



**Universiteit
Leiden**
The Netherlands

International Union of Basic and Clinical Pharmacology. CXII: adenosine receptors: a further update

IJzerman, A.P.; Jacobson, K.A.; Müller, C.E.; Cronstein, B.N.; Cunha, R.A.

Citation

IJzerman, A. P., Jacobson, K. A., Müller, C. E., Cronstein, B. N., & Cunha, R. A. (2022). International Union of Basic and Clinical Pharmacology. CXII: adenosine receptors: a further update. *Pharmacological Reviews*, 74(2), 340-372. doi:10.1124/pharmrev.121.000445

Version: Publisher's Version
License: [Leiden University Non-exclusive license](#)
Downloaded from: <https://hdl.handle.net/1887/3505427>

Note: To cite this publication please use the final published version (if applicable).

ASSOCIATE EDITOR: ELIOT OHLSTEIN

International Union of Basic and Clinical Pharmacology. CXII: Adenosine Receptors: A Further Update^S

Adriaan P. IJzerman, Kenneth A. Jacobson, Christa E. Müller, Bruce N. Cronstein, and Rodrigo A. Cunha

Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands (A.P.IJ.); National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Molecular Recognition Section, Bethesda, Maryland (K.A.J.); Universität Bonn, Bonn, Germany (C.E.M.); New York University School of Medicine, New York, New York (B.N.C.); and Center for Neurosciences and Cell Biology and Faculty of Medicine, University of Coimbra, Coimbra, Portugal (R.A.C.)

Abstract	341
Significance Statement	341
I. Introduction	341
II. Receptor Ligands (for Structures, See Figs. 1, 2, 3, and 5)	342
A. Adenosine Receptor Agonists	342
B. Adenosine Receptor Antagonists	347
C. Allosteric Modulators of Adenosine Receptors	350
D. Inosine and Guanosine	350
III. Receptor Binding Kinetics	350
A. Orthosteric Ligands and Adenosine Receptor Binding Kinetics	351
B. Orthosteric Ligands Binding Covalently to Adenosine Receptors	352
C. Allosteric Ligands and Adenosine Receptor Binding Kinetics	352
D. Adenosine Receptor Target Binding Kinetics – Conclusions	352
IV. Receptor Structures	353
A. Resolution	353
B. Ligand Binding Site	353
C. NMR Studies	355
V. Cellular Pharmacology – Biased Signaling of Adenosine Receptors	356
VI. Pharmacology – Novel Developments	357
A. Therapeutic Targeting of Adenosine Receptors	357
B. Therapeutic Targeting of Peripheral Adenosine Receptors	357
1. Adenosine A ₁ Receptors and Congestive Heart Failure	357
2. Adenosine A _{2A} Receptors and Cancer	357
3. Adenosine Receptors and Autoimmune and Inflammatory Diseases	358
4. Adenosine Receptors and Infectious Diseases	358
5. Adenosine A _{2A} Receptors and Retinal Disease	358
6. Adenosine Receptors and Bone	359
7. Adenosine Receptors and Cartilage	359
8. Adenosine Receptors and Fibrosis	359
9. Adenosine A _{2A} Receptors and Sickle Cell Disease	359
10. Summary	359
C. Therapeutic Targeting of Central Nervous System Adenosine Receptors	359

Address correspondence to: Adriaan P. IJzerman, LACDR, Leiden University, PO Box 9502, 2300RA Leiden, The Netherlands.
E-mail: ijzerman@lacdr.leidenuniv.nl

This research was supported in part by the Intramural Research Program of the National Institutes of Health [National Institute of Diabetes and Digestive and Kidney Diseases]. This work was supported by National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grant ZIADK31117]; the Deutsche Forschungs Gemeinschaft [Grants FOR2372, SFB1328, and GRK1873]; La Caixa Foundation [Grant LCF/PR/HP17/52190001]; Centro 2020 [Grants CENTRO-01-0145-FEDER-000008:BrainHealth 2020 and CENTRO-01-0246-FEDER-000010]; and Fundação para a Ciência e a Tecnologia [Grant UIDB/04539/2020].

Financial disclosure: Bruce N. Cronstein is founder and Chairman of the Scientific Advisory Board of Regenosine, LLC.
dx.doi.org/10.1124/pharmrev.121.000445.

1. Acute Brain Dysfunction – Ischemia and Epilepsy	360
2. Neurodegenerative Diseases – Parkinson’s and Motor Diseases	360
3. Neurodegenerative Diseases – Alzheimer’s Disease and Cognitive Dysfunction	360
4. Neuropsychiatric Diseases – Major Depression and Suicide	361
5. Other Neuropsychiatric Diseases	361
6. Brain Aging	361
VII. Current and Recent Clinical Trials	362
A. Clinical Trials of Adenosine Receptor Agonists	362
B. Clinical Trials of Adenosine Receptor Antagonists	362
VIII. Concluding Remarks	364
Acknowledgments	365
Authorship Contributions	365
References	365

Abstract—Our previous International Union of Basic and Clinical Pharmacology report on the nomenclature and classification of adenosine receptors (2011) contained a number of emerging developments with respect to this G protein-coupled receptor subfamily, including protein structure, protein oligomerization, protein diversity, and allosteric modulation by small molecules. Since then, a wealth of new data and results has been added, allowing us to explore novel concepts such as target binding kinetics and biased signaling of adenosine receptors, to examine a multitude of receptor structures and novel ligands, to gauge new pharmacology, and to evaluate clinical trials with adenosine receptor ligands. This

review should therefore be considered a further update of our previous reports from 2001 and 2011.

Significance Statement—Adenosine receptors (ARs) are of continuing interest for future treatment of chronic and acute disease conditions, including inflammatory diseases, neurodegenerative afflictions, and cancer. The design of AR agonists (“biased” or not) and antagonists is largely structure based now, thanks to the tremendous progress in AR structural biology. The A_{2A}- and A_{2B}AR appear to modulate the immune response in tumor biology. Many clinical trials for this indication are ongoing, whereas an A_{2A}AR antagonist (istradefylline) has been approved as an anti-Parkinson agent.

I. Introduction

A decade has passed since our last International Union of Basic and Clinical Pharmacology report on the nomenclature and classification of adenosine receptors appeared (Fredholm et al., 2011), after the first one in 2001 (Fredholm et al., 2001). The field has matured to the extent that the recommendations on the nomenclature stand firmly and require neither changes nor refinements. Substantial developments, however, took place (Fredholm et al., 2021), and these alone warrant a further update already. The adenosine A_{2A} receptor (A_{2A}AR) has become a test case for G protein-coupled receptor (GPCR) structure elucidation, whereas structures of the adenosine A₁ receptor (A₁AR) have also become available. The structures have been obtained through either X-ray crystallography or a more recent development, cryo-electron microscopy (EM). These together constitute a huge variety, most of which were determined with different antagonist ligands, a few with agonistic ligands with or without (parts of the) G protein present, and one with a partial agonist. Secondly, the increasing awareness that the

study of target binding kinetics reveals more details on the interaction between ligand and receptor has had its effect on the further and more detailed kinetic characterization of adenosine receptor ligands, both agonists and antagonists. Moreover, there is ample attention again for novel ligands interacting with adenosine receptors. Some of these newer and older ligands possess a preference for biased signaling (i.e., the preferred coupling to particular signaling pathways), most notably through different G proteins or β -arrestin. Furthermore, there is an in-depth analysis of the (patho)pharmacological aspects of adenosine receptors and their ligands, both in the periphery and the central nervous system (CNS), leading to an evaluation of the receptors’ relevance in diverse disease states including COVID-19 infection and in aging. The report is concluded with a (nonexhaustive) overview of the clinical trials with adenosine receptor ligands in the last ten years. Disappointing were the outcomes for A₁AR partial agonists, with lack of efficacy in heart failure noted in advanced phase 2b clinical studies. On the other hand, an A_{2A}AR antagonist was approved in the United States as a new anti-Parkinsonian drug,

ABBREVIATIONS: ADA, adenosine deaminase; AdoK, adenosine kinase; AR, adenosine receptor; CCPA, 2-chloro-N⁶-cyclopentyladenosine; CNS, central nervous system; EM, electron microscopy; GFR, glomerular filtration rate; GPCR, G protein-coupled receptor; h, human; K_D, equilibrium dissociation constant; MECA, methylcarboxamidoadenosine; NASH, nonalcoholic steatohepatitis; NECA, 5'-N-ethylcarboxamidoadenosine; PAM, positive allosteric modulator; PD, Parkinson’s disease; PDB, Protein Data Bank; RT, residence time; TM, transmembrane domain; WT, wild-type.

and the role of adenosine receptors in immunology has led to a surge of ongoing studies in immunology, particularly with A_{2A} AR, A_{2B} AR, or dual A_{2A} AR/ A_{2B} AR antagonists.

II. Receptor Ligands (for Structures, See Figs. 1, 2, 3, and 5)

Adenosine receptors (ARs) have become established drug targets. Adenosine (1, Fig. 1) itself is used as an injectable diagnostic for cardiac imaging to dilate the coronary arteries via A_{2A} AR activation of patients who cannot exercise on a treadmill (Singh and McKintosh, 2020). The short half-life of under 10 seconds prevents severe side effects of concomitant A_1 AR activation, such as cardiac block. Moreover, adenosine is applied in supraventricular tachycardia due to its antiarrhythmic effects (Singh and McKintosh, 2020). The A_{2A} AR-selective agonist regadenoson (2, Table 1), used for the same purpose, displays a longer half-life of 2–3 minutes and is of benefit for patients who develop bronchospasms upon treatment with adenosine (Thomas et al., 2017; Patel and Alzahrani, 2020). The natural products caffeine (3) and theophylline (4), xanthine alkaloids present in plants (e.g., *Coffea arabica* and *Camellia sinensis*), are moderately potent, nonselective AR antagonists (see Table 2 for receptor affinities) that have been used for thousands of years (Daly, 2007; Müller and Jacobson, 2011b; van Dam et al., 2020). There is epidemiologic evidence linking coffee and tea consumption with different health benefits (Grosso et al., 2016; Poole et al., 2017; van Dam et al., 2020). Caffeine, probably the most widely applied psychoactive drug in the world and broadly used for recreational purposes, is therapeutically applied as a central nervous system (CNS) stimulant, for preterm infants to support breathing function, and in combination therapeutics with analgesics to treat pain and colds (Abo-Salem et al., 2004; Lipton et al., 2017; Alhersh et al., 2020; Evans et al., 2020; van Dam et al., 2020). Several ongoing clinical trials (see also Chapter VII) are evaluating caffeine for various indications including cognition, pain, obesity, cataract prevention, and others. Theophylline, which is less brain-permeant than caffeine, is used for the treatment of asthma, but due to its narrow therapeutic window and the availability of safer and more potent alternative therapeutics, it has lost its importance and nowadays serves as a third-line treatment of add-on therapy only (Barnes, 2003; Tilley, 2011; Journey and Bentley, 2020). Both caffeine and theophylline also interact with other targets (e.g., they inhibit phosphodiesterases), but many of these effects are only observed at high, nonphysiologic concentrations. Most of the desired effects of caffeine and theophylline can in fact be explained by a blockade of ARs. It has to be noted that both xanthine derivatives are about equally

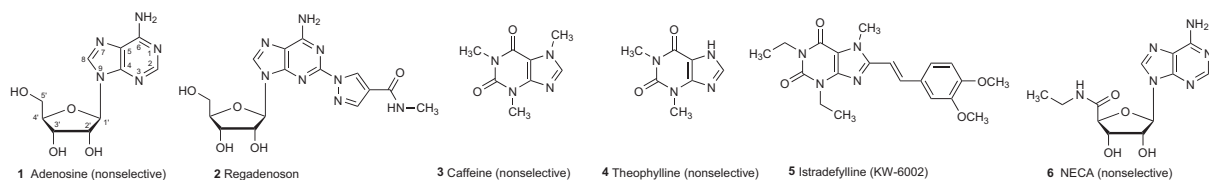
potent at all four human AR subtypes, but they are inactive at rodent A_3 ARs (see Table 2). The A_{2A} AR-selective antagonist istradefylline (5), a xanthine derivative that is structurally derived from caffeine, has been approved for the treatment of Parkinson's disease (PD) in combination with levodopa, initially only in Japan (in 2013) but now also in the United States (in 2019), whereas the approval process in Europe is in progress (Takahashi et al., 2018; Chen and Cunha, 2020; Jenner et al., 2021). Due to intensive research for several decades aimed at developing AR ligands, a large number of subtype-selective agonists and antagonists has been developed (for reviews, see Müller and Jacobson, 2011a; Jacobson and Müller, 2016; Jacobson et al., 2019; Jacobson et al., 2021). The rather modest success in drug approvals despite a large number of clinical trials discouraged scientists and pharmaceutical companies. However, the recent approval of the A_{2A} AR antagonist istradefylline in the United States and, in particular, the 'gold rush fever' in immunology centered around adenosine as an immunosuppressant (Boison and Yegutkin, 2019; Borah et al., 2019; Allard et al., 2020; Willingham et al., 2020; Thompson and Powell, 2021) have newly energized and fueled the field.

This chapter will provide guidance in selecting tool compounds for research on ARs. Rather than presenting a comprehensive collection of AR ligands for which the reader be referred to previous review articles selected (Fredholm et al., 2011; Müller and Jacobson, 2011a; Jacobson and Müller, 2016; Jacobson et al., 2021), preferably well characterized ligands will be discussed that are recommended as tool compounds. Whenever possible, not only data for human ARs but also those for rat and mouse orthologs will be listed since considerable species differences have been observed in some cases, which are most pronounced for the A_3 AR subtype (Alnouri et al., 2015; Du et al., 2018). For most receptor subtypes, at least two different agonists and antagonists will be included. In addition, useful physicochemical and pharmacokinetic data have been collected if available.

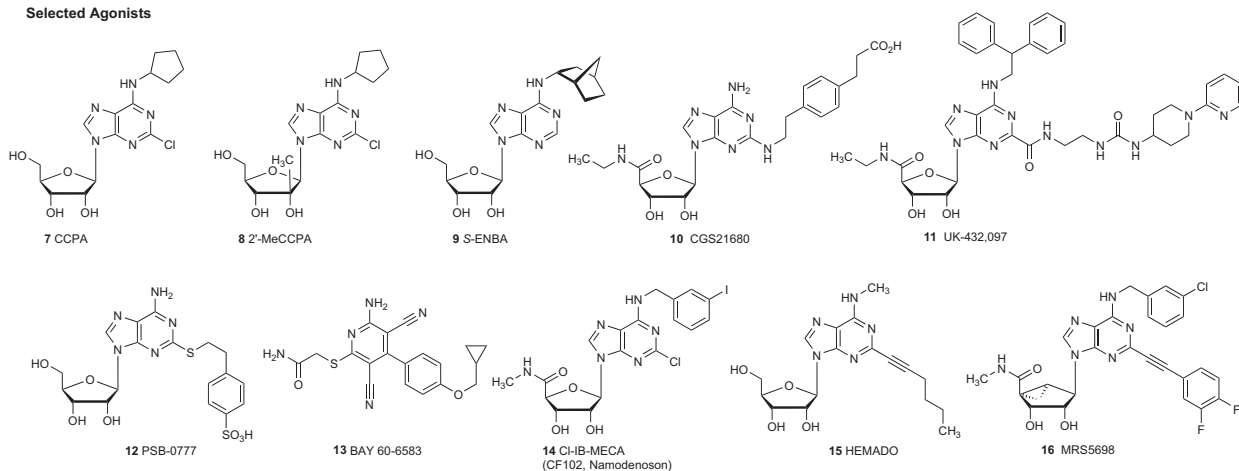
A. Adenosine Receptor Agonists

The physiologic agonist adenosine (1) is more potent at A_1 -, A_{2A} - and A_3 ARs than at A_{2B} ARs in most settings (see Table 1). However, reliable radioligand binding data cannot be obtained since adenosine is present in tissues, cells, and even cell membrane preparations and is constantly produced (e.g., from released ATP by ectonucleotidases) (Zimmermann, 2021). Therefore, it usually has to be removed, which is achieved by preincubation or addition of adenosine deaminase (ADA). Thus, ADA and its reaction product inosine are typically present during incubation with radioligand and test compound. ADA itself can allosterically modulate ARs (Gracia et al., 2013). In

Prototypic ligands and ligands on the market



Selected Agonists



Selected Antagonists

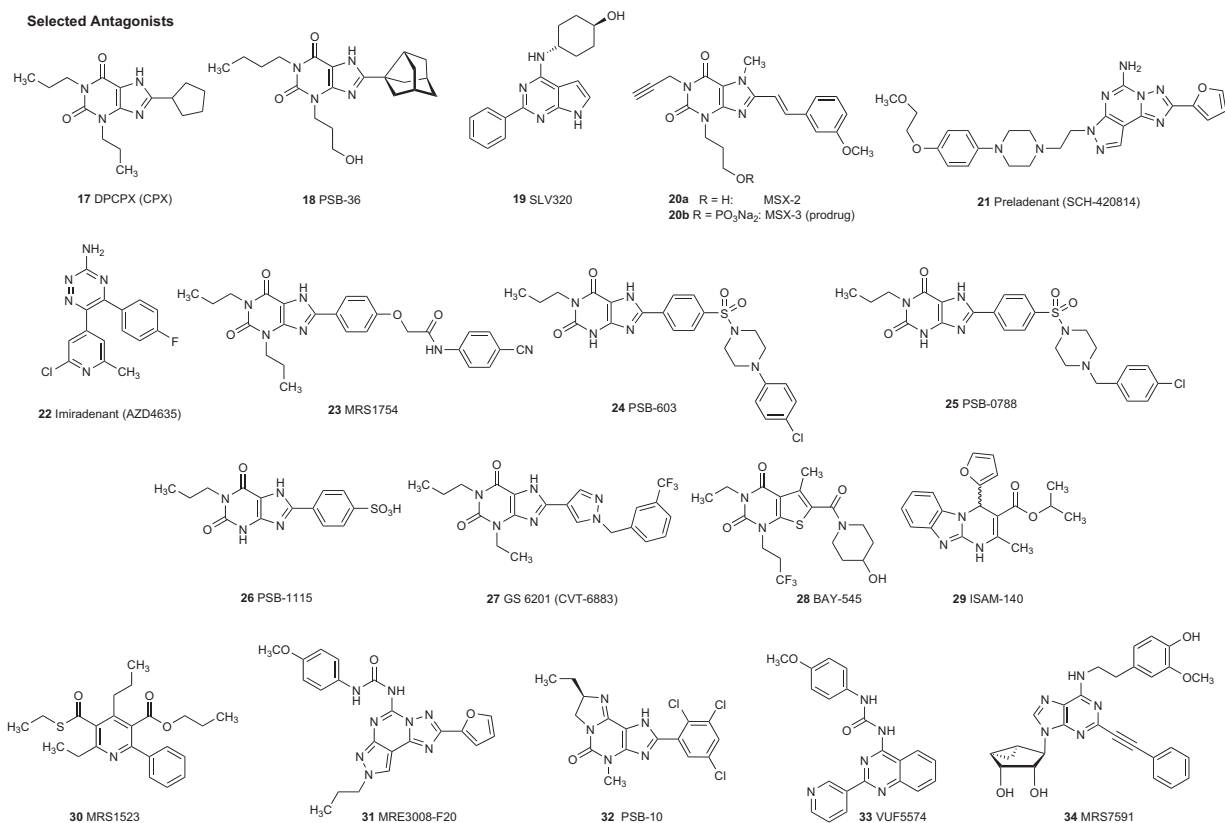


Fig. 1. Selected ligands for studying ARs.

contrast to radioligand binding data, potencies determined in functional, G protein-dependent assays such as cAMP accumulation studies depend on receptor

expression levels and receptor reserve, and concentration-effect curves are shifted to the left with increased receptor expression levels (Fujioka and Omori, 2012).

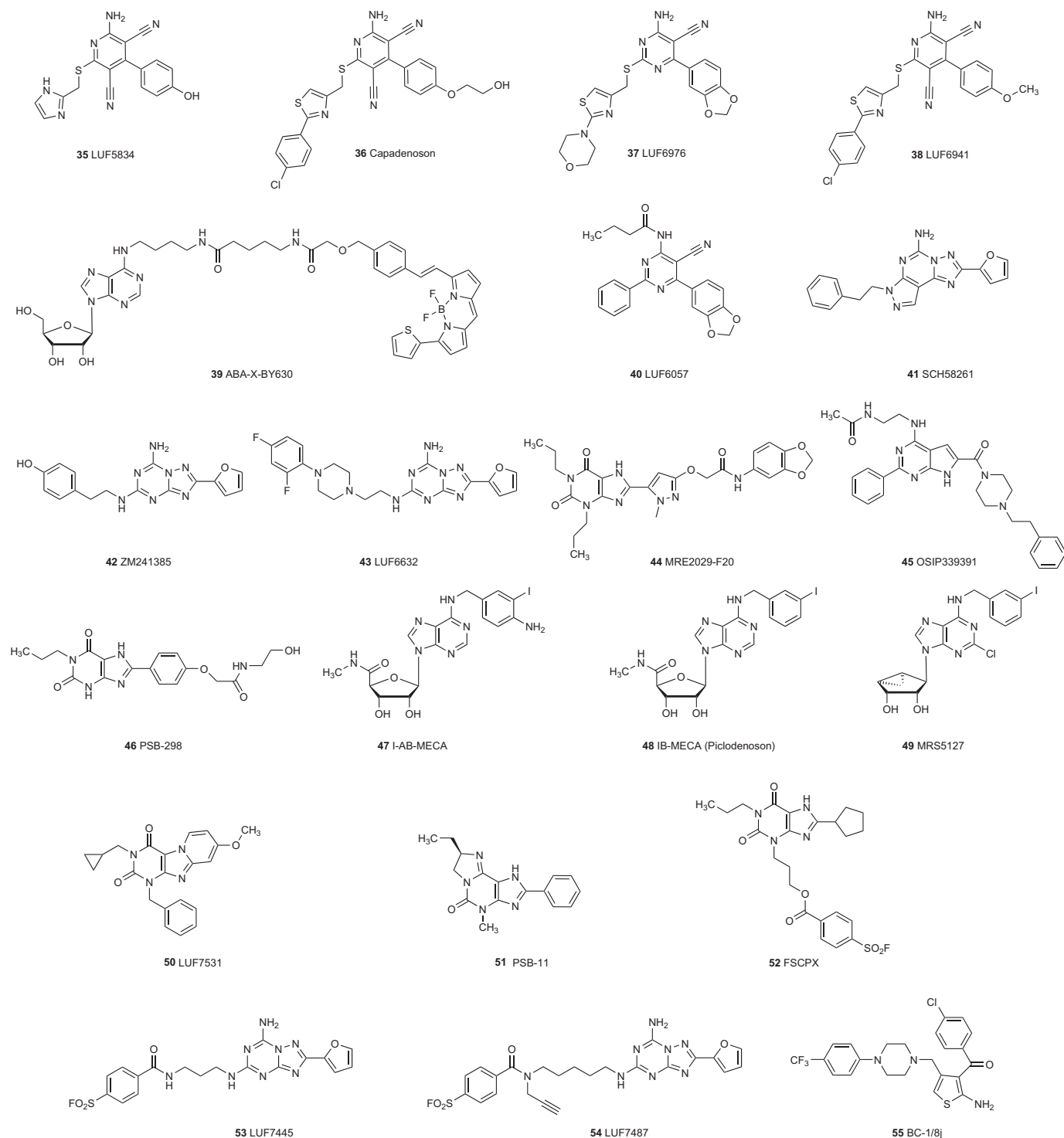
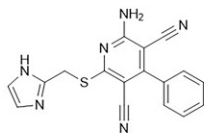


Fig. 2. Ligands investigated in kinetic studies.

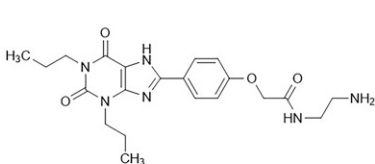
Therefore, EC_{50} values of agonists obtained in different cellular systems are not comparable. As mentioned before, adenosine has a short half-life being metabolized by ADA or adenosine kinase (AdoK) after removal by cellular uptake through nucleoside transporters, which can additionally influence results. For that reason, metabolically (more) stable adenosine analogs have been

developed. Nevertheless, it becomes increasingly clear that synthetic ligands do not necessarily induce the same effects at a certain receptor as the cognate agonist (e.g., regarding the activation of intracellular signaling pathways) (see also Chapter V). Therefore, if possible, adenosine should always be included in pharmacological studies besides more stable and selective synthetic

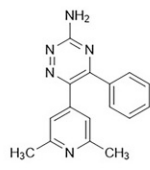
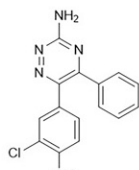
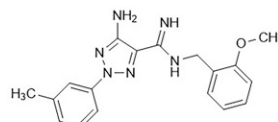
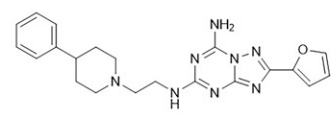
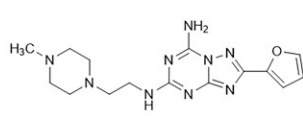
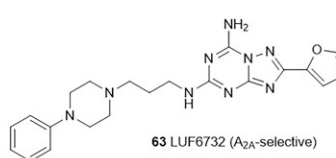
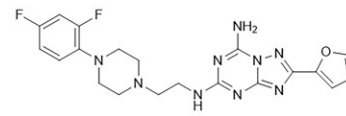
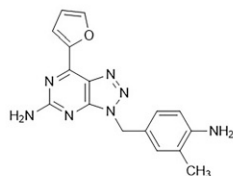
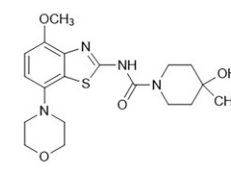
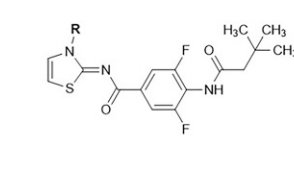
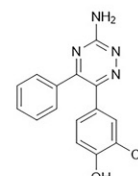
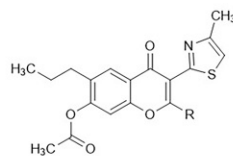
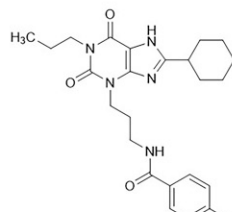
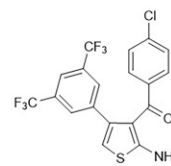
Agonists in receptor structures

56 LUF5833 (partial A_{2A} AR agonist)

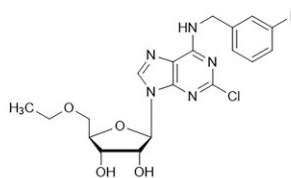
Antagonists or PAM in receptor structures



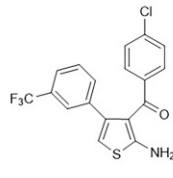
57 XAC

58 T4G (A_{2A} -selective)59 T4E/4e (A_{2A} -selective)60 8D1 (A_{2A} -selective)61 LUF6805 (A_{2A} -selective)62 LUF6806 (A_{2A} -selective)63 LUF6732 (A_{2A} -selective)64 LUF6632 (A_{2A} -selective)65 Vipadenant (A_{2A} -selective)66 Tozadenant (SYN-115, A_{2A} -selective)67 R = H: A_{2A} -selective antagonist released from phosphate prodrug
68 R = $-\text{CH}_2\text{OPO}_3\text{H}_2$: LUAA47070 (water-soluble prodrug of 67)69 compound 4e (A_{2A} -selective)70 R = H: 4d (high-affinity A_{2A} AR antagonist)71 R = CH_3 : 5d (low-affinity A_{2A} AR antagonist)72 DU172 (moderately A_1 -selective)73 MIPS521 (A_1 -PAM)

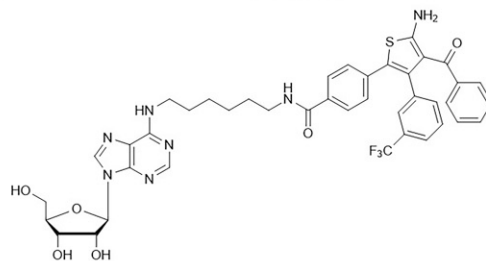
Ligands associated with biased signaling



74 LUF5589



75 VCP520



76 VCP746

Fig. 3. Ligands in 3D receptor structures and ligands in biased signaling studies. (Note: In 67 and 68, the X-ray structure of “LUAA47070” was not obtained with the prodrug LUAA47070 but with the A_{2A} AR antagonist that is released from the prodrug upon hydrolysis.)

agonists. The closely related adenosine analog 5'-N-ethylcarboxamidoadenosine (NECA, 6) cannot be metabolized by ADA or AdoK. Similar to adenosine, NECA is significantly more potent at A_1 -, A_{2A} -, and A_3 ARs than at A_{2B} ARs. There is a lack of potent, selective, and fully

efficacious A_{2B} AR agonists; NECA is still one of the more potent full agonists at the A_{2B} AR and represents a useful tool to study A_{2B} ARs in combination with selective antagonists for the other AR subtypes (Verzijl and IJzerman, 2011; Müller et al., 2018; Franco et al., 2021b).

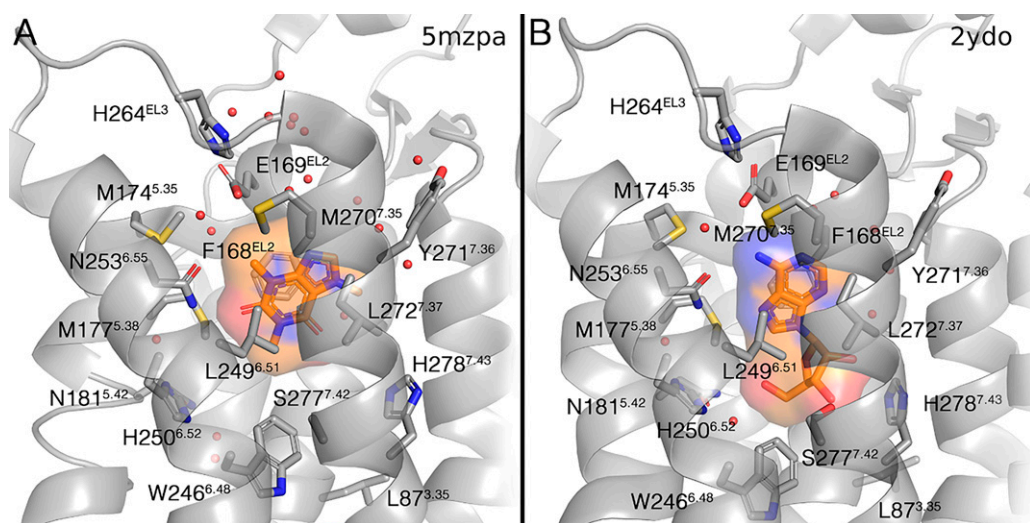


Fig. 4. Overview of the $A_{2A}AR$ binding site, showing the first frame of two movies: (A) antagonist (Supplemental Video 1) and (B) agonist (Supplemental Video 2). All residues within 2Å of a given ligand in an $A_{2A}AR$ structure were considered as the binding pocket, and this selection was maintained in all frames. The residues are labeled according to the wild-type (WT) sequence, and modifications made to the receptor (e.g., the thermostabilizing mutant S277^{7.42}A) are not taken into account; note that no labeling is used in the supplemental movie files. The Ballesteros-Weinstein numbering is given in superscript. Ligands are shown in orange, with a volumetric occupancy surface-colored on the atom type. Water atoms in the binding site are shown as red dots, and the sodium ion (when present) as a purple sphere. If alternate coordinates were given in the extracted PDB file, the 'A' coordinates were maintained, except in the case of caffeine, in which case we generated two separate frames (referred to as 5mzpa and 5mzpb) to show the two binding modes in the crystal structure. Only distinctly different binding modes of ZM241385 (as present in 4E1Y and 3PWH) are included in the movie.

Potent, truly selective A_1AR agonists have been developed by N⁶-substitution of adenosine (see Table 1 and Fig. 2). 2-chloro-N⁶-cyclopentyladenosine (CCPA, 7) is suitable for rat and mouse studies, where it shows > 100-fold selectivity versus the other AR subtypes, whereas it is less selective in humans versus the A_3AR subtype (46-fold). For studies at the human A_1AR , its 2'-methyl-substituted derivative 2'-MeCCPA (8) can be used, which is more selective (>300-fold) in humans (Franchetti et al., 2009). Data at rat and mouse ARs are not available for this compound. Another potent and selective A_1AR agonist is (S)-ENBA (9), possessing a bulky bicyclo[2.2.1]hept-2-yl moiety at the N⁶-position that confers A_1AR selectivity.

$A_{2A}AR$ -selective agonists have been obtained by introducing large, bulky substituents into the 2-position of adenosine or NECA, in some cases in combination with an additional bulky N⁶-substituent. Most of the developed compounds are only moderately selective in humans versus the A_1 - or A_3AR subtypes. CGS21680 (10) is a potent and $A_{2A}AR$ -selective agonist in rat and mouse but shows only moderate selectivity in humans (vs. A_1 - and A_3AR s; see Table 1). However, in some studies on mouse brain, 10 has been observed to additionally bind to A_1AR s (Lopes et al., 2004). The reason for this observation is still unclear; one explanation could be the formation of heteromeric receptor complexes showing a different pharmacology. The 2,N⁶-disubstituted NECA derivative 11 (UK-432,097; Table 1) is potent at the human $A_{2A}AR$ and was reported to also be selective. Compound 11 is a relatively large and lipophilic molecule that is less water-soluble than other adenosine

derivatives and analogs. It showed a long receptor residence time of 250 minutes at 5°C (see Table 3), which probably contributed to its successful cocrystallization with the human $A_{2A}AR$ (Xu et al., 2011). PSB-0777 (12), bearing a phenylsulfonate group, is well soluble in water and has been useful for injection or for local application in the gut since it is not perorally absorbed due to its negative charge. It shows high selectivity in rats but not in humans and is thus useful for studies in rodents. Regadenoson (2) is only moderately potent but selective in humans and is clinically used as a diagnostic (see above). Importantly, in tissues with higher A_1AR versus $A_{2A}AR$ density such as the brain, (moderately selective) $A_{2A}AR$ agonists often bind to and activate A_1AR rather than $A_{2A}AR$ (Zhang et al., 1994; Cunha et al., 1996; Halldner et al., 2004; Pliássova et al., 2020). Thus, potent and really selective $A_{2A}AR$ agonists to target central $A_{2A}AR$ s are still required.

So far, potent and selective full agonists for the $A_{2B}AR$ are not available. BAY 60-6583 (13), a non-nucleoside aminopyridine derivative, behaves as a partial $A_{2B}AR$ agonist (Hinz et al., 2014) but was shown to act as an antagonist at other AR subtypes (Alnouri et al., 2015). In the presence of high adenosine concentrations, it can even inhibit $A_{2B}AR$ activation (Hinz et al., 2014; Alnouri et al., 2015). Data obtained with 13 are therefore difficult to interpret. BAY 60-6583 may induce a different $A_{2B}AR$ conformation than adenosine or NECA; for example, it has been shown that BAY 60-6583 does not induce calcium mobilization via $A_{2B}AR$ -mediated G_q protein activation in human embryonic kidney (HEK) cells with low endogenous $A_{2B}AR$ expression in contrast to adenosine or NECA

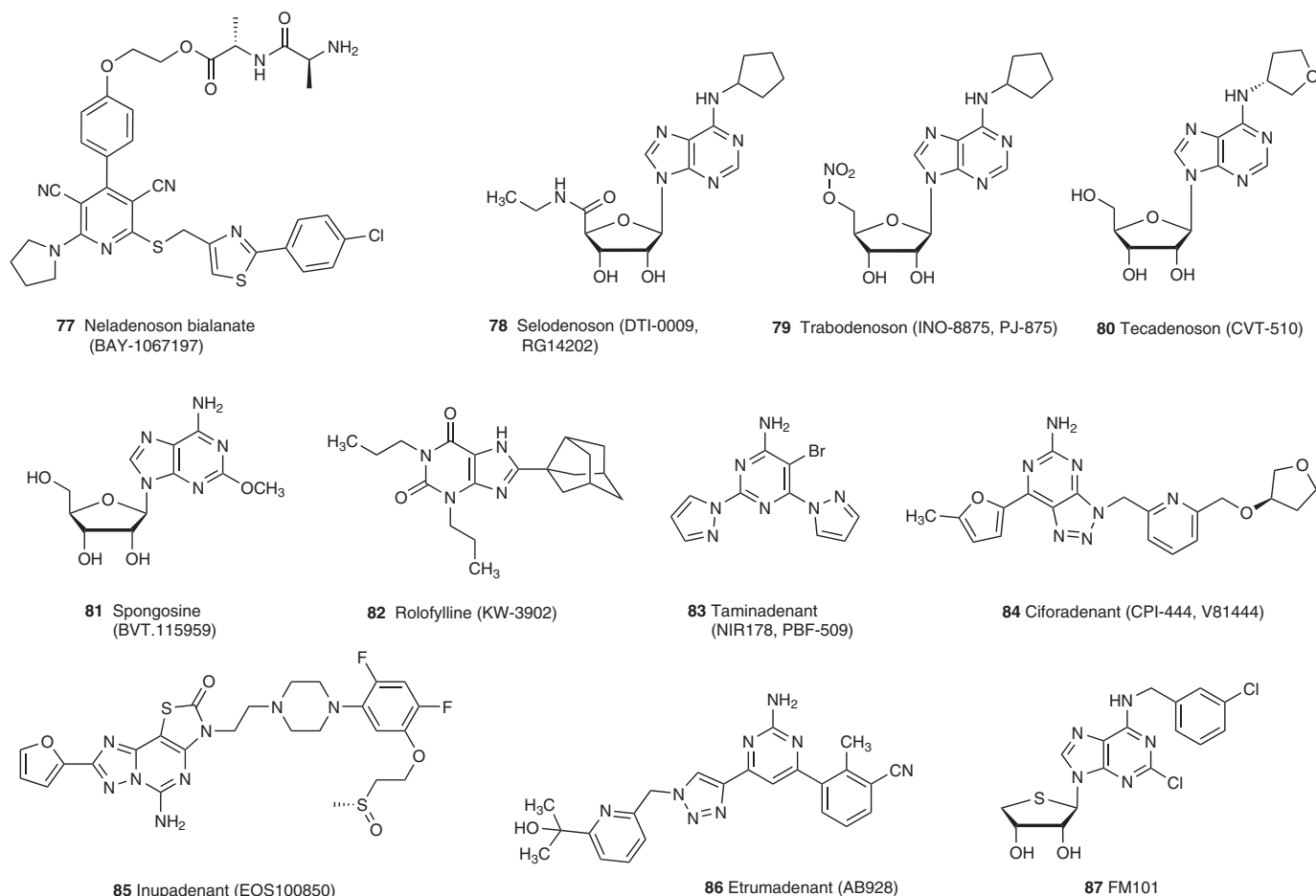


Fig. 5. Ligands in clinical studies.

(Hinz et al., 2014; Gao et al., 2018b). Thus, a potent, selective, efficacious, and unbiased A_{2B} AR agonist is urgently needed. Instead of the partial agonist BAY 60-6583, the full, nonselective agonist NECA (6) may be used in the presence of antagonists for the other AR subtypes.

For the A_3 AR, potent and selective agonists are available. Cl-IB-MECA (14, CF102, namodenoson), a 2-chloro- N^6 -iodobenzyl-substituted methylcarboxamidoadenosine (MECA) derivative, is being evaluated in clinical trials for the treatment of hepatocellular carcinoma and nonalcoholic steatohepatitis (NASH). For pharmacological studies, especially in mice, the doses of 14 have to be carefully chosen in order not to activate the A_1 AR as well (see Table 1). HEMADO (15) is similarly potent and selective in humans. A potent and at the same time selective A_3 AR agonist, in human as well as in mouse, is MRS5698 (16).

B. Adenosine Receptor Antagonists

Many potent A_1 AR-selective antagonists have been developed based on caffeine and theophylline as lead structures, such as DPCPX (17, CPX) and PSB-36 (18) (Müller and Jacobson, 2011b). Whereas DPCPX shows only moderate selectivity in humans, PSB-36 is highly selective in all three species: human, rat, and mouse

(Alnouri et al., 2015). SLV320 (19) is an A_1 AR antagonist with a 7-deaza-adenine core structure bearing a cyclohexyl moiety at the exocyclic amino function (Kalk et al., 2007). The compound is potent and selective in humans and displays similar potency in rat, but complete data in rat and mouse are not available.

The xanthine derivative istradefylline (5) was the first A_{2A} AR antagonist to be approved as a drug (Shimada et al., 1992; Takahashi et al., 2018). Its potency and selectivity for the A_{2A} AR is similar in human, rat, and mouse. Although it is highly selective versus the A_{2B} - and A_3 AR subtypes, selectivity versus the A_1 AR is somewhat lower (50- to 70-fold) (see Table 2). Like many other A_{2A} AR antagonists, it is moderately water-soluble. In addition, the double bond of its styryl residue can undergo light-induced *E/Z*-isomerization in dilute solution and is prone to light-induced dimerization in the solid state; therefore, it needs to be protected from light (Nonaka et al., 1993; Hockemeyer et al., 2004). The same is true for MSX-3 (20), a phosphate prodrug of MSX-2, which is, however, well soluble in water (Sauer et al., 2000; Faivre et al., 2018). The A_{2A} AR selectivity of MSX-2 is higher than that of istradefylline (see Table 2). The nonxanthine A_{2A} AR

TABLE 1
Affinities of selected adenosine receptor agonists

		K_i or EC_{50} (nM) ^a			
		A_1	A_{2A}	A_{2B}^b	A_3
<i>Nonselective Agonists</i>					
1	Adenosine ^c	ca. 100 (h) 73 (r)	310 (h) 150 (r)	15,000 (h) 5,100 (r)	290 (h) 6,500 (r)
6	NECA	14 (h) 5.1 (r) 2.49 (m)	20 (h) 9.7 (r) 43.4 (m)	1,890 (h) 1,110 (r) 656 (m)	25 (h) 113 (r) 13.2 (m)
<i>A₁AR-Selective Agonists</i>					
7	CCPA	0.83 (h) 1.3 (r) 0.269 (m)	2270 (h) 950 (r) 988 (m)	18,800 (h) 6,160 (r) 25,300 (m)	38 (h) 237 (r) 15.6 (m)
8	2'-MeCCPA	3.3 (h)	9,580 (h)	37,600 (h)	1.150 (h)
9	(S)-ENBA	n.d. (h) 0.34 (r)	n.d. (h) 477 (r)	n.d.	282 (h) 915 (r)
<i>A_{2A}AR-Selective Agonists</i>					
10	CGS21680	289 (h) 1800 (r) 961 (m)	27 (h) 19 (r) 13.7 (m)	>10,000 (h) >10,000 (r) >10,000 (m)	67 (h) 584 (r) 93.0 (m)
11	UK-432,097	n.d.	4	n.d.	n.d.
12	PSB-0777	541 (h) ≥10,000 (r) >10,000 (h)	360 (h) 44.4 (r) 290 (h)	>10,000 (h) >10,000 (h)	>10,000 (h) >10,000 (h)
2	Regadenoson				
<i>A_{2B}AR-Selective (Partial) Agonist</i>					
13	BAY 60-6583	387 (h) 514 (r) 351 (m)	>10,000 (h) >10,000 (r) >10,000 (m)	3–10 (h, EC_{50}) 114 (h) 100 (r) 136 (m)	223 (h) 2,750 (r) 3,920 (m)
<i>A₃AR-Selective Agonists</i>					
14	Cl-IB-MECA (CF102, Namodenoson)	220 (h) 280 (r) 35 (m)	5360 (h) 470 (r) 290 (m)	>10,000 (h) 1,210 (r) 44,300 (m)	1.4 (h) 0.33 (r) 0.18 (m)
15	HEMADO	330 (h)	1200 (h)	>30,000 (h)	1.10 (h)
16	MRS5698	>10,000 (h) >10,000 (m)	>10,000 (h) >10,000 (m)	assumed to be inactive	3.49 (h) 3.08 (m)

h, human; K_i , inhibition constant; m, mouse; n.d., no data; r, rat.

^adata (if available from K_i values from radioligand binding assays) are taken from the literature cited in the text.

^bmost A_{2B} AR data are from functional studies (cAMP accumulation).

^cadenosine data are from functional studies (cAMP accumulation).

antagonist preladenant (21, SCH-420814) is one of the most potent and selective A_{2A} AR antagonists. It has been evaluated in clinical trials for PD and was found to be well tolerated but did not show significant beneficial effects (Stocchi et al., 2017; LeWitt et al., 2020). As observed with istradefylline, the study design is most critical for these types of clinical PD studies and may have contributed to the negative outcome in the case of preladenant (Hauser et al., 2015). AZD4635 (22, imaradenant) is a potent A_{2A} AR antagonist with moderate selectivity versus the A_{2B} - and A_3 ARs subtypes. Despite its relatively low molecular weight (315.7 g/mol) the compound is not readily soluble in water.

In recent years many A_{2B} AR-selective antagonists have been developed (Müller et al., 2018). The xanthine derivative MRS1754 (23) is a potent and selective A_{2B} AR antagonist in humans but not in rats and mice, where it additionally blocks the A_1 AR (Kim et al., 2000; Alnouri et al., 2015). One of the most potent and selective A_{2B} AR antagonists in all three species is the 8-sulfophenylxanthine derivative PSB-603 (24) (Borrmann et al., 2009; Alnouri et al., 2015). The

compound is metabolically highly stable in human, rat, and mouse. Its main drawback, however, is its low water solubility. The related A_{2B} AR antagonist PSB-0788 (25) (Borrmann et al., 2009; Alnouri et al., 2015) is better soluble, especially under weakly acidic conditions since it bears a basic nitrogen atom that can be protonated. However, it is less metabolically stable and therefore less suitable for in vivo studies. PSB-0788 is moderately selective for A_{2B} - versus A_1 ARs in mouse (only about 60-fold) but highly A_{2B} AR-selective in human and rat. PSB-1115 (26) was developed as an A_{2B} AR antagonist with high water solubility due to its sulfonate group (Hayallah et al., 2002). Although the compound is potent and selective in human, it is not selective in rat and mouse and additionally blocks rodent A_1 ARs (see Table 2) (Alnouri et al., 2015). The xanthine derivative GS6201 (27, CVT6883) which shows good potency and selectivity for human A_{2B} ARs (Elzein et al., 2008), was evaluated in a phase 1 clinical trial for pulmonary diseases, but further development has not been reported (Kalla and Zablocki, 2009). The compound displayed a half-life of 4 hours and a peroral

TABLE 2
Affinities of selected, useful adenosine receptor antagonists

		K_i (nM) ^a			
		A ₁	A _{2A}	A _{2B}	A ₃
<i>Nonselective Antagonists</i>					
3	Caffeine	44,900 (h) 41,000 (r) 50,700 (m)	23,400 (h) 43,000 (r) 11,100 (m)	33,800 (h) 30,000 (r) 23,000 (m)	13,300 (h) >100,000 (r) >100,000 (m)
4	Theophylline	6,770 (h) 14,000 (r)	6,700 (h) 22,000 (r)	9,070 (h) 15,100 (r) 5,630 (m)	22,300 (h) >100,000 (r)
<i>A₁AR-Selective Antagonists</i>					
17	DPCPX (CPX)	3.0 (h) 0.50 (r) 0.413 (m)	129 (h) 157 (r) 263 (m)	51 (h) 186 (r) 86.2 (m)	243 (h) >10,000 (r) >10,000 (m)
18	PSB-36	0.7 (h) 0.124 (r) 1.58 (m)	980 (h) 552 (r) 697 (m)	187 (h) 350 (r) 704 (m)	2,300 (h) 6,500 (r) >10,000 (m)
19	SLV320	1.00 (h) 2.51 (r)	398 (h)	3,981 (h) 501 (r)	200 (h)
<i>A_{2A}AR-Selective Antagonists</i>					
5	Istradefylline (KW6002)	841 (h) 230 (r) 438 (m)	12 (h) 4.46 (r) 6.83 (m)	>10,000 (h) 5,940 (r) 3,590 (m)	4,470 (h) >10,000 (r) >10,000 (m)
20	MSX-3 / MSX-2 (Data are for MSX-2)	2,500 (h) 900 (r)	5.38 (h) 8.04 (r)	>10,000 (h)	>10,000 (h)
21	Preladenant (SCH-420814)	>1,000 (h) >1,000 (h) 462 (m)	0.9 (h) 0.986 (r) 0.241 (m)	>1,000 (h) >1,000 (m) >1,000 (r)	>1,000 (h) >1,000 (m) >1,000 (r)
22	Imaradenant (AZD4635)	160 (h)	1.7 (h)	64 (h)	>10,000 (h)
<i>A_{2B}AR-Selective Antagonists</i>					
23	MRS1754	403 (h) 16.8 (r) 1.45 (m)	503 (h) 612 (r) >10,000 (m)	1.97 (h) 12.8 (r) 3.12 (m)	570 (h) >1,000 (m) >1,000 (r)
24	PSB-603	>10,000 (h) >10,000 (r) 42.4 (m)	>10,000 (h) >10,000 (r) >10,000 (m)	0.553 (h) 0.355 (r) 0.265 (m)	>10,000 (h) >10,000 (r) >10,000 (m)
25	PSB-0788	2,240 (h) 386 (r) 118 (m)	333 (h) 1,730 (r) 235 (m)	0.393 (h) 2.12 (r) 1.90 (m)	>1,000 (h) >10,000 (r) >10,000 (m)
26	PSB-1115	>10,000 (h) 2,200 (r) 591 (m)	3790 (h) 24,000 (r) >10,000 (m)	53.4 (h) 3,140 (r) 1,940 (m)	>10,000 (h) >10,000 (r) >10,000 (m)
27	GS 6201 (CVT-6883)	1,940 (h)	3,280 (h)	22 (h)	1,070 (h)
28	BAY-545	>1,000; 1,300 (h) n.d. (r) >6,700 (m)	>1,000; 820 (h) 750 (r) 470 (m)	59–97 (h) 280 (r) 400 (m)	>10,000 (h) n.d. (r) >6,700 (m)
29	ISAM-140	>1,000 (h)	>1,000 (h)	3.49 (h)	>1,000 (h)
<i>A₃AR-Selective Antagonists</i>					
30	MRS1523	>10,000 (h) 15,600 (r) >10,000 (m)	3660 (h) 2050 (r) >10,000 (m)	>10,000 (h) >10,000 (r) >10,000 (m)	18.9 (h) 113 (r) 731 (m)
31	MRE3008-F20	1200 (h)	141 (h)	2100 (h)	0.82 (h)
32	PSB-10	1,700 (h) 805 (r)	2,700 (h) 6,040 (r)	30,000 (h)	0.441 (h) 17,000 (r)
33	VUF5574	≥10,000 (r)	≥10,000 (r)	n.d.	4.03 (h)
34	MRS7591 ^b	>10,000 (h) 590 (m)	>10,000 (h) n.d.	n.d. n.d.	10.9 (h) 17.8 (m)

h, human; K_i, inhibition constant; m, mouse; n.d., no data; r, rat.

^adata are taken from the literature cited in the text.

^bpartial agonistic activity if receptor is highly expressed.

bioavailability of 35% in rat (Elzein et al., 2008); potency and selectivity in rodents have not been reported. BAY-545 (28) is a recently published A_{2B}AR antagonist with a new scaffold identified by high-throughput screening, although its thienopyrimidinedione structure resembles the xanthine scaffold (Härter

et al., 2019). The compound shows moderate affinity compared with other developed A_{2B}AR antagonists and is more potent at human than at rat and mouse A_{2B}ARs. It is more than 10-fold selective in human but is nonselective in mouse and rat (Härter et al., 2019). Another novel scaffold, a pyrimido[1,2-*a*]benzimidazole,

is represented by ISAM-140 (29), an A_{2B} AR antagonist that shows high potency and selectivity in human. Unfortunately, data from other species are not available (El Maatougui et al., 2016). Subsequently, related dihydropyrimidine derivatives have been developed that are similarly potent and selective (Majellaro et al., 2021).

The A_3 AR typically shows large species differences for antagonists (Müller, 2003; Jacobson and Müller, 2016). Most published antagonists that are very potent at human A_3 ARs are inactive at the rodent (rat and mouse) orthologs. One of the best A_3 AR antagonists for rodent studies is MRS1523 (30). The compound is only moderately potent but very selective in human (>100-fold) and at least somewhat selective in rat (18-fold vs. A_{2A} , >100-fold vs. the other AR subtypes) and mouse (at least 14-fold vs. the other subtypes) (van Rhee et al., 1996; Li et al., 1998; Müller and Jacobson, 2011a; Alnouri et al., 2015; Jacobson and Müller, 2016). Further potent A_3 AR antagonists, including MRE3008-F20 (31) (Baraldi et al., 2012; Borea et al., 2015), PSB-10 (32) (Ozola et al., 2003; Alnouri et al., 2015), and VUF5574 (33) (van Muijlwijk-Koezen et al., 2000) are highly potent and selective in human but virtually inactive at rodent A_3 ARs (see Table 2). As species differences are more pronounced for A_3 AR antagonists than for agonists, most of which are derivatives or analogs of adenosine, compounds with a truncated, furanyl, or carbocyclic moiety in place of the ribose ring of adenosine were investigated and optimized (Jeong et al., 2007; Lee et al., 2010; Nayak et al., 2014; An et al., 2020). Such adenosine analogs show reduced intrinsic activity or even block the receptors. Appropriate substitution on the adenine ring led to MRS7591 (34) showing high affinity for both human and mouse A_3 ARs and good selectivity in human (>1000-fold) (Tosh et al., 2020). Selectivity in mouse has only been assessed against the A_1 AR (33-fold). It has to be kept in mind that compound 50 behaved as a (weakly efficacious) partial agonist (Tosh et al., 2020).

C. Allosteric Modulators of Adenosine Receptors

The development of allosteric modulators for GPCRs in general is an emerging field of research (Müller et al., 2012; Gao and Jacobson, 2013; Wootten et al., 2013). Positive allosteric modulators (PAMs) increasing the potency or efficacy of agonists, and negative allosteric modulators (NAMs) acting as noncompetitive antagonists, have been reported for various AR subtypes, especially for the A_1 AR. The AR-PAMs that have been developed so far display only moderate potency or selectivity, and their usefulness is still unclear (Fredholm et al., 2011; Göblyös and IJzerman, 2011; Jacobson et al., 2011; Müller et al., 2012; Nguyen et al., 2016; Barresi et al., 2021). Interestingly, in a recent cryo-EM structure of the A_1 AR, a PAM (MIPS521) was found to

be localized in an extrahelical domain (Draper-Joyce et al., 2021). MIPS521's analgesic properties were evaluated in the same paper, reminiscent of earlier attempts to profile another PAM as a potential painkiller (Kiesman et al., 2009).

D. Inosine and Guanosine

Adenosine is metabolized to inosine by adenosine deaminases (ADA-1 and -2). Inosine has been reported by several groups to interact with ARs (e.g., with A_{2A} AR and A_3 AR) but only at very high, nonphysiologic concentrations (>100 μ M) (Welihinda et al., 2016). On the other hand, inosine (Lovász et al., 2021) as well as the nucleoside guanosine (Di Liberto et al., 2016) clearly show pharmacological effects, at least some of which seemed to be exerted by interaction with GPCRs. However, it is unlikely that these effects are mediated by direct activation of ARs. As an example, the hypothermic effects of inosine disappear completely in mice lacking either all four ARs or the A_3 AR (Xiao et al., 2019). Alternatively, they may be due to inhibition of adenosine uptake through the equilibrative nucleoside transporter 1 (ENT1). Indirect effects are also conceivable (e.g., through allosteric modulation). Further research on inosine and guanosine as extracellular signaling molecules in their own right is warranted.

III. Receptor Binding Kinetics

It has been recognized in recent years that the study of target binding kinetics is crucial to reduce attrition rates in drug discovery (Copeland, 2016). Over the decades medicinal chemists have successfully synthesized lead compounds displaying high, often (sub)nanomolar affinity for a given target, including ARs. However, kinetic aspects of the ligand-receptor interaction have been studied in lesser detail. Although these can be very informative, the extra effort to obtain values for association (k_{on}) and dissociation (k_{off}) rate constants was and is substantial. This is because kinetic assays tend to be laborious although more efficient approaches (Guo et al., 2013) and methods are being developed, including scintillation proximity assays (Xia et al., 2016) and bioluminescence resonance energy transfer (BRET)-based ligand binding studies (Bouzo-Lorenzo et al., 2019; White et al., 2019). On the other hand, systematically evaluating the binding kinetics of a series of lead compounds that are otherwise chemically or biologically similar provides additional parameters for triage and advancement of molecules in the drug discovery process (Guo et al., 2016a; 2017). For instance, assessment of the lifetime of a ligand-receptor complex, coined residence time ($RT = 1/k_{off}$) (Copeland et al., 2006), has been shown predictive for drug efficacy and selectivity, including on ARs (Swinney,

2006a,b; Guo et al., 2014a; Zhang, 2015; Tonge, 2018). Drugs with long target RT are likely to produce a longer duration of action by more gradually reducing the decline of target occupancy than those with short RT (Dahl and Akerud, 2013; de Witte et al., 2016). Furthermore, a direct correlation between receptor RT and functional efficacy has been observed in some cases (Sykes et al., 2009; Guo et al., 2012). A thorough review of the kinetic characteristics of AR ligands, both orthosteric ligands and allosteric modulators, has recently appeared (Guo et al., 2017); hence, we will only provide a concise summary and update here.

A. Orthosteric Ligands and Adenosine Receptor Binding Kinetics

In Table 3, kinetic data [association and dissociation rate constants, kinetic equilibrium dissociation constants (K_D), and residence times] for (orthosteric) agonists and antagonists of the human adenosine receptors (hARs) are summarized. Their chemical structures, if not listed in Fig. 1, are assembled in Fig. 2. Most experiments were radioligand binding assays performed on membrane preparations, whereas lower than physiologic temperatures were employed in most cases due to practical limitations of the (radio)labeled probe used, such as a (too) fast dissociation at higher temperatures.

There have been a few attempts to use surface plasmon resonance instrumentation for kinetic assays on hA_{2A}AR, but these have not become routinely available since solubilized and purified receptor material is needed (Bocquet et al., 2015; Errey et al., 2015).

It is often thought that association rate constants for bimolecular encounters readily reach high values that are diffusion-limited only ($10^{10} \sim 10^{11} \text{ M}^{-1} \cdot \text{min}^{-1}$) (Smoluchowski, 1918; Alberty and Hammes, 1958). However, this is only true for reactant molecules that have isotropic reactivity, whereas the interaction between ligand and receptor, including ARs, is of a more constrained nature (e.g., due to the stereospecificity of recognition). This is obvious from Table 3, in which association rate constants vary from 5.0×10^5 (11, UK432,097) to 6.4×10^8 (41, SCH58261) $\text{M}^{-1} \cdot \text{min}^{-1}$, an over 1000-fold difference but still far from diffusion control. The latter compound is another selective A_{2A}AR antagonist that has been extensively characterized in rodents. Association rate constants appear correlated with the onset of clinical action, in vivo target occupancy, and target rebinding (Vauquelin, 2018). However, this has not been demonstrated for AR ligands yet.

The dissociation rate constants or, for convenience, residence times also vary significantly, up to >5000-fold. There are ligands with ultra-short residence

TABLE 3
Association and dissociation rate constants of selected AR ligands

Compound	Target (h, human; r, rat)	Temp (°C)	k_{on} ($\text{M}^{-1} \cdot \text{min}^{-1}$)	k_{off} (min^{-1})	RT (min)	Kinetic K_D (nM) ^a	Reference	
7	CCPA	hA ₁ AR	25	9.6×10^6	1.2	0.9	131	(Guo et al., 2014b)
6	NECA	hA ₁ AR	25	9.0×10^5	0.47	2.1	522	(Guo et al., 2014b)
35	LUF5834	hA ₁ AR	25	2.0×10^8	0.92	1.1	4.6	(Guo et al., 2014b)
36	Capadenoson	hA ₁ AR	25	2.4×10^7	0.036	28	1.5	(Louvel et al., 2015)
37	LUF6976	hA ₁ AR	25	3.9×10^8	0.87	1.1	2.2	(Louvel et al., 2014)
38	LUF6941	hA ₁ AR	25	2.6×10^6	0.0076	132	2.9	(Louvel et al., 2015)
39	ABA-X-BY630	hA ₁ AR ^b	37	2.6×10^7	2.0	0.5	77	(May et al., 2010)
17	DPCPX	hA ₁ AR	25	1.4×10^8	0.21	4.8	1.5	(Guo et al., 2013)
17	DPCPX	rA ₁ R	25	9.6×10^7	0.045	22.2	0.50	(Guo et al., 2017)
10	CGS21680	hA _{2A} AR	25	5.0×10^4	0.02	50.0	380	(Guo et al., 2017)
10	CGS21680	rA _{2A} AR	23	2.1×10^7	0.033	30.3	1.6	(Guo et al., 2017)
40	LUF6057	hA ₁ AR	25	4.8×10^8	3.0	0.3	6.3	(Guo et al., 2013)
6	NECA	hA _{2A} AR	4	1.9×10^6	0.053	19	28	(Guo et al., 2015)
11	UK432,097	hA _{2A} AR	5	5.0×10^5	0.004	250	8.0	(Guo et al., 2012)
41	SCH58261	hA _{2A} AR	25	6.4×10^8	1.5	0.67	2.3	(Dionisotti et al., 1997)
42	ZM241385	hA _{2A} AR	4	1.3×10^8	0.014	71	0.11	(Guo et al., 2014c)
43	LUF6632	hA _{2A} AR	4	3.4×10^7	0.0031	323	0.091	(Guo et al., 2014c)
20a	MSX-2	rA _{2A} AR (brain striatal membranes)	23	14.5×10^7	0.2839	3.52	1.95	(Müller et al., 2000)
6	NECA	hA _{2B} AR	4	n.d.	2.201	0.45	n.d.	(Hinz et al., 2018)
23	MRS1754	hA _{2B} AR	25	2.2×10^7	0.027	37	1.2	(Ji et al., 2001)
44	MRE2029-F20	hA _{2B} AR	4	1.7×10^7	0.031	32	1.8	(Baraldi et al., 2004)
45	OSIP339391	hA _{2B} AR	22	9.5×10^7	0.039	26	0.41	(Stewart et al., 2004)
24	PSB-603	hA _{2B} AR	21	11.4×10^7	0.02279	44	0.652	(Borrmann et al., 2009)
46	PSB-298	hA _{2B} AR	25	3.76×10^7	0.9533	1.05	25	(Bertarelli et al., 2006)
47	I-AB-MECA	hA ₃ AR	37	6.1×10^7	0.042	24	0.69	(Gao et al., 2001)
48	IB-MECA	hA ₃ AR	10	3.5×10^7	0.011	95	0.30	(Xia et al., 2018)
14	2-Cl-IB-MECA	hA ₃ AR	10	2.4×10^7	0.0043	231	0.18	(Xia et al., 2018)
49	MRS5127	hA ₃ AR	25	2.4×10^8	0.51	2.0	2.1	(Auchampach et al., 2010)
16	MRS5698	hA ₃ AR	10	7.8×10^6	5.1×10^{-4}	1961	0.068	(Xia et al., 2018)
31	MRE3008-F20	hA ₃ AR	4	7.6×10^7	0.042	24	0.55	(Varani et al., 2000)
50	LUF7531 (empd 2)	hA ₃ AR	10	1.7×10^8	0.0036	315	0.021	(Xia et al., 2017)
51	PSB-11	hA ₃ AR	25	2.35×10^8	0.2082	4.80	0.46	(Müller et al., 2002)

n.d., no data.

^a(kinetic) $K_D = k_{off}/k_{on}$.

^bwhole cells.

times [e.g., only seconds for antagonists LUF6057 (40, A₁AR) and SCH58261 (41, A_{2A}AR)], whereas agonists UK432,097 (11, A_{2A}AR), Cl-IB-MECA (14, A₃AR), and MRS5698 (16, A₃AR) as well as antagonists LUF6632 (43, A_{2A}AR) and LUF7531 (50, A₃AR) engage with the receptor for hours. In a recent study, Hothersall and coworkers (2017) identified UK432,097 analogs that displayed even longer target occupancy on hA_{2A}AR. Differences in RT for a number of A_{2A}AR antagonists have been linked to their differential modulation of the salt bridge strength between amino acids Glu¹⁶⁹ and His²⁶⁴ in the egress pathway at the extracellular side of the receptor (Guo et al., 2016b; Segala et al., 2016).

B. Orthosteric Ligands Binding Covalently to Adenosine Receptors

Ligands that react covalently with ARs can be regarded as having infinite RT as long as the chemical bond between ligand and receptor “survives.” Over the decades, such ligands have been developed as probes mostly (e.g., to identify the molecular weight of AR molecules, block the physiologic function of ARs or, more recently, help in AR structure elucidation). It remains to be investigated whether such ligands might have relevant therapeutic value.

Thus, both chemoreactive and photoaffinity agonists and antagonists were synthesized in early A₁AR studies and evaluated for their binding irreversibility using various assays and degrees of sophistication (Choca et al., 1985; Klotz et al., 1985; Earl et al., 1988; Patel et al., 1988; Stiles and Jacobson, 1988; Jacobson et al., 1989a; Boring et al., 1991; Scammells et al., 1994; Srinivas et al., 1996; Beauglehole et al., 2000; van Muijlwijk-Koezen et al., 2001; Jorg et al., 2016). Of these, FSCPX (52) has been most widely used, and a close derivative of it, DU172 (72) (Beauglehole et al., 2000), appeared crucial for the crystallographic structure elucidation of hA₁AR (Glukhova et al., 2017) (Chapter IV). DU172, through its fluorosulfonyl moiety, forms a covalent bond with amino acid Y271^{7,36} at the extracellular end of the seventh transmembrane domain (TM7) of the receptor.

Likewise, similar efforts have been performed on A_{2A}AR for agonists (Jacobson et al., 1989b; Barrington et al., 1990; Jacobson et al., 1992; Niiya et al., 1993; Luthin et al., 1995; Moss et al., 2014) and antagonists (Ji et al., 1993; Muranaka et al., 2017; Yang et al., 2017). One of the covalent antagonists, LUF7445 (53), was equipped with a click handle to act as a chemical probe (54, LUF7487) for A_{2A}AR (Yang et al., 2018). This chemical biology approach allowed, among others, receptor visualization in hA_{2A}AR-expressing cell membranes.

The A_{2B}AR has not been subjected to covalent labeling yet, whereas the A₃AR has been the target for

such studies, sampling both irreversibly binding agonist (Ji et al., 1994) and antagonist ligands (Li et al., 1999; Baraldi et al., 2001; Yang et al., 2019).

C. Allosteric Ligands and Adenosine Receptor Binding Kinetics

Ligands binding to an allosteric site distinct from the AR orthosteric binding pocket (see also Chapter II) may influence the binding kinetics of orthosteric ligands. Indeed, on many occasions it has been shown that positive allosteric modulators (PAMs) for the A₁AR retard the dissociation rate of orthosteric A₁AR agonists, as summarized in a number of reviews (Göblyös and IJzerman, 2011; Kimatrai-Salvador et al., 2013; Guo et al., 2017). For instance, one of the more potent A₁AR PAMs, BC-1/compound 8j (55) (Romagnoli et al., 2008), increased the residence time of CCPA up to 200-fold from 0.9 minutes (Table 2) to 172 minutes (Guo et al., 2014b). Unfortunately, the often modest, micromolar potency of PAMs and other allosteric ligands for ARs has so far precluded the assessment of the binding kinetics of these ligands per se.

D. Adenosine Receptor Target Binding Kinetics – Conclusions

Kinetic parameters are an additional factor in assessing the quality and nature of new chemical entities. Nearly all compounds in Table 3 have high affinity, but their kinetics can be very different. A striking example is the pair of LUF6976 (37, K_D = 2.2 nM for A₁AR, RT = 1.1 minutes) and LUF6941 (38, K_D = 2.9 nM for A₁AR, RT = 132 minutes), showing identical affinity but a more than 100-fold difference in residence time. Thus, many compounds are considered equivalent on the basis of affinity alone, whereas a further differentiation or even triage may be possible depending on their kinetic characteristics. For instance, A_{2A}AR antagonists are currently in clinical trials as potential adjuvants in cancer immunotherapy (see Chapter VII) to block adenosine’s unwanted anti-inflammatory and immunosuppressive effects (Hatfield and Sitkovsky, 2016). The local adenosine concentration in the tumor may be so high that short-RT antagonists cannot productively compete, whereas a long-RT antagonist may lead to sufficient target engagement even in the presence of elevated adenosine concentrations. Likewise, A_{2A}AR antagonists have been developed for the treatment of PD in combination with levodopa/dopaminergic agonists, although clinical success has been limited so far (Morelli et al., 2009; Hickey and Stacy, 2012). In that setting, a compound with a long receptor RT could have some advantages, as it might yield a reduction in the “wear-off” effect (e.g., of levodopa in between doses) (Hickey and Stacy, 2012). Thus, information obtained from a kinetic perspective may provide additional rationales for the design of new AR ligands. At

the same time, one needs to realize that pharmacokinetic aspects are also governing in vivo effects and that an integration of aspects of target binding kinetics and of pharmacokinetics is required (Daryae and Tonge, 2019).

IV. Receptor Structures

Over the last decade, the elucidation of receptor architecture has been one of the hallmarks in GPCR research (Venkatakrishnan et al., 2013). The A_{2A}AR was one of the first structures solved, through X-ray crystallography (Jaakola et al., 2008), and since then many adenosine receptor structures have been reported (see Table 4 and references therein). Typical characteristics of GPCRs such as their thermolability and fragility have dictated the use of highly engineered proteins and protein constructs for structure elucidation as well as of highly sophisticated technologies (Grishammer, 2017). At least three approaches have been used widely. First, thermostabilization of GPCRs (Magnani et al., 2016), including the A_{2A}AR, has yielded material sufficient for crystallization by combining amino acid mutations to raise the protein melting temperature. Secondly, fusion of the A_{2A}AR with proteins that crystallize “easily,” such as T4 lysozyme (T4L) (Jaakola et al., 2008) or apocytochrome b₅₆₂RIL (bRIL) (Liu et al., 2012), has been instrumental to generate crystalline material. Thirdly, complexation of the A_{2A}AR with antibodies raised against epitopes of the receptor provided sufficient stability to render X-ray crystallography feasible (Hino et al., 2012). In recent years, cryogenic electron microscopy (EM), particularly single-particle cryo-EM (Cheng, 2018; Ceska et al., 2019), has been employed to study membrane protein structures as well, including agonist-bound structures of the A₁AR (Draper-Joyce et al., 2018; 2021) and A_{2A}AR (Garcia-Nafria et al., 2018) in complex with G protein variants.

A. Resolution

The overall resolution of the AR crystal structures varies between 1.7 Å and 3.6 Å (see Table 4). A high resolution (lower Å values) provides more structural details, particularly the presence or absence of explicit water molecules. It has been shown that a minimum resolution of ~3.0 Å is required to see any water molecules in a protein crystal structure, whereas on average one water molecule per amino acid residue can be detected at 2.0 Å (Carugo and Bordo, 1999). This means that most adenosine receptor crystal structures lack information on the role that water molecules play in ligand binding. However, >60 explicit water molecules are observed in the 1.8 Å resolution A_{2A}AR-ZM241385 (42) complex (4EIY, Table 4), showing a wide distribution throughout the protein, including the ligand binding site, in which water molecules hydrogen-bond to both ligand and

amino acid residues (Liu et al., 2012). In fact, the receptor structure is suggestive of a water-filled pore or channel. The channel has two bottlenecks around Trp246^{6,48} and Tyr288^{7,53}, slightly less in size than the diameter of one water molecule. These amino acids are part of two general motifs related to GPCR activation, the “rotamer/toggle switch” and the NPxxY sequence, respectively. Interestingly, recent developments show that molecular dynamics and other calculations can make up for the absence of water molecules in a low-resolution protein structure (Matter and Gussregen, 2018). Due to its high resolution, the same 4EIY structure was the first to show the presence of an allosterically binding sodium ion, interacting in a cavity containing a strongly conserved aspartic acid (Asp52^{2,50}). As this domain is generic among most class A GPCRs, it is expected that other GPCRs bind sodium ions at this site as well (e.g., as was demonstrated for the human δ -opioid receptor) (Fenalti et al., 2014).

B. Ligand Binding Site

The ARs’ orthosteric binding site (i.e., the binding site for endogenous agonist adenosine) accommodates a range of ligands with diverse scaffolds and different sizes (see Table 4; Fig. 4). In fact, the A_{2A}AR is the GPCR with the most structures available in the Protein Data Bank (PDB) (Vass et al., 2018), allowing an unprecedented view on the conformational flexibility of the ligand binding site.

In total >15 different antagonists have been cocrystallized with hA_{2A}AR so far (Table 4), compared with just one structure with ZM241385 (PDB: 3EML) in the previous update. The receptor binding site appears flexible as these antagonists take slightly different positions therein (see Supplemental Video 1). The four agonists cocrystallized with hA_{2A}AR until now (Table 4) all have a ribose moiety, pointing deeper into the ligand binding pocket and displacing explicit water molecules present in the antagonist-occupied receptor structures (see Supplemental Video 2). The agonist-bound structures crystallized in the absence of G protein are now regarded as representing intermediate states in the process of receptor activation. The presence of an engineered G protein makes the cytoplasmic end of TM6 move away considerably from the receptor core by ~14 Å compared with the other agonist-bound structures, with little impact on the extracellular side of the receptor and the ligand binding pocket (Carpenter et al., 2016; Garcia-Nafria et al., 2018). This is most pronounced for the NECA (6)-bound cryo-EM structure with engineered G protein and nanobody Nb35 (Garcia-Nafria et al., 2018). The thermodynamic contributions of a single, conserved water molecule bridging the 2'-hydroxyl and 3-aza groups of adenosine were analyzed, which led to the design of a modified, potent agonist containing a mimic of this water (Matricon et al., 2020). Recently, the first X-ray structure of A_{2A}AR with

TABLE 4
Reported structures of adenosine receptor subtypes

PDB	Engineering	Ligand	Resolution (Å)	Technique	Remarks	Reference
<i>A_{2A}AR Antagonist Structures</i>						
3PWH	TS	ZM241385 (42)	3.3	X-ray		(Dore et al., 2011)
3REY	TS	XAC (57)	3.3	X-ray		(Dore et al., 2011)
3RFM	TS	Caffeine (3)	3.6	X-ray		(Dore et al., 2011)
3UZA	TS	T4G (58)	3.3	X-ray	T4G: 6-(2,6-dimethylpyridin-4-yl)-5-phenyl-1,2,4-triazin-3-amine	(Congreve et al., 2012)
3UZC	TS	T4E (59)	3.3	X-ray	T4E: 4-(3-amino-5-phenyl-1,2,4-triazin-6-yl)-2-chlorophenol	(Congreve et al., 2012)
3EML	FP (T4L)	ZM241385	2.6	X-ray		(Jaakola et al., 2008)
4E1Y	FP (bRIL)	ZM241385	1.8	X-ray		(Liu et al., 2012)
5UIG	FP (bRIL)	8D1 (60)	3.5	X-ray	8D1: 5-amino-N-[(2-methoxyphenyl)methyl]-2-(3-methylphenyl)-2H-1,2,3-triazole-4-carboximidamide	(Sun et al., 2017)
5K2A	FP (bRIL)	ZM241385	2.5	X-ray/SFX/XFEL, sulfur SAD phasing	SFX: serial femtosecond crystallography; XFEL: X-ray free-electron laser; SAD: single-wavelength anomalous diffraction	(Batyuk et al., 2016)
5K2B	FP (bRIL)	ZM241385	2.5	X-ray/SFX/XFEL, MR phasing	MR: molecular replacement	(Batyuk et al., 2016)
5K2C	FP (bRIL)	ZM241385	1.9	X-ray/SFX/XFEL, sulfur SAD phasing and phase extension		(Batyuk et al., 2016)
5K2D	FP (bRIL)	ZM241385	1.9	X-ray/SFX/XFEL, MR phasing		(Batyuk et al., 2016)
5VRA	FP (bRIL)	ZM241385	2.4	X-ray in situ	in situ: film sandwich plates at room temperature	(Broecker et al., 2018)
5JTB	FP (bRIL)	ZM241385	2.8	X-ray/I-SAD	I-SAD: iodide-single-wavelength anomalous diffraction	(Melnikov et al., 2017)
5UVI	FP (bRIL)	ZM241385	3.2	X-ray millisecond	millisecond: serial millisecond crystallography using synchrotron radiation	(Martin-Garcia et al., 2017)
6AQF	FP (bRIL)	ZM241385	2.5	X-ray		(Eddy et al., 2018b)
7RM5	FP (bRIL)	ZM241385	2.8	Microcrystal electron diffraction		(Martynowycz et al., 2021)
5NM2	TS-FP (bRIL)	ZM241385	2.0	X-ray millisecond (cryo)		(Weinert et al., 2017)
5NLX	TS-FP (bRIL)	ZM241385	2.1	X-ray millisecond (room temp)		(Weinert et al., 2017)
5NM4	TS-FP (bRIL)	ZM241385	1.7	X-ray femtosecond (room temp)	Serial femtosecond crystallography using XFEL	(Weinert et al., 2017)
5MZJ	TS-FP (bRIL)	Theophylline (4)	2.0	X-ray		(Cheng et al., 2017)
5MZP	TS-FP (bRIL)	Caffeine (3)	2.1	X-ray		(Cheng et al., 2017)
5N2R	TS-FP (bRIL)	PSB-36 (18)	2.8	X-ray		(Cheng et al., 2017)
5IU4	TS-FP (bRIL)	ZM241385	1.7	X-ray		(Segala et al., 2016)
5IU7	TS-FP (bRIL)	12c (61, LUF6805)	1.9	X-ray	12c: 2-(furan-2-yl)-N ⁵ -(2-(4-phenylpiperidin-1-yl)ethyl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine	(Segala et al., 2016)
5IU8	TS-FP (bRIL)	12f (62, LUF6806)	2.0	X-ray	12f: 2-(furan-2-yl)-N ⁵ -(2-(4-methylpiperazin-1-yl)ethyl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine	(Segala et al., 2016)
5IUA	TS-FP (bRIL)	12b (63, LUF6732)	2.2	X-ray	12b: 2-(furan-2-yl)-N ⁵ -(3-(4-phenylpiperazin-1-yl)propyl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine	(Segala et al., 2016)
5IUB	TS-FP (bRIL)	12x (64, LUF6632)	2.1	X-ray	12x: N ⁵ -(2-(4-(2,4-difluorophenyl)piperazin-1-yl)ethyl)-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine	(Segala et al., 2016)
5OLG	TS-FP (bRIL)	ZM241385	1.9	X-ray, soaking	soaking of ligand to displace theophylline in the crystals	(Rucktooa et al., 2018)
5OLH	TS-FP (bRIL)	Vipadenant (65)	2.6	X-ray, soaking for 24 hr		(Rucktooa et al., 2018)
5OLO	TS-FP (bRIL)	Tozadenant (66)	3.1	X-ray, soaking for 24 hr		(Rucktooa et al., 2018)
5OLV	TS-FP (bRIL)	LUAA47070 (analog) (67/68)	2.0	X-ray, soaking for 24 hr		(Rucktooa et al., 2018)

(continued)

TABLE 4—Continued

PDB	Engineering	Ligand	Resolution (Å)	Technique	Remarks	Reference
5OLZ	TS-FP (bRIL)	4e (69)	1.9	X-ray	4e: 4-(3-amino-5-phenyl-1,2,4-triazin-6-yl)-2-chlorophenol	(Rucktooa et al., 2018)
5OM1	TS-FP (bRIL)	4e	2.1	X-ray, soaking for 1 hr		(Rucktooa et al., 2018)
5OM4	TS-FP (bRIL)	4e	2.0	X-ray, soaking for 24 hr		(Rucktooa et al., 2018)
6LPJ/K/L	FP (bRIL)	ZM241385	1.8–2.0	Serial femtosecond crystallography using XFEL	EROCOC ₁₇₊₄ as crystallization matrix	(Ihara et al., 2020)
6ZDR	TS-FP (bRIL)	Chromone 4d (70)	1.9	X-ray		(Jespers et al., 2020)
6ZDV	TS-FP (bRIL)	Chromone 5d (71)	2.1	X-ray		(Jespers et al., 2020)
6GT3	TS-FP (bRIL)	AZD4635 (22)	2.0	X-ray		(Borodovsky et al., 2020)
6S0Q	TS-FP (bRIL)	ZM241385	2.7	Native SAD	SAD: single-wavelength anomalous diffraction	(Nass et al., 2020)
3VG9	antibody-stab	ZM241385	2.7	X-ray		(Hino et al., 2012)
3VGA	antibody-stab	ZM241385	3.1	X-ray		(Hino et al., 2012)
<i>A_{2A}AR Agonist Structures</i>						
2YDO	TS	Adenosine (1)	3.0	X-ray		(Lebon et al., 2011)
2YDV	TS	NECA (6)	2.6	X-ray		(Lebon et al., 2011)
4UG2	TS	CGS21680 (10)	2.6	X-ray		(Lebon et al., 2015)
4UHR	TS	CGS21680	2.6	X-ray		(Lebon et al., 2015)
3QAK	FP (T4L)	UK432097 (11)	2.7	X-ray	WT receptor	(Xu et al., 2011)
5WF5	FP (bRIL)	UK432097	2.6	X-ray	D52N mutant	(White et al., 2018)
5WF6	FP (bRIL)	UK432097	2.9	X-ray	S91A mutant	(White et al., 2018)
5G53	truncated and tagged WT	NECA (6)	3.4	X-ray	with engineered G protein (mini-Gs)	(Carpenter et al., 2016)
6GDG	FP (thioredoxin)	NECA	4.1	cryo-EM	with engineered G protein (mini-Gs-β ₁₇₂); also includes nanobody Nb35	(Garcia-Nafria et al., 2018)
7ARO	TS-FP (bRIL)	LUF5833 (56)	3.1	X-ray	LUF5833 is a partial agonist	(Amelia et al., 2021)
<i>A₁AR Antagonist Structures</i>						
5N2S	TS	PSB-36 (18)	3.3	X-ray		(Cheng et al., 2017)
5UEN	FP (bRIL)	DU172 (72)	3.2	X-ray		(Glukhova et al., 2017)
<i>A₁AR Agonist Structures</i>						
6D9H	tagged WT receptor	Adenosine (1)	3.6	cryo-EM	with engineered G _{i2} protein	(Draper-Joyce et al., 2018)
7LD3/4	tagged WT receptor	Adenosine (1) +/- MIPS521 (73)	3.3–3.4	cryo-EM	with engineered G _{i2} protein	(Draper-Joyce et al., 2021)

FP, fusion protein; PDB, Protein Data Bank four-digit entry number; TS, thermostabilization. Chemical structures of ligands are depicted in Fig. 3, if not already in Figs. 1 and 2.

a nonriboside, 3,5-dicyanopyridine partial agonist (56, LUF5833) was reported (Amelia et al., 2021).

The structure elucidation of the hA₁AR is another achievement. Two crystal structures of antagonist-bound receptor are available (Cheng et al., 2017; Glukhova et al., 2017), whereas one cryo-EM structure with an agonist (adenosine, 1) bound has been reported, the latter in the presence of an engineered G_i protein (Draper-Joyce et al., 2018). The latter structure was later complemented with a positive allosteric modulator, MIPS521 (73), as well, the binding site of which appeared to be extrahelically located involving TM domains 1, 6, and 7 (Draper-Joyce et al., 2021). In the antagonist structures, it was noted that there are differences in the extracellular loop regions, particularly the second one, relative to the hA_{2A}AR structure. The ligand binding cavity is relatively wide, again in comparison with hA_{2A}AR. Differences in pocket shape between the two receptors may determine selectivity

more than the (very similar) amino acids lining the pockets. There is a tightening of the orthosteric binding site induced by an ~4 Å inward movement of the extracellular ends of TMs 1 and 2 in the adenosine-bound, active structure compared with the antagonist-bound, inactive hA₁AR. At the intracellular surface, the engineered G protein present causes a 10.5 Å outward movement of TM6 in the hA₁AR, quite comparable to the similar large shift in active hA_{2A}AR.

All agonists and antagonists are anchored by two amino acids in particular in both receptors [i.e., Asn253^{6.55} (numbering as in hA_{2A}AR) and Phe168 in EL2]. A further summary of relevant amino acids for ligand binding, also focusing on selectivity issues between ARs, has been provided recently (Jespers et al., 2018).

C. NMR Studies

Although X-ray crystallography and cryo-EM methods provide important information on AR architecture, NMR

spectroscopy has the potential to reveal additional structural dynamics data. Two main approaches have been used so far: 1) indole ^{15}N - ^1H chemical shifts are monitored after introducing extrinsic (^{15}N -labeled) tryptophan residues at relevant sites, or 2) by incorporating ^{19}F reporter tags onto cysteine residues in the protein, ^{19}F - ^1H resonances are assessed. In both cases, distinct conformational $\text{A}_{2\text{A}}\text{AR}$ states were observed upon interaction with G protein (Prosser et al., 2017; Huang et al., 2021), cations (Ye et al., 2018), full and partial agonists (Eddy et al., 2018a; 2021; Sušac et al., 2018), and allosteric modulators/sites (Eddy et al., 2018b). The next challenge will be to begin and address other aspects of AR dynamics and functioning, such as the impact of the lipid membrane environment (Guixà-González et al., 2017).

V. Cellular Pharmacology – Biased Signaling of Adenosine Receptors

Each GPCR potentially couples to multiple G proteins, as was demonstrated for the endogenous $\text{A}_{2\text{A}}\text{AR}$ (Cunha et al., 1999) and $\text{A}_{2\text{B}}\text{AR}$ (Gao et al., 2018b), and to non-G protein dependent pathways, such as β -arrestins (Michel and Charlton, 2018; Vecchio et al., 2018). In some cases, the net effect of each of these signaling cascades induced by the same endogenously expressed AR in different cells may be opposite, such as with the $\text{A}_{2\text{B}}\text{AR}$ (Gao et al., 2018b). In theory, the ability of a GPCR agonist to consistently distinguish among multiple intracellular signaling pathways provides advantages when used in a therapeutic mode if the preferred pathway is associated with the beneficial action at the receptor. Such an agonist is termed biased, which implies a nonequivalence in the potency or efficacy across the signaling pathways. In principle, side effects that are associated with the nonpreferred pathways would be avoided. Signaling bias might also affect the kinetics of GPCR trafficking, as internalized receptors can also signal, or gene transcription.

Biased signaling depends on multiple active GPCR conformations, each of which would couple to its own spectrum of second messenger pathways. Thus, biased agonists, also at ARs, achieve signaling selectivity by interacting with or stabilizing a subset of the possible active receptor conformations, and this subset has characteristic pharmacology distinct from other conformations of the same receptor (Verzijl and IJzerman, 2011). Biased agonism has been reported at adenosine A_1 -, $\text{A}_{2\text{B}}$ -, and A_3 ARs for both nucleoside agonists and two classes of non-nucleoside AR agonists, the 3,5-dicyanopyridines and 5-cyanopyrimidines (Langemeijer et al., 2013). Tissue-dependent $\text{A}_{2\text{A}}\text{AR}$ signaling was observed in neurons of different brain areas through engineered optogenetic signaling (Li et al., 2015). Inhibitors of GPCR signaling could be biased as well, for example, as shown for the A_1AR

using a suramin derivative (Kudlacek et al., 2002). Allosteric GPCR modulators, such as A_1AR enhancers (PAMs) in the 2-amino-3-benzoylthiophene family or A_3AR PAMs in the imidazoquinolinamine family, can show biased effects on agonist-induced signaling (Gao et al., 2011).

A_1AR : In a broad screen of AR agonists, nucleoside agonist LUF5589 (74, 2-chloro-5'-O-ethyl- N^6 -(3-iodobenzyl)adenosine) tended toward a signaling bias for the G_i protein-dependent pathway in comparison with the β -arrestin pathway (Langemeijer et al., 2013). Biased agonism at the A_1AR was also explored by Baltos and coworkers (2016). PAM VCP520 (75) potentiated A_1AR agonist-induced Ca^{2+} mobilization more effectively than extracellular signal-regulated kinase 1/2 activation (Valant et al., 2010). Identification of biased agonism (i.e., cardioprotective efficacy without hemodynamic side effect) associated with an A_1AR PAM conjugated to an agonist, VCP746 (76), suggested that this bitopic ligand might be bridging orthosteric and allosteric sites on the receptor (Valant et al., 2014). A recent structure determined for A_1AR (Glukhova et al., 2017) shows that it possesses at least one allosteric site, potentially the site that has been exploited to promote biased agonism (Valant et al., 2014).

$\text{A}_{2\text{A}}\text{AR}$: The biased signaling of $\text{A}_{2\text{A}}\text{AR}$ is rather peculiar among ARs since it seems to be a property of the environment of the $\text{A}_{2\text{A}}\text{AR}$, probably related to the numerous G protein-interacting proteins that are associated with $\text{A}_{2\text{A}}\text{AR}$. In fact, at least six G protein-interacting proteins (actinin, calmodulin, NECAB2, translin-associated protein X, ARNO/cytohesin-2, and ubiquitin-specific protease-4) have been reported to interact with the long $\text{A}_{2\text{A}}\text{AR}$ C terminus (Keuerleber et al., 2011). The exploitation of constructs with an altered C-terminal tail revealed a biased $\text{A}_{2\text{A}}\text{AR}$ -mediated signaling with PSB-0777 (12) and LUF5834 (35) (Navarro et al., 2020). Also, inosine has been proposed to activate $\text{A}_{2\text{A}}\text{AR}$ in a biased manner in CHO-K1 cells heterologously expressing h $\text{A}_{2\text{A}}\text{AR}$ (Welihinda et al., 2016). Still, $\text{A}_{2\text{A}}\text{AR}$ agonists with biased properties have been scarcely explored, although they would be of clear interest to potentially optimize immunomodulatory functions without cytotoxic or vascular effects.

$\text{A}_{2\text{B}}\text{AR}$: Nucleoside agonists distinguish among different G protein-dependent signaling pathways of the $\text{A}_{2\text{B}}\text{AR}$ (Gao et al., 2014). Extracellular signal-regulated kinase 1/2 activation may result from β -arrestin mobilization or from G_q - or G_s -protein coupling. In fact, entirely different signaling pathways are activated depending on whether the receptor is endogenously occurring or introduced by transfection (Gao et al., 2018b). $\text{A}_{2\text{B}}\text{AR}$ activation in muscle and brown fat had a beneficial effect on energy expenditure and

increased muscle mass, suggesting the application of selective A_{2B} AR agonists that principally activate cAMP for treating obesity (Gnad et al., 2020). A_{2B} AR activation also reduces cardiac fibrosis via the PKC δ to p38-MAPK pathway and protects the ischemic heart by stabilizing HIF-1 α (Campos-Martins et al., 2021). Thus, translational opportunities are conceivable if selective and biased A_{2B} AR agonists could be developed for these signaling pathways.

A_3 AR: Storme and coworkers (2018), using an engineered arrestin-reporter cell line, compared the G_i -dependent and β -arrestin2-dependent signaling of 19 nucleoside agonists at the A_3 AR to show a tendency toward weak bias for the G protein pathway in a few analogs. Similar conclusions were reported in an earlier study comparing known A_3 AR agonists (Gao and Jacobson, 2008), which noted differences in the kinetics of receptor activation.

VI. Pharmacology – Novel Developments

This chapter addresses select aspects of AR pharmacology. In this update, we focus on the therapeutic targeting of ARs and elaborate on their relevance in disease states. In the previous update, dimerization/oligomerization of ARs was particularly emphasized (Fredholm et al., 2011). This potentially critical variable to selectively modulate AR activity has been detailed in a number of recent reviews (Vecchio et al., 2018; Ferré and Ciruela, 2019; Franco et al., 2021a).

A. Therapeutic Targeting of Adenosine Receptors

Adenosine receptors have been targeted in the treatment of a number of (peripheral and CNS) diseases including PD, cardiac arrhythmias, asthma, and infant apnea (Kreutzer and Bassler, 2014). Adenosine receptors are also targeted for diagnostic studies of coronary circulation in individuals unable to manage a treadmill. Over the years, targeting adenosine receptors has been tested in animal models of diabetes, inflammatory diseases, wound healing, sickle cell disease, congestive heart failure, Alzheimer's disease, depression, and grand mal epilepsies, as well as in human trials. Other potential disease targets for agents targeted to adenosine receptors have recently been identified. Below we identify some of the most promising applications for adenosine receptor agents described over the past 10 years (Borea et al., 2018). It should be kept in mind, however, that a knowledge gap exists between advanced animal studies, which are many, and the limited number of reports on native human cells and tissues. From a translational perspective toward successful clinical studies, it seems essential to close this gap.

B. Therapeutic Targeting of Peripheral Adenosine Receptors

1. *Adenosine A_1 Receptors and Congestive Heart Failure.* Adenosine, generated within the kidney and acting at A_1 AR, induces vasoconstriction of afferent arterioles reducing renal blood flow and glomerular filtration rate (GFR), further stimulating renin release. Moreover, activation of A_1 AR increases proximal tubular reabsorption of sodium ions (Vallon et al., 2006). In congestive heart failure A_1 AR activation was postulated to play a role in the reduced GFR and sodium retention that characterize congestive heart failure, and it was suggested that blockade of A_1 AR could alleviate the symptoms of congestive heart failure by increasing GFR and promoting sodium elimination (Vallon et al., 2008). When tested in the clinic, however, short courses of rolofylline, a selective A_1 AR antagonist, provided no benefit in the treatment of congestive heart failure, and a number of patients suffered seizures, a known potential adverse effect of A_1 AR antagonists (Massie et al., 2010). Subsequently, it was noted that A_1 AR stimulation could enhance cardiac myocyte function by improving mitochondrial function and the function of the Ca²⁺-ATPase (SERCA2a), and the use of a partial agonist could potentially avoid the cardiac dysrhythmias induced by A_1 AR (full) agonists (Voors et al., 2018). Unfortunately, the partial A_1 AR agonist neladenoson (BAY1067197) did not improve exercise tolerance (see also Chapter VII) in patients with heart failure (Shah et al., 2019).

2. *Adenosine A_{2A} Receptors and Cancer.* Severe impairment of the cellular immune system was first associated with deficiency of adenosine deaminase in 1972 (Giblett et al., 1972). Whereas adenosine deaminase deficiency is toxic to T cells, in many subsequent studies the immunosuppressive effects of adenosine at concentrations that are not toxic to T cells have been further confirmed. Moreover, the A_{2A} AR has been implicated as the mediator by which adaptive immunity is suppressed (Huang et al., 1997), the T cell subtypes affected have been identified, and the intracellular signaling mechanisms have been investigated (Cronstein and Sitkovsky, 2017). The impact of A_{2A} AR in cancer development is best heralded by the pioneering report in which melanoma and lymphoma cell lines were completely rejected in A_{2A} AR knockout mice (Ohta et al., 2006) through a mechanism involving the control of the antitumor effects of CD8 T cells. Although it had previously been established that high concentrations of adenosine were present in the extracellular fluid of solid tumors (Blay et al., 1997), the significance of that finding was not fully appreciated until the report by Ohta and colleagues. Moreover, a number of more recent studies suggest that A_{2A} AR antagonists interact with anti-PD1 and anti-CTLA4 therapy to further

enhance tumor immunity and promote tumor regression (Iannone et al., 2014; Beavis et al., 2015; Gessi et al., 2017). Indeed, A_{2A} AR antagonists bolster cytokine release by CAR-T cells increasing their antitumor efficiency (Beavis et al., 2017). Currently, a number of A_{2A} AR, A_{2B} AR, and dual antagonists are at various stages of clinical development (see Chapter VII) (Yu et al., 2020). In addition, other therapeutic approaches targeting adenosine production from adenine nucleotides by ecto-5'-nucleotidase (CD73) are making their way to the clinic as well (Congreve et al., 2018).

3. Adenosine Receptors and Autoimmune and Inflammatory Diseases. The potential anti-inflammatory effects of adenosine, acting at A_{2A} AR, have been known since 1983 (Cronstein et al., 1983). Subsequently adenosine, acting at both A_{2A} AR and A_3 AR, was shown to mediate many of the anti-inflammatory and immunosuppressive effects of low-dose methotrexate therapy, the gold standard in the therapy of rheumatoid arthritis and psoriasis (Cronstein and Sitkovsky, 2017). Administration of A_{2A} AR agonists, although potentially useful for treatment of inflammatory diseases, would likely have too many side effects to be tolerated, mainly due to their strong hypotensive action, so other approaches have been taken. Thus, one approach has been to develop a pro-drug of an A_{2A} AR agonist that is liberated by the action of ecto-5'-nucleotidase (CD73). Such an agent was shown to suppress inflammatory arthritis in animal models (Flögel et al., 2012) and suggests a promising approach to development of new anti-inflammatory agents.

In contrast, A_3 AR agonists do not appear to have the same potential for systemic toxicity, as receptor expression is not as widespread as for the A_{2A} AR. Thus, relatively selective A_3 AR agonists have been tested in both animal models and the clinic for their anti-inflammatory effects. Potential clinical utility with minimal toxicity has been reported for A_3 AR agonists in the treatment of rheumatoid arthritis, psoriasis, and liver conditions, and thus agents remain in development for the treatment of these autoimmune disorders (reviewed in Jacobson et al., 2018).

4. Adenosine Receptors and Infectious Diseases. The anti-inflammatory and immunosuppressive effects of adenosine, acting at A_{2A} AR, have not gone unnoticed by microorganisms. Thus, adenosine has been identified as a virulence factor in *Candida albicans* (Smail et al., 1992; Rodrigues et al., 2016), *Staphylococcus aureus*, (Thammavongsa et al., 2009), and *Streptococcus suis* (Liu et al., 2014) that mitigates the effects of the host immune and inflammatory response on these microorganisms. *Leishmania amazonensis* also exploits the adenosine system to elude detection by dendritic cells, in this case through A_{2B} AR (Figueiredo et al., 2021). To date, A_{2A} AR or A_{2B} AR have not

been targeted as a means to enhance host responses to microorganisms for the treatment of infectious diseases for resistant organisms.

In contrast, it is increasingly clear that much of the injury associated with infections comes as a result of the active host response to the infection with tissue damage in affected tissues, much like the tissue injury triggered by inflammatory and autoimmune diseases. First postulated as a potential therapy for COVID-19 pneumonia (Abouelkhair, 2020; Falcone et al., 2020), Correale and colleagues (2020) reported on the beneficial effects of administration of aerosolized adenosine in patients with COVID-19 pneumonia. They treated 14 patients with COVID-19 interstitial pneumonitis with aerosolized adenosine and observed improved oxygenation in 13 of 14 patients (compared with 7 of 52 control patients) and improved imaging studies, although the RNA load of SARS-CoV-2 increased in 13 of 14 patients. There was one death in the adenosine-treated patients compared with 11 of 52 patients in the historic control group. Bronchospasm was observed in one of the treated patients. The authors concluded that aerosolized adenosine might be a useful adjunct to other therapies for the treatment of SARS-CoV-2 pneumonia and might be similarly effective in other types of viral pneumonia. Although it is likely that the actions of adenosine in viral pneumonitis are mediated by the actions of an adenosine receptor, it is unclear which receptor(s) that might be, although the actions of A_{2A} AR, A_{2B} AR, and A_3 AR could account for the anti-inflammatory effects observed, as noted above.

5. Adenosine A_{2A} Receptors and Retinal Disease. The retinopathy of prematurity is the most common cause of childhood blindness. A_{2A} AR stimulation in the retina promotes retinal vascular overgrowth, and results of recent studies indicate that A_{2A} ARs play a significant role in the development of oxygen toxicity-induced retinal angiogenesis (Taomoto et al., 2000; Liu et al., 2010; 2017). Caffeine, which is commonly used to treat apnea in neonates, was recently shown to prevent oxygen toxicity-induced retinal angiogenesis in animal models and has been suggested as a therapeutic approach to prevent retinopathy of prematurity (Zhang et al., 2017), an effect mimicked by the selective antagonism of A_{2A} ARs (Zhou et al., 2018). The antagonism of A_{2A} ARs also emerges as a novel promising strategy to dampen the local inflammatory processes involved in the degeneration of ganglion neurons in ischemic eye diseases and glaucoma that are a prevalent cause of blindness in the elderly (Liu et al., 2016; Madeira et al., 2016; Boia et al., 2017). A_1 AR agonists, which prevent neuronal damage from pressure and ischemia in animal models, have been tested in the treatment of glaucoma but failed in phase 3 trials to reduce intraocular pressure

better than placebo (ClinicalTrials.gov Identifier: NCT02565173).

6. Adenosine Receptors and Bone. Adenosine A_1 -, A_{2A} -, and A_{2B} -ARs play a role in regulating bone biology by modulating osteoclast differentiation and bone remodeling as well as osteoblast differentiation and production of new bone (Strazzulla and Cronstein, 2016). A_1 AR stimulation is required for osteoclast differentiation, and A_1 AR knockout mice have mild osteopetrosis (Kara et al., 2010a,b). In contrast, A_{2A} AR and A_{2B} AR stimulation diminish osteoclast differentiation and stimulate new bone formation by osteoblasts (Mediero et al., 2012b; 2013; 2015b; Corciulo et al., 2016). More importantly, an A_1 AR antagonist, an A_{2A} AR agonist, or dipyridamole, which blocks adenosine uptake via the equilibrative nucleoside transport protein ENT1 (SLC29A1) and thereby increases extracellular adenosine levels, stimulate bone regeneration in critical bone defects, whether applied topically or as a coating for 3D-printed β -tricalcium phosphate scaffolds (Mediero et al., 2015b; Ishack et al., 2017). Currently, dipyridamole-coated scaffolds are undergoing preclinical testing for restoration of bone.

Despite the remarkable success of joint replacement therapy, approximately 25% of implanted hip and knee prostheses will require revision due to erosion of the bone surrounding the prosthesis (Bozic et al., 2010). Application of A_{2A} AR agonists markedly 1) diminishes the inflammation due to prosthesis wear particles, the most common cause of bone destruction leading to prosthetic joint replacement, and 2) by inhibiting osteoclast differentiation, diminishes wear particle-induced bone destruction in a murine model (Mediero et al., 2012a). Moreover, weekly low doses of methotrexate, a commonly used anti-inflammatory drug that inhibits inflammation by increasing local adenosine concentrations, similarly alleviates wear particle-induced bone destruction in mice (Mediero et al., 2015a) by an A_{2A} AR-dependent mechanism.

7. Adenosine Receptors and Cartilage. In recent studies in both mice (Corciulo et al., 2017) and humans (St Hilaire et al., 2011), premature development of osteoarthritis has been described, and in mice, loss of A_{2A} ARs leads to spontaneous development of osteoarthritis (Corciulo et al., 2017), indicating that endogenous adenosine production acts in an autocrine fashion to maintain chondrocyte homeostasis. Moreover, treatment of rats with post-traumatic osteoarthritis with intra-articular injections of liposomal adenosine preparations prevents progression of osteoarthritis (Corciulo et al., 2017). Similarly, loss of A_3 ARs leads to the development of osteoarthritis in mice (Shkhyan et al., 2018), and treatment of chemically induced osteoarthritis with an A_3 AR agonist inhibits development of osteoarthritis (Bar-Yehuda

et al., 2009). These events suggest that targeting A_{2A} ARs or A_3 ARs in the joint may be useful approaches to the treatment of osteoarthritis, a disabling condition affecting as many as 150 million people worldwide.

8. Adenosine Receptors and Fibrosis. Fibrosis is a common condition in a number of organs, and recent studies indicate that blockade of A_{2A} ARs can diminish excessive fibrosis in the skin, liver, and other organs in response to injury, ionizing radiation, or exposure to toxins (Shaikh and Cronstein, 2016). Indeed, in recent studies, topical application of an A_{2A} AR antagonist prevents both scarring and radiation fibrosis in the skin (Perez-Aso et al., 2012; 2016). In some organs, A_{2B} AR blockade can also diminish fibrosis (Shaikh and Cronstein, 2016), but recent studies suggest that in Peyronie's disease, which involves fibrosis of the shaft of the penis, A_{2B} AR stimulation prevents myofibroblast production of collagen (Mateus et al., 2018), suggesting that an A_{2B} AR agonist could prevent the development of Peyronie's disease.

9. Adenosine A_{2A} Receptors and Sickle Cell Disease. Patients with sickle cell disease suffer from focal areas of vascular obstruction leading to localized regions of poor perfusion and resulting ischemia. In these hypoxic foci, invariant natural killer T cells can induce further tissue injury, and A_{2A} AR stimulation inhibits invariant natural killer T cell function and tissue injury. Studies in humanized mice with sickle cell disease demonstrated that infusion of an A_{2A} AR agonist, regadenoson, reduced the tissue injury associated with sickle cell disease (Nathan et al., 2012). Although preclinical studies showed promise in these patients, the results of a clinical trial of regadenoson infusions for sickle cell disease did not show any evidence of shortened hospital stay or reduction in respiratory symptoms or opioid use (ClinicalTrials.gov Identifier: NCT01788631).

10. Summary. ARs are expressed ubiquitously in the periphery and play a variety of roles. ARs remain targets for clinical development despite recent failures in treatment of congestive heart failure and sickle cell disease. Immunostimulatory blockade of A_{2A} AR for the treatment of cancer shows real promise in early clinical trials, and development of other adenosine receptor targets is moving out of the laboratory into the clinic.

C. Therapeutic Targeting of Central Nervous System Adenosine Receptors

Although ARs are present throughout the human body, their density is far greater in the brain. Accordingly, manipulating ARs upon moderate intake of caffeine (Fredholm et al., 1999) mainly results in brain-associated effects, typified by increased arousal and attention with faster reaction time, decreased fatigue, more efficient working memory and memory recall, and better mood (Smith et al., 2005; McLellan et al.,

2016). These effects of caffeine are mostly mediated by brain ARs, namely A_1 ARs and A_{2A} ARs (Fredholm et al., 2005), as heralded by the elimination of the effects of caffeine on synaptic transmission and plasticity upon blockade of A_1 AR and A_{2A} AR (Lopes et al., 2019). Although also present in glia cells, A_1 AR and A_{2A} AR are mostly colocalized in excitatory synapses where they cooperate to encode information salience in neuronal circuits through a combined A_1 AR-mediated inhibition of synaptic transmission (decreasing noise) and an A_{2A} AR-mediated facilitation of synaptic plasticity (increasing encoding) (Cunha, 2016).

1. Acute Brain Dysfunction – Ischemia and Epilepsy. Apart from their physiologic role, ARs also have an impact on brain dysfunction and damage, in accordance with the universal utilization of ATP (Rodrigues et al., 2015) and adenosine (Cunha, 2001) to signal stress or increased cellular workload in the brain. Thus, in conditions of metabolic stress such as upon ischemic stroke, both the acute A_1 AR activation and A_{2A} AR blockade afford a robust neuroprotection but through different mechanisms. A_1 AR activation increases the hurdle for onset of brain dysfunction by hyperpolarizing neurons. In contrast, A_{2A} AR blockade restrains neurodegeneration, probably as a result of the combined inhibition of glutamate release and decreased activation of N-methyl-D-aspartate (NMDA) receptors (Cunha, 2016), together with an attenuation of neuroinflammation (Rebola et al., 2011) and decreased neuronal apoptosis (Silva et al., 2007). A similar dual and opposite control by A_1 ARs and A_{2A} ARs occurs upon abnormal increased workload typified by epileptic conditions (Tescarollo et al., 2020). Acute A_1 AR activation attenuates the onset of seizures and, conversely, acute A_1 AR inhibition decreases seizures threshold, whereas A_{2A} ARs control seizure-induced neurodegeneration (Canas et al., 2018). This dual control of the onset and evolution of brain damage by A_1 ARs and A_{2A} ARs prompts the suggestion that a combined activation of A_1 ARs and blockade of A_{2A} ARs might have a superior efficacy to limit acute brain damage (Cunha, 2005). However, timing of intervention might be of key importance since A_1 ARs desensitize and their function decreases in the injured brain, which may result in paradoxical effects (Jacobson et al., 1996). In contrast, central A_{2A} ARs are upregulated in noxious brain condition (Cunha, 2016), justifying the interest in A_{2A} AR antagonists to control brain damage.

2. Neurodegenerative Diseases – Parkinson's and Motor Diseases. The particularly high density of A_{2A} ARs in the basal ganglia and their tight antagonistic interaction with dopamine D_2 receptors typified by the formation of A_{2A} AR- D_2 receptor heteromers (Ferré and Ciruela, 2019) prompted targeting A_{2A} ARs to alleviate dopaminergic depletion characteristic of PD. Indeed, A_{2A} AR antagonists dampen PD features in animal models, and the regular consumption of

moderate doses of caffeine attenuates PD features in humans (Schwarzschild et al., 2006). As mentioned above, this preclinical evidence, together with the safety profile of A_{2A} AR antagonists, supported the US Food and Drug Administration's recent approval of istradefylline as an add-on therapy to manage PD patients (Chen and Cunha, 2020). This offers novel possibilities of carrying out phase 4 trials to directly test the role of A_{2A} ARs in the control of nonmotor PD symptoms, such as cognitive deficits and mood dysfunction. It should be mentioned that the clinical trajectory of istradefylline has been long and windy. After its introduction in Japan in 2013, market access to the United States has only recently (in 2019) been granted after an earlier rejection and is limited to treating "off" episodes with levodopa only. Other A_{2A} AR antagonists such as preladenant have failed to obtain market authorization from the Food and Drug Administration, as clinical efficacy was not convincingly demonstrated. This might be due to our insufficient knowledge of the role that different A_{2A} AR populations have in the control of altered motor function and to lack of patient stratification in the clinical studies.

Selective A_{2A} AR antagonists also attenuate other motor conditions, such as catalepsy and tremor (Salamone et al., 2008), akathisia (Varty et al., 2008), dystonia (Maltese et al., 2017), cocaine or MK801-induced psychomotor activity (Shen et al., 2008; Yu et al., 2008), cerebrosplinal type 3 ataxia or Machado-Joseph's disease (Gonçalves et al., 2013; Gonçalves et al., 2017), or amyotrophic lateral sclerosis (Ng et al., 2015). Their impact on Huntington's disease is less clear and might depend on the phase of the disease (Popoli et al., 2008). This broader ability of A_{2A} AR antagonists to control different motor disorders that might not directly result from dopaminergic depletion prompts the involvement of a control of glutamate excitotoxicity rather than only the control of dopamine D_2 receptors (Schiffmann et al., 2007; Cunha, 2016).

3. Neurodegenerative Diseases – Alzheimer's Disease and Cognitive Dysfunction. The pharmacological or the genetic blockade of A_{2A} ARs prevents memory deficits in different animal models of Alzheimer's disease (Canas et al., 2009; Laurent et al., 2016; Viana da Silva et al., 2016). A_{2A} AR antagonism also prevents memory dysfunction associated with other conditions, such as convulsions (Cognato et al., 2010), diabetes (Duarte et al., 2012), hypoxia (Chen et al., 2018), traumatic brain injury (Zhao et al., 2017), demyelination conditions (Akbari et al., 2018), repeated stress or depression (Batalha et al., 2013; Kaster et al., 2015; Machado et al., 2017), PD (Hu et al., 2016; Carmo et al., 2019), or cannabis exposure (Mouro et al., 2019). A_{2A} ARs are not only necessary but

actually sufficient to impair memory since their increased activity driven by genetic (Temido-Ferreira et al., 2020), optogenetic (Li et al., 2015), or pharmacological strategies (Pagnussat et al., 2015) impairs memory in normal animals. This converges with several findings in humans, namely: 1) Caffeine intake prevents cognitive deterioration upon aging (Ritchie et al., 2007; Dong et al., 2020) and is inversely associated with the onset (Eskelinen et al., 2009; Sugiyama et al., 2016) or neuropathological hallmarks of dementia (Gelber et al., 2011); 2) A_{2A} ARs are upregulated in the brains of demented patients (Temido-Ferreira et al., 2020); and 3) A_{2A} AR polymorphisms are associated with memory phenotypes (Beste et al., 2012; Horgusluoglu-Moloch et al., 2017). However, it is still unknown if A_{2A} AR antagonists ameliorate memory deficits in dementia patients.

4. Neuropsychiatric Diseases – Major Depression and Suicide. Caffeine, A_{2A} AR antagonists, and the genetic deletion of A_{2A} ARs selectively in forebrain neurons abrogate the onset of depressive-like symptoms and can also reverse these symptoms in mice subject to chronic unpredictable stress (Kaster et al., 2015). Accordingly, coffee intake is inversely correlated with the incidence of depression (Grosso et al., 2016; Lucas et al., 2011) and its major consequence suicide (Lucas et al., 2014), and the incidence of major depression is associated with A_{2A} AR haplotypes (Oliveira et al., 2019). In parallel, the upregulation of A_1 ARs bolsters the resilience toward depressive-like behavior and, conversely, knocking out A_1 ARs increased depressive-like behavior and eliminated the antidepressant effects of sleep deprivation (Serchov et al., 2015). Furthermore, A_1 ARs in the amygdala are also involved in neuroimmune-driven depression (Fan et al., 2019).

5. Other Neuropsychiatric Diseases. The elegant work of Chen and colleagues revealed a temporal ability of A_{2A} ARs to modulate instrumental behavior, formatting the sensitivity to goal-directed valuation (Li et al., 2016; 2018). Thus, A_{2A} AR-mediated overactivation of striatopallidal neurons disrupts the homeostatic control of goal-directed behavior, with impaired decision-making and behavioral disinhibition with loss of flexibility, which are at the core of psychiatric symptoms (Li et al., 2016; 2020; He et al., 2020). Indeed, A_{2A} ARs control addiction (Ferré, 2016; Borroto-Escuela et al., 2018) and preservative and obsessive-compulsive behaviors (Bleickardt et al., 2014; Asaoka et al., 2019) that are transversal to most neuropsychiatric diseases. Accordingly, A_{2A} AR polymorphisms are associated with anxiety (Alsene et al., 2003; Fraporti et al., 2019), depression (Oliveira et al., 2019), phobia (Deckert et al., 1998; Hamilton et al., 2004), preservative/obsessive disorders (Freitag et al., 2010; Janik et al., 2015), or addictive profiles (Kobayashi et al., 2010). This A_{2A} AR-mediated control of behavioral inhibition may be

associated with their ability to modulate arousal (Lazarus et al., 2012) and enhanced motivation (He et al., 2020), which are founding behaviors of decision-making and cognitive performance.

6. Brain Aging. Aging is by far the major risk factor for most prevalent chronic brain diseases, namely depressive, cerebrovascular, and neurodegenerative diseases. The adenosine modulation system in the forebrain is modified upon aging with a decreased density and functional efficiency of A_1 ARs (Sperlagh et al., 1997; Sebastião et al., 2000; Costenla et al., 2011) and an increased density and efficiency of A_{2A} ARs (Rebola et al., 2003; Canas et al., 2009; Costenla et al., 2011). These alterations posit a contribution of the adenosine modulation system to the deterioration of brain function since hyperactivity of A_{2A} ARs is sufficient to trigger brain dysfunction (Li et al., 2015; Carvalho et al., 2019; Temido-Ferreira et al., 2020) and a hypofunction of A_1 ARs increases excitability (noise) of brain networks and bolsters the spreading of excitotoxicity (Tescarollo et al., 2020). Indeed, the intake of caffeinated coffee is inversely associated with memory deterioration upon aging (Hameleers et al., 2000; Ritchie et al., 2007; van Gelder et al., 2007; Arab et al., 2011; Dong et al., 2020), which was shown in animal models to be reverted by caffeine and by selective A_{2A} AR antagonists (Prediger et al., 2005). This stresses the particular association between increased A_{2A} AR activity with the deterioration of brain function upon aging, which might underlie the increased susceptibility for the emergence of age-associated brain diseases.

Hyperactivity of A_{2A} ARs in the aged brain is further reinforced by the parallel increase of the pathway responsible for the formation of the pool of adenosine selectively associated with the activation of A_{2A} ARs, namely ecto-5'-nucleotidase (CD73)-mediated formation of ATP-derived extracellular adenosine (Augusto et al., 2013; Carmo et al., 2019; Gonçalves et al., 2019). In fact, different studies reported a robust increase of the activity of CD73 in the aged brain (Fuchs, 1991; Cunha et al., 2001; Mackiewicz et al., 2006), as best heralded by the inverse association of CD73 activity with the probability of reaching centenarian ages (Crooke et al., 2017). In parallel, the activity of AdoK is decreased in the aged brain (Mackiewicz et al., 2006). This bolsters the availability of the extracellular adenosine (Cunha et al., 2001; Murillo-Rodriguez et al., 2004), possibly to compensate the decreased density of A_1 ARs. Indeed, the alteration of purinergic metabolism seems to be a prominent characteristic—a metabolic fingerprint of the aging process in different organisms (Furman et al., 2017; Gao et al., 2018a). Precocious modifications of adenosine metabolism occur in the brain of aged rodents (Ivanisevic et al., 2016) and in an

animal model of accelerated aging (Sanchez-Melgar et al., 2020).

VII. Current and Recent Clinical Trials

Both AR agonists and antagonists have been in clinical trials dating back to the late 1960s for a wide range of conditions (Borah et al., 2019; Jacobson et al., 2019). Although most of these clinical trials were unsuccessful, the therapeutic focus of AR-based therapeutics has shifted since the early days, and new trials are underway for more recently identified indications (Table 5; Fig. 5). There is reason for optimism that this situation can be remedied in future clinical trials based on current pharmaceutical technology. Firstly, the availability of high-resolution experimental structures of two of the adenosine receptors allows the discovery and optimization of compounds of extremely high selectivity for each of the four receptors. Furthermore, the lack of efficacy in clinical trials often results from inadequate pharmacokinetics, which has been greatly improved in the recent generation of adenosine receptor ligands, which are also much more structurally diverse than in the past. Additionally, the expanded range of allosteric modulators of the adenosine receptors and indirect adenosine modulators (e.g., enzyme and transport inhibitors) promises to provide clinical candidate molecules that are more temporally and spatially selective than orthosteric agonists. More specifically, further research may lead to new therapeutics, including: A_{2A} - and A_{2B} AR antagonists for treating cancer and neurodegenerative conditions such as Parkinson's disease and Alzheimer's disease; A_{2B} - and A_3 AR agonists for treating chronic conditions such as obesity and NASH, respectively; A_1 - and A_3 AR agonists for treating chronic pain; A_3 AR antagonists for treating glaucoma; and A_{2A} - and A_{2B} AR agonists for treating musculoskeletal conditions such as osteoporosis. These are potential therapeutic approaches that have matured scientifically in recent years.

A. Clinical Trials of Adenosine Receptor Agonists

AR agonists and partial agonists have been considered for pharmaceutical development in the treatment of: pain, seizures, arrhythmias, atrial fibrillation, diabetes, chronic heart failure, glaucoma (A_1 AR); hypertension (and diagnosis), inflammation, atrial fibrillation, ischemic conditions, sickle cell disease (A_{2A} AR); neurodegeneration, inflammation, hepatocellular carcinoma, ischemic conditions, NASH, and chronic neuropathic pain (A_3 AR). There are no clinical trials of A_{2B} AR agonists, but their use as antidiabetic agents or in cardioprotection, lung injury, diabetes, pulmonary hypertension, and other vascular conditions has been suggested (Eckle et al., 2007; Kosco et al., 2013; Merighi et al., 2015; Bessa-Gonçalves et al., 2018).

The antiarrhythmic effects of adenosine acting at the A_1 AR led to its approval for treating supraventricular tachycardia (Jacobson et al., 2019). More selective nucleoside-based A_1 AR agonists were also considered for this application, but their clinical trials failed. A partial A_1 AR agonist, CVT-3619, was predicted to display fewer side effects as an antiarrhythmic agent, but the clinical trial was discontinued (Jacobson et al., 2019). A clinical trial of A_1 AR agonist selodenoson (78, DTI-0009) for atrial fibrillation was discontinued. Non-nucleosides have also been developed as A_1 AR agonists. 3,5-Dicyanopyridine derivative capadenoson was in a clinical trial for heart failure, but it was later supplanted by an ongoing trial of neladenoson (77, BAY1067197), a newer, more selective prodrug derivative of the same structural class (Shah et al., 2019). However, the compound failed to meet the clinical endpoint. A phase 3 trial of A_1 AR agonist trabodenoson (79) also failed to demonstrate efficacy (Jacobson and Civan, 2016). A_1 AR agonists, including selective agonist GW493838 and intrathecally administered adenosine, and a PAM (T-62) were also studied for their application in pain, but three clinical trials failed to demonstrate efficacy (Miao et al., 2018; Jacobson et al., 2019).

The vasodilatory effects of A_{2A} AR agonists have resulted in the approval of adenosine (1) itself and regadenoson (2, CVT-3146) for coronary stress imaging in patients not suitable for exercise-induced vasodilation. However, clinical trials for chronic obstructive pulmonary disease, asthma, and sickle cell disease, based on the anti-inflammatory effects of A_{2A} AR agonists, were unsuccessful (Jacobson et al., 2019).

A_3 AR activation has been the subject of many clinical trials, and several are ongoing (Jacobson et al., 2019). Two prototypical A_3 AR agonists, IB-MECA (48, CF101, piclodenoson) and CI-IB-MECA (14, CF102, namodenoson) have progressed to clinical phases 3 and 2 for autoimmune inflammatory diseases and liver diseases, respectively. A_3 AR agonists also have protective effects in models of chronic pain. Piclodenoson is in phase 3 trials for rheumatoid arthritis/psoriasis. Namodenoson is in phase 2 trials for hepatocellular carcinoma and nonalcoholic steatohepatitis (NASH).

B. Clinical Trials of Adenosine Receptor Antagonists

AR antagonists have been considered for pharmaceutical development in the treatment of: asthma, renal dysfunction (A_1 AR); neurodegeneration, cancer (A_{2A} AR); cancer, asthma, diabetes (A_{2B} AR); glaucoma, psoriasis, NASH, and ulcerative colitis (A_3 AR). There are not yet therapeutic antibodies proposed for use in blocking ARs.

Various clinical trials of A_1 AR antagonists for treatment of heart failure were eventually discontinued. These A_1 AR antagonists include rolofylline (82,

TABLE 5
Representative adenosine receptor modulators in clinical trials, currently and previously, according to clinicaltrials.gov

Compound	Action	Activity	Phase, National Clinical Trial Number
<i>Agonists</i>			
Adenosine (1)	Nonselective agonist	headache/migraine ADCY5-related dyskinesia	-, 04577443 -, 04469283
Neladenoson bialanate (77, BAY1067197)	A ₁ AR agonist	heart failure	2, 02040233, PARSIFAL 2, 02992288, PANTHEON 2, 03098979, PANACHE
Selodenoson (78, DTI-0009, RG14202)	A ₁ AR agonist	atrial fibrillation	2, 00040001
Trabodenoson (79, INO-8875; PJ-875)	A ₁ AR agonist	glaucoma	3, 02565173
Regadenoson (2, CVT 3146)	A _{2A} AR agonist	sickle cell anemia COVID-19	2, 01788631 1/2, 0460609
Tecadenoson (80, CVT-510)	A _{2A} AR agonist	pulmonary hypertension	-, 02220634
Spongosine (81, BVT.115959)	A _{2A} AR agonist	glioma (disrupting BBB)	1, 03971734
UK-432,097 (11)	A _{2A} AR agonist	atrial fibrillation	2, 00713401
Piclodenoson (48, IB-MECA, CF-101)	A ₃ AR agonist	diabetic nerve pain	2, 00452777
Namodenoson (14, Cl-IB-MECA, CF-102)	A ₃ AR agonist	chronic obstructive pulmonary disease	2, 00430300
		rheumatoid arthritis	3, 02647762
		psoriasis	3, 03168256
		COVID-19	2, 04333472
		hepatocellular carcinoma	2, 02128958
		NASH (non-alcoholic steatohepatitis)	2, 02927314
<i>Antagonists</i>			
Caffeine (3)	Nonselective antagonist	hypoxic-ischemic encephalopathy ADCY5-related dyskinesia	1, 03913221 -, 04469283
		Alzheimer's disease	3, 04570085
		radiation-induced fibrosis	2, 03768492
		glaucoma	-, 03675412
Theophylline (4)	Nonselective antagonist	acute kidney injury	3, 03897335
		smell in COVID-19	2, 04789499
		depression	1, 04309877
		anaesthesia recovery	1, 04151381
Rolofylline (82, KW-3902)	A ₁ AR antagonist	congestive heart failure	3, 00328692 (PROTECT-1) 3, 00354458 (PROTECT-2)
SLV320 (19)	A ₁ AR antagonist	heart failure and renal dysfunction	2, 00744341 2, 00160134
		combined with furosemide	-, 00568009 ^a
PBF-680	A ₁ AR antagonist	asthma	2, 03774290, ADENOASMA
Istradefylline (5, KW-6002)	A _{2A} AR antagonist	Parkinson's disease (alone)	2, 00250393
		Parkinson's disease (with L-dopa)	3, 00955526, 6002-009 3, 01968031
Preladenant (21, MK-3814, SCH 420814)	A _{2A} AR antagonist	Parkinson's disease	3, 01155479, PARADYSE
		antipsychotic drug side effects	2, 00686699, P04628
		advanced solid tumors (alone and in combination with pembrolizumab)	1, 0309916
BIIB014 (65)	A _{2A} AR antagonist	Parkinson's disease	2, NCT00438607
Tozadenant (66)	A _{2A} AR antagonist	Parkinson's disease	3, NCT03051607
Taminadenant (83, NIR178, PBF- 509)	A _{2A} AR antagonist	Parkinson's disease, non-small cell lung cancer (with PDR001b)	1, 02111330 1/2, 02403193, AdenONCO
Ciforadenant (84, CPI-444, V81444)	A _{2A} AR antagonist	various cancers (with PDR001)	3, 03207867
		advanced cancers (in combination with CD73 blocker, CPI-0006)	1, 03454451
		(in combination with PD-L1/PD-1 ^b)	1/2, 03337698
Imaradenant (22, AZD4635, HTL1071)	A _{2A} AR antagonist	cancer, alone	1, 03980821
		(in combination with CD73 blocker, MEDI9447d)	1/2, 03381274
		(in combination with anticancer drugs)	1, 02740985 2, 04089553
Inupadenant (85, EOS100850)	A _{2A} AR antagonist	solid tumors	1, 03873883
Etrumadenant (86, AB928)	A _{2A} /A _{2B} antagonist	various cancers (with AB122 ^b)	1, 03629756
		(in combination with anticancer drugs)	1/2, 04381832
PBF-1129	A _{2B} AR antagonist	non-small cell lung cancer	1, 03274479
PBF-677	A ₃ AR antagonist	glaucoma	1, 02639975
		ulcerative colitis	2, 03773952, ADENOIBD
PBF-1650	A ₃ AR antagonist	psoriasis, NASH	1, 03798236, ADENOIMMUNE
FM101 (87)	A ₃ AR antag/part agonist	glaucoma	1/2, 04585100
		NASH	2, 04710524

Note that this list is not all-inclusive (e.g., dipyrindamole has been omitted). Other compounds are reviewed elsewhere (Borah et al., 2019; Jacobson et al., 2019). Structures, when disclosed, are shown in Figs. 1, 2, 3, and 5.

^aterminated additional enrollment criteria made patient recruitment unfeasible.

^bcheckpoint inhibitor.

^ctriple negative breast cancer, pancreatic ductal adenocarcinoma, non-small cell lung cancer, renal cell cancer, urothelial cancer, head and neck cancer, diffused large B cell lymphoma, microsatellite stable colon cancer, non-Hodgkin lymphoma.

^doleclumab.

KW-3902) for congestive heart failure and SLV320 (19) for renal dysfunction/heart failure.

Initially, when A_{2A} AR antagonists were first reported in the early 1990s, the principal target was PD. Several selective antagonists were in clinical trials, some of which indicated a relatively modest effect, whereas others did not reach statistical significance. Antagonists in this group were tozadenant (66), preladenant (21), and istradefylline (5). A phase 3 trial of tozadenant was discontinued after five fatalities from agranulocytosis had occurred. The caffeine-like antagonist istradefylline was first approved in Japan for use as a cotherapy in treating PD to reduce off-time. Additional clinical evidence of a beneficial effect of istradefylline has accrued, leading to its recent approval in the United States, as mentioned before (Chen and Cunha, 2020).

Currently, the most excitement surrounds the use of A_{2A} AR antagonists as adjuvants in cancer immunotherapy. Adenosine forms an immunosuppressive “cloud” in the tumor microenvironment for both solid malignancies and hematologic cancer (Sek et al., 2018). The adenosine acts through both A_{2A} AR and A_{2B} AR to induce an anti-inflammatory phenotype in T cells, macrophages, and other cells; and antagonists have a beneficial effect when combined with immunotherapy (Congreve et al., 2018). Ongoing clinical trials include: NIR178 (83, PBF-509, now taminadenant) for PD, NSCLC, and various other cancers in combination with a checkpoint inhibitor; CPI-444 (84, formerly V81444, now ciforadenant) for advanced cancers in combination with a checkpoint inhibitor; phase 1/1b study of inupadenant (85, EOS100850) for solid tumors (NCT03873883) (Houthuys et al., 2018); PBF-1129 for non-small cell lung cancer (NSCLC, structure not disclosed); and mixed A_{2A} AR/ A_{2B} AR antagonist AB928 (86, now etrumadenant) for various cancers in combination with checkpoint inhibitor AB122. A_{2A} AR antagonist preladenant (21) has been repurposed from a failed phase 3 trial in PD to treating advanced solid tumors (also in combination with pembrolizumab) but the data did not support study endpoints. A_{2A} AR antagonist AZD4635 (22, formerly HTL1071, now imaradenant) was developed by rational drug design based on A_{2A} AR X-ray structures. Its envisioned application was attention deficit hyperactivity disorder (ADHD), but it is currently being applied to cancer immunotherapy (Borodovsky et al., 2020).

VIII. Concluding Remarks

The number of scientific publications with “adenosine receptor” as a topic in the Web of Science database has remained relatively stable over the years. With close to 4500 articles in each of the two decades covered by this and the previous report, one captures the field as relatively mature and significant. This number is quite comparable to other GPCRs (e.g., ~4000 in the last

decade for serotonin/5-HT and ~6000 for dopamine), more than for histamine receptors (~1000) but fewer than for chemokine receptors (close to 13,000 publications in the last decade). Even a relatively comprehensive report like this one can only pinpoint to some of these many references, however, with a focus on particular topics that have gained traction over the years. We invite readers to draw our attention to other focal points for future inclusion. Such focal points in this report not or hardly covered before are target binding kinetics, receptor structure, and biased signaling. It turns out that high-affinity ligands, be it agonists or antagonists, may have very divergent kinetic profiles. Some have relatively short residence times (defined as $1/k_{off}$) at one or more of the four AR subtypes, whereas others show long residence times, up to several hours. It should be mentioned that many of these studies have been performed at lower than physiologic temperature, which impedes a reliable estimation of in vivo residence times and target engagement. The ultimate in this respect is covalent binding, a feature that is now well established in clinically approved kinase inhibitors but that has not found much application yet in clinical studies of GPCRs. With only one receptor structure discussed in the previous report, remarkable progress has been made, particularly with respect to the A_{2A} AR. By combinations of fusion proteins and thermostabilizing receptor mutations, this receptor has become one of the more easily accessed GPCRs for structure elucidation. Today it has become possible, as with cytosolic proteins, to soak/exchange receptor crystals to enable multiple crystal structures at the same time. On the other hand, the A_{2B} AR and A_3 AR have not been successfully subjected to structure elucidation. Biased signaling has been a hot topic and heavily studied aspect of GPCR signaling in the last decade, but less so for ARs. Compared with the opioid receptor field, for example, most AR agonists appear to display less outspoken preferences, also with a less obvious separation between desired and side effects. Still, the potential of developing adenosine receptor agonists that are biased for particular signaling pathways associated with treatment modalities promises to alleviate the problem of side effects of adenosine agonists, as noted in multiple clinical trials. However, in most cases, the precise G protein-dependent or independent pathways involved in the salutary effects of adenosine agonists are unexplored. Recently, an A_1 AR agonist that was reported to selectively activate G_{ob} versus the other five $G_{\alpha_i/o}$ subtypes and without β -arrestin recruitment was discovered (Wall et al., 2020). It appears to act as a potent analgesic without sedation or cardiorespiratory depression. This can serve as a model for applying biased signaling to other adenosine receptor subtypes. Finally, clinical development takes a long time, and this is also true for AR ligands. Istradefylline

was only recently allowed access to the United States (Food and Drug Administration) and is in the approval process for the European market (European Medicines Agency) for the treatment of motor effects in PD long after its introduction in Japan. It had been reviewed as an early clinical candidate in our 2001 report, whereas in the 2011 update it was again mentioned as a clinical candidate, then in large phase 3 trials. This suggests that there will be room for a fourth update a decade from now.

Acknowledgments

Figure 4 and Supplemental Videos 1 and 2 were assembled and configured by Willem Jespers (Uppsala University, Sweden/Leiden University, The Netherlands), which is gratefully acknowledged.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: IJzerman, Jacobson, Müller, Cronstein, Cunha.

References

- Abo-Salem OM, Hayallah AM, Bilkei-Gorzo A, Filipek B, Zimmer A, and Müller CE (2004) Antinociceptive effects of novel A_{2B} adenosine receptor antagonists. *J Pharmacol Exp Ther* **308**:358–366.
- Abouelkhair MA (2020) Targeting adenosinergic pathway and adenosine A_{2A} receptor signaling for the treatment of COVID-19: a hypothesis. *Med Hypotheses* **144**:110012.
- Akbari A, Khalili-Fomeshi M, Ashrafpour M, Moghadannia AA, and Ghasemi-Kasman M (2018) Adenosine A_{2A} receptor blockade attenuates spatial memory deficit and extent of demyelination areas in lyolecithin-induced demyelination model. *Life Sci* **205**:63–72.
- Alberty RA and Hammes GG (1958) Application of the theory of diffusion-controlled reactions to enzyme kinetics. *J Phys Chem* **62**:154–159.
- Alhersh E, Abushanab D, Al-Shaibi S, and Al-Badriyeh D (2020) Caffeine for the treatment of apnea in the neonatal intensive care unit: a systematic overview of meta-analyses. *Paediatr Drugs* **22**:399–408.
- Allard B, Allard D, Buisseret L, and Stagg J (2020) The adenosine pathway in immuno-oncology. *Nat Rev Clin Oncol* **17**:611–629.
- Alnouri MW, Jepards S, Casari A, Schiedel AC, Hinz S, and Müller CE (2015) Selectivity is species-dependent: characterization of standard agonists and antagonists at human, rat, and mouse adenosine receptors. *Purinergic Signal* **11**:389–407.
- Alsene K, Deckert J, Sand P, and de Wit H (2003) Association between A_{2A} receptor gene polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacology* **28**:1694–1702.
- Amelia T, van Veldhoven JPD, Falsini M, Liu R, Heitman LH, van Westen GJP, Segala E, Verdon G, Cheng RKY, Cooke RM, et al. (2021) Crystal structure and subsequent ligand design of a nonriboside partial agonist bound to the adenosine A_{2A} receptor. *J Med Chem* **64**:3827–3842.
- An S, Kim G, Kim HJ, Ahn S, Kim HY, Ko H, Hyun YE, Nguyen M, Jeong J, Liu Z, et al. (2020) Discovery and structure-activity relationships of novel template, truncated 1'-homologated adenosine derivatives as pure dual PPAR γ / δ modulators. *J Med Chem* **63**:16012–16027.
- Arab L, Biggs ML, O'Meara ES, Longstreth WT, Crane PK, and Fitzpatrick AL (2011) Gender differences in tea, coffee, and cognitive decline in the elderly: the Cardiovascular Health Study. *J Alzheimers Dis* **27**:553–566.
- Aasaoka N, Nishitani N, Kinoshita H, Nagai Y, Hatakama H, Nagayasu K, Shirakawa H, Nakagawa T and Kaneko S (2019) An adenosine A_{2A} receptor antagonist improves multiple symptoms of repeated quinpirole-induced psychosis. *eNeuro* **6**:0366-18.2019.
- Auchampach JA, Gizewski ET, Wan TC, de Castro S, Brown Jr GG, and Jacobson KA (2010) Synthesis and pharmacological characterization of [(125)I]MRS5127, a high affinity, selective agonist radioligand for the A₃ adenosine receptor. *Biochem Pharmacol* **79**:967–973.
- Augusto E, Matos M, Sévigny J, El-Tayeb A, Bynoe MS, Müller CE, Cunha RA, and Chen JF (2013) Ecto-5'-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A_{2A} receptor functions. *J Neurosci* **33**:11390–11399.
- Baltos JA, Paoletta S, Nguyen AT, Gregory KJ, Tosh DK, Christopoulos A, Jacobson KA, and May LT (2016) Structure-activity analysis of biased agonism at the human adenosine A₃ receptor. *Mol Pharmacol* **90**:12–22.
- Bar-Yehuda S, Rath-Wolfson L, Del Valle L, Ochaion A, Cohen S, Patoka R, Zozulya G, Barer F, Atar E, Piña-Oviedo S, et al. (2009) Induction of an antiinflammatory effect and prevention of cartilage damage in rat knee osteoarthritis by CF101 treatment. *Arthritis Rheum* **60**:3061–3071.
- Baraldi PG, Cacciari B, Moro S, Romagnoli R, Ji Xd, Jacobson KA, Gessi S, Borea PA, and Spalluto G (2001) Fluorosulfonyl- and bis-(beta-chloroethyl)amino-phenylamino functionalized pyrazolo[4,3-e][1,2,4-triazolo[1,5-c]pyrimidine derivatives: irreversible antagonists at the human A₃ adenosine receptor and molecular modeling studies. *J Med Chem* **44**:2735–2742.
- Baraldi PG, Preti D, Borea PA, and Varani K (2012) Medicinal chemistry of A₃ adenosine receptor modulators: pharmacological activities and therapeutic implications. *J Med Chem* **55**:5676–5703.
- Baraldi PG, Tabrizi MA, Preti D, Bovero A, Fruttarolo F, Romagnoli R, Moorman AR, Gessi S, Merighi S, Varani K, et al. (2004) [3H]-MRE 2029-F20, a selective antagonist radioligand for the human A_{2B} adenosine receptors. *Bioorg Med Chem Lett* **14**:3607–3610.
- Barnes PJ (2003) Theophylline: new perspectives for an old drug. *Am J Respir Crit Care Med* **167**:813–818.
- Barresi E, Giacomelli C, Marchetti L, Baglini E, Salerno S, Greco G, Da Settimo F, Martini C, Trincavelli ML, and Taliani S (2021) Novel positive allosteric modulators of A_{2B} adenosine receptor acting as bone mineralisation promoters. *J Enzyme Inhib Med Chem* **36**:286–294.
- Barrington WW, Jacobson KA, and Stiles GL (1990) Glycoprotein nature of the A₂-adenosine receptor binding subunit. *Mol Pharmacol* **38**:177–183.
- Batalha VL, Pego JM, Fontinha BM, Costenla AR, Valadas JS, Baqi Y, Radjainia H, Müller CE, Sebastião AM, and Lopes LV (2013) Adenosine A_{2A}(2A) receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. *Mol Psychiatry* **18**:320–331.
- Batyuk A, Galli L, Ishchenko A, Han GW, Gati C, Popov PA, Lee MY, Stauch B, White TA, Barty A, et al. (2016) Native phasing of x-ray free-electron laser data for a G protein-coupled receptor. *Sci Adv* **2**:e1600292.
- Beaughole AR, Baker SP, and Scammells PJ (2000) Fluorosulfonyl-substituted xanthines as selective irreversible antagonists for the A₁(1)-adenosine receptor. *J Med Chem* **43**:4973–4980.
- Beavis PA, Henderson MA, Giuffrida L, Mills JK, Sek K, Cross RS, Davenport AJ, John LB, Mardiana S, Slaney CY, et al. (2017) Targeting the adenosine 2A receptor enhances chimeric antigen receptor T cell efficacy. *J Clin Invest* **127**:929–941.
- Beavis PA, Milenkovski N, Henderson MA, John LB, Allard B, Loi S, Kershaw MH, Stagg J, and Darcy PK (2015) Adenosine receptor 2A blockade increases the efficacy of anti-PD-1 through enhanced antitumor T-cell responses. *Cancer Immunol Res* **3**:506–517.
- Bertarelli DC, Diekmann M, Hayallah AM, Rüsing D, Iqbal J, Preiss B, Verspohl EJ, and Müller CE (2006) Characterization of human and rodent native and recombinant adenosine A_{2B} receptors by radioligand binding studies. *Purinergic Signal* **2**:559–71 DOI: <https://doi.org/10.1007/s11302-006-9012-4>.
- Bessa-Gonçalves M, Bragança B, Martins-Dias E, Correia-de-Sá P, and Fontes-Sousa AP (2018) Is the adenosine A_{2B} 'biased' receptor a valuable target for the treatment of pulmonary arterial hypertension? *Drug Discov Today* **23**:1285–1292.
- Beste C, Stock AK, Ness V, Epplen JT, and Arning L (2012) Differential effects of ADORA2A gene variations in pre-attentive visual sensory memory subprocesses. *Eur Neuropharmacol* **22**:555–561.
- Blay J, White TD, and Hoskin DW (1997) The extracellular fluid of solid carcinomas contains immunosuppressive concentrations of adenosine. *Cancer Res* **57**:2602–2605.
- Bleickardt CJ, Kazdoba TM, Jones NT, Hunter JC, and Hodgson RA (2014) Antagonism of the adenosine A_{2A} receptor attenuates akathisia-like behavior induced with MP-10 or aripiprazole in a novel non-human primate model. *Pharmacol Biochem Behav* **118**:36–45.
- Bocquet N, Kohler J, Hug MN, Kusznir EA, Rufer AC, Dawson RJ, Hennig M, Ruf A, Huber W, and Huber S (2015) Real-time monitoring of binding events on a thermostabilized human A_{2A} receptor embedded in a lipid bilayer by surface plasmon resonance. *Biochim Biophys Acta* **1848**:1224–1233.
- Boia R, Elvas F, Madeira MH, Aires ID, Rodrigues-Neves AC, Tralhão P, Szabó EC, Baqi Y, Müller CE, Tomé AR, et al. (2017) Treatment with A_{2A} receptor antagonist KW6002 and caffeine intake regulate microglia reactivity and protect retina against transient ischemic damage. *Cell Death Dis* **8**:e3065.
- Boison D and Yegutkin GG (2019) Adenosine metabolism: emerging concepts for cancer therapy. *Cancer Cell* **36**:582–596.
- Borah P, Deka S, Mailavaram RP, and Deb PK (2019) P1 receptor agonists/antagonists in clinical trials - potential drug candidates of the future. *Curr Pharm Des* **25**:2792–2807.
- Borea PA, Gessi S, Merighi S, Vincenzi F, and Varani K (2018) Pharmacology of adenosine receptors: the state of the art. *Physiol Rev* **98**:1591–1625.
- Borea PA, Varani K, Vincenzi F, Baraldi PG, Tabrizi MA, Merighi S, and Gessi S (2015) The A₃ adenosine receptor: history and perspectives. *Pharmacol Rev* **67**:74–102.
- Boring DL, Ji XD, Zimmel J, Taylor KE, Stiles GL, and Jacobson KA (1991) Trifunctional agents as a design strategy for tailoring ligand properties: irreversible inhibitors of A₁ adenosine receptors. *Bioconjug Chem* **2**:77–88.
- Borodovsky A, Barbon CM, Wang Y, Ye M, Prickett L, Chandra D, Shaw J, Deng N, Sachsenmeier K, Clarke JD, et al. (2020) Small molecule AZD4635 inhibitor of A_{2A}R signaling rescues immune cell function including CD103⁺ dendritic cells enhancing anti-tumor immunity. *J Immunother Cancer* **8**:e000417.
- Borrmann T, Hinz S, Bertarelli DC, Li W, Florin NC, Scheiff AB, and Müller CE (2009) 1-alkyl-8-(piperazine-1-sulfonyl)phenylxanthines: development and characterization of adenosine A_{2B} receptor antagonists and a new radioligand with subnanomolar affinity and subtype specificity. *J Med Chem* **52**:3994–4006.
- Borroto-Escuela DO, Wydra K, Filip M, and Fuxe K (2018) A_{2A}R-D₂R Heteroreceptor complexes in cocaine reward and addiction. *Trends Pharmacol Sci* **39**:1008–1020.
- Bouzo-Lorenzo M, Stoddart LA, Xia L, IJzerman AP, Heitman LH, Briddon SJ, and Hill SJ (2019) A live cell NanoBRET binding assay allows the study of ligand-binding kinetics to the adenosine A₃ receptor. *Purinergic Signal* **15**:139–153.
- Bozic KJ, Kurtz SM, Lau E, Ong K, Chiu V, Vail TP, Rubash HE, and Berry DJ (2010) The epidemiology of revision total knee arthroplasty in the United States. *Clin Orthop Relat Res* **468**:45–51.
- Broecker J, Morizumi T, Ou WL, Klingel V, Kuo A, Kissick DJ, Ishchenko A, Lee MY, Xu S, Makarov O, et al. (2018) High-throughput in situ X-ray screening of

- and data collection from protein crystals at room temperature and under cryogenic conditions. *Nat Protoc* **13**:260–292.
- Campos-Martins A, Bragança B, Correia-de-Sá P, and Fontes-Sousa AP (2021) Pharmacological tuning of adenosine signal nuances underlying heart failure with preserved ejection fraction. *Front Pharmacol* **12**:724320.
- Canas PM, Porciuncula LO, Cunha GM, Silva CG, Machado NJ, Oliveira JM, Oliveira CR, and Cunha RA (2009) Adenosine A_{2A} receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway. *J Neurosci* **29**:14741–14751.
- Canas PM, Porciuncula LO, Simoes AP, Augusto E, Silva HB, Machado NJ, Gonçalves N, Alfaro TM, Gonçalves FQ, Araujo IM, Real JJ, Coelho JE, Andrade GM, Almeida RD, Chen JF, Kofalvi A, Agostinho P and Cunha RA (2018) Neuronal adenosine a_{2a} receptors are critical mediators of neurodegeneration triggered by convulsions. *eNeuro* **5**:0385-18.2018.
- Carmo M, Gonçalves FQ, Canas PM, Oses JP, Fernandes FD, Duarte FV, Palmeira CM, Tomé AR, Agostinho P, Andrade GM, et al. (2019) Enhanced ATP release and CD73-mediated adenosine formation sustain adenosine A_{2A} receptor over-activation in a rat model of Parkinson's disease. *Br J Pharmacol* **176**:3666–3680.
- Carpenter B, Nehmé R, Warne T, Leslie AG, and Tate CG (2016) Structure of the adenosine A(2A) receptor bound to an engineered G protein. *Nature* **536**:104–107.
- Carugo O and Bordo D (1999) How many water molecules can be detected by protein crystallography? *Acta Crystallogr D Biol Crystallogr* **55**:479–483.
- Carvalho K, Faivre E, Pietrowski MJ, Marques X, Gomez-Murcia V, Deleau A, Huin V, Hansen JN, Kozlov S, Danis C, et al.; NeuroCEB Brain Bank (2019) Exacerbation of C1q dysregulation, synaptic loss and memory deficits in tau pathology linked to neuronal adenosine A_{2A} receptor. *Brain* **142**:3636–3654.
- Ceska T, Chung CW, Cooke R, Phillips C, and Williams PA (2019) Cryo-EM in drug discovery. *Biochem Soc Trans* **47**:281–293.
- Chen JF and Cunha RA (2020) The belated US FDA approval of the adenosine A_{2A} receptor antagonist istradefylline for treatment of Parkinson's disease. *Purinergic Signal* **16**:167–174.
- Chen PZ, He WJ, Zhu ZR, E GJ, Xu G, Chen DW, and Gao YQ (2018) Adenosine A_{2A} receptor involves in neuroinflammation-mediated cognitive decline through activating microglia under acute hypobaric hypoxia. *Behav Brain Res* **347**:99–107.
- Cheng RKY, Segala E, Robertson N, Defforian F, Dore AS, Errey JC, Fiez-Vandal C, Marshall FH and Cooke RM (2017) Structures of human A1 and A2A adenosine receptors with xanthines reveal determinants of selectivity. *Structure* **25**:1275–1285.e4.
- Cheng Y (2018) Single-particle cryo-EM-How did it get here and where will it go. *Science* **361**:876–880.
- Choca JI, Kwatra MM, Hoseney MM, and Green RD (1985) Specific photoaffinity labelling of inhibitory adenosine receptors. *Biochem Biophys Res Commun* **131**:115–121.
- Cognato GP, Agostinho PM, Hockemeyer J, Müller CE, Souza DO, and Cunha RA (2010) Caffeine and an adenosine A(2A) receptor antagonist prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life. *J Neurochem* **112**:453–462.
- Congreve M, Andrews SP, Doré AS, Hollenstein K, Hurrell E, Langmead CJ, Mason JS, Ng JW, Tehan B, Zhukov A, et al. (2012) Discovery of 1,2,4-triazine derivatives as adenosine A(2A) antagonists using structure based drug design. *J Med Chem* **55**:1898–1903.
- Congreve M, Brown GA, Borodovsky A, and Lamb ML (2018) Targeting adenosine A_{2A} receptor antagonism for treatment of cancer. *Expert Opin Drug Discov* **13**:997–1003.
- Copeland RA (2016) The drug-target residence time model: a 10-year retrospective. *Nat Rev Drug Discov* **15**:87–95.
- Copeland RA, Pompliano DL, and Meek TD (2006) Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov* **5**:730–739.
- Corciulo C, Lendhey M, Wilder T, Schoen H, Cornelissen AS, Chang G, Kennedy OD, and Cronstein BN (2017) Endogenous adenosine maintains cartilage homeostasis and exogenous adenosine inhibits osteoarthritis progression. *Nat Commun* **8**:15019.
- Corciulo C, Wilder T, and Cronstein BN (2016) Adenosine A2B receptors play an important role in bone homeostasis. *Purinergic Signal* **12**:537–547.
- Correale P, Caracciolo M, Bilotta F, Conte M, Cuzzola M, Falcone C, Mangano C, Falzea AC, Iuliano E, Morabito A, et al. (2020) Therapeutic effects of adenosine in high flow 21% oxygen aerosol in patients with Covid19-pneumonia. *PLoS One* **15**:e0239692.
- Costenla AR, Diógenes MJ, Canas PM, Rodrigues RJ, Nogueira C, Maroco J, Agostinho PM, Ribeiro JA, Cunha RA, and de Mendonça A (2011) Enhanced role of adenosine A(2A) receptors in the modulation of LTP in the rat hippocampus upon ageing. *Eur J Neurosci* **34**:12–21.
- Cronstein BN, Kramer SB, Weissmann G, and Hirschhorn R (1983) Adenosine: a physiological modulator of superoxide anion generation by human neutrophils. *J Exp Med* **158**:1160–1177.
- Cronstein BN and Sitkovsky M (2017) Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. *Nat Rev Rheumatol* **13**:41–51.
- Crooke A, Martínez-Henández J, Martínez-López J, Cruz-Jentoft A, Huetter-Toral F, and Pintor J (2017) Low expression of CD39 and CD73 genes in centenarians compared with octogenarians. *Immun Ageing* **14**:11.
- Cunha RA (2001) Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. *Neurochem Int* **38**:107–125.
- Cunha RA (2005) Neuroprotection by adenosine in the brain: from A(1) receptor activation to A(2A) receptor blockade. *Purinergic Signal* **1**:111–134.
- Cunha RA (2016) How does adenosine control neuronal dysfunction and neurodegeneration? *J Neurochem* **139**:1019–1055.
- Cunha RA, Constantino MD, Fonseca E, and Ribeiro JA (2001) Age-dependent decrease in adenosine A1 receptor binding sites in the rat brain. Effect of cis unsaturated free fatty acids. *Eur J Biochem* **268**:2939–2947.
- Cunha RA, Constantino MD, and Ribeiro JA (1999) G protein coupling of CGS 21680 binding sites in the rat hippocampus and cortex is different from that of adenosine A1 and striatal A2A receptors. *Naunyn Schmiedebergs Arch Pharmacol* **359**:295–302.
- Cunha RA, Johansson B, Constantino MD, Sebastião AM, and Fredholm BB (1996) Evidence for high-affinity binding sites for the adenosine A_{2A} receptor agonist [3H] CGS 21680 in the rat hippocampus and cerebral cortex that are different from striatal A_{2A} receptors. *Naunyn Schmiedebergs Arch Pharmacol* **353**:261–271.
- Dahl G and Akerud T (2013) Pharmacokinetics and the drug-target residence time concept. *Drug Discov Today* **18**:697–707.
- Daly JW (2007) Caffeine analogs: biomedical impact. *Cell Mol Life Sci* **64**:2153–2169.
- Daryaei F and Tonge PJ (2019) Pharmacokinetic-pharmacodynamic models that incorporate drug-target binding kinetics. *Curr Opin Chem Biol* **50**:120–127.
- de Witte WEA, Danhof M, van der Graaf PH, and de Lange ECM (2016) In vivo target residence time and kinetic selectivity: the association rate constant as determinant. *Trends Pharmacol Sci* **37**:831–842.
- Deckert J, Nöthen MM, Franke P, Delmo C, Fritze J, Knapp M, Maier W, Beckmann H, and Propping P (1998) Systematic mutation screening and association study of the A1 and A2a adenosine receptor genes in panic disorder suggest a contribution of the A2a gene to the development of disease. *Mol Psychiatry* **3**:81–85.
- Di Liberto V, Mudò G, Garozzo R, Frinchi M, Fernandez-Dueñas V, Di Iorio P, Ciccarelli R, Caciagli F, Condorelli DF, Ciruela F, et al. (2016) The guanine-based purinergic system: the tale of an orphan neuromodulation. *Front Pharmacol* **7**:158.
- Dionisotti S, Ongini E, Zocchi C, Kull B, Arslan G, and Fredholm BB (1997) Characterization of human A_{2A} adenosine receptors with the antagonist radioligand [3H]-SCH 58261. *Br J Pharmacol* **121**:353–360.
- Dong X, Li S, Sun J, Li Y, and Zhang D (2020) Association of coffee, decaffeinated coffee and caffeine intake from coffee with cognitive performance in older adults: National Health and Nutrition Examination Survey (NHANES) 2011–2014. *Nutrients* **12**:840.
- Dore AS, Robertson N, Errey JC, Ng I, Hollenstein K, Tehan B, Hurrell E, Bennett K, Congreve M, Magnani F, Tate CG, Weir M and Marshall FH (2011) Structure of the adenosine A(2A) receptor in complex with ZM241385 and the xanthines XAC and caffeine. *Structure* **19**:1283–1293.
- Draper-Joyce CJ, Bhola R, Wang J, Bhattarai A, Nguyen ATN, Cowie-Kent I, O'Sullivan K, Chia LY, Venugopal H, Valant C, et al. (2021) Positive allosteric mechanisms of adenosine A₁ receptor-mediated analgesia. *Nature* **597**:571–576.
- Draper-Joyce CJ, Khoshouei M, Thal DM, Liang YL, Nguyen ATN, Furness SGB, Venugopal H, Baltos JA, Plitzko JM, Danev R, et al. (2018) Structure of the adenosine-bound human adenosine A₁ receptor-G_i complex. *Nature* **558**:559–563.
- Du L, Gao ZG, Paoletta S, Wan TC, Giewzski ET, Barbour S, van Veldhoven JPD, IJzerman AP, Jacobson KA, and Auchampach JA (2018) Species differences and mechanism of action of A₃ adenosine receptor allosteric modulators. *Purinergic Signal* **14**:59–71.
- Duarte JM, Agostinho PM, Carvalho RA, and Cunha RA (2012) Caffeine consumption prevents diabetes-induced memory impairment and synaptotoxicity in the hippocampus of NONcZNO10/LTJ mice. *PLoS One* **7**:e21899.
- Earl CQ, Patel A, Craig RH, Daluge SM, and Linden J (1988) Photoaffinity labeling adenosine A1 receptors with an antagonist 125I-labeled aryl azide derivative of 8-phenylxanthine. *J Med Chem* **31**:752–756.
- Eckle T, Krahn T, Grenz A, Köhler D, Mittelbronn M, Ledent C, Jacobson MA, Osswald H, Thompson LF, Unertl K, et al. (2007) Cardioprotection by ecto-5'-nucleotidase (CD73) and A2B adenosine receptors. *Circulation* **115**:1581–1590.
- Eddy MT, Gao ZG, Mannes P, Patel N, Jacobson KA, Katritch V, Stevens RC, and Wüthrich K (2018a) Extrinsic tryptophans as NMR probes of allosteric coupling in membrane proteins: application to the A_{2A} adenosine receptor. *J Am Chem Soc* **140**:8228–8235.
- Eddy MT, Lee MY, Gao ZG, White KL, Didenko T, Horst R, Audet M, Stanczak P, McClary KM, Han GW, et al. (2018b) Allosteric coupling of drug binding and intracellular signaling in the A_{2A} adenosine receptor. *Cell* **172**:68–80.e12.
- Eddy MT, Martin BT and Wüthrich K (2021) A(2A) adenosine receptor partial agonism related to structural rearrangements in an activation microswitch. *Structure* **29**:170–176.e3.
- El Maatougui A, Azuaje J, González-Gómez M, Miguez G, Crespo A, Carbajales C, Escalante L, García-Mera X, Gutiérrez-de-Terán H, and Sotelo E (2016) Discovery of potent and highly selective A2B adenosine receptor antagonist chemotypes. *J Med Chem* **59**:1967–1983.
- Elzein E, Kalla RV, Li X, Perry T, Gimbel A, Zeng D, Lustig D, Leung K, and Zablocki J (2008) Discovery of a novel A2B adenosine receptor antagonist as a clinical candidate for chronic inflammatory airway diseases. *J Med Chem* **51**:2267–2278.
- Errey JC, Doré AS, Zhukov A, Marshall FH, and Cooke RM (2015) Purification of stabilized GPCRs for structural and biophysical analyses. *Methods Mol Biol* **1335**:1–15.
- Eskelinen MH, Ngandu T, Tuomilehto J, Soininen H, and Kivipelto M (2009) Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study. *J Alzheimers Dis* **16**:85–91.
- Evans J, Richards JR, and Battisti AS (2020) Caffeine, in *StatPearls*, StatPearls Publishing LLC, Treasure Island, FL.
- Faivre E, Coelho JE, Zornbach K, Malik E, Baqi Y, Schneider M, Cellai L, Carvalho K, Sebda S, Figeac M, et al. (2018) Beneficial effect of a selective adenosine A_{2A} receptor antagonist in the APPsw/PS1dE9 mouse model of Alzheimer's disease. *Front Mol Neurosci* **11**:235.
- Falcone C, Caracciolo M, Correale P, Macheda S, Vadalà EG, La Scala S, Tescione M, Danieli R, Ferrarelli A, Tarsitano MG, et al. (2020) Can adenosine fight COVID-19 acute respiratory distress syndrome? *J Clin Med* **9**:3045.

- Fan KQ, Li YY, Wang HL, Mao XT, Guo JX, Wang F, Huang LJ, Li YN, Ma XY, Gao ZJ, et al. (2019) Stress-induced metabolic disorder in peripheral CD4⁺ T cells leads to anxiety-like behavior. *Cell* **179**:864–879.e19.
- Fenalti G, Giguere PM, Katritch V, Huang XP, Thompson AA, Cherezov V, Roth BL, and Stevens RC (2014) Molecular control of δ -opioid receptor signalling. *Nature* **506**:191–196.
- Ferré S (2016) Mechanisms of the psychostimulant effects of caffeine: implications for substance use disorders. *Psychopharmacology (Berl)* **233**:1963–1979.
- Ferré S and Ciruela F (2019) Functional and neuroprotective role of striatal adenosine A_{2A} receptor heterotetramers. *J Caffeine Adenosine Res* **9**:89–97.
- Figueiredo AB, de Oliveira E Castro RA, Nogueira-Paiva NC, Moreira F, Gonçalves FQ, Soares RP, Castro-Borges W, Silva GG, Cunha RA, Gonçalves T, et al. (2021) Clustering of adenosine A_{2B} receptors with ectonucleotidases in caveolin-rich lipid rafts underlies immunomodulation by *Leishmania amazonensis*. *FASEB J* **35**:e21509.
- Flögel U, Burghoff S, van Lent PL, Temme S, Galbarz L, Ding Z, El-Tayeb A, Huels S, Bönner F, Borg N, et al. (2012) Selective activation of adenosine A_{2A} receptors on immune cells by a CD73-dependent prodrug suppresses joint inflammation in experimental rheumatoid arthritis. *Sci Transl Med* **4**:146ra108.
- Franchetti P, Cappellacci L, Vita P, Petrelli R, Lavecchia A, Kachler S, Klotz KN, Marabese I, Luongo L, Maione S, et al. (2009) N6-cycloalkyl- and N6-bicycloalkyl-C5'(C2')-modified adenosine derivatives as high-affinity and selective agonists at the human A₁ adenosine receptor with antinociceptive effects in mice. *J Med Chem* **52**:2393–2406.
- Franco R, Córdomi A, Llinas Del Torrent C, Lillo A, Serrano-Marin J, Navarro G, and Pardo L (2021a) Structure and function of adenosine receptor heteromers. *Cell Mol Life Sci* **78**:3957–3968.
- Franco R, Rivas-Santisteban R, Reyes-Resina I, and Navarro G (2021b) The old and new visions of biased agonism through the prism of adenosine receptor signaling and receptor/receptor and receptor/protein interactions. *Front Pharmacol* **11**:628601.
- Fraportti TT, Contini V, Tovo-Rodrigues L, Recamonde-Mendoza M, Rovaris DL, Rohde LA, Hutz MH, Salatino-Oliveira A, and Genro JP (2019) Synergistic effects between ADORA2A and DRD2 genes on anxiety disorders in children with ADHD. *Prog Neuropsychopharmacol Biol Psychiatry* **93**:214–220.
- Fredholm BB, Bättig K, Holmén J, Nehlig J, and Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* **51**:83–133.
- Fredholm BB, Chen JF, Cunha RA, Svenningsson P, and Vaugeois JM (2005) Adenosine and brain function. *Int Rev Neurobiol* **63**:191–270.
- Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, and Linden J (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* **53**:527–552.
- Fredholm BB, IJzerman AP, Jacobson KA, Linden J, and Müller CE (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. *Pharmacol Rev* **63**:1–34.
- Fredholm BB, Frenguelli BG, Hills R, IJzerman AP, Jacobson KA, Klotz KN, Linden J, Müller CE, Schwabe U, Stiles GL (2021). Adenosine receptors in GtoPdb v.2021.2. *IUPHAR/BPS Guide to Pharmacology CITE 2021* DOI: <https://doi.org/10.2218/gtopdb/F3/2021.2>.
- Freitag CM, Agelopoulos K, Huy E, Rothermundt M, Krakowitzky P, Meyer J, Deckert J, von Gontard A, and Hohoff C (2010) Adenosine A_{2A} receptor gene (ADORA2A) variants may increase autistic symptoms and anxiety in autism spectrum disorder. *Eur Child Adolesc Psychiatry* **19**:67–74.
- Fuchs JL (1991) 5'-Nucleotidase activity increases in aging rat brain. *Neurobiol Aging* **12**:523–530.
- Fujioka M and Omori N (2012) Subtleities in GPCR drug discovery: a medicinal chemistry perspective. *Drug Discov Today* **17**:1133–1138.
- Furman D, Chang J, Lartigue L, Bolen CR, Haddad F, Gaudilliere B, Ganio EA, Fragiadakis GK, Spitzer MH, Douchet I, et al. (2017) Expression of specific inflammasome gene modules stratifies older individuals into two extreme clinical and immunological states. *Nat Med* **23**:174–184.
- Gao AW, Smith RL, van Weeghel M, Kamble R, Janssens GE, and Houtkooper RH (2018a) Identification of key pathways and metabolic fingerprints of longevity in *C. elegans*. *Exp Gerontol* **113**:128–140.
- Gao ZG, Balasubramanian R, Kiselev E, Wei Q, and Jacobson KA (2014) Probing biased/partial agonism at the G protein-coupled A_{2B} adenosine receptor. *Biochem Pharmacol* **90**:297–306.
- Gao ZG, Inoue A, and Jacobson KA (2018b) On the G protein-coupling selectivity of the native A_{2B} adenosine receptor. *Biochem Pharmacol* **151**:201–213.
- Gao ZG and Jacobson KA (2008) Translocation of arrestin induced by human A₃ adenosine receptor ligands in an engineered cell line: comparison with G protein-dependent pathways. *Pharmacol Res* **57**:303–311.
- Gao ZG and Jacobson KA (2013) Allosteric modulation and functional selectivity of G protein-coupled receptors. *Drug Discov Today Technol* **10**:e237–e243.
- Gao ZG, Van Muijlwijk-Koezen JE, Chen A, Müller CE, IJzerman AP, and Jacobson KA (2001) Allosteric modulation of A₃ adenosine receptors by a series of 3-(2-pyridinyl)isoquinoline derivatives. *Mol Pharmacol* **60**:1057–1063.
- Gao ZG, Verzijl D, Zweemer A, Ye K, Göblyös A, IJzerman AP, and Jacobson KA (2011) Functionally biased modulation of A₃ adenosine receptor agonist efficacy and potency by imidazoquinolinamide allosteric enhancers. *Biochem Pharmacol* **82**:658–668.
- García-Nafria J, Lee Y, Bai X, Carpenter B, and Tate CG (2018) Cryo-EM structure of the adenosine A_{2A} receptor coupled to an engineered heterotrimeric G protein. *eLife* **7**:e35946.
- Gelber RP, Petrovitch H, Masaki KH, Ross GW, and White LR (2011) Coffee intake in midlife and risk of dementia and its neuropathologic correlates. *J Alzheimers Dis* **23**:607–615.
- Gessi S, Bencivenni S, Battistello E, Vincenzi F, Colotta V, Catarzi D, Varano F, Merighi S, Borea PA, and Varani K (2017) Inhibition of A_{2A} adenosine receptor signaling in cancer cells proliferation by the novel antagonist TP455. *Front Pharmacol* **8**:888.
- Giblet ER, Anderson JE, Cohen F, Pollara B, and Meuwissen HJ (1972) Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet* **2**:1067–1069.
- Glukhova A, Thal DM, Nguyen AT, Vecchio EA, Jörg M, Scammells PJ, May LT, Sexton PM, and Christopoulos A (2017) Structure of the adenosine A₁ receptor reveals the basis for subtype selectivity. *Cell* **168**:867–877.e13.
- Gnad T, Navarro G, Lahesmaa M, Reverte-Salisa L, Copperi F, Cordomi A, Naumann J, Hochhäuser A, Haufs-Brusberg S, Wenzel D, et al. (2020) Adenosine/A_{2B} receptor signaling ameliorates the effects of aging and counteracts obesity. *Cell Metab* **32**:56–70.e7.
- Göblyös A and IJzerman AP (2011) Allosteric modulation of adenosine receptors. *Biochim Biophys Acta* **1808**:1309–1318.
- Gonçalves FQ, Lopes JP, Silva HB, Lemos C, Silva AC, Gonçalves N, Tomé AR, Ferreira SG, Canas PM, Rial D, et al. (2019) Synaptic and memory dysfunction in a β -amyloid model of early Alzheimer's disease depends on increased formation of ATP-derived extracellular adenosine. *Neurobiol Dis* **132**:104570.
- Gonçalves N, Simões AT, Cunha RA, and de Almeida LP (2013) Caffeine and adenosine A_{2A} receptor inactivation decrease striatal neuropathology in a lentiviral-based model of Machado-Joseph disease. *Ann Neurol* **73**:655–666.
- Gonçalves N, Simões AT, Prediger RD, Hirai H, Cunha RA, and Pereira de Almeida L (2017) Caffeine alleviates progressive motor deficits in a transgenic mouse model of spinocerebellar ataxia. *Ann Neurol* **81**:407–418.
- Gracia E, Farré D, Cortés A, Ferrer-Costa C, Orozco M, Mallol J, Lluís C, Canela EI, McCormick PJ, Franco R, et al. (2013) The catalytic site structural gate of adenosine deaminase allosterically modulates ligand binding to adenosine receptors. *FASEB J* **27**:1048–1061.
- Grishammer R (2017) New approaches towards the understanding of integral membrane proteins: A structural perspective on G protein-coupled receptors. *Protein Sci* **26**:1493–1504.
- Grosso G, Micek A, Castellano S, Pajak A, and Galvano F (2016) Coffee, tea, caffeine and risk of depression: a systematic review and dose-response meta-analysis of observational studies. *Mol Nutr Food Res* **60**:223–234.
- Guixà-González R, Albasanz JL, Rodríguez-Espigares I, Pastor M, Sanz F, Martí-Solano M, Manna M, Martínez-Seara H, Hildebrand PW, Martín M, et al. (2017) Membrane cholesterol access into a G-protein-coupled receptor. *Nat Commun* **8**:14505.
- Guo D, Heitman LH, and IJzerman AP (2015) *Importance of Drug Target Residence Time at G Protein-Coupled Receptors*, Wiley-VCH Verlag GmbH & Co., Weinheim, Germany.
- Guo D, Heitman LH, and IJzerman AP (2016a) The added value of assessing ligand-receptor binding kinetics in drug discovery. *ACS Med Chem Lett* **7**:819–821.
- Guo D, Heitman LH, and IJzerman AP (2017) Kinetic aspects of the interaction between ligand and G protein-coupled receptor: the case of the adenosine receptors. *Chem Rev* **117**:38–66.
- Guo D, Hillger JM, IJzerman AP, and Heitman LH (2014a) Drug-target residence time—a case for G protein-coupled receptors. *Med Res Rev* **34**:856–892.
- Guo D, Mulder-Krieger T, IJzerman AP, and Heitman LH (2012) Functional efficacy of adenosine A_{2A} receptor agonists is positively correlated to their receptor residence time. *Br J Pharmacol* **166**:1846–1859.
- Guo D, Pan AC, Dror RO, Mocking T, Liu R, Heitman LH, Shaw DE, and IJzerman AP (2016b) Molecular basis of ligand dissociation from the adenosine A_{2A} receptor. *Mol Pharmacol* **89**:485–491.
- Guo D, van Dorp EJ, Mulder-Krieger T, van Veldhoven JP, Brussee J, IJzerman AP, and Heitman LH (2013) Dual-point competition association assay: a fast and high-throughput kinetic screening method for assessing ligand-receptor binding kinetics. *J Biomol Screen* **18**:309–320.
- Guo D, Venhorst SN, Massink A, van Veldhoven JP, Vauquelin G, IJzerman AP, and Heitman LH (2014b) Molecular mechanism of allosteric modulation at GPCRs: insight from a binding kinetics study at the human A₁ adenosine receptor. *Br J Pharmacol* **171**:5295–5312.
- Guo D, Xia L, van Veldhoven JP, Hazeu M, Mocking T, Brussee J, IJzerman AP, and Heitman LH (2014c) Binding kinetics of ZM241385 derivatives at the human adenosine A_{2A} receptor. *ChemMedChem* **9**:752–761.
- Halldner L, Lopes LV, Daré E, Lindström K, Johansson B, Ledent C, Cunha RA, and Fredholm BB (2004) Binding of adenosine receptor ligands to brain of adenosine receptor knock-out mice: evidence that CGS 21680 binds to A₁ receptors in hippocampus. *Naunyn Schmiedeberg's Arch Pharmacol* **370**:270–278.
- Hameleers PA, Van Boxtel MP, Hogervorst E, Riedel WJ, Houx PJ, Buntinx F, and Jolles J (2000) Habitual caffeine consumption and its relation to memory, attention, planning capacity and psychomotor performance across multiple age groups. *Hum Psychopharmacol* **15**:573–581.
- Hamilton SP, Slager SL, De Leon AB, Heiman GA, Klein DF, Hodge SE, Weissman MM, Fyer AJ, and Knowles JA (2004) Evidence for genetic linkage between a polymorphism in the adenosine 2A receptor and panic disorder. *Neuropsychopharmacology* **29**:558–565.
- Härter M, Kalthof B, Delbeck M, Lustig K, Gerisch M, Schulz S, Kast R, Meibom D, and Lindner N (2019) Novel non-xanthine antagonist of the A_{2B} adenosine receptor: from HTS hit to lead structure. *Eur J Med Chem* **163**:763–778.
- Hatfield SM and Sitkovsky M (2016) A_{2A} adenosine receptor antagonists to weaken the hypoxia-HIF-1 α driven immunosuppression and improve immunotherapies of cancer. *Curr Opin Pharmacol* **29**:90–96.
- Hauser RA, Stocchi F, Rascol O, Huyck SB, Capece R, Ho TW, Sklar P, Lines C, Michelson D, and Hewitt D (2015) Preladenant as an adjunctive therapy with levodopa in Parkinson disease: two randomized clinical trials and lessons learned. *JAMA Neurol* **72**:1491–1500.
- Havallah AM, Sandoval-Ramírez J, Reith U, Schobert U, Preiss B, Schumacher B, Daly JW, and Müller CE (2002) 1,8-disubstituted xanthine derivatives: synthesis of potent A_{2B}-selective adenosine receptor antagonists. *J Med Chem* **45**:1500–1510.

- He Y, Li Y, Pu Z, Chen M, Gao Y, Chen L, Ruan Y, Pan X, Zhou Y, Ge Y, et al. (2020) Striatopallidal pathway distinctly modulates goal-directed valuation and acquisition of instrumental behavior via striatopallidal output projections. *Cereb Cortex* **30**:1366–1381.
- Hickey P and Stacy M (2012) Adenosine A_{2A} antagonists in Parkinson's disease: what's next? *Curr Neurol Neurosci Rep* **12**:376–385.
- Hino T, Arakawa T, Iwanari H, Yurugi-Kobayashi T, Ikeda-Suno C, Nakada-Nakura Y, Kusano-Arai O, Weyand S, Shimamura T, Nomura N, et al. (2012) G-protein-coupled receptor inactivation by an allosteric inverse-agonist antibody. *Nature* **482**:237–240.
- Hinz S, Alnouri WM, Pleiss U, and Müller CE (2018) Tritium-labeled agonists as tools for studying adenosine A_{2B} receptors. *Purinergic Signal* **14**:223–233 DOI: <https://doi.org/10.1007/s11302-018-9608-5>.
- Hinz S, Lacher SK, Seibt BF, and Müller CE (2014) BAY60-6583 acts as a partial agonist at adenosine A_{2B} receptors. *J Pharmacol Exp Ther* **349**:427–436.
- Hockemeyer J, Burbiel JC, and Müller CE (2004) Multigram-scale syntheses, stability, and photoreactions of A_{2A} adenosine receptor antagonists with 8-strylxanthine structure: potential drugs for Parkinson's disease. *J Org Chem* **69**:3308–3318.
- Horgusluoglu-Moloch E, Nho K, Risacher SL, Kim S, Foroud T, Shaw LM, Trojanowski JQ, Aisen PS, Petersen RC, Jack Jr CR, et al.; Alzheimer's Disease Neuroimaging Initiative (ADNI) (2017) Targeted neurogenesis pathway-based gene analysis identifies ADORA2A associated with hippocampal volume in mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging* **60**:92–103.
- Hothersall JD, Guo D, Sarda S, Sheppard RJ, Chen H, Keur W, Waring MJ, Ijzerman AP, Hill SJ, Dale IL, et al. (2017) Structure-activity relationships of the sustained effects of adenosine A_{2A} receptor agonists driven by slow dissociation kinetics. *Mol Pharmacol* **91**:25–38.
- Houthuys E, Marillier R, Derégnaucourt T, Brouwer M, Basilico P, Pirson R, Marchante J, Prasad S, Hermant A, Nywouame F, et al. (2018) Abstract LB-291: EOS100850, an insurmountable and non-brain penetrant A_{2A} receptor antagonist, inhibits adenosine-mediated T cell suppression, demonstrates anti-tumor activity and exhibits best-in class characteristics, in AACR Annual Meeting; 2018 Apr 14–18; Chicago. American Association for Cancer Research, Philadelphia.
- Hu Q, Ren X, Liu Y, Li Z, Zhang L, Chen X, He C, and Chen JF (2016) Aberrant adenosine A_{2A} receptor signaling contributes to neurodegeneration and cognitive impairments in a mouse model of synucleinopathy. *Exp Neurol* **283** (Pt A):213–223.
- Huang S, Apasov S, Koshiba M, and Sitkovsky M (1997) Role of A_{2A} extracellular adenosine receptor-mediated signaling in adenosine-mediated inhibition of T-cell activation and expansion. *Blood* **90**:1600–1610.
- Huang SK, Pandey A, Tran DP, Villanueva NL, Kitao A, Sunahara RK, Slijo A and Prosser RS (2021) Delineating the conformational landscape of the adenosine A_{2A} receptor during G protein coupling. *Cell* **184**:1884–1894.e14.
- Iannone R, Miele L, Maiolino P, Pinto A, and Morello S (2014) Adenosine limits the therapeutic effectiveness of anti-CTLA4 mAb in a mouse melanoma model. *Am J Cancer Res* **4**:172–181.
- Ihara K, Hato M, Nakane T, Yamashita K, Kimura-Someya T, Hosaka T, Ishizuka-Katsura Y, Tanaka R, Tanaka T, Sugahara M, et al. (2020) Isoprenoid-chained lipid EROCC₁₇₋₄: a new matrix for membrane protein crystallization and a crystal delivery medium in serial femtosecond crystallography. *Sci Rep* **10**:19305.
- Ishack S, Mediero A, Wilder T, Ricci JL, and Cronstein BN (2017) Bone regeneration in critical bone defects using three-dimensionally printed β -tricalcium phosphate/hydroxyapatite scaffolds is enhanced by coating scaffolds with either dipyridamole or BMP-2. *J Biomed Mater Res B Appl Biomater* **105**:366–375.
- Ivanisevic J, Stauch KL, Petraschek M, Benton HP, Epstein AA, Fang M, Gorantla S, Tran M, Hoang L, Kuczy ME, et al. (2016) Metabolic drift in the aging brain. *Aging (Albany NY)* **8**:1000–1020.
- Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, and Stevens RC (2008) The 2.6 angstrom crystal structure of a human A_{2A} adenosine receptor bound to an antagonist. *Science* **322**:1211–1217.
- Jacobson KA, Barone S, Kammula U, and Stiles GL (1989a) Electrophilic derivatives of purines as irreversible inhibitors of A₁ adenosine receptors. *J Med Chem* **32**:1043–1051.
- Jacobson KA and Civan MM (2016) Ocular purine receptors as drug targets in the eye. *J Ocul Pharmacol Ther* **32**:534–547.
- Jacobson KA, Gao ZG, Göblyös A, and Ijzerman AP (2011) Allosteric modulation of purine and pyrimidine receptors. *Adv Pharmacol* **61**:187–220.
- Jacobson KA, Ijzerman AP, and Müller CE (2021) Medicinal chemistry of P₂ and adenosine receptors: common scaffolds adapted for multiple targets. *Biochem Pharmacol* **187**:114311.
- Jacobson KA, Merighi S, Varani K, Borea PA, Baraldi S, Aghazadeh Tabrizi M, Romagnoli R, Baraldi PG, Ciancetta A, Tosh DK, et al. (2018) A₃ adenosine receptors as modulators of inflammation: from medicinal chemistry to therapy. *Med Res Rev* **38**:1031–1072.
- Jacobson KA and Müller CE (2016) Medicinal chemistry of adenosine, P_{2Y} and P_{2X} receptors. *Neuropharmacology* **104**:31–49.
- Jacobson KA, Pannell LK, Ji XD, Jarvis MF, Williams M, Hutchison AJ, Barrington WW, and Stiles GL (1989b) Agonist derived molecular probes for A₂ adenosine receptors. *J Mol Recognit* **2**:170–178.
- Jacobson KA, Stiles GL, and Ji XD (1992) Chemical modification and irreversible inhibition of striatal A_{2A} adenosine receptors. *Mol Pharmacol* **42**:123–133.
- Jacobson KA, Tosh DK, Jain S, and Gao ZG (2019) Historical and current adenosine receptor agonists in preclinical and clinical development. *Front Cell Neurosci* **13**:124.
- Jacobson KA, von Lubitz DK, Daly JW, and Fredholm BB (1996) Adenosine receptor ligands: differences with acute versus chronic treatment. *Trends Pharmacol Sci* **17**:108–113.
- Janik P, Berdyński M, Safranow K, and Żekanowski C (2015) Association of ADORA1 rs2228079 and ADORA2A rs7515176 polymorphisms with Gilles de la Tourette Syndrome in the Polish population. *PLoS One* **10**:e0136754.
- Jenner P, Mori A, Aradi SD and Hauser RA (2021) Istradefylline - a first generation adenosine A_{2A} antagonist for the treatment of Parkinson's disease. *Expert Rev Neurother* **21**:317–333.
- Jeong LS, Choe SA, Gunaga P, Kim HO, Lee HW, Lee SK, Tosh DK, Patel A, Palaniappan KK, Gao ZG, et al. (2007) Discovery of a new nucleoside template for human A₃ adenosine receptor ligands: D-4'-thioadenosine derivatives without 4'-hydroxymethyl group as highly potent and selective antagonists. *J Med Chem* **50**:3159–3162.
- Jespers W, Schiedel AC, Heitman LH, Cooke RM, Kleene L, van Westen GJP, Gloriam DE, Müller CE, Sotelo E, and Gutiérrez-de-Terán H (2018) Structural mapping of adenosine receptor mutations: ligand binding and signaling mechanisms. *Trends Pharmacol Sci* **39**:75–89.
- Jespers W, Verdon G, Azuaje J, Majellaro M, Keränen H, García-Mera X, Congreve M, Deflorian F, de Graaf C, Zhukov A, et al. (2020) X-ray crystallography and free energy calculations reveal the binding mechanism of A_{2A} adenosine receptor antagonists. *Angew Chem Int Ed Engl* **59**:16536–16543.
- Ji X, Kim YC, Ahern DG, Linden J, and Jacobson KA (2001) [3H]MRS 1754, a selective antagonist radioligand for A(2B) adenosine receptors. *Biochem Pharmacol* **61**:657–663.
- Ji XD, Gallo-Rodríguez C, and Jacobson KA (1993) 8-(3-Isothiocyano-2-ethylphenyl)caffeine is a selective, irreversible inhibitor of striatal A(2)-adenosine receptors. *Drug Dev Res* **29**:292–298.
- Ji XD, Gallo-Rodríguez C, and Jacobson KA (1994) A selective agonist affinity label for A₃ adenosine receptors. *Biochem Biophys Res Commun* **203**:570–576.
- Jörg M, Glukhova A, Abdul-Ridha A, Vecchio EA, Nguyen AT, Sexton PM, White PJ, May LT, Christopoulos A, and Scammells PJ (2016) Novel irreversible agonists acting at the A₁ adenosine receptor. *J Med Chem* **59**:11182–11194.
- Journey JD and Bentley TP (2020) Theophylline Toxicity, in *StatPearls*, StatPearls Publishing LLC, Treasure Island, FL.
- Kalk P, Eggert B, Relle K, Godes M, Heiden S, Sharkovska Y, Fischer Y, Ziegler D, Bielenberg GW, and Hochoer B (2007) The adenosine A₁ receptor antagonist SLV320 reduces myocardial fibrosis in rats with 5/6 nephrectomy without affecting blood pressure. *Br J Pharmacol* **151**:1025–1032.
- Kalla RV and Zablocki J (2009) Progress in the discovery of selective, high affinity A(2B) adenosine receptor antagonists as clinical candidates. *Purinergic Signal* **5**:21–29.
- Kara FM, Chitu V, Sloane J, Axelrod M, Fredholm BB, Stanley ER, and Cronstein BN (2010a) Adenosine A₁ receptors (A1Rs) play a critical role in osteoclast formation and function. *FASEB J* **24**:2325–2333.
- Kara FM, Doty SB, Boskey A, Goldring S, Zaidi M, Fredholm BB, and Cronstein BN (2010b) Adenosine A(1) receptors regulate bone resorption in mice: adenosine A(1) receptor blockade or deletion increases bone density and prevents ovariectomy-induced bone loss in adenosine A(1) receptor-knockout mice. *Arthritis Rheum* **62**:534–541.
- Kaster MP, Machado NJ, Silva HB, Nunes A, Ardaiz AP, Santana M, Baqi Y, Müller CE, Rodrigues AL, Porciúncula LO, et al. (2015) Caffeine acts through neuronal adenosine A_{2A} receptors to prevent mood and memory dysfunction triggered by chronic stress. *Proc Natl Acad Sci USA* **112**:7833–7838.
- Keulerleber S, Gsandtner I, and Freissmuth M (2011) From cradle to twilight: the carboxyl terminus directs the fate of the A(2A)-adenosine receptor. *Biochim Biophys Acta* **1808**:1350–1357.
- Kiesman WF, Elzein E, and Zablocki J (2009) A₁ adenosine receptor antagonists, agonists, and allosteric enhancers. *Handb Exp Pharmacol* **193**:25–58.
- Kim YC, Ji X, Melman N, Linden J, and Jacobson KA (2000) Anilide derivatives of an 8-phenylxanthine carboxylic congener are highly potent and selective antagonists at human A(2B) adenosine receptors. *J Med Chem* **43**:1165–1172.
- Kimatrai-Salvador M, Baraldi PG, and Romagnoli R (2013) Allosteric modulation of A₁-adenosine receptor: a review. *Drug Discov Today Technol* **10**:e285–e296.
- Klotz KN, Cristalli G, Grifantini M, Vittori S, and Lohse MJ (1985) Photoaffinity labeling of A₁-adenosine receptors. *J Biol Chem* **260**:14659–14664.
- Kobayashi H, Ujike H, Iwata N, Inada T, Yamada M, Sekine Y, Uchimura N, Iyo M, Ozaki N, Itokawa M, et al. (2010) The adenosine A_{2A} receptor is associated with methamphetamine dependence/psychosis in the Japanese population. *Behav Brain Funct* **6**:50.
- Koscsó B, Trepakov A, Csóka B, Németh ZH, Pacher P, Eltzhig HK, and Haskó G (2013) Stimulation of A_{2B} adenosine receptors protects against trauma-hemorrhagic shock-induced lung injury. *Purinergic Signal* **9**:427–432.
- Kreutzer K and Bessler D (2014) Caffeine for apnea of prematurity: a neonatal success story. *Neonatology* **105**:332–336.
- Kudlacek O, Waldhoer M, Kassack MU, Nickel P, Salmi JI, Freissmuth M, and Nanoff C (2002) Biased inhibition by a suramin analogue of A₁-adenosine receptor/G protein coupling in fused receptor/G protein tandems: the A₁-adenosine receptor is predominantly coupled to G α in human brain. *Naunyn-Schmiedeberg Arch Pharmacol* **365**:8–16.
- Langemeijer EV, Verzijl D, Dekker SJ, and Ijzerman AP (2013) Functional selectivity of adenosine A₁ receptor ligands? *Purinergic Signal* **9**:91–100.
- Laurent C, Burnouf S, Ferry B, Batalha VL, Coelho JE, Baqi Y, Malik E, Marciniak E, Parrot S, Van der Jeugd A, et al. (2016) A_{2A} adenosine receptor deletion is protective in a mouse model of Touppathy. *Mol Psychiatry* **21**:149.
- Lazarus M, Huang ZL, Lu J, Urade Y, and Chen JF (2012) How do the basal ganglia regulate sleep-wake behavior? *Trends Neurosci* **35**:723–732.
- Lebon G, Edwards PC, Leslie AG, and Tate CG (2015) Molecular determinants of CGS21680 binding to the human adenosine A_{2A} receptor. *Mol Pharmacol* **87**:907–915.
- Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, Leslie AG, and Tate CG (2011) Agonist-bound adenosine A_{2A} receptor structures reveal common features of GPCR activation. *Nature* **474**:521–525.

- Lee HW, Kim HO, Choi WJ, Choi S, Lee JH, Park SG, Yoo L, Jacobson KA, and Jeong LS (2010) Design, synthesis, and binding of homologated truncated 4'-thioadenosine derivatives at the human A₃ adenosine receptors. *Bioorg Med Chem* **18**:7015–7021.
- LeWitt PA, Aradi SD, Hauser RA, and Rascol O (2020) The challenge of developing adenosine A_{2A} antagonists for Parkinson disease: istradefylline, preladenant, and tozadenant. *Parkinsonism Relat Disord* **80** (Suppl 1):S54–S63.
- Li AH, Chang L, Ji Xd, Melman N, and Jacobson KA (1999) Functionalized congeners of 1,4-dihydropyridines as antagonist molecular probes for A₃ adenosine receptors. *Bioconjug Chem* **10**:667–677.
- Li AH, Moro S, Melman N, Ji XD, and Jacobson KA (1998) Structure-activity relationships and molecular modeling of 3, 5-diacyl-2,4-dialkylpyridine derivatives as selective A₃ adenosine receptor antagonists. *J Med Chem* **41**:3186–3201.
- Li P, Rial D, Canas PM, Yoo JH, Li W, Zhou X, Wang Y, van Westen GJ, Payen MP, Augusto E, et al. (2015) Optogenetic activation of intracellular adenosine A_{2A} receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory. *Mol Psychiatry* **20**:1339–1349.
- Li Y, He Y, Chen M, Pu Z, Chen L, Li P, Li B, Li H, Huang ZL, Li Z, et al. (2016) Optogenetic activation of adenosine A_{2A} receptor signaling in the dorsomedial striatopallidal neurons suppresses goal-directed behavior. *Neuropsychopharmacology* **41**:1003–1013.
- Li Y, Pan X, He Y, Ruan Y, Huang L, Zhou Y, Hou Z, He C, Wang Z, Zhang X, et al. (2018) Pharmacological blockade of adenosine A_{2A} but not A₁ receptors enhances goal-directed valuation in satiety-based instrumental behavior. *Front Pharmacol* **9**:393.
- Li Y, Ruan Y, He Y, Cai Q, Pan X, Zhang Y, Liu C, Pu Z, Yang J, Chen M, et al. (2020) Striatopallidal adenosine A_{2A} receptors in the nucleus accumbens confer motivational control of goal-directed behavior. *Neuropharmacology* **168**:108010.
- Lipton RB, Diener HC, Robbins MS, Garas SY, and Patel K (2017) Caffeine in the management of patients with headache. *J Headache Pain* **18**:107.
- Liu P, Pian Y, Li X, Liu R, Xie W, Zhang C, Zheng Y, Jiang Y, and Yuan Y (2014) Streptococcus suis adenosine synthase functions as an effector in evasion of PMN-mediated innate immunity. *J Infect Dis* **210**:35–45.
- Liu W, Chun E, Thompson AA, Chubukov P, Xu F, Katritch V, Han GW, Roth CB, Heitman LH, IJzerman AP, et al. (2012) Structural basis for allosteric regulation of GPCRs by sodium ions. *Science* **337**:232–236.
- Liu X, Huang P, Wang J, Yang Z, Huang S, Luo X, Qi J, Shen X, and Zhong Y (2016) The effect of A_{2A} receptor antagonist on microglial activation in experimental glaucoma. *Invest Ophthalmol Vis Sci* **57**:776–786.
- Liu XL, Zhou R, Pan QQ, Jia XL, Gao WN, Wu J, Lin J, and Chen JF (2010) Genetic inactivation of the adenosine A_{2A} receptor attenuates pathologic but not developmental angiogenesis in the mouse retina. *Invest Ophthalmol Vis Sci* **51**:6625–6632.
- Liu Z, Yan S, Wang J, Xu Y, Wang Y, Zhang S, Xu X, Yang Q, Zeng X, Zhou Y, et al. (2017) Endothelial adenosine A_{2A} receptor-mediated glycolysis is essential for pathological retinal angiogenesis. *Nat Commun* **8**:584.
- Lopes JP, Pliássova A, and Cunha RA (2019) The physiological effects of caffeine on synaptic transmission and plasticity in the mouse hippocampus selectively depend on adenosine A₁ and A_{2A} receptors. *Biochem Pharmacol* **166**:313–321.
- Lopes LV, Halldner L, Rebola N, Johansson B, Ledent C, Chen JF, Fredholm BB, and Cunha RA (2004) Binding of the prototypical adenosine A_{2A} receptor agonist CGS 21680 to the cerebral cortex of adenosine A₁ and A_{2A} receptor knockout mice. *Br J Pharmacol* **141**:1006–1014.
- Louvel J, Guo D, Agliardi M, Mocking TA, Kars R, Pham TP, Xia L, de Vries H, Brussee J, Heitman LH, et al. (2014) Agonists for the adenosine A₁ receptor with tunable residence time. A Case for nonribose 4-amino-6-aryl-5-cyano-2-thiopyrimidines. *J Med Chem* **57**:3213–3222.
- Louvel J, Guo D, Soethoudt M, Mocking TA, Lenselink EB, Mulder-Krieger T, Heitman LH, and IJzerman AP (2015) Structure-kinetics relationships of Capadenoson derivatives as adenosine A₁ receptor agonists. *Eur J Med Chem* **101**:681–691.
- Lovási M, Németh ZH, Gause WC, Beesley J, Pacher P, and Haskó G (2021) Inosine monophosphate and inosine differentially regulate endotoxemia and bacterial sepsis. *FASEB J* **35**:e21935.
- Lucas M, Mirzaei F, Pan A, Okereke OI, Willett WC, O'Reilly ÉJ, Koenen K, and Ascherio A (2011) Coffee, caffeine, and risk of depression among women. *Arch Intern Med* **171**:1571–1578.
- Lucas M, O'Reilly EJ, Pan A, Mirzaei F, Willett WC, Okereke OI, and Ascherio A (2014) Coffee, caffeine, and risk of completed suicide: results from three prospective cohorts of American adults. *World J Biol Psychiatry* **15**:377–386.
- Luthin DR, Lee KS, Okonkwo D, Zhang P, and Linden J (1995) Photoaffinity labeling with 2-(2-(4-azido-3-(125I)-iodophenyl)ethylamino)adenosine and autoradiography with 2-(2-(4-amino-3-(125I)iodophenyl)ethylamino)adenosine of A_{2A} adenosine receptors in rat brain. *J Neurochem* **65**:2072–2079.
- Machado NJ, Simões AP, Silva HB, Ardais AP, Kaster MP, Garçon P, Rodrigues DI, Pochmann D, Santos AI, Araújo IM, et al. (2017) Caffeine reverts memory but not mood impairment in a depression-prone mouse strain with up-regulated adenosine A_{2A} receptor in hippocampal glutamate synapses. *Mol Neurobiol* **54**:1552–1563.
- Mackiewicz M, Nikonova EV, Zimmermann JE, Romer MA, Cater J, Galante RJ, and Pack AI (2006) Age-related changes in adenosine metabolic enzymes in sleep/wake regulatory areas of the brain. *Neurobiol Aging* **27**:351–360.
- Madeira MH, Boia R, Elvas F, Martins T, Cunha RA, Ambrósio AF, and Santiago AR (2016) Selective A_{2A} receptor antagonist prevents microglia-mediated neuroinflammation and protects retinal ganglion cells from high intraocular pressure-induced transient ischemic injury. *Transl Res* **169**:112–128.
- Magnani F, Serrano-Vega MJ, Shibata Y, Abdul-Hussein S, Lebon G, Miller-Gallacher J, Singhal A, Strega A, Thomas JA, and Tate CG (2016) A mutagenesis and screening strategy to generate optimally thermostabilized membrane proteins for structural studies. *Nat Protoc* **11**:1554–1571.
- Majellaro M, Jespers W, Crespo A, Núñez MJ, Novio S, Azaúe J, Prieto-Díaz R, Gioé C, Alispahic B, Brea J, et al. (2021) 3,4-Dihydropyrimidin-2(1H)-ones as antagonists of the human A_{2B} adenosine receptor: optimization, structure-activity relationship studies, and enantiospecific recognition. *J Med Chem* **64**:458–480.
- Maltese M, Martella G, Imbriani P, Schuermans J, Billion K, Sciamanna G, Farook F, Pontiero G, Tassone A, Santoro M, et al. (2017) Abnormal striatal plasticity in a DYT11/SGCE myoclonus dystonia mouse model is reversed by adenosine A_{2A} receptor inhibition. *Neurobiol Dis* **108**:128–139.
- Martin-Garcia JM, Conrad CE, Nelson G, Stander N, Zatzepin NA, Zook J, Zhu L, Geiger J, Chun E, Kissick D, et al. (2017) Serial millisecond crystallography of membrane and soluble protein microcrystals using synchrotron radiation. *IUCrJ* **4**:439–454.
- Martynowicz MW, Shiriaeva A, Ge X, Hattne J, Nannenga BL, Cherezov V, and Gonen T (2021) MicroED structure of the human adenosine receptor determined from a single nanocrystal in LCP. *Proc Natl Acad Sci U S A* **118**:e2106041118.
- Massie BM, O'Connor CM, Metra M, Ponikowski P, Teerlink JR, Cotter G, Weatherly BD, Cleland JG, Givertz MM, Voors A, et al.; PROTECT Investigators and Committees (2010) Rolofylline, an adenosine A₁-receptor antagonist, in acute heart failure. *N Engl J Med* **363**:1419–1428.
- Mateus M, Ilg MM, Stebbeds WJ, Christopher N, Muneer A, Ralph DJ, and Celtek S (2018) Understanding the role of adenosine receptors in the myofibroblast transformation in Peyronie's disease. *J Sex Med* **15**:947–957.
- Matricon P, Suresh RR, Gao ZG, Panel N, Jacobson KA, and Carlsson J (2020) Ligand design by targeting a binding site water. *Chem Sci (Camb)* **12**:960–968.
- Matter H and Güssregen S (2018) Characterizing hydration sites in protein-ligand complexes towards the design of novel ligands. *Bioorg Med Chem Lett* **28**:2343–2352.
- May LT, Self TJ, Briddon SJ, and Hill SJ (2010) The effect of allosteric modulators on the kinetics of agonist-G protein-coupled receptor interactions in single living cells. *Mol Pharmacol* **78**:511–523.
- McLellan TM, Caldwell JA, and Lieberman HR (2016) A review of caffeine's effects on cognitive, physical and occupational performance. *Neurosci Biobehav Rev* **71**:294–312.
- Mediero A, Frenkel SR, Wilder T, He W, Mazumder A, and Cronstein BN (2012a) Adenosine A_{2A} receptor activation prevents wear particle-induced osteolysis. *Sci Transl Med* **4**:135ra65.
- Mediero A, Kara FM, Wilder T, and Cronstein BN (2012b) Adenosine A_{2A} receptor ligation inhibits osteoclast formation. *Am J Pathol* **180**:775–786.
- Mediero A, Perez-Aso M, and Cronstein BN (2013) Activation of adenosine A_{2A} receptor reduces osteoclast formation via PKA- and ERK1/2-mediated suppression of NFκB nuclear translocation. *Br J Pharmacol* **169**:1372–1388.
- Mediero A, Perez-Aso M, Wilder T, and Cronstein BN (2015a) Brief report: Methotrexate prevents wear particle-induced inflammatory osteolysis via activation of the adenosine A_{2A} receptor. *Arthritis Rheumatol* **67**:849–855.
- Mediero A, Wilder T, Perez-Aso M, and Cronstein BN (2015b) Direct or indirect stimulation of adenosine A_{2A} receptors enhances bone regeneration as well as bone morphogenetic protein-2. *FASEB J* **29**:1577–1590.
- Melnikov I, Polovinkin V, Kovalev K, Gushchin I, Shevtsov M, Shevchenko V, Mishin A, Alekseev A, Rodriguez-Valera F, Borschhevskiy V, et al. (2017) Fast iodide-SAD phasing for high-throughput membrane protein structure determination. *Sci Adv* **3**:e1602952.
- Merighi S, Borea PA, and Gessi S (2015) Adenosine receptors and diabetes: focus on the A_{2B} adenosine receptor subtype. *Pharmacol Res* **99**:229–236.
- Miao Y, Bhattarai A, Nguyen ATN, Christopoulos A, and May LT (2018) Structural basis for binding of allosteric drug leads in the adenosine A₁ receptor. *Sci Rep* **8**:16836.
- Michel MC and Charlton SJ (2018) Biased agonism in drug discovery—is it too soon to choose a path? *Mol Pharmacol* **93**:259–265.
- Morelli M, Carta AR, and Jenner P (2009) Adenosine A_{2A} receptors and Parkinson's disease. *Handb Exp Pharmacol* **193**:589–615.
- Moss SM, Jayasekara PS, Paoletta S, Gao ZG, and Jacobson KA (2014) Structure-based design of reactive nucleosides for site-specific modification of the A_{2A} adenosine receptor. *ACS Med Chem Lett* **5**:1043–1048.
- Mouro FM, Köfalvi A, André LA, Baqi Y, Müller CE, Ribeiro JA, and Sebastião AM (2019) Memory deficits induced by chronic cannabinoid exposure are prevented by adenosine A_{2A}R receptor antagonism. *Neuropharmacology* **155**:10–21.
- Müller CE (2003) Medicinal chemistry of adenosine A₃ receptor ligands. *Curr Top Med Chem* **3**:445–462.
- Müller CE, Baqi Y, Hinz S, and Namasivayam V (2018) Medicinal chemistry of A_{2B} adenosine receptors, in *The Adenosine Receptors* (Borea PA, Varani K, Gessi S, Merighi S, and Vincenzi F, eds) in *The Receptors*, vol 34, pp 137–168, Springer International Publishing, Cham, Switzerland.
- Müller CE, Diekmann M, Thorand M, and Ozola V (2002) [(3H)8-Ethyl-4-methyl-2-phenyl-(8R)-4,5,7,8-tetrahydro-1H-imidazo[2,1-i]-purin-5-one ((3H)PSB-11), a novel high-affinity antagonist radioligand for human A₃ adenosine receptors. *Bioorg Med Chem Lett* **12**:501–503 DOI: [https://doi.org/10.1016/s0960-894x\(01\)00785-5](https://doi.org/10.1016/s0960-894x(01)00785-5).
- Müller CE and Jacobson KA (2011a) Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochim Biophys Acta* **1808**:1290–1308.
- Müller CE and Jacobson KA (2011b) Xanthines as adenosine receptor antagonists. *Handb Exp Pharmacol* **200**:151–199.
- Müller CE, Maurinsh J, and Sauer R (2000) Binding of [3H]MSX-2/3-(3-hydroxypropyl)-7-methyl-8-(m-methoxystyryl)-1-propargylxanthine to rat striatal membranes—a new, selective antagonist radioligand for A_{2A} adenosine receptors. *Eur J Pharm Sci* **10**:259–65 DOI: [https://doi.org/10.1016/s0928-0987\(00\)00064-6](https://doi.org/10.1016/s0928-0987(00)00064-6).
- Müller CE, Schiedel AC, and Baqi Y (2012) Allosteric modulators of rhodopsin-like G protein-coupled receptors: opportunities in drug development. *Pharmacol Ther* **135**:292–315.

- Muranaka H, Momose T, Handa C, and Ozawa T (2017) Photoaffinity labeling of the human A2A adenosine receptor and cross-link position analysis by mass spectrometry. *ACS Med Chem Lett* **8**:660–665.
- Murillo-Rodríguez E, Blanco-Centurion C, Gerashchenko D, Salin-Pascual RJ, and Shiromani PJ (2004) The diurnal rhythm of adenosine levels in the basal forebrain of young and old rats. *Neuroscience* **123**:361–370.
- Nass K, Cheng R, Vera L, Mozzanica A, Redford S, Ozerov D, Basu S, James D, Knopp G, Cirelli C, et al. (2020) Advances in long-wavelength native phasing at X-ray free-electron lasers. *IUCr* **7**:965–975.
- Nathan DG, Field J, Lin G, Neuberger D, Majerus E, Onyekwere O, Keefer J, Okam M, Ross A, and Linden J (2012) Sickle cell disease (SCD), iNKT cells, and regadenoson infusion. *Trans Am Clin Climatol Assoc* **123**:312–317, discussion 317–318.
- Navarro G, Gonzalez A, Campanacci S, Rivas-Santisteban R, Reyes-Resina I, Casajuana-Martin N, Cordomi A, Pardo L, and Franco R (2020) Experimental and computational analysis of biased agonism on full-length and a C-terminally truncated adenosine A_{2A} receptor. *Comput Struct Biotechnol J* **18**:2723–2732.
- Nayak A, Chandra G, Hwang I, Kim K, Hou X, Kim HO, Sahu PK, Roy KK, Yoo J, Lee Y, et al. (2014) Synthesis and anti-renal fibrosis activity of conformationally locked truncated 2-hexynyl-N(6)-substituted-(N)-methanocarba-nucleosides as A₃ adenosine receptor antagonists and partial agonists. *J Med Chem* **57**:1344–1354.
- Ng SK, Higashimori H, Tolman M, and Yang Y (2015) Suppression of adenosine 2a receptor (A_{2A}R)-mediated adenosine signaling improves disease phenotypes in a mouse model of amyotrophic lateral sclerosis. *Exp Neurol* **267**:115–122.
- Nguyen AT, Vecchio EA, Thomas T, Nguyen TD, Aurelio L, Scammells PJ, White PJ, Sexton PM, Gregory KJ, May LT, et al. (2016) Role of the second extracellular loop of the adenosine A₁ receptor on allosteric modulator binding, signaling, and cooperativity. *Mol Pharmacol* **90**:715–725.
- Niwa K, Jacobson KA, Silvia SK, and Olsson RA (1993) Covalent binding of a selective agonist irreversibly activates guinea pig coronary artery A₂ adenosine receptors. *Naunyn Schmiedeberg Arch Pharmacol* **347**:521–526.
- Nonaka Y, Shimada J, Nonaka H, Koike N, Aoki N, Kobayashi H, Kase H, Yamaguchi K, and Suzuki F (1993) Photoisomerization of a potent and selective adenosine A₂ antagonist, (E)-1,3-Dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine. *J Med Chem* **36**:3731–3733.
- Ohta A, Gorelik E, Prasad SJ, Ronchese F, Lukashev D, Wong MK, Huang X, Caldwell S, Liu K, Smith P, et al. (2006) A_{2A} adenosine receptor protects tumors from antitumor T cells. *Proc Natl Acad Sci USA* **103**:13132–13137.
- Oliveira S, Ardaís AP, Bastos CR, Gazal M, Jansen K, de Mattos Souza L, da Silva RA, Kaster MP, Lara DR, and Ghisleni G (2019) Impact of genetic variations in ADORA2A gene on depression and symptoms: a cross-sectional population-based study. *Purinergic Signal* **15**:37–44.
- Ozola V, Thorand M, Diekmann M, Qurishi R, Schumacher B, Jacobson KA, and Müller CE (2003) 2-Phenylimidazo[2,1-*l*]purin-5-ones: structure-activity relationships and characterization of potent and selective inverse agonists at human A₃ adenosine receptors. *Bioorg Med Chem* **11**:347–356.
- Pagnussat N, Almeida AS, Marques DM, Nunes F, Chenet GC, Botton PH, Mioranzza S, Loss CM, Cunha RA, and Porciúncula LO (2015) Adenosine A_{2A} receptors are necessary and sufficient to trigger memory impairment in adult mice. *Br J Pharmacol* **172**:3831–3845.
- Patel A, Craig RH, Daluge SM, and Linden J (1988) 125I-BW-A844U, an antagonist radioligand with high affinity and selectivity for adenosine A₁ receptors, and 125I-azido-BW-A844U, a photoaffinity label. *Mol Pharmacol* **33**:585–591.
- Patel JJ and Alzahrani T (2020) Myocardial perfusion scan, in *StatPearls*, StatPearls Publishing LLC, Treasure Island, FL.
- Perez-Aso M, Chiriboga L, and Cronstein BN (2012) Pharmacological blockade of adenosine A_{2A} receptors diminishes scarring. *FASEB J* **26**:4254–4263.
- Perez-Aso M, Mediero A, Low YC, Levine J, and Cronstein BN (2016) Adenosine A_{2A} receptor plays an important role in radiation-induced dermal injury. *FASEB J* **30**:457–465.
- Plássova A, Henriques M, Silva H, Agostinho P, Cunha R, and Ferreira S (2020) Control of NMDA receptor-mediated currents by adenosine A₁ and A_{2A} receptors within the basolateral amygdala. *J Caffeine Adenosine Res* **10**:61–70.
- Poole R, Kennedy OJ, Roderick P, Fallowfield JA, Hayes PC, and Parkes J (2017) Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. *BMJ* **359**:j5024.
- Popoli P, Blum D, Domenici MR, Burnouf S, and Chern Y (2008) A critical evaluation of adenosine A_{2A} receptors as potentially “druggable” targets in Huntington’s disease. *Curr Pharm Des* **14**:1500–1511.
- Prediger RD, Fernandes D, and Takahashi RN (2005) Blockade of adenosine A_{2A} receptors reverses short-term social memory impairments in spontaneously hypertensive rats. *Behav Brain Res* **159**:197–205.
- Prosser RS, Ye L, Pandey A, and Oraziotti A (2017) Activation processes in ligand-activated G protein-coupled receptors: a case study of the adenosine A_{2A} receptor. *BioEssays* **39**:1700072.
- Rebola N, Coelho JE, Costenla AR, Lopes LV, Parada A, Oliveira CR, Soares-da-Silva P, de Mendonça A, and Cunha RA (2003) Decrease of adenosine A₁ receptor density and of adenosine neuromodulation in the hippocampus of kindled rats. *Eur J Neurosci* **18**:820–828.
- Rebola N, Simões AP, Canas PM, Tomé AR, Andrade GM, Barry CE, Agostinho PM, Lynch MA, and Cunha RA (2011) Adenosine A_{2A} receptors control neuroinflammation and consequent hippocampal neuronal dysfunction. *J Neurochem* **117**:100–111.
- Ritchie K, Carrière I, de Mendonça A, Portet F, Dartigues JF, Rouaud O, Barberger-Gateau P, and Ancelin ML (2007) The neuroprotective effects of caffeine: a prospective population study (the Three City Study). *Neurology* **69**:536–545.
- Rodrigues L, Miranda IM, Andrade GM, Mota M, Cortes L, Rodrigues AG, Cunha RA, and Gonçalves T (2016) Blunted dynamics of adenosine A_{2A} receptors is associated with increased susceptibility to *Candida albicans* infection in the elderly. *Oncotarget* **7**:62862–62872.
- Rodrigues RJ, Tomé AR, and Cunha RA (2015) ATP as a multi-target danger signal in the brain. *Front Neurosci* **9**:148.
- Romagnoli R, Baraldi PG, Carrion MD, Cara CL, Cruz-Lopez O, Iaconinoto MA, Preti D, Shryock JC, Moorman AR, Vincenzi F, et al. (2008) Synthesis and biological evaluation of 2-amino-3-(4-chlorobenzoyl)-4-[N-(substituted) piperazin-1-yl]thiophenes as potent allosteric enhancers of the A₁ adenosine receptor. *J Med Chem* **51**:5875–5879.
- Rucktooa P, Cheng RKY, Segala E, Geng T, Errey JC, Brown GA, Cooke RM, Marshall FH, and Doré AS (2018) Towards high throughput GPCR crystallography: in meso soaking of adenosine A_{2A} receptor crystals. *Sci Rep* **8**:41.
- Salamone JD, Betz AJ, Ishiwari K, Felsted J, Madson L, Mirante B, Clark K, Font L, Korbey S, Sager TN, et al. (2008) Tremorolytic effects of adenosine A_{2A} antagonists: implications for parkinsonism. *Front Biosci* **13**:3594–3605.
- Sánchez-Melgar A, Albasanz JL, Pallàs M, and Martín M (2020) Adenosine metabolism in the cerebral cortex from several mice models during aging. *Int J Mol Sci* **21**:7300.
- Sauer R, Maurinsh J, Reith U, Fülle F, Klotz KN, and Müller CE (2000) Water-soluble phosphate prodrugs of 1-propargyl-8-styrylxanthine derivatives, A_{2A}-selective adenosine receptor antagonists. *J Med Chem* **43**:440–448.
- Scammells PJ, Baker SP, Belardinelli L, and Olsson RA (1994) Substituted 1,3-dipropylxanthines as irreversible antagonists of A₁ adenosine receptors. *J Med Chem* **37**:2704–2712.
- Schiffmann SN, Fisone G, Moresco R, Cunha RA, and Ferré S (2007) Adenosine A_{2A} receptors and basal ganglia physiology. *Prog Neurobiol* **83**:277–292.
- Schwarzschild MA, Agnati L, Fuxe K, Chen JF, and Morelli M (2006) Targeting adenosine A_{2A} receptors in Parkinson’s disease. *Trends Neurosci* **29**:647–654.
- Sebastião AM, Cunha RA, de Mendonça A, and Ribeiro JA (2000) Modification of adenosine modulation of synaptic transmission in the hippocampus of aged rats. *Br J Pharmacol* **131**:1629–1634.
- Segala E, Guo D, Cheng RK, Bortolato A, Deflorian F, Doré AS, Errey JC, Heitman LH, IJzerman AP, Marshall FH, et al. (2016) Controlling the dissociation of ligands from the adenosine A_{2A} receptor through modulation of salt bridge strength. *J Med Chem* **59**:6470–6479.
- Sek K, Mølcik C, Stewart GD, Kats L, Darcy PK, and Beavis PA (2018) Targeting adenosine receptor signaling in cancer immunotherapy. *Int J Mol Sci* **19**:3837.
- Serchov T, Clement HW, Schwarz MK, Iasevoli F, Toshi DK, Idzko M, Jacobson KA, de Bartolomeis A, Normann C, Biber K, et al. (2015) Increased signaling via adenosine A₁ receptors, sleep deprivation, imipramine, and ketamine inhibit depressive-like behavior via induction of Homer1a. *Neuron* **87**:549–562.
- Shah SJ, Voors AA, McMurray JJV, Kitzman DW, Viethen T, Bomfim Wirtz A, Huang E, Pap AF, and Solomon SD (2019) Effect of neladenoson bialanate on exercise capacity among patients with heart failure with preserved ejection fraction: a randomized clinical trial. *JAMA* **321**:2101–2112.
- Shaikh G and Cronstein B (2016) Signaling pathways involving adenosine A_{2A} and A_{2B} receptors in wound healing and fibrosis. *Purinergic Signal* **12**:191–197.
- Shen HY, Coelho JE, Ohtsuka N, Canas PM, Day YJ, Huang QY, Rebola N, Yu L, Boison D, Cunha RA, et al. (2008) A critical role of the adenosine A_{2A} receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A_{2A} receptor knock-outs. *J Neurosci* **28**:2970–2975.
- Shimada J, Suzuki F, Nonaka H, Ishii A, and Ichikawa S (1992) (E)-1,3-dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthines: potent and selective adenosine A₂ antagonists. *J Med Chem* **35**:2342–2345.
- Shkhyan R, Lee S, Gullo F, Li L, Peleli M, Carlstrom M, Chagin AS, Banks NW, Limfat S, Liu NQ, et al. (2018) Genetic ablation of adenosine receptor A₃ results in articular cartilage degeneration. *J Mol Med (Berl)* **96**:1049–1060.
- Silva CG, Porciúncula LO, Canas PM, Oliveira CR, and Cunha RA (2007) Blockade of adenosine A_{2A} receptors prevents staurosporine-induced apoptosis of rat hippocampal neurons. *Neurobiol Dis* **27**:182–189.
- Singh S and McKintosh R (2020) Adenosine, in *StatPearls*, StatPearls Publishing LLC, Treasure Island, FL.
- Copyright © 2020, StatPearls Publishing LLC., Treasure Island (FL).
- Smail EH, Cronstein BN, Meshulam T, Esposito AL, Ruggeri RW, and Diamond RD (1992) In vitro, *Candida albicans* releases the immune modulator adenosine and a second, high-molecular weight agent that blocks neutrophil killing. *J Immunol* **148**:3588–3595.
- Smith A, Sutherland D, and Christopher G (2005) Effects of repeated doses of caffeine on mood and performance of alert and fatigued volunteers. *J Psychopharmacol* **19**:620–626.
- Smoluchowski MV (1918) Versuch einer mathematischen Theorie der Koagulationskinetik kolloider Lösungen. *Z Phys Chem* **92**:129–168.
- Sperlagh B, Zsilla G, Baranyi M, Kékes-Szabó A, and Vizi ES (1997) Age-dependent changes of presynaptic neuromodulation via A₁-adenosine receptors in rat hippocampal slices. *Int J Dev Neurosci* **15**:739–747.
- Srinivas M, Shryock JC, Scammells PJ, Ruble J, Baker SP, and Belardinelli L (1996) A novel irreversible antagonist of the A₁-adenosine receptor. *Mol Pharmacol* **50**:196–205.
- St Hilaire C, Ziegler SG, Markello TC, Brusco A, Groden C, Gill F, Carlson-Donohoe H, Lederman RJ, Chen MY, Yang D, et al. (2011) NT5E mutations and arterial calcifications. *N Engl J Med* **364**:432–442.
- Stewart M, Steinig AG, Ma C, Song JP, McKibben B, Castelhamo AL, and MacLennan SJ (2004) [3H]OSIP339391, a selective, novel, and high affinity antagonist radioligand for adenosine A_{2B} receptors. *Biochem Pharmacol* **68**:305–312.
- Stiles GL and Jacobson KA (1988) High affinity acylating antagonists for the A₁ adenosine receptor: identification of binding subunit. *Mol Pharmacol* **34**:724–728.
- Stocchi F, Rascol O, Hauser RA, Huyck S, Tzontcheva A, Capece R, Ho TW, Sklar P, Lines C, Michelson D, et al.; Preladenant Early Parkinson Disease Study

- Group (2017) Randomized trial of preladenant, given as monotherapy, in patients with early Parkinson disease. *Neurology* **88**:2198–2206.
- Storme J, Toshi DK, Gao ZG, Jacobson KA, and Stove CP (2018) Probing structure-activity relationship in β -arrestin2 recruitment of diversely substituted adenosine derivatives. *Biochem Pharmacol* **158**:103–113.
- Strazzulla LC and Cronstein BN (2016) Regulation of bone and cartilage by adenosine signaling. *Purinergic Signal* **12**:583–593.
- Sugiyama K, Tomata Y, Kaiho Y, Honkura K, Sugawara Y, and Tsuji I (2016) Association between coffee consumption and incident risk of disabling dementia in elderly Japanese: the Ohsaki cohort 2006 study. *J Alzheimers Dis* **50**:491–500.
- Sun B, Bachhawat P, Chu ML, Wood M, Ceska T, Sands ZA, Mercier J, Lebon F, Kobilka TS, and Kobilka BK (2017) Crystal structure of the adenosine A_{2A} receptor bound to an antagonist reveals a potential allosteric pocket. *Proc Natl Acad Sci USA* **114**:2066–2071.
- Sušac L, Eddy MT, Didenko T, Stevens RC, and Wüthrich K (2018) A_{2A} adenosine receptor functional states characterized by ¹⁹F-NMR. *Proc Natl Acad Sci USA* **115**:12733–12738.
- Swinney DC (2006a) Biochemical mechanisms of new molecular entities (NMEs) approved by United States FDA during 2001–2004: mechanisms leading to optimal efficacy and safety. *Curr Top Med Chem* **6**:461–478.
- Swinney DC (2006b) Can binding kinetics translate to a clinically differentiated drug? From theory to practice. *Lett Drug Des Discov* **3**:569–574.
- Sykes DA, Dowling MR, and Charlton SJ (2009) Exploring the mechanism of agonist efficacy: a relationship between efficacy and agonist dissociation rate at the muscarinic M3 receptor. *Mol Pharmacol* **76**:543–551.
- Takahashi M, Fujita M, Asai N, Saki M, and Mori A (2018) Safety and effectiveness of istradefylline in patients with Parkinson's disease: interim analysis of a post-marketing surveillance study in Japan. *Expert Opin Pharmacother* **19**:1635–1642.
- Taomoto M, McLeod DS, Merges C, and Luty GA (2000) Localization of adenosine A_{2A} receptor in retinal development and oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci* **41**:230–243.
- Temido-Ferreira M, Ferreira DG, Batalha VL, Marques-Morgado I, Coelho JE, Pereira P, Gomes R, Pinto A, Carvalho S, Canas PM, et al. (2020) Age-related shift in LTD is dependent on neuronal adenosine A_{2A} receptors interplay with mGluR5 and NMDA receptors. *Mol Psychiatry* **25**:1876–1900.
- Tescarollo FC, Rombo DM, DeLiberto LK, Fedele DE, Alharfoush E, Tomé AR, Cunha RA, Sebastião AM, and Boison D (2020) Role of adenosine in epilepsy and seizures. *J Caffeine Adenosine Res* **10**:45–60.
- Thammavongsa V, Kern JW, Missiakas DM, and Schneewind O (2009) *Staphylococcus aureus* synthesizes adenosine to escape host immune responses. *J Exp Med* **206**:2417–2427.
- Thomas GS, Cullom SJ, Kitt TM, Feaheny KM, Ananthasubramaniam K, Gropler RJ, Jain D, and Thompson RC (2017) The EXERT trial: “EXercise to Regadenoson in Recovery Trial”: a phase 3b, open-label, parallel group, randomized, multicenter study to assess regadenoson administration following an inadequate exercise stress test as compared to regadenoson without exercise for myocardial perfusion imaging using a SPECT protocol. *J Nucl Cardiol* **24**:788–802.
- Thompson EA and Powell JD (2021) Inhibition of the adenosine pathway to potentiate cancer immunotherapy: potential for combinatorial approaches. *Annu Rev Med* **72**:331–348.
- Tilley SL (2011) Methylxanthines in asthma. *Handb Exp Pharmacol* **200**:439–456.
- Tonge PJ (2018) Drug-target kinetics in drug discovery. *ACS Chem Neurosci* **9**:29–39.
- Toshi DK, Salmaso V, Rao H, Bitant A, Fisher CL, Lieberman DI, Vorbrüggen H, Reitman ML, Gavrilova O, Gao ZG, et al. (2020) Truncated (N)-methanocarba nucleosides as partial agonists at mouse and human A₃ adenosine receptors: affinity enhancement by N⁶-(2-phenylethyl) substitution. *J Med Chem* **63**:4334–4348.
- Valant C, Aurelio L, Urmaliya VB, White P, Scammells PJ, Sexton PM, and Christopoulos A (2010) Delineating the mode of action of adenosine A1 receptor allosteric modulators. *Mol Pharmacol* **78**:444–455.
- Valant C, May LT, Aurelio L, Chuo CH, White PJ, Baltos JA, Sexton PM, Scammells PJ, and Christopoulos A (2014) Separation of on-target efficacy from adverse effects through rational design of a bitopic adenosine receptor agonist. *Proc Natl Acad Sci USA* **111**:4614–4619.
- Vallon V, Miracle C, and Thomson S (2008) Adenosine and kidney function: potential implications in patients with heart failure. *Eur J Heart Fail* **10**:176–187.
- Vallon V, Mühlbauer B, and Osswald H (2006) Adenosine and kidney function. *Physiol Rev* **86**:901–940.
- van Dam RM, Hu FB, and Willett WC (2020) Coffee, caffeine, and health. *N Engl J Med* **383**:369–378.
- van Gelder BM, Buijsse B, Tjhuis M, Kalmijn S, Giampaoli S, Nissinen A, and Kromhout D (2007) Coffee consumption is inversely associated with cognitive decline in elderly European men: the FINE Study. *Eur J Clin Nutr* **61**:226–232.
- van Muijlwijk-Koeze JE, Timmerman H, van der Goot H, Menge WM, Frijtag Von Drabbe Künzel J, de Groot M, and IJzerman AP (2000) Isoquinoline and quinoxaline urea analogues as antagonists for the human adenosine A₃ receptor. *J Med Chem* **43**:2227–2238.
- van Muijlwijk-Koeze JE, Timmerman H, van der Sluis RP, van de Stolpe AC, Menge WM, Beukers MW, van der Graaf PH, de Groot M, and IJzerman AP (2001) Synthesis and use of FSCPX, an irreversible adenosine A1 antagonist, as a ‘receptor knock-down’ tool. *Bioorg Med Chem Lett* **11**:815–818.
- van Rhee AM, Jiang JL, Melman N, Olah ME, Stiles GL, and Jacobson KA (1996) Interaction of 1,4-dihydropyridine and pyridine derivatives with adenosine receptors: selectivity for A₃ receptors. *J Med Chem* **39**:2980–2989.
- Varani K, Merighi S, Gessi S, Klotz KN, Leung E, Baraldi PG, Cacciari B, Romagnoli R, Spalluto G, and Borea PA (2000) [(3)H]MRE 3008F20: a novel antagonist radioligand for the pharmacological and biochemical characterization of human A₃ adenosine receptors. *Mol Pharmacol* **57**:968–975.
- Varty GB, Hodgson RA, Pond AJ, Grzelak ME, Parker EM, and Hunter JC (2008) The effects of adenosine A_{2A} receptor antagonists on haloperidol-induced movement disorders in primates. *Psychopharmacology (Berl)* **200**:393–401.
- Vass M, Kooistra AJ, Yang D, Stevens RC, Wang MW, and de Graaf C (2018) Chemical diversity in the G protein-coupled receptor superfamily. *Trends Pharmacol Sci* **39**:494–512.
- Vauquelin G (2018) Link between a high *k_{on}* for drug binding and a fast clinical action: to be or not to be? *MedChemComm* **9**:1426–1438.
- Vecchio EA, Baltos JA, Nguyen ATN, Christopoulos A, White PJ, and May LT (2018) New paradigms in adenosine receptor pharmacology: allosteric oligomerization and biased agonism. *Br J Pharmacol* **175**:4036–4046.
- Venkatakrishnan AJ, Deupi X, Lebon G, Tate CG, Schertler GF, and Babu MM (2013) Molecular signatures of G-protein-coupled receptors. *Nature* **494**:185–194.
- Verzija D and IJzerman AP (2011) Functional selectivity of adenosine receptor ligands. *Purinergic Signal* **7**:171–192.
- Viana da Silva S, Haber MG, Zhang P, Bethge P, Lemos C, Gonçalves N, Gorlewicz A, Malezieux M, Gonçalves FQ, Grosjean N, et al. (2016) Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A_{2A} receptors. *Nat Commun* **7**:11915.
- Voors AA, Shah SJ, Bax JJ, Butler J, Gheorghide M, Hernandez AF, Kitzman DW, McMurray JJV, Wirtz AB, Lanius V, et al. (2018) Rationale and design of the phase 2b clinical trials to study the effects of the partial adenosine A1-receptor agonist neladenoson bialanate in patients with chronic heart failure with reduced (PANTHEON) and preserved (PANACHE) ejection fraction. *Eur J Heart Fail* **20**:1601–1610.
- Wall MJ, Hill E, Huckstep R, Barkan K, Deganutti G, Leuenberger M, Preti B, Winfield I, Wei H, Imlach W, et al. (2020) A biased adenosine A1R agonist elicits analgesia without cardiorespiratory depression. *bioRxiv*:2020.2004.023945.
- Weinert T, Olieric N, Cheng R, Brünle S, James D, Ozerov D, Gashi D, Vera L, Marsh M, Jaeger K, et al. (2017) Serial millisecond crystallography for routine room-temperature structure determination at synchrotrons. *Nat Commun* **8**:542.
- Welihinda AA, Kaur M, Greene K, Zhai Y, and Amento EP (2016) The adenosine metabolite inosine is a functional agonist of the adenosine A_{2A} receptor with a unique signaling bias. *Cell Signal* **28**:552–560.
- White CW, Johnstone EKM, See HB, and Pfeifer KDG (2019) NanoBRET ligand binding at a GPCR under endogenous promotion facilitated by CRISPR/Cas9 genome editing. *Cell Signal* **54**:27–34.
- White KL, Eddy MT, Gao ZG, Han GW, Lian T, Deary A, Patel N, Jacobson KA, Katritch V and Stevens RC (2018) Structural connection between activation microswitch and allosteric sodium site in GPCR signaling. *Structure* **26**:259–269.e5.
- Willingham SB, Hotson AN, and Miller RA (2020) Targeting the A_{2A}R in cancer: early lessons from the clinic. *Curr Opin Pharmacol* **53**:126–133.
- Wooden D, Christopoulos A, and Sexton PM (2013) Emerging paradigms in GPCR allosteric: implications for drug discovery. *Nat Rev Drug Discov* **12**:630–644.
- Xia L, Burger WAC, van Veldhoven JPD, Kuiper BJ, van Duijl TT, Lenselink EB, Paasman E, Heitman LH, and IJzerman AP (2017) Structure-affinity relationships and structure-kinetics relationships of pyrido[2,1-f]purine-2,4-dione derivatives as human adenosine A₃ receptor antagonists. *J Med Chem* **60**:7555–7568.
- Xia L, de Vries H, IJzerman AP, and Heitman LH (2016) Scintillation proximity assay (SPA) as a new approach to determine a ligand's kinetic profile. A case in point for the adenosine A1 receptor. *Purinergic Signal* **12**:115–126.
- Xia L, Kyrizaki A, Toshi DK, van Duijl TT, Roorda JC, Jacobson KA, IJzerman AP, and Heitman LH (2018) A binding kinetics study of human adenosine A₃ receptor agonists. *Biochem Pharmacol* **153**:248–259.
- Xiao C, Liu N, Jacobson KA, Gavrilova O, and Reitman ML (2019) Physiology and effects of nucleosides in mice lacking all four adenosine receptors. *PLoS Biol* **17**:e3000161.
- Xu F, Wu H, Katritch V, Han GW, Jacobson KA, Gao ZG, Cherezov V, and Stevens RC (2011) Structure of an agonist-bound human A_{2A} adenosine receptor. *Science* **332**:322–327.
- Yang X, Dong G, Michiels TJM, Lenselink EB, Heitman L, Louvel J, and IJzerman AP (2017) A covalent antagonist for the human adenosine A_{2A} receptor. *Purinergic Signal* **13**:191–201.
- Yang X, Michiels TJM, de Jong C, Soethoudt M, Dekker N, Gordon E, van der Stelt M, Heitman LH, van der Es D, and IJzerman AP (2018) An affinity-based probe for the human adenosine A_{2A} receptor. *J Med Chem* **61**:7892–7901.
- Yang X, van Veldhoven JPD, Offringa J, Kuiper BJ, Lenselink EB, Heitman LH, van der Es D, and IJzerman AP (2019) Development of covalent ligands for G protein-coupled receptors: a case for the human adenosine A₃ receptor. *J Med Chem* **62**:3539–3552.
- Ye L, Neale C, Slička A, Lyda B, Pichugin D, Tsuchimura N, Larda ST, Pomès R, García AE, Ernst OP, et al. (2018) Mechanistic insights into allosteric regulation of the A_{2A} adenosine G protein-coupled receptor by physiological cations. *Nat Commun* **9**:1372.
- Yu F, Zhu C, Xie Q, and Wang Y (2020) Adenosine A_{2A} receptor antagonists for cancer immunotherapy. *J Med Chem* **63**:12196–12212.
- Yu L, Shen HY, Coelho JE, Araújo IM, Huang QY, Day YJ, Rebola N, Canas PM, Rapp EK, Ferrara J, et al. (2018) Adenosine A_{2A} receptor antagonists exert motor and neuroprotective effects by distinct cellular mechanisms. *Ann Neurol* **63**:338–346.
- Zhang G, Franklin PH, and Murray TF (1994) Activation of adenosine A1 receptors underlies anticonvulsant effect of CGS21680. *Eur J Pharmacol* **255**:239–243.
- Zhang R (2015) Pharmacodynamics: which trails are your drugs taking? *Nat Chem Biol* **11**:382–383.
- Zhang S, Zhou R, Li B, Li H, Wang Y, Gu X, Tang L, Wang C, Zhong D, Ge Y, et al.

- (2017) Caffeine preferentially protects against oxygen-induced retinopathy. *FASEB J* **31**:3334–3348.
- Zhao ZA, Zhao Y, Ning YL, Yang N, Peng Y, Li P, Chen XY, Liu D, Wang H, Chen X, et al. (2017) Adenosine A_{2A} receptor inactivation alleviates early-onset cognitive dysfunction after traumatic brain injury involving an inhibition of tau hyperphosphorylation. *Transl Psychiatry* **7**:e1123.
- Zhou R, Zhang S, Gu X, Ge Y, Zhong D, Zhou Y, Tang L, Liu XL, and Chen JF (2018) Adenosine A_{2A} receptor antagonists act at the hyperoxic phase to confer protection against retinopathy. *Mol Med* **24**:41.
- Zimmermann H (2021) Ectonucleoside triphosphate diphosphohydrolases and ecto-5'-nucleotidase in purinergic signaling: how the field developed and where we are now. *Purinergic Signal* **17**:117–125.