mg daily represents the first choice dosage to reduce off-time, and 40 mg daily could be used to improve UPDRS motor scores.[253] Clinical trials performed with KW6002 have also studied the tolerability and safety profile. The most common adverse effects are an aggravation of dyskinesia, dizziness, insomnia, nausea and vomiting, headache and hallucination.[254,255] No clinical differences have been found in systolic or diastolic blood pressure, heart rate, respiratory rate, and body weight.[3,256] As mentioned in section 5, KW6002 has been licensed for use as an anti-parkinsonian drug in Japan. Nevertheless, because of concern about the efficacy findings that do not seem to entirely support the clinical utility of this molecule, the US Food and Drug Administration denied its approval in 2008, highlighting the need for further clinical investigation. In contrast to its promising preclinical profile in animal models of PD, the drug did not provide unambiguous evidence of improving PD-related motor symptoms when administered alone in human patients.[101] A possible reason may be the need for higher doses of istradefylline when administered in monotherapy regimen. Other doubts have emerged as far as the choice of the active internal comparator in these studies. Moreover, the intrinsic photosensitivity of the molecule may limit the effectiveness of drug formulations (see section 5). Further studies involving a larger number of patients could, in the future, clarify the real effectiveness of this compound.[101]

KW6002 also has been patented as a therapeutic agent for behavioral disorders, anxiety and higher brain dysfunction, in combination with dopaminergic agents, MAO-B inhibitors, or COMT inhibitors for PD, restless leg syndrome, and attention deficit hyperactivity disorder. It could be used in combination with antidepressant agents, such as the serotonin and/or norepinephrine reuptake inhibitors for depression, and for diseases accompanied by chronic muscular/skeletal pain, and drug dependence.[234]

Preclinical studies and a Phase I clinical trial have established good tolerability and safety based on pharmacokinetic data of different doses of ST1535.[257] The pharmacokinetic profile of this compound at multiple doses in chronic pharmacological treatment has been studied in PD patients.[166] In *in vivo* experiments, ST1535 is able to increase spontaneous motor activity in mice and to antagonize haloperidol-induced catalepsy.[163] ST1535, when administered alone, produces a dose-related increase in locomotor activity and tends to reverse motor disability.[164,258] ST1535 antagonizes catalepsy induced by intracerebroventricular administration of A_{2A} agonists in mice. In addition, the oral administration of ST1535 potentiates the effect of L-DOPA in reducing haloperidol-induced catalepsy.[259] Sub-chronic ST1535 and L-DOPA does not induce sensitization to turning behavior or abnormal involuntary movements during the course of treatment, indicating a low dyskinetic potential for the drug. The acute administration of ST1535 has reduced jaw tremors in PD, representing a potential compound with long-lasting activity for the treatment of this disorder.[260] Moreover, the effects of two metabolites of ST1535, originating *in vivo* from enzymatic oxidation, have been investigated, suggesting that the long duration of action of this A_{2A} antagonist could be due in part to the presence of these metabolites.[166]

The benzothiazole derivative Tozadenant (SYN115) is a potent and selective non-xanthine A_{2A} antagonist.[261] A Phase II clinical trial with this compound as monotherapy or in combination with L-DOPA has been performed in PD patients and an improvement in tapping speed and in two UPDRS measures of bradykinesia were reported.[262] SYN115 produced dose-responsive decreases in cerebral blood flow in different regions of the brain, such as thalamic cerebral blood flow.[262] An international double-blind, Phase II trial has been performed to evaluate the effect of different dosages of SYN115 on the reduction of off-

time.[262] SYN115 is generally well tolerated, with low incidence of adverse effects such as dyskinesia, nausea, dizziness, constipation, insomnia, and falls.[263]

Preclinical pharmacokinetic studies of the non-xanthine compound vipadenant (V2006) show good oral bioavailability, long plasma half-life, and good brain penetration in rat models.[171] The results from a Phase I trial indicate that the pharmacokinetics of V2006 are appropriate for monotherapy or in combination with other drugs.[264] Clinical trials based on PET have revealed that this compound was delivered to the brain in concentrations closely related to dose and plasma levels.[265] In a Phase II clinical trial, different doses of V2006 administered orally have suggested that this compound was promising even though it was discontinued following preclinical toxicology studies that indicated side-effect issues.[101]

As an alternative approach, Phase I clinical trials have been performed in PD patients to investigate the safety, tolerability, and pharmacokinetics of another A_{2A} antagonist, V81444.[101] In particular, two Phase I studies with V81444 have been completed and demonstrated that this compound had an acceptable pharmacokinetic, safety, and tolerability profile. A receptor occupancy study has revealed that high levels of A_{2A} antagonists could be achieved at doses that were well tolerated without safety concerns.[101] In healthy volunteers this compound has a good safety and tolerability profile and the blocking of the A_{2A}ARs could be achieved with a single dose of V81444. A Phase II clinical trial to evaluate the pharmacokinetics, safety and tolerability of V81444 is being conducted with a specific evaluation on drug-related adverse events.[101]

Preclinical studies have been performed to assess the pharmacological, pharmacokinetic, safety, and toxicological profile of PBF509, a non-xanthine A_{2A} antagonist that is characterized by an excellent response in PD animal models. A Phase I clinical trial to

verify the safety, tolerability and pharmacokinetic profile of PBF509 has been just completed in healthy male volunteers using a placebo-controlled, single oral, escalating dose design.[101]

Preladenant (SCH420814) is a selective, orally active A_{2A} antagonist discovered by scientists at Schering-Plough. Preclinical studies performed in different animal models reported a good safety profile and tolerability at different doses.[266] A transient increase in systolic and diastolic blood pressure occurred after SCH420814 treatment, not associated with a delay of cardiac repolarization.[267] Preladenant showed good dose-dependent efficacy *in vivo* in the haloperidol-induced rat catalepsy assay and reversed the rat hypolocomotion induced by A_{2A} agonists.[28] Early clinical trials have been performed in PD patients where SCH420814, as mono-therapy or added to L-DOPA therapy, produced an improvement in motor function and a sustained efficacy in improving on-time and reducing off-time.[268] However, following Phase III trials that failed to demonstrate efficacy when compared to placebo, Merck (who acquired the drug during a merger with Schering-Plough) decided to discontinue the extension phases of the trials and has indicated that they will not pursue regulatory filings. SCH420814 has also been patented for treating symptoms of anxiety, including social phobia, panic attack, agoraphobia, obsessive compulsive disorder, and post-traumatic stress disorders.[261]

After reviewing the literature on the potential therapeutic applications of A_{2A} antagonists in peripheral system, a clear pattern to their meaning does not emerge, suggesting that further work is needed to identify a specific relationship for the pharmacological use of A_{2A} antagonists in peripheral pathologies. Recently, Ivachtchenko and coworkers have suggested that a series of istradefylline analogues acting as selective A_{2A} antagonists may find utility as adjuvants to intensify the immune response with oncovaccines and in adoptive immunotherapy.[28, 269]

Different research and diagnostic studies have been presented in the literature showing the potential use of selective, high affinity radiolabeled A_{2A} antagonists as PET radiotracers for imaging A_{2A}ARs in humans.[270] PET tracers for A_{2A}ARs and their detailed biological evaluation in rodents, nonhuman primates, and humans have been recently reviewed. including several new lead structures that could be potential candidates for radiolabeling and mapping of A_{2A}ARs in healthy and diseased cerebral tissue.[270] Interestingly, PET can play an important role in measuring radiolabeled A_{2A} antagonists non-invasively in the brain, such as with [^{11}C]SCH442416 (see compound **8**, figure 3) and [^{11}C]KW-6002, both used for the *in vivo* PET imaging of A_{2A}ARs.[28,114] [^{11}C]SCH442416 has been successfully used to investigate the relationships between steady-state plasma levels and the link between dose and receptor occupancy of vipadenant in healthy male volunteers.[28] A phase I PET trial using [^{11}C]KW6002 showed that A_{2A}AR occupancy increased in a dose dependent manner, reaching more than 90% occupancy at 5 mg/day.[101] A new PET radiotracer ,[^{18}F]MNI444 (^{18}F-labeled **9**, figure 3), has been used to evaluate receptor occupancy with A_{2A}AR targeted therapies, especially for CNS pathologies and full tracer characterization in whole body imaging.[271] In addition, the relationship between plasma levels and A_{2A}AR occupancy by tozadenant and preladenant has been reported.[272]

7. Concluding remarks and future perspectives

The investigation of A_{2A}ARs and their pharmacological modulation is a rapidly growing area of research, with an important impact on the drug discovery process. There is now extensive evidence that A_{2A}ARs are involved in several physiological processes and different pathologies. The present review documents the state of knowledge of A_{2A}ARs and of selected antagonists acting on these receptors. A wide range of information, based on molecular and cellular pharmacology, signal transduction, new drug discoveries, and clinical

applications has been reported. From a medicinal chemistry point of view, we have identified some common chemical scaffolds that can be recognized among the plethora of molecules above described and have summarized these in tables V-VIII. Here we presented the state of the art with regard to the current advancement of A_{2A} antagonists in drug development. For each of the identified templates, we have indicated the general mono- or multi-target profile, the most representative molecules, and corresponding advantages and disadvantages. During the reviewing process of this work additional examples of dual acting ligands (A_{2A}AR/D2) have been reported.[273]

Detailed understanding of the chemical aspects and molecular biology of the A_{2A}ARs provides a basis for specifically targeted pharmacotherapies based on adenosine modulation. The identification of potent and selective A_{2A} antagonists opens new frontiers for the elucidation of specific and selective therapeutic potentials. The characterization of intracellular pathways involving A_{2A}ARs supports the belief that the modulation of these signaling pathways will lead to considerable advances in the management of a number of disease states. The A_{2A} antagonists appear to play a prominent role in neurodegenerative diseases, such as PD. Clinical trials using A_{2A} antagonists have clearly indicated their ability to reduce off-time in PD patients, although this pharmacological approach may require the use of additional adjunctive drugs such as MAO-B inhibitors, COMT inhibitors, dopamine agonists, or L-DOPA. The concomitant administration of A_{2A} antagonists and L-DOPA appears to have significant effects in PD patients, mediating an improvement of parkinsonian motor deficits and reducing different adverse effects such as dyskinesia, dizziness, insomnia, nausea, headache and hallucination. Additional results from PD patients in early stage disease are expected to elucidate whether A_{2A} antagonists are suitable for administration as monotherapy against motor symptoms and dyskinesia.

The neuroprotective potential of combined therapy, promoted by different single-drug effects, points to the possibility of multidrug therapies in PD with minor side effects. The incorporation of A_{2A} antagonists into PD pharmacological treatment with the capability to improve human health could represent an important therapeutic strategy. Moreover, A_{2A}AR heteromers may be used as interesting selective targets for drug development, considering the neuronal localization of receptor heteromers and the distinct ligand affinity of a receptor depending on its partner in the heteromer. Future development of therapies that target other neurotransmitter systems including adenosine, MAO-B inhibitors, glutamatergic or adrenergic antagonists and serotoninergic agents could become a useful strategy to reduce doses required and motor complications.

Finally, the design of compounds with optimized affinity and/or functional activity toward multiple targets (A_1/A_{2A}ARs , MAO-B) involved in the pathogenesis of PD has been examined. The first triple-modulators acting on the rat isoforms of the three molecular targets (see for example the tetrahydropyrazino-purinedione derivative **35**) could play a fundamental role in the preclinical validation of this multi-target approach. These findings could also open unexpected therapeutic perspectives for adenosine antagonists whose development was originally discarded due to a low A_1/A_{2A} selectivity profile. Compounds that exhibited good drug-like properties and pharmacokinetics, especially, could be candidates for re-evaluation in the drug discovery process. Undoubtedly, the SAR optimization of a single molecule against multiple targets is a major and exciting challenge to be addressed. However, increasing evidence indicates that the polypharmacological approach could be preferred to the current polypharmaceutical approach for the treatment of different pathological conditions, such as neurodegenerative diseases and cancer. It can be speculated that the contradictory data about the clinical efficacy of a monotherapy based on selective A_{2A} antagonists (see for example

Istradefylline or Preladenant) may be due to the ineffective modulation of a single drug target in the case of a multifactorial diseases. Based on the chemical and pharmacological results analyzed in this review, it would appear that the future advancement of multi-target tools will assess the real translational potential of the polypharmacological approach compared to mono- and combination therapies, with improvements in clinical utility for the treatment of complex multifactorial disorders.The future advancement of the dual A_1/A_{2A} antagonist ST1535 in Phase II/III trials will help to determine the beneficial effects of combined A_1 and A_{2A} blockade on PD-related cognitive impairment.

8. Abbreviations

Aβ = amyloid-β-peptide
AD = Alzheimer's disease
ADME = absorption, distribution, metabolism, and excretion
ADP = adenosine diphosphate
ARs = adenosine receptors
ARNO = adenosine diphosphate-ribosylation factor nucleotide site opener
BRET = bioluminescence technique
CNS = central nervous system
COMT = catechol-O-methyltransferase
CREB = cAMP response element binding protein
cAMP = cyclic adenosine monophosphate
DARPP32 = dopamine- and cAMP-regulated phosphoprotein
DRs = dopamine receptors
EL = extracellular loop
ERK = extracellular signal regulated kinases
GABA = gamma-aminobutyric acid
GDP = guanosine diphosphate
GPCRs = G protein coupled receptors
GTP = guanosine triphosphate
HD = Huntington's disease
IL = intracellular loop
KO = knockout
L-DOPA = L-3,4-dihydroxyphenylalanine
MAO-B = monoamine oxidase-B
MAPK = mitogen activated protein kinases
mGlu5 = metabotropic glutamate receptor type 5
NECA = 5'-*N*-Ethylcarboxamidoadenosine
NF-κB = nuclear factor kappa B
NMDA = N-methyl-D-aspartate

PD = Parkinson’s disease
PET = positron emission tomography
PKA = protein kinase A
PKC = protein kinase C
PTPs = pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidines
RET = resonance energy transfer technique
SAR = structure activity relationship
SCI = spinal cord injury
TM = transmembrane
TRAX = translin-associated protein X
UPDRS = Unified Parkinson's Disease Rating Scale
USP = ubiquitin-specific protease
UTR = untranslated region
WT = wild-type

BIOSKETCH

Delia Preti received her degree in Pharmaceutical Chemistry and Technology (2001) and Ph.D. in Pharmaceutical Sciences (2005) from Ferrara University, Italy. She is currently research associate at the Department of Chemical and Pharmaceutical Sciences of the same University. She has mainly worked on the design and synthesis of AR ligands and TRPA1 channel modulators within industrial collaborations.

Pier Giovanni Baraldi received his degree in Chemistry in 1974 from the University of Ferrara where he is currently full professor of Medicinal Chemistry. He has published more than 400 scientific papers including about 50 patents focusing his research activity on the design and synthesis of minor groove alkylating agents, combretastatin analogs, ligands for ARs and TRP channel modulators.

Allan Ray Moorman received his B.S. in Pharmacy (University of Wyoming, 1976), Ph.D. in Medicinal Chemistry (University of Kansas, 1981), and postdoctoral training in mechanistic enzymology (Brandeis University). He spent more than 30 years in the pharmaceutical industry, focused on the discovery and development of novel pharmaceuticals

targeted for such diverse therapeutic targets as ARs, benzodiazepine, cannabinoid, and opioid receptors, antibacterials, and antivirals, and contributing to more than 50 scientific publications in international journals and more than 30 patents. He is currently the president of Alta Vetta Pharmaceutical Consulting in Durham, North Carolina.

Pier Andrea Borea received his degree in Chemistry from the University of Ferrara in 1967. He is currently President of the Evaluation Board of the University of Ferrara. He contributed to some 400 publications in international journals and about 20 chapters in international books. His main field of interest is represented by the study, at the molecular level, of drug-receptor interactions.

Katia Varani received her Doctoral degree in Biology in 1988 and her Ph.D. degree in Cellular and Molecular Pharmacology in 1995 from the University of Ferrara. She is currently Associate Professor of Pharmacology in the Medical Sciences Department of the University of Ferrara. Her research activities primarily focus on the pharmacological, biochemical, and molecular study of adenosine receptors.

FIGURE LEGENDS

Figure 1. Principal signaling pathways activated by A_{2A}AR and D_2DR. These receptors in turn activate or inhibit adenylate cyclase (AC) by coupling with a stimulatory G protein α subunit (Gαs) or inhibitory G protein α subunit (Gαi), respectively. Cyclic adenosine monophosphate (cAMP) modulates different pathways: a) cAMP-regulated guanine nucleotide exchange factor 1 (cAMP-GEF1) involving a Ras related protein (RAP-1a) and a family of three serine/threonine-specific protein kinases (B-Raf) that stimulate an extracellular signal-regulated kinase (ERK) to phosphorylate E-26-like transcription factor-1 (Elk-1); b) the activation of cAMP-dependent protein kinase (PKA) is able to trigger three pathways involving the activation of cAMP binding protein (CREB); the specific guanine nucleotide exchange factor (BETA-PIX) linked to the cell division control protein 42 (CDC42) mediating the activation of different kinases such as an atypical PKC (PARD6), the isotope zeta of PKC (PKC-zeta), and a proapoptotic protein (BAD); other protein kinases (PKs) involved are: a serin-threonin PK (PAK1), a PK of STE11 family (MEKK1) and a c-Jun N terminal kinases (JNK), a mitogen-activated protein kinase kinase (MEKK4), MEK6, a p38 mitogen-activated protein kinase (p38MAPK) that activate a transcription factor (ATF-2); the third pathway involves a cAMP-regulated neuronal phosphoprotein (DARP-32) and its phosphorylation; c) a PDZ domain-containing guanine nucleotide exchange factor 1 (PDZ-GEF1) pathway involves a GTP-asi (H-Ras), and a phosphoinositide 3-kinase (PI3K) that phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3), protein kinase B or PKB (Akt), IKK-α or inhibitor of NF-κB that permit the translocation of nuclear factor kB (NF-kB) in the nucleus. Moreover, the βγ complex of D_2DR is linked to phospholipase C (PLC) and IP_3 production that stimulates the release of calcium from endoplasmic reticulum into the cytoplasm.

Figure 2. Structures of A_{2A} antagonists of historical interest.

Figure 3. Pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidines (PTPs) as A_{2A} antagonists.

Figure 4. SAR optimization of PTPs leading to Preladenant.

Figure 5. PTP-related tricycles as A_{2A} antagonists.

Figure 6. Indenopyrimidines (**30**-**33**) and tricyclic xanthines (**34**-**36**) identified as A_1/A_{2A}/MAO-B multi-target ligands.

Figure 7. Xanthine-based derivatives identified as A_{2A} selective antagonists or A_{2A}/MAO-B dual ligands.

Figure 8. Purine derivatives identified as A_{2A} antagonists or A_1/A_{2A} dual ligands.

Figure 9. Triazolo-triazines/pyrimidines identified as A_{2A} antagonists.

Figure 10. Thienopyrimidines identified as A_{2A} antagonists.

Figure 11. Benzofurans identified as A_{2A} antagonists.

Figure 12. Benzothiazoles identified as A_{2A} antagonists.

Figure 13. Benzothiazinone, chromone, aminoquinoxaline and aminoquinoline derivatives as examples of 6,6-bicycles with A_{2A} antagonistic activity.

Figure 14. Pyridines and Pyrimidines identified as A_{2A} antagonists.

Figure 15. Triazines identified as A_{2A} antagonists.

Figure 16. Pyrazines identified as A_{2A} antagonists.

Figure 17. 5-Membered heterocycles identified as A_{2A} antagonists.

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Table I. Affinity and selectivity of the most representative A_{2A} antagonists characterized by a tricyclic system.

	K_i (nM) [a]						
	A_1ARs	**A_{2A}ARs**	**A_{2B}ARs**	**A_3ARs**	**A_1/A_{2A}**	**A_{2B}/A_{2A}**	**A_3/A_{2A}**
2, [5,28]	3.5	0.15	71	51	23	473	340
CGS15943	6 (r)[b]	1.2 (r)			5.0 (r)		
4, [5]	3.3 (r)	1.2 (r)			2.8		
8FBPTP							
5, [5]	594	1.1	> 10000	> 10000	540	> 9091	> 9091
SCH58261							
6, [5]	350	1.2	> 10000	>10000	292	> 8333	> 8333
SCH63390							
7, [114]	1111	0.048	> 10000	> 10000	23146	> 208333	> 208333
SCH442416	1815 (r)	0.50 (r)		> 10000 (r)	3630 (r)		> 20000
9[115]	43	12		60	3.4		5
10[117]	253	1.5		> 10000	169		> 6670
	741 (r)	0.94 (r)			788 (r)		
11[118]	4927	4.6	> 10000	> 10000	1064	> 2160	> 2160
12[118]	2160	0.22	> 10000	> 10000	9818	> 45454	> 45454
13[118]	558	1.1	> 10000	> 10000	507	> 9091	> 9091
14[118]	369	3.8	> 10000	> 10000	97	> 2631	> 2631
15, [101,121]	1474	1.1	> 1700	> 1000	1340		
Preladenant							
16, [121]	> 960	0.6			> 1600		

SCH412348							
17[125]	1062	0.5			2124		
18[125]	406	2.4			169		
19[126]	680	5.4			126		
20[126]	192	3.2			60		
21[126]	880	1.9			463		
22[126]	358	2.0			179		
23[127]	602	0.9			669		
24[128]		0.1-10					
25[129]		5					
26[129]		1					
27, [132]	29000	6.3			4603		
PTTP							
28[134]	1404	23			60		
29[135]		0.4					
30[137]	1.8[c]	1.0 [c]			1.8		
31[137]	17 [c]	4.1 [c]			4.1		
32, [143]	49 [c]	7.5	230 [c]	9200 [c]	7.4		
JNJ40255293		6.5 [c]					
33[136]	59 [c]	8.2 [c]			7.1		
34[144]	605	417	> 1000	4400	1.5	> 2.4	> 11
	1000 (r)	641 (r)			1.6 (r)		
35[145]	791	1510	> 1000	>100	0.50	0.7	0.07
	351 (r)	322 (r)			1.1 (r)		

36[145]	217	268	> 1000	>300	0.80	3.7	1.1
	111 (r)	603 (r)			0.20 (r)		

[a] K_i values from competition binding assays to human ARs unless otherwise specified.

[b] r = rat.

[c] IC_{50} values from cAMP functional assays.

Table II. Affinity and selectivity of the most representative A_{2A} antagonists characterized by a bicyclic system.

	K_i (nM)[a]						
	A_1ARs	**A_{2A}ARs**	**A_{2B}ARs**	**A_3ARs**	**A_1/A_{2A}**	**A_{2B}/A_{2A}**	**A_3/A_{2A}**
1,[28]	44900	23400	20500	> 100000 (r)[b]	1.9	0.9	
Caffeine							
3, [28]	255	0.8	50	> 10000	319	63	> 12500
ZM241385							
37, [147,148,152]	>10000	71		2500	141		35
KF17837	62 (r)	1.0 (r)			62 (r)		
	1500 (gp)[b]						
38, [7,168]	2830	36	1800	> 3000	79	50	> 83
KW6002	230 (r)	2.2 (r)			104 (r)		
39, [7,145]	> 10000	38	8200	> 10000	> 263	216	> 263
CSC	28200 (r)	54 (r)			522 (r)		
40, [150]	2500	5.4	> 10000[c]	> 10000	463		> 1852
MSX2	900 (r)	8.0 (r)			112 (r)		
43[156]	> 10000	45	> 10000[c]	> 30000	> 222		> 667
44,[160]	10000	260		10000			
ST3564	(59%)[d]			(60%)[d]			
45[157]		59 (r)					
46[157]		114 (r)					
47, [161]	72	6.6	352	> 1000	11	53	> 152

ST1535							
48[161]	80	4.7	2330		17	496	
49[167]	3288	6			548		
50[167]	1780	3.1			574		
51, [168,169]	17	1.1	112	1472	15	102	1338
VER6947							
52, [168,169]	170	1.7	141	1931	100	83	1136
VER7835							
53, [171]	68	1.3	63	1005	52	48	773
Vipadenant							
54[175]	1967	9					
55[177]	5.6	0.56	728				
56[178]	5.3	55	1000 (42%)[f]	> 1000			
57[180]	1300 (r)	3 (r)			433 (r)		
58[180]	10000	100	28000	> 10000	100	280	100
	1100 (r)	14 (r)			78 (r)		
59[181]	820 (r)	4 (r)			205 (r)		
60[182]	800 (r)	2 (r)			400 (r)		
61[183]	3300 (r)	0.20 (r)			16500 (r)		
62[183]	1200 (r)	30 (r)			40 (r)		
63[184]	1350	17	10,700[c]	10000 (73%)[d]	80		592
64[133]	1447	1.5			965		

65[189]	185	3.3	1110	238	56	336	72
66[190]	1680[c]	29[c]					
67[193]	290 [c]	7.2[c]					
68[190]	1010 [c]	36 [c]					
69[193]	222[c]	11[c]					
70[195]	1733	25			69		
71, [197,198]	208	1.4	865	476	148	618	340
VER6623	913 (r)	17 (r)		5974 (r)	54 (r)		351 (r)
72[199]		100 (58%)[e]					
73[199]		100 (100%)[e]					
74[199]	1000 (4%) [f]	100 (73%)[e]	1000 (21%) [f]				
75[201]		0.5					
76[202]		1					
77[203]		0.50					
78[203]		0.50					
79,[28]	1350	5.0	700	1570	270	140	314
SYN115							
80[206]	> 7000	4.1			> 1707		
81[210]	2.8	0.0038			737		
82[212]	≥ 10000 > 1000 (r)	70 285 (r)	178	> 1000	≥ 144 > 3.5 (r)	2.6	> 14

83[213]	2500	40	> 1000	> 1000	63	> 14	> 14
	229 (r)	423 (r)			0.50 (r)		
84[214]	> 1500	15			> 100		
85[215]	5000	40	> 10000	> 10000	125	> 250	> 250

[a] K_i values from competition binding assays to human ARs unless otherwise specified.

[b] r = rat; gp = guinea pig.

[c] IC_{50} values from cAMP functional assays.

[d] % of inhibition at 10 μM.

[e] % of inhibition at 100 nM.

[f] % of inhibition at 1 μM.

Table III. Affinity and selectivity of the most representative A_{2A} antagonists characterized by a monocyclic system.

	K_i (nM)[a]						
	A_1ARs	**A_{2A}ARs**	**A_{2B}ARs**	**A_3ARs**	**A_1/A_{2A}**	**A_{2B}/A_{2A}**	**A_3/A_{2A}**
87,[217] LUF6080	12	1.0	34	> 1000	12	34	> 1000
88[217]	34	41	> 1000	> 1000	0.8	> 24	> 24
89[218]	266	2.7			98		
		26 (r)[b]					
90[218]	1730	2.0			265		
		14 (r)					
91[221]	850	135			6.3		
92[221,222]	850	12			71		
		131 (r)					
93[221]	850	17			50		
94[221]	2000	9			222		
95[222]	630	2.4			262		
		41 (r)					
96[226]	85	0.44			193		
		1.50 (r)					
97[227]	162	4.7	145	19	34	31	4
		48 (r)					
98[229]		0.22					

99[231]	43	1.7	460	1740	25	270	1023
100[230]	133	2.5	3185	366	53	1274	146
101[232]		1.0					
102[214]	59	1.0			59		
103[214]	30	1.6			19		
104[234]	32	3.5			9.1		
105[234]	135	2.2			61		
106[234]	> 10000	16			> 625		
107[234]	> 10000	68			> 147		
108[236]	4.9	0.84			5.8		
109[236]	0.61	0.23			2.6		
110, [237]	9.0	1.8			5		
ASP5854							
111[233]	500	200	> 10000	600	2.5	> 50	3
112[233]	>10000	200	> 10000	300	> 50	> 50	1.5
113[242]	410	5.9	260	> 10000	69	44	> 1695

[a] K_i values from competition binding assays to human ARs unless otherwise specified.

[b] r = rat.

Table IV. Adenosine receptor affinities in comparison with MAO-A and MAO-B inhibitory potencies of potential multi target ligands.

	K_i (nM)[a]					
	A_1ARs	**A_{2A}ARs**	**A_{2B}ARs**	**A_3ARs**	**MAO-B**[b] IC_{50} (nM)	**MAO-A**[b] IC_{50} (nM)
Tricyclic Systems						
34[144]	605 1000 (r)	417 641 (r)	> 1000	4400	1800	> 10000
35[145]	791 351 (r)	1510 322 (r)	> 1000	>100	197 260 (r)	> 10000 (h)
36[145]	217 111 (r)	268 603 (r)	> 1000	>300	508 3000 (r)	> 10000 (h)
Bicyclic Systems						
38, [7,145,158,168] Istradefylline	2830 230 (r)	36 2.2 (r)	1800	> 3000	> 10000	> 10000
39, [7,145,158] CSC	> 10000 28200 (r)	38 54 (r)	8200	> 10000	235[c] 70 (b)[c]	> 10000
44,[160] ST3564	10000 (59%)	260		10000 (60%)	200	10000
45[157]		59 (r)			38 (b)	
46[157]		104 (r)			96 (b)	
83[213]	2500 229 (r)	40 423 (r)	> 1000	> 1000	35 186 (r)	> 10000

[a] K_i values from competition binding assays to human ARs unless otherwise specified.

[b] IC_{50} values from monoamine oxidase (MAO) assays with human, rat (r) or baboon liver (b) enzymes.

[c] K_i values from monoamine oxidase (MAO) assays with human or baboon liver (b) mitochondrial enzymes.

Table V. Tricyclic A_{2A} antagonists in drug development.

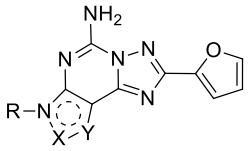
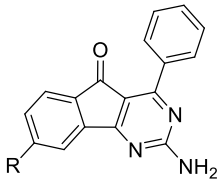
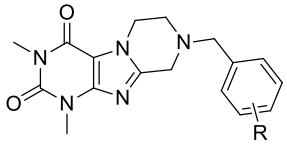
GENERAL STRUCTURE	MONO- OR MULTI-TARGET PROFILE	REPRESENTATIVE LIGANDS	CLASS ADVANTAGES	CLASS DISADVANTAGES
	Mainly A_{2A} selective antagonists	**PRELADENANT** (SI hA_1/hA_{2A} = 1340) Phase III Discontinued	1) High A_{2A}AR binding affinity and selectivity 2) High preclinical efficacy in animal model of PD (rodents and primates) alone or in combination with L-DOPA 3) Useful for *in vivo* imaging techniques (PET)	1) Controversial clinical efficacy especially in monotherapy regimen 2) Low solubility 3) The unsubstituted furan ring confers metabolic reactivity
	A_1/A_{2A} dual antagonists	**JNJ40255293** (SI hA_1/hA_{2A} = 7.4) Preclinical investigation	1) High *in vivo* potency 2) Good brain exposure 3) Promising PK profile in different species 4) In animal models of PD, JNJ40255293 displays beneficial effects on cognitive functions through A_1ARs blockade.	1) Some derivatives display genotoxicity due to metabolic instability
	A_1/A_{2A}/MAO-B triple ligands	**35** (SI rA_1/rA_{2A} = 1.1) In vitro biological testing	1) Good water solubility 2) Promising tools for the investigation of translational potential of the polypharmacological approach	1) Relatively low affinity toward A_1/A_{2A} and inhibitory potency against MAO-B that need further optimization.

Table VI. Bicyclic A_{2A} antagonists in drug development (part I).

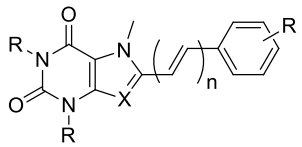
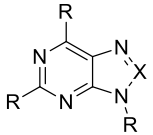
GENERAL STRUCTURE	MONO- OR MULTI-TARGET PROFILE	REPRESENTATIVE LIGANDS	CLASS ADVANTAGES	CLASS DISADVANTAGES
	A_{2A} selective antagonists	**ISTRADEFYLLINE** (SI hA_1/hA_{2A} = 141) (SI rA_1/rA_{2A} = 62) Approved in Japan while discontinued by FDA	1) Preclinical efficacy in animal model of PD alone or in combination with L-DOPA	1) Poor water solubility and light sensitivity 2) Controversial clinical efficacy against PD especially in monotherapy regimen
	A_{2A}/MAO-B dual ligands	**ST3564** Preclinical investigation	1) Oral in vivo efficacy in animal model of PD	1) Light sensitivity 2) Relatively low affinity toward A_{2A} and inhibitory potency against MAO-B that need further optimization. 3)Improvable in vivo potency against PD
	A_1/A_{2A} dual antagonists	**ST1535** (SI hA_1/hA_{2A} = 11) Phase I	1) Good preclinical efficacy in animal model of PD (rodents and primates) 2) Preclinical efficacy on cognitive aspects of PD. 3) Long-lasting pharmacological activity (active metabolites) 4) Good tolerability/safety profile in humans	1) Further investigation of ST1535 treatment of PD in phase II/III trials is needed.
		VIPADENANT (SI hA_1/hA_{2A} = 52) Phase II Discontinued	1) Clinical efficacy both alone and in combination with L-DOPA 2) Good PK profile in rodents and non-human primates	1) Toxicity in humans

Table VII. Bicyclic A_{2A} antagonists in drug development (part II).

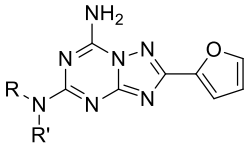
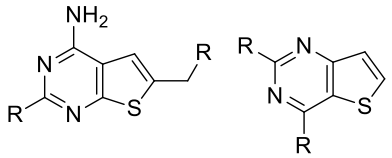
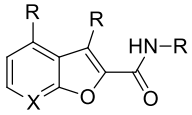
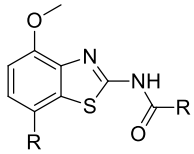
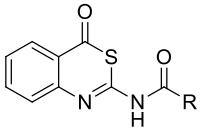
GENERAL STRUCTURE	MONO- OR MULTI-TARGET PROFILE	REPRESENTATIVE LIGANDS	CLASS ADVANTAGES	CLASS DISADVANTAGES
	Mainly A_{2A} selective antagonists	**ZM241385** Preclinical investigation	1) Good A_{2A} in vitro affinity and selectivity. 2) ZM241385 was largely employed for the preclinical characterization of A_{2A}AR.	1) Poor oral bioavailability for a significant number of compounds 2) The unsubstituted furan ring confers metabolic reactivity 3) Discrepancy between affinity, *in vivo* potency, and metabolic stability
	Weak selectivity for the A_{2A}AR	**VER6623** Preclinical investigation	1) Good in vivo efficacy in animal model of PD	1) VER6623 exhibits species specific affinity between h and r A_{2A}AR 2) Some derivatives display short duration of action in vivo and poor oral bioavailability
	Weak selectivity for the A_{2A}AR	**74** Preclinical investigation	1) Promising in vivo efficacy in animal model of PD	1) Further in vitro/vivo investigation are recommended to assess the receptor selectivity and the real potential of this chemotype
	A_{2A} antagonists	**TOZADENANT** (SI hA_1/hA_{2A} = 270) Phase II	1) Preclinical and clinical efficacy against PD both alone and in combination with L-DOPA 2) Good tolerability/safety profile in humans	1) Further investigation of tozadenant treatment of PD in phase III trials is needed.
	A_1/A_{2A}/MAO-B triple ligands in rat	**83** (SI hA_1/hA_{2A} = 63) (SI rA_1/rA_{2A} = 0.5) In vitro biological testing	1) Promising tools for the investigation of translational potential of the polypharmacological approach	1) Relatively low affinity toward A_1/A_{2A} and inhibitory potency against MAO-B that need further optimization.

Table VIII. Monocyclic A_{2A} antagonists in drug development.

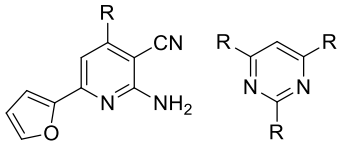
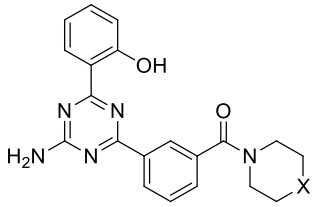
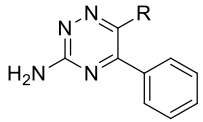
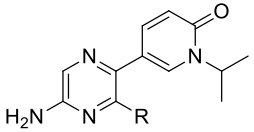
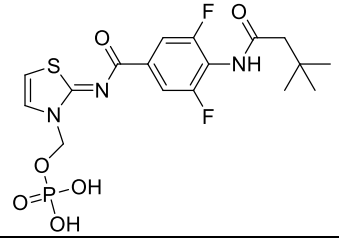
GENERAL STRUCTURE	MONO- OR MULTI-TARGET PROFILE	REPRESENTATIVE LIGANDS	CLASS ADVANTAGES	CLASS DISADVANTAGES
	A_1/A_{2A} dual antagonists or compounds with weak selectivity for the A_{2A}AR	**98-100** Preclinical Investigation (SI hA_1/hA_{2A} = 25-53)	1) The optimization effort led to 2-amino pyrimidines (i.e. **99** and **100**) with very promising PK properties and *in vivo* oral activity against PD	1) Some derivatives exhibit species specific affinity between h and r A_{2A}AR 2) Some derivatives are weak inhibitors of the hERG channel 3) Metabolic liability
	A_1/A_{2A} dual antagonists	**102-103** In vitro biological testing (SI hA_1/hA_{2A} = 19-59)	1) Potential A_1/A_{2A} dual acting compounds	1) Further in vitro/vivo investigation are recommended to assess the receptor selectivity and the real potential of this chemotype
	A_1/A_{2A} dual antagonists	**104** Preclinical Investigation (SI hA_1/hA_{2A} = 9.1)	1) Good PK and ADME profile. 2) No inhibition of hERG channel. 3) Oral in vivo efficacy in animal model of PD	1) Lack of clinical data assessing efficacy in humans
	A_1/A_{2A} dual antagonists	**ASP5853** Preclinical Investigation (SI hA_1/hA_{2A} = 5)	1) Good preclinical efficacy in animal model of PD (rodents and primates) 2) Preclinical efficacy on cognitive aspects of PD.	1) Lack of clinical data assessing efficacy in humans
	Weak selectivity for the A_{2A}AR	**LuAA47070** Preclinical Investigation (SI hA_1/hA_{2A} = 69)	1) Good PK and ADME profile. 2) Oral in vivo efficacy in animal model of PD 3) potentially useful for the treatment of PD and of some of the motivational symptoms of depression.	1) Lack of clinical data assessing efficacy in humans

Figure 1

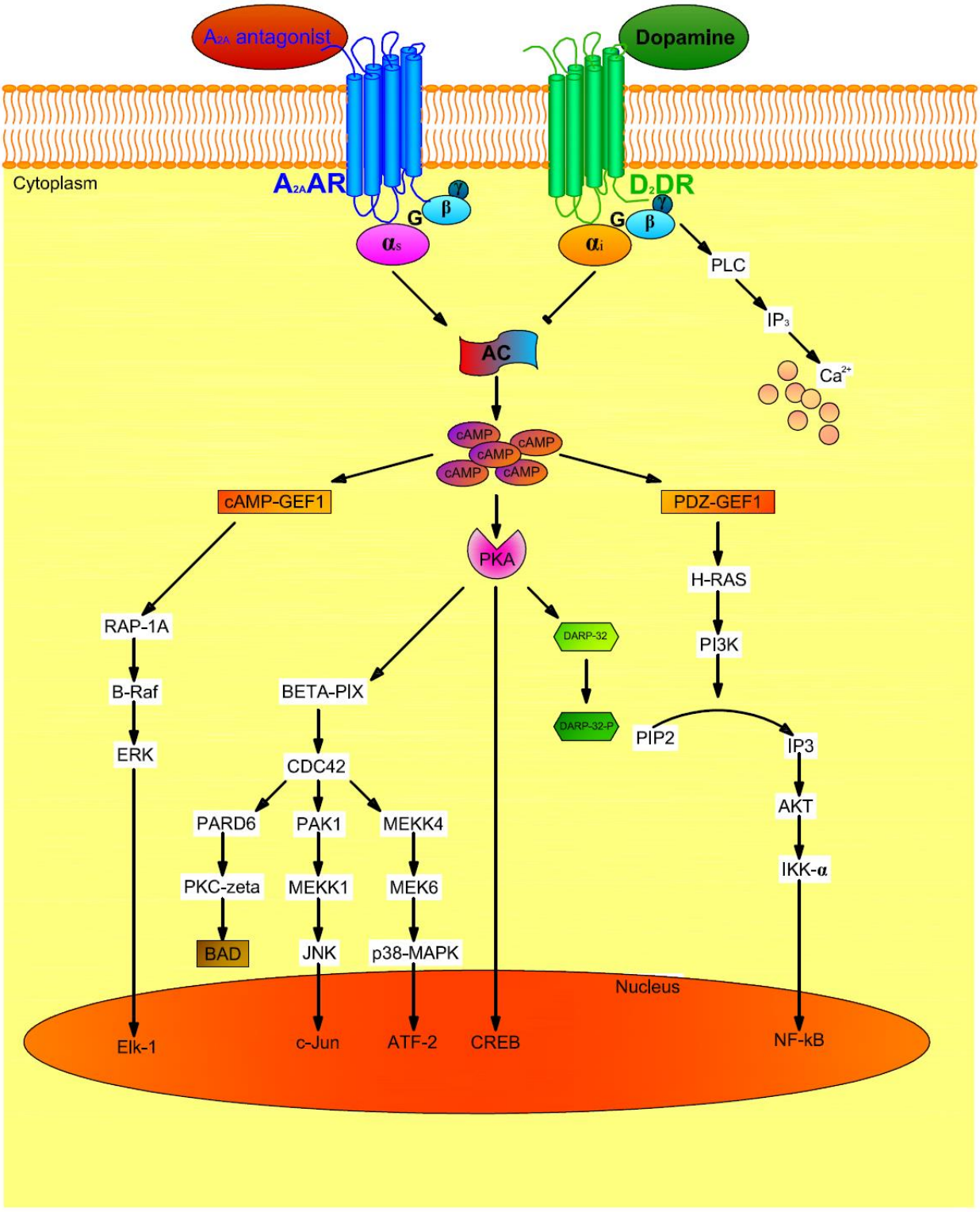
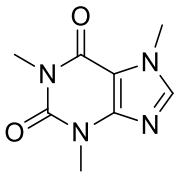
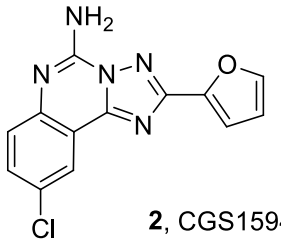

A2A antagonist
Dopamine
Cytoplasm
A2AAR
D2DR
G
β
γ
αs
αi
PLC
IP3
Ca2+
AC
cAMP
cAMP-GEF1
PDZ-GEF1
PKA
H-RAS
RAP-1A
DARP-32
PI3K
B-Raf
BETA-PIX
DARP-32-P
PIP2
IP3
ERK
CDC42
AKT
PARD6
PAK1
MEKK4
IKK-α
PKC-zeta
MEKK1
MEK6
BAD
JNK
p38-MAPK
Nucleus
Elk-1
c-Jun
ATF-2
CREB
NF-kB

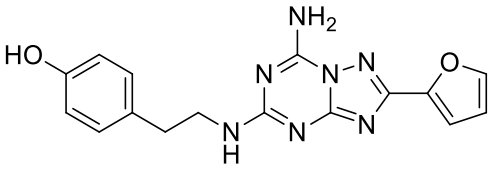
Figure 2



1, CAFFEINE

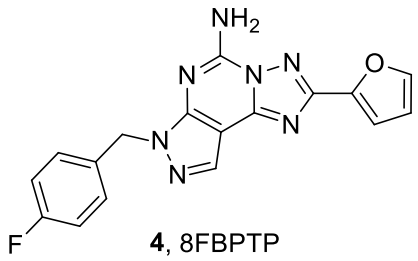


2, CGS15943

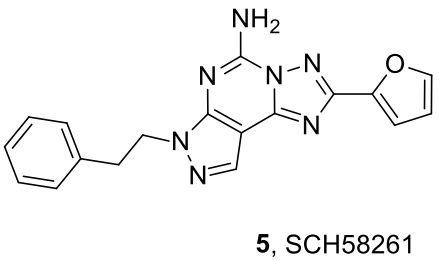


3, ZM241385

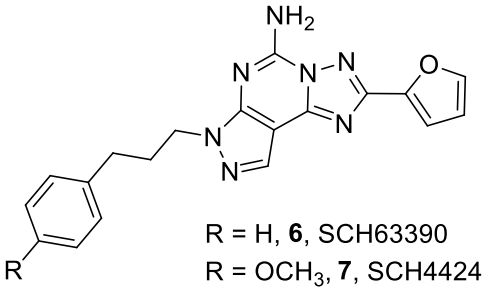
Figure 3



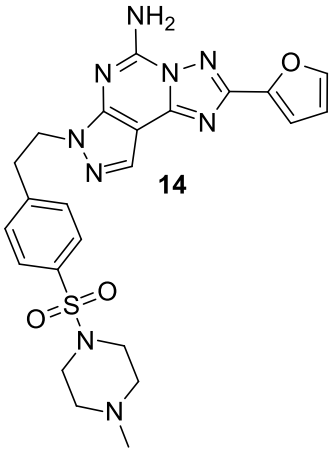
4, 8FBPTP



5, SCH58261

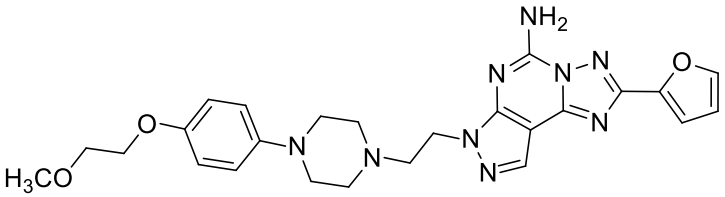


R = H, **6**, SCH63390
R = OCH_3, **7**, SCH442416
R = $O^{11}CH_3$, **8**, [^{11}C]SCH442416
R = OCH_2CH_2F, **9**
R = OH, **10**
R = COOH, **11**
R = NH_2, **12**
R = $N(CH_2CH_2OH)_2$, **13**

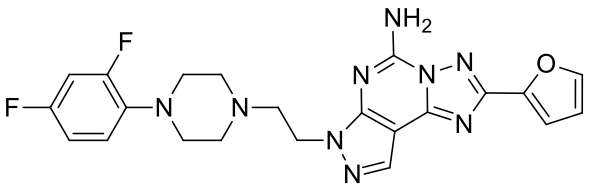


14

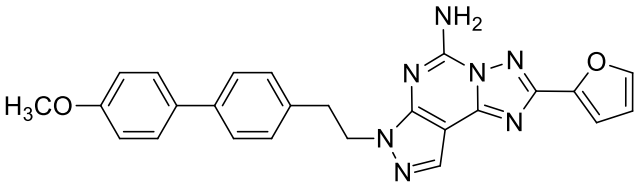
Figure 4



15, Preladenant
SCH420814



16, SCH412348



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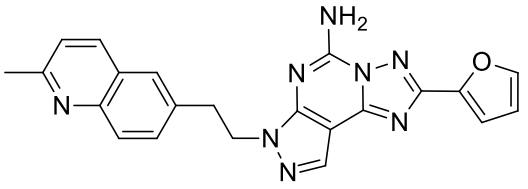

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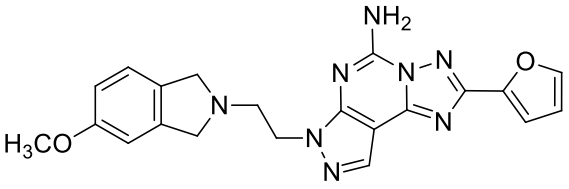
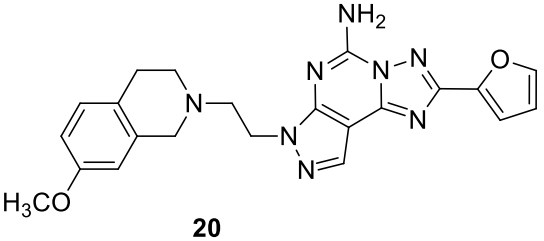


19



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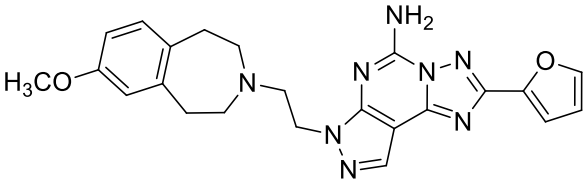

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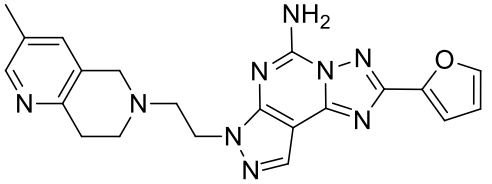
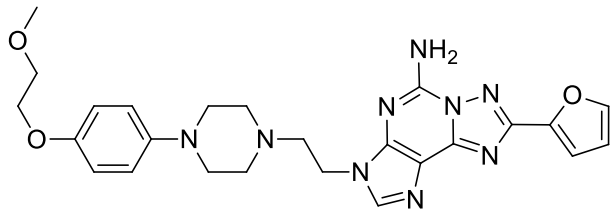


22

Figure 5



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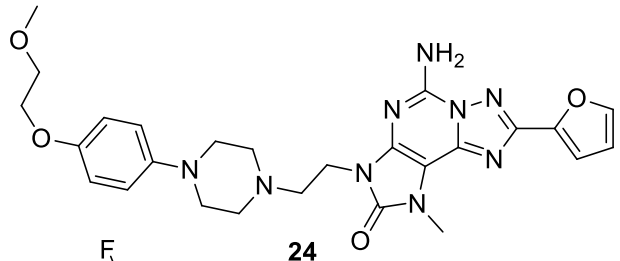

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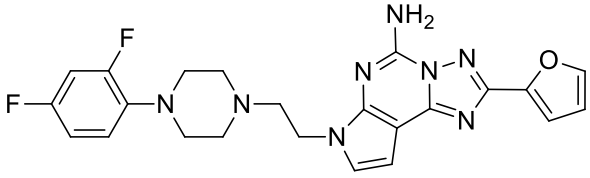

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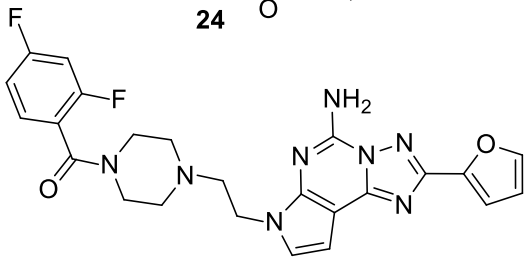

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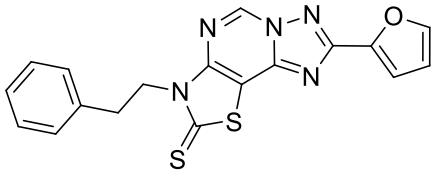
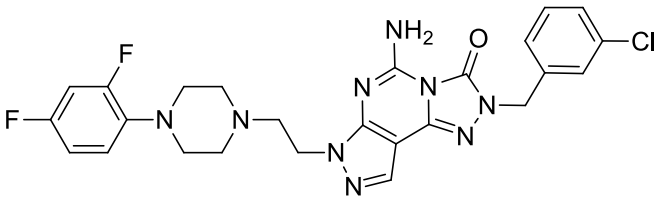


27, PTTP



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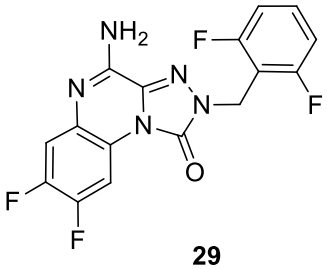
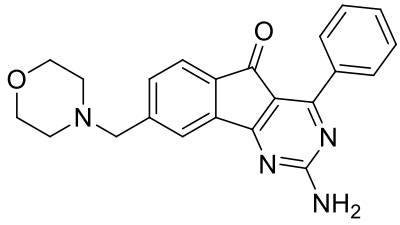


29

Figure 6



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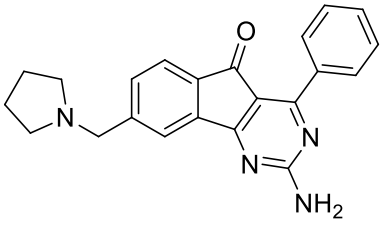
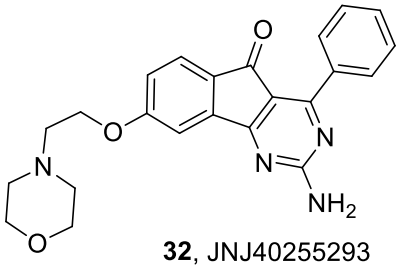
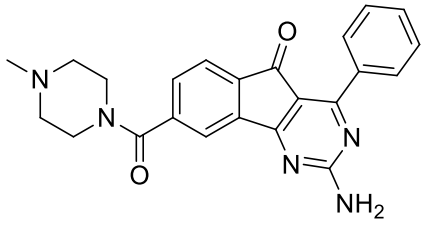


31



32, JNJ40255293



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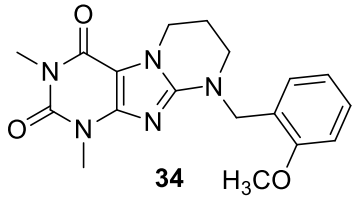
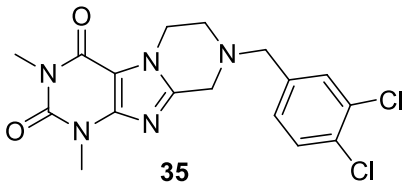


34



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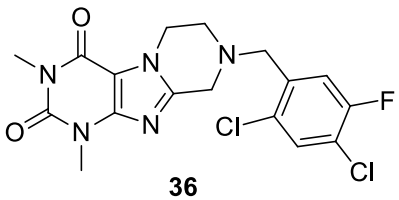
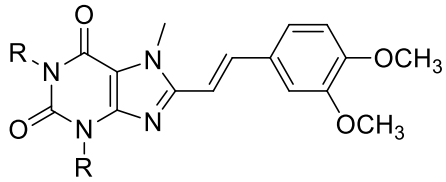
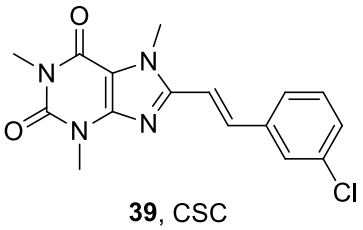


36

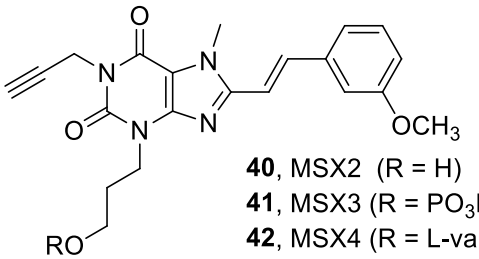
Figure 7



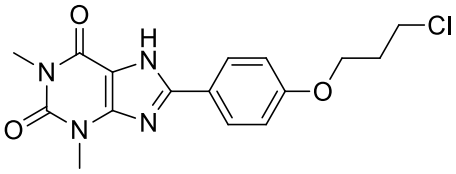
37, KF17837 (R = propyl)
38, Istradefylline/KW6002 (R = ethyl)



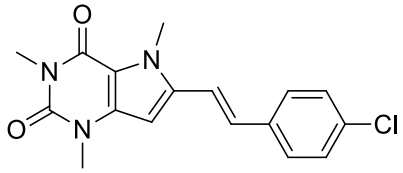
39, CSC



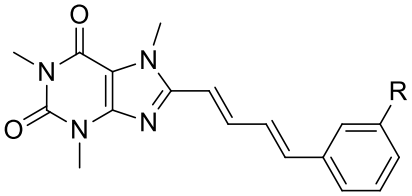
40, MSX2 (R = H)
41, MSX3 (R = PO_3Na_2)
42, MSX4 (R = L-valine ester)



43



44, ST3564



45 (R = Br)
46 (R = Cl)

Figure 8

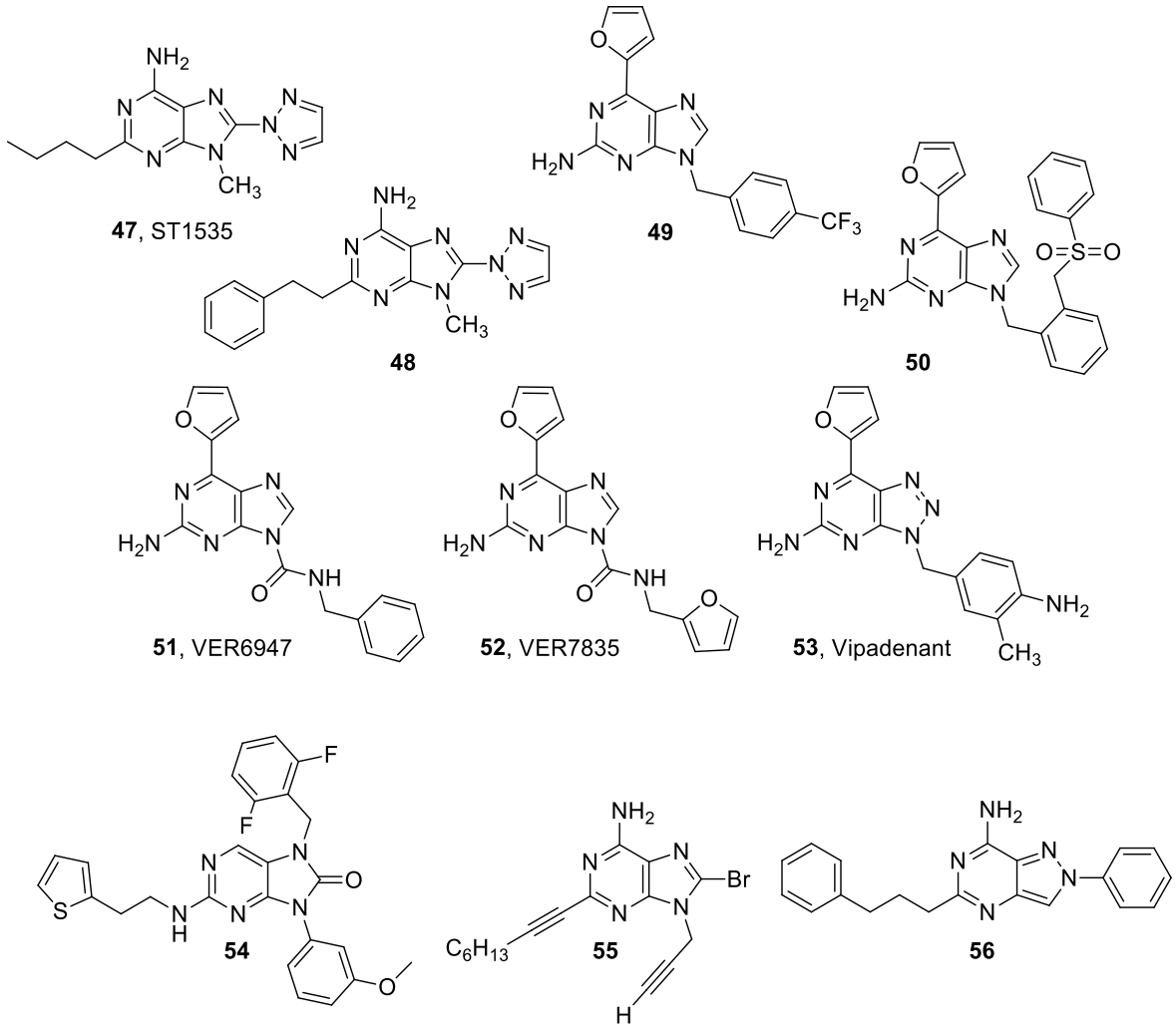

47, ST1535
48
49
50
51, VER6947
52, VER7835
53, Vipadenant
54
55
56

Figure 9

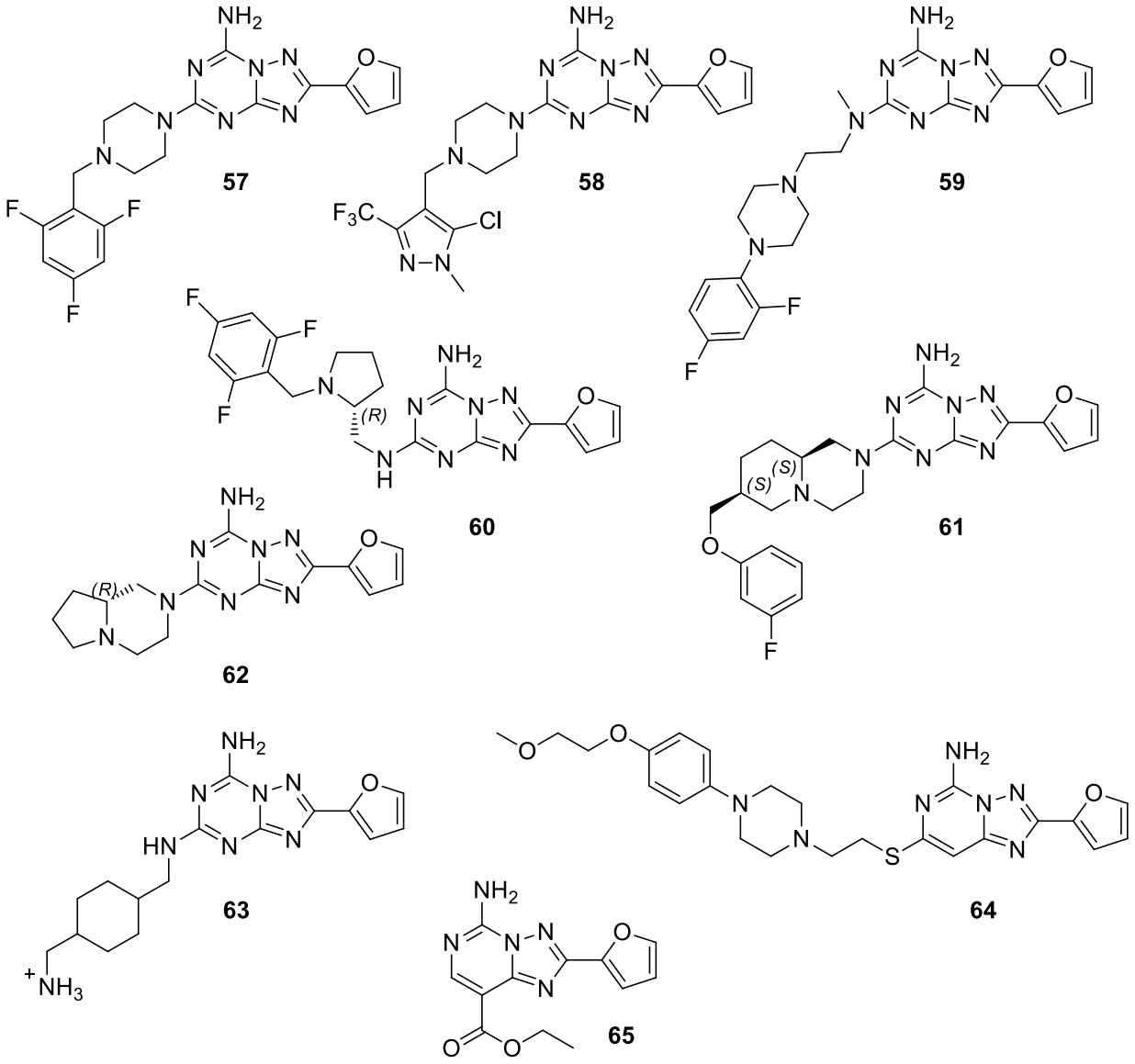
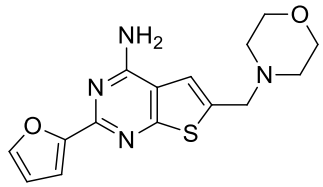
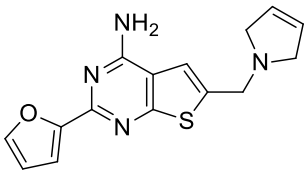


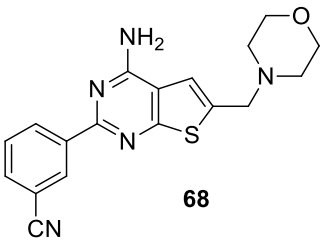
Figure 10



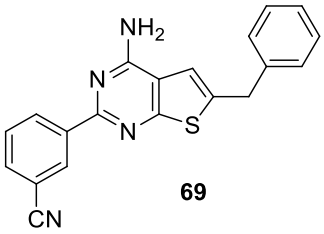
66



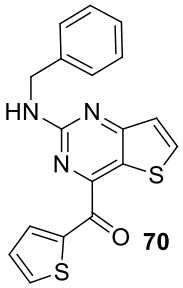
67



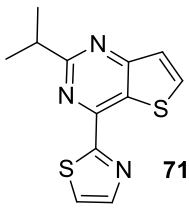
68



69

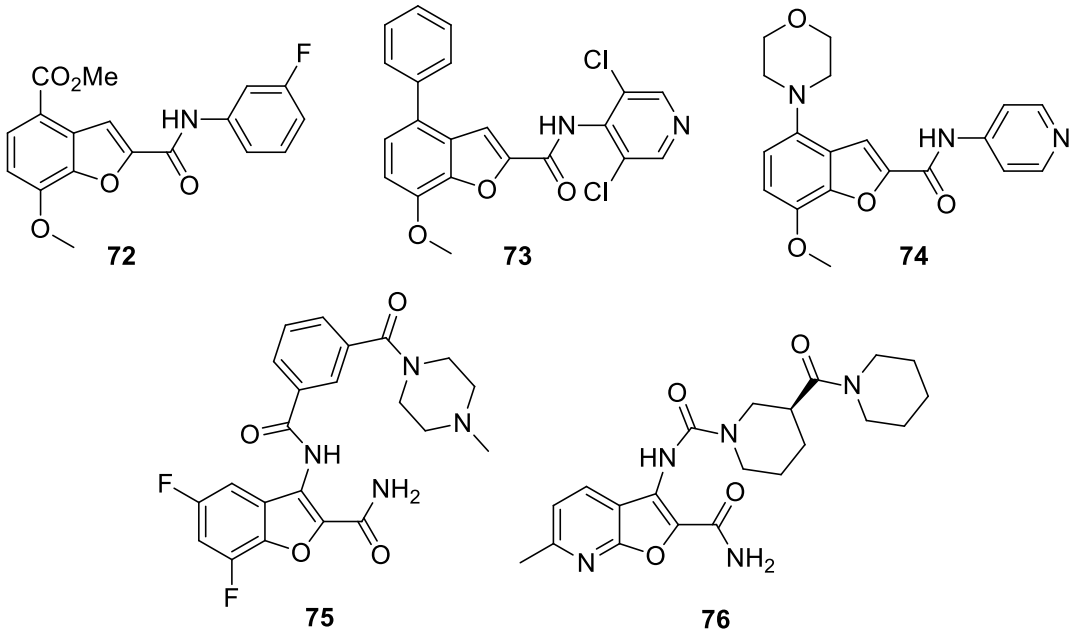


70



71, VER6623

Figure 11


CO2Me
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F
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72
Cl
HN
N
O
O
Cl
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73
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N
HN
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O
O
74
O
N
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NH
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NH2
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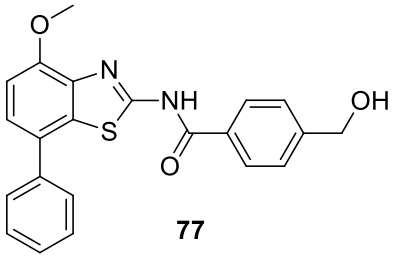
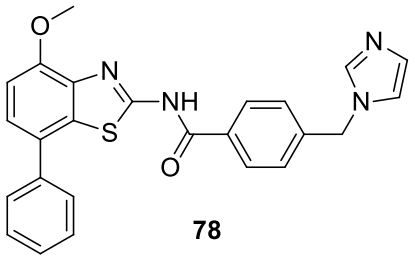
75
O
N
O
HN
N
O
N
O
NH2
76

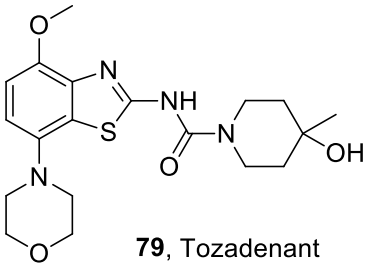
Figure 12



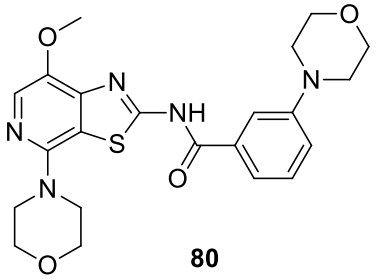
77



78



79, Tozadenant
SYN115



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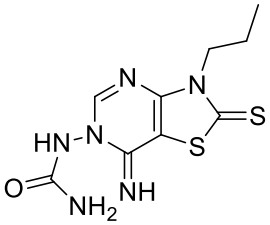
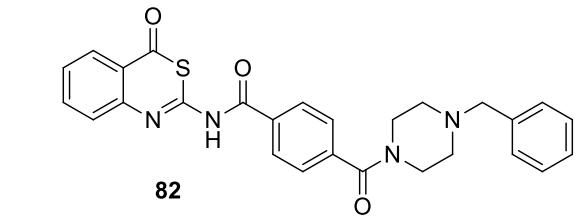
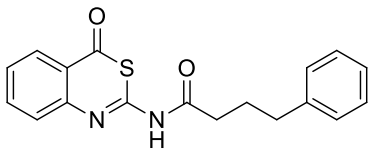


81

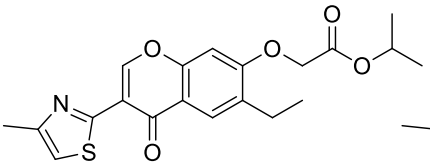
Figure 13



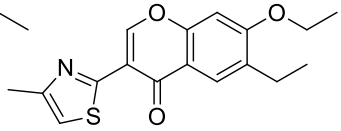
82



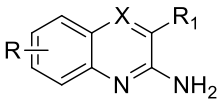
83



84



85



86

Figure 14

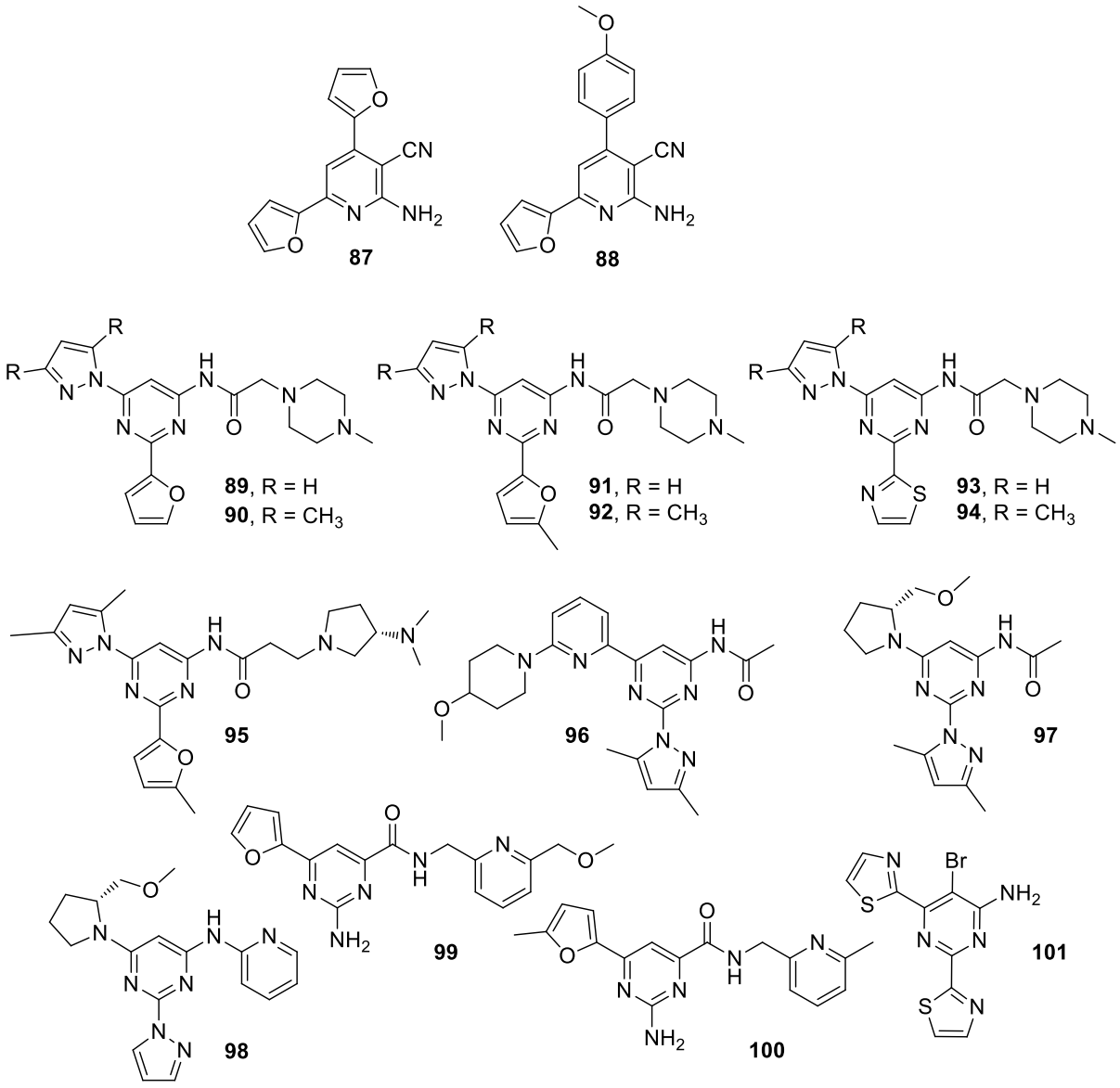
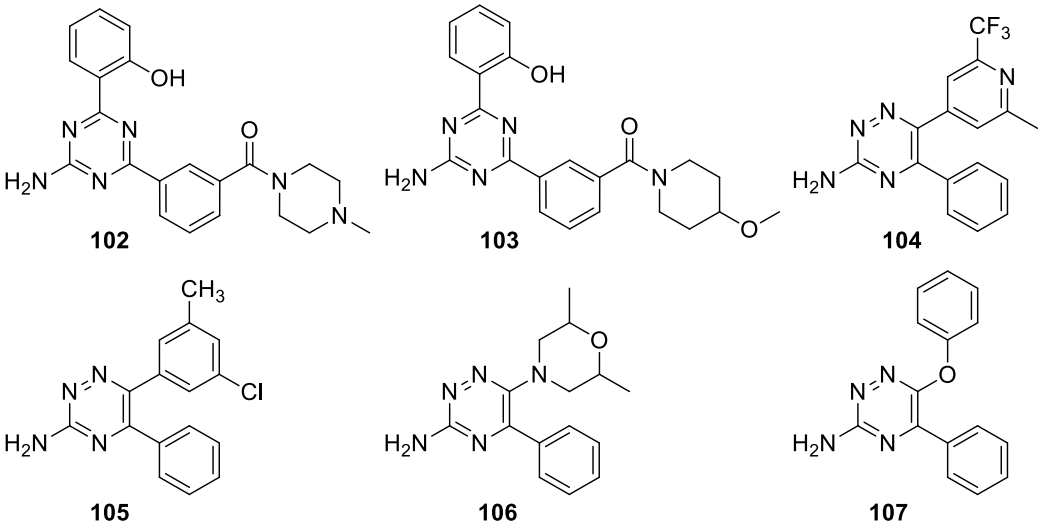

87
88
89, R = H
90, R = CH3
91, R = H
92, R = CH3
93, R = H
94, R = CH3
95
96
97
98
99
100
101

Figure 15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N
N
N
H_2N
O
N
N
102
OH
N
N
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H_2N
O
N
O
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CF_3
N
N
N
N
H_2N
104
CH_3
N
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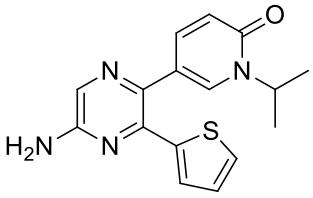
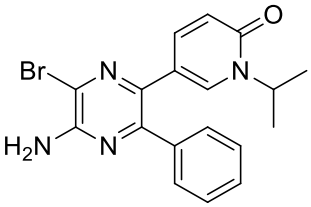
106
O
N
N
N
H_2N
107

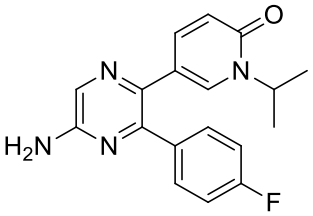
Figure 16



108

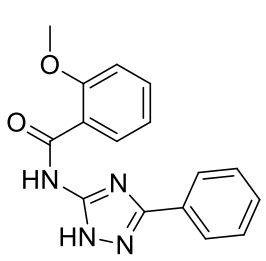


109

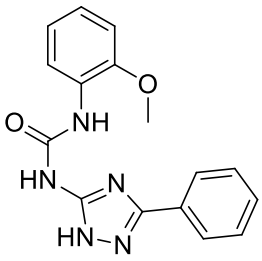


110, ASP5854

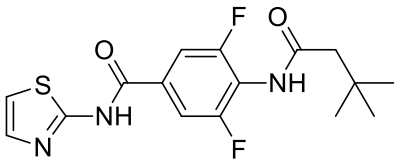
Figure 17



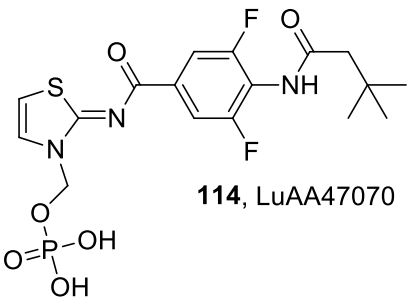
111



112



113



114, LuAA47070