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Drug-target residence time : a case for the adenosine A1 and A2A receptors

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Chapter 1

General introduction

About this thesis

Drug design and discovery is a daunting task. To date, the pharmaceutical industry is continuously experiencing high attrition rates in research and development (R&D) of drug candidates. One of the major reasons for drug failure is lack of efficacy.¹ Apparently, the current evaluation strategies, often measurements based on examining a compound's affinity and selectivity, are not sufficient to predict *in vivo* drug efficacy. A more predictive experimental paradigm is, therefore, highly desired. Several recent reviews, in an *a posteriori* perspective, showed that many marketed drugs—in other words successful examples—favor certain kinetic aspects.²⁻⁴ In particular, the residence time of a drug on its target may be more relevant for the *in vivo* efficacy of medicines than their typical *in vitro* equilibrium binding constants, e.g., K_i values. However, this hypothesis is merely supported from retrospective studies. Only a few kinetics-directed studies have been performed so far. As such, more insight into ligand-receptor binding kinetics, in an *a priori* manner, might be beneficial in the early phases of drug discovery.

1.1 Drug discovery and development pipeline

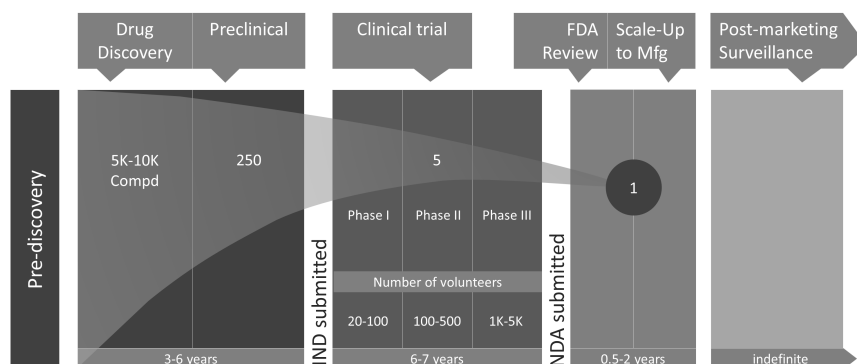


Figure 1.1 | Figure is adapted from Drug Discovery and Development: Understanding the R&D Process, www.innovation.org; CBO, Research and Development in the Pharmaceutical Industry, 2006.

Figure 1.1 represents a summary of the sequential steps that are essential for a drug to progress through the R&D pipeline. This process on average takes 10-15 years. It usually starts from a large number of hits (e.g. 5,000-10,000) which are selected from high-throughput screening, a step that enables running more than 50,000 compounds a day with complex work-stations.⁵ In a typical scenario, approximately 250 candidates (leads) are selected for preclinical testing. Following a program with 3-6 years' duration, about 5 candidate drugs will progress into clinical trials of three consecutive phases (I-III) to examine their efficacy and safety among an increasing number of volunteers and patients. Finally, only one will receive approval, if at all. The average cost to research and develop a successful drug is estimated to be \$800 million to \$1 billion.

1.2 Lack of efficacy: the main reason of drug attrition

An investigation into the causes of drug attrition is very necessary and instructive in the identification of strategies and tactics to improve the

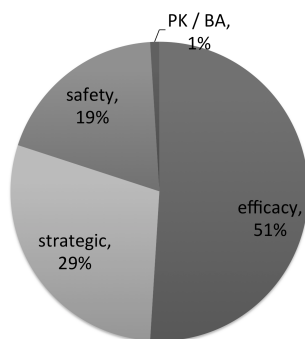


Figure 1.2 | Phase II failures: 2008-2010. The 108 failures are divided according to reasons for failure when reported (87 drugs). Figure is adapted from Arrowsmith, 2011.¹

efficiency of drug development. In a recent survey of 108 reported phase II failures from 2008 to 2010 (Figure 1.2),¹ 44 drugs had failed due to insufficient efficacy, which accounted for 51% of 87 cases with reported reasons for failure. Following this, 29% (25 out of 87) were due to strategic reasons and 19% (17 out of 87) were due to clinical or preclinical safety concerns. Pharmacokinetics (PK) or bioavailability (BA) issues are seldom reported.¹ Apparently, lack of efficacy is the main reason for drug attrition. This, in part, results from the insufficiently predictive efficacy in animal models for translation into clinical efficacy. The development of more predictive animal models—where possible—is therefore needed. Yet, what might be more important is to develop experimental paradigms that are more predictive of clinical outcomes in much earlier phases of drug design and discovery.

1.3 Drug-target residence time: the ‘time’ to fix the leaky drug candidate pipeline?

Drug-target residence time (RT) is the duration a drug stays in a complex with the target, which is defined as the reciprocal of the dissociation rate constant of the drug-target complex ($1 / k_{\text{off}}$).⁶ Recently, awareness of the importance of residence time has started to increase since accumulating evidence suggested that the *in vivo* efficacy of a ligand may be attributed

to the time it resides at its receptor.^{2,7-9} However, this parameter has so far been overlooked or neglected in the traditional drug discovery process. Currently, mostly steady-state metrics have been used in practice, e.g. affinity or potency, as predictor for *in vivo* drug efficacy. Yet, the aforementioned high attrition rate and the insufficient *in vivo* efficacy led to the realization that this equilibrium-based strategy might be too simplistic. Thus, introducing analysis of binding kinetics in the pipeline of drug research might provide a better predictor for *in vivo* efficacy in the earlier phases of drug design and discovery, and should be explored better. This added knowledge might foster a better strategy in the triage and advancement of drug candidates towards the clinic.

Clinical benefits of drugs with optimized residence time

Supposed that marketed drugs are the most successful clinical candidates, many of them have been shown, in retrospect, to favor certain kinetic aspects. In a survey of 50 drugs on 12 different drug targets by Swinney, about 70% of long residence time therapeutics displayed higher efficacy than comparable faster dissociating drugs.¹⁰ One example is the well-known HIV-1 protease inhibitor, Darunavir, which is an extremely effective drug due to its RT of more than 14 days (over 340 h).¹¹ Examples in the field of G protein-coupled receptors (GPCRs) include tiotropium,¹² the muscarinic M_3 receptor antagonist for clinical indication of Chronic Obstructive Pulmonary Disease (COPD),¹³ maraviroc, an allosteric inhibitor of the CCR5 receptor for HIV/AIDS,¹⁴ and ‘sartan’ antagonists for the angiotensin II receptor to treat hypertension.¹⁵ Notably, tiotropium is non-selective in its affinity for the five subtypes of muscarinic receptors. However, due to its very slow dissociation kinetics from the M_3 receptor compared to the other subtypes it is kinetically selective. On the other hand, there are cases where mechanism-based or ‘on-target’ toxicity outweighs the therapeutic advantages of long receptor occupancy. In this situation, a fast dissociating compound displaying short-lived receptor intervention is preferred. This is the case for some antipsychotic D_2 dopamine receptor antagonists, where long RT is undesirable due to on-target toxicity associated with strong extrapyramidal motor effects. The clinical benefits of optimized RT will be further discussed in Chapter 2.

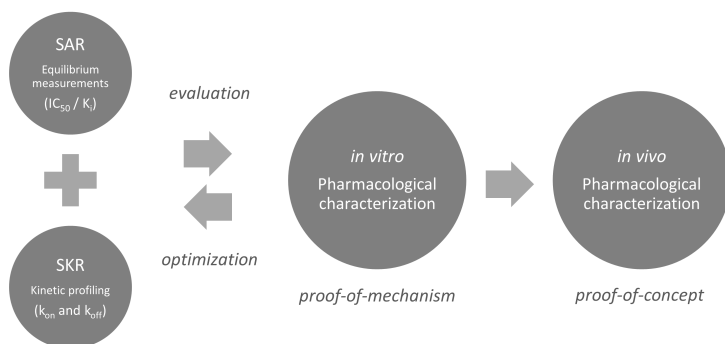


Figure 1.3 | Structure-kinetics relationships (SKR) and structure-affinity relationships (SAR)—an *a priori* approach for the design of chemical entities with desired kinetics.

Finding new chemical entities with optimized kinetics

The examples mentioned above are prospective drivers for design and development of new candidate compounds with appealing kinetic profiles for proof of concept. Figure 1.3 describes such a scheme of a kinetics-directed process in combination with the affinity-based evaluation. It includes the kinetic profiling (on-rate and off-rate) of compounds of interest (SKR, structure-kinetics relationships), complementary to the traditional equilibrium measurements (SAR, structure-affinity relationships), such as IC_{50} or K_i , and the characterization of pharmacological responses upon the formation of the ligand-receptor binary complex. The information obtained from the latter further stimulates a second round of optimization. This quick synthesis and evaluation loop can lead to more candidate compounds with desired kinetics profile to fuel further *in vivo* proof-of-concept experiments.

1.4 Adenosine receptors—the prototypic targets

Adenosine receptors (ARs) are used as prototypical targets for binding kinetics investigations in the present thesis. These receptors belong to the superfamily of G protein-coupled receptors (GPCRs) and have four subtypes: A_1 , A_{2A} ,

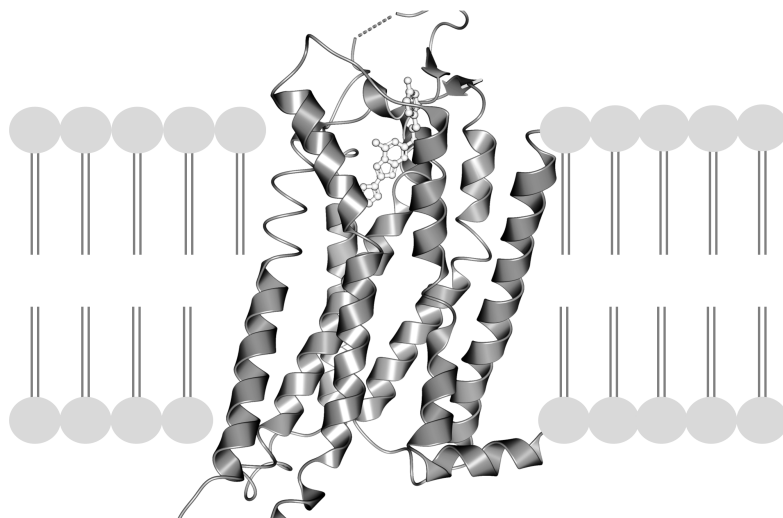


Figure 1.4 | Schematic representation of the adenosine A_{2A} receptor in complex with the antagonist ZM241385 embedded in a lipid bilayer. The structure is based on the first crystallographic structure of the adenosine A_{2A} receptor (PDB: 3EML). The receptor contains heptahelical transmembrane motifs connected by three intracellular and three extracellular loops, with the amino terminus located at the extracellular side and the carboxyl terminus at the intracellular side.

A_{2B} and A_3 . Figure 1.4 is a schematic representation of the adenosine A_{2A} receptor in complex with the antagonist ZM241385 which is based on the first crystallographic structure of this receptor (PDB: 3EML). Like all GPCRs, it contains heptahelical transmembrane motifs connected by three intracellular and three extracellular loops, with the amino terminus located at the extracellular side and the carboxyl terminus at the intracellular side. The adenosine A_1 and A_3 receptors are mainly coupled to the enzyme adenylyl cyclase in an inhibitory fashion via a G_i protein, whereas the A_{2A} and A_{2B} receptors stimulate this enzyme via a G_s protein.¹⁶ Adenosine receptors are promising therapeutic targets. They are involved in a wide range of conditions, including cerebral and cardiac ischemic diseases, sleep disorders, immune and inflammatory disorders and cancer.¹⁷ As such, decades of medicinal chemistry research has resulted in a plethora of AR ligands, which are good starting points for binding kinetics investigations. In this thesis we will focus on the A_1 R and A_{2A} R.

A₁ receptor

The A₁R is widely expressed throughout the body.¹⁸ Its activation inhibits adenylyl cyclase activity, activates potassium channels (including K_{ATP} channels in neurons and the myocardium), blocks transient calcium channels and increases intracellular calcium and phospholipase C (PLC).¹⁹ This receptor can exert many physiologically important effects in different organs and cells. For instance, A₁R activation in the cardiovascular system reduces heart rate and atrial contractility, and attenuates the stimulatory actions of catecholamines on the heart.²⁰ However, for long-term indications, selective A₁R agonists are required to avoid side effects related to other AR subtypes such as hypotension (A_{2A}R).^{17,19,20} A₁R inhibition in the nervous system was reported to have potential therapeutic effects in divergent conditions, such as dementia, hyperactivity, anxiety, schizophrenia and sleep disorders.^{16,21,22}

A_{2A} receptor

The A_{2A}R is highly expressed in the brain striatum, leukocytes and blood platelets, and is also found in the heart, lung and blood vessels.¹⁶ Its activation stimulates the cyclic AMP-protein kinase A (PKA) pathway by coupling to a G_s protein in peripheral tissues or G_{olf} in the brain.²³⁻²⁵ In the central nervous system, A_{2A}R plays significant roles in regulating motor activity, psychiatric behaviors, the sleep-wake cycle and neuronal cell death. In peripheral tissues, A_{2A}R is involved in the modulation of inflammation, myocardial oxygen consumption, coronary blood flow, angiogenesis and the control of cancer pathogenesis.¹⁹ Thus, activation of A_{2A}R has clinical relevance in various pathological conditions such as respiratory disorders and inflammation,^{26,27} while inhibition of this receptor may have potential therapeutic roles for syndromes such as insomnia, pain, depression, drug addiction and Parkinson's disease (PD).²⁸⁻³⁰

Status quo: translation of current knowledge towards clinical progress

The translation of the current knowledge of adenosine receptors to clinical progress has been disappointingly slow. After decades of research, only two adenosine receptor agonists have been approved by the US Food and Drug Administration (FDA). They are adenosine itself (Adenocard; Astellas Pharma) for restoring normal heart rhythm in patients with paroxysmal supraventricular tachycardia (PSVT) and an A_1 R agonist regadenoson (Lexiscan; Astellas Pharma) for myocardial perfusion imaging in patients with suspected coronary artery disease.^{17,31} Currently, several trials are in progress, which are summarized in Table 1.1.¹⁹ Revisiting the database with a kinetic perspective may provide additional knowledge for future medicinal chemistry projects. The proof of mechanism in pre-clinical trials—for instance, preladenant's long duration-of-action (> 12 h in a rat model)³²—could still direct us to find compounds bearing chemical resemblance for similar or better kinetic behavior, even though the starting compound has been discontinued in clinical trials. Such strategy is exemplified in Chapter 4. On the other hand, investigating the residence time of UK432,097, an A_{2A} R agonist that in Phase II clinical trials was not efficacious for COPD, may provide clues for its failure.

Long or short receptor residence time for AR ligands?

The question whether to aim for long or short residence time A_1 R and A_{2A} R ligands needs careful consideration in the early phase of drug design and discovery. The same holds for all GPCRs and other drug targets as well. It is thus necessary to weigh both potential beneficial and detrimental outcomes upon longer or shorter AR occupancy. For example, myocardial A_1 Rs have been shown to inhibit a number of myocardial pathologies associated with ischemia and reperfusion injury.^{33,34} However, such desired A_1 R-mediated protective and regenerative cardiovascular effects might be counteracted by unintended side-effects like bradycardia or atrioventricular block, due to the broad physiologic spectrum of cardiac responses upon myocardial

Table 1.1 | List of ongoing or recently completed phase IIb-III clinical trials targeting A₁ and A_{2A} receptors. The table summarizes the status found at the ClinicalTrials.gov website, accessed at December 6, 2013, and was adapted from Chen et al.¹⁹

<i>Compound</i>	<i>Name of the study</i>	<i>Status</i>
Adenosine	Salvage: postconditioning after STEMI	Phase II ongoing
	Prophylactic intra-coronary adenosine to prevent post coronary artery stenting myonecrosis	Phase III terminated
Caffeine	Clinical study of caffeine for apnea of prematurity	Phase III completed
	Magnetic resonance imaging (MRI) and neurodevelopmental outcomes in preterm infants following administration of high-dose caffeine	Phase IV Recruiting
	Caffeine for motor manifestations of Parkinson's disease	Phase II Completed
	Caffeine for excessive daytime somnolence in Parkinson's disease	Phase II Completed
GW493838	The study of GW493838, an adenosine A ₁ agonist, in peripheral neuropathic pain	Phase II terminated
INO-8875	A dose-escalation study designed to evaluate the tolerability, safety, pharmacokinetics (PK), and efficacy of chronic topical ocular application of INO-8875 in adults with ocular hypertension or primary open-angle glaucoma	Phase II Completed
CV-3146 (regadenoson)	ADVANCE MPI 2: study of regadenoson versus Adenoscan® in patients undergoing myocardial perfusion imaging (MPI)	Phase III Completed
	Regadenoson blood flow in type I diabetes (RABIT1D)	Phase IV Completed
ALT-146e (apadenoson)	Study of the safety and efficacy of apadenoson for detection of myocardial perfusion defects using SPECT MPI (ASPECT)	Phase III terminated
UK432,097	Safety and efficacy of UK432,097 administration in patients with chronic obstructive pulmonary disease	Phase II terminated
BG9928 (tonapofylline)	Phase 2b study to assess the safety and tolerability of IV tonapofylline in subjects with acute decompensated heart failure (ADHF) and renal insufficiency (TRIDENT-1)	Phase II completed
KW-3902 (Rolofylline)	PROTECT-1: a study of the selective A ₁ adenosine receptor antagonist KW-3902 for patients hospitalized with acute HF and volume overload to assess treatment effect on congestion and renal function	Phase III completed
	PROTECT-2: a study of the selective A ₁ adenosine receptor antagonist KW-3902 for patients hospitalized with acute HF and volume overload to assess treatment effect on congestion and renal function	Phase III completed
KW-6002 (istradefylline)	Study of KW-6002 (istradefylline) for the treatment of Parkinson's disease in patients taking Levodopa (6002-009)	Phase III completed
	Long-term safety study of istradefylline in patients with Parkinson's disease	Phase III completed
Preladenant	A placebo- and active-controlled study of preladenant in early Parkinson's disease (P05664 AM5)	Phase III terminated
	A placebo- and active-controlled study of preladenant in subjects with moderate to severe Parkinson's disease (Study P04938 AM5)	Phase III terminated
SYN-115	Safety and efficacy study of SYN115 in Parkinson's patients using L-DOPA to treat end of dose wearing off	Phase III completed

A₁R activation.^{20,35} Receptor desensitization is another potential issue when long residence time A₁ receptor agonists are considered. Therefore, a short intervention might be preferred to overcome the restrictions mentioned above. There are also cases where long residence time AR ligands might be beneficial. One example could be the A_{2A}R antagonists for the treatment of Parkinson's disease (PD) in combination with levodopa (the gold-standard medicine to boost dopamine levels in the brain). However, many PD patients' symptoms return in between doses, when levodopa's effects wear off—the fluctuation known as 'off' time.³⁶ In light of this, a slowly dissociating A_{2A}R antagonist may have the advantage of decreasing the 'off' time compared to a short residence time compound. Additionally, longer receptor residence time on one AR subtype can provide so-called kinetic selectivity over the others. This feature could be of great use for further optimizing those less-selective AR ligands in terms of affinity (i.e. the non-selective AR agonist NECA¹⁷). Such an advantage is well exemplified by tiotropium, the M₃R antagonist highly selective over other muscarinic subtypes in terms of binding kinetics while less so in terms of affinity. To better define the optimal range of a target's residence time, it is necessary to obtain a series of candidate molecules having a divergent frame of residence times—for instance, the A_{2A}R antagonists described in Chapter 4 – for later proof-of-concept tests.

1.5 Objectives and overview of this thesis

Investigating ligand-receptor binding kinetics, in particular the residence time, is a leading theme in this thesis, which will be explored by several case studies on adenosine A₁ and A_{2A} receptors.

In Chapter 2, the state of the art in drug-GPCR residence time is reviewed. We summarized a series of ligands for four prototypic GPCRs that have reported kinetic data, and extensively discussed these data from a molecular mechanism of action to *in vivo* pharmacology. In this chapter we also reviewed and summarized the emerging (*in vitro*) methods for studying binding kinetics. Among other assays, a newly developed kinetic screening method is listed, the so-called dual-point competition association assay. This assay enables fast and high-throughput assessment of ligand-receptor binding kinetics and is further described in Chapter 3.

At the start of this project, few SKR studies had been reported in the

field of GPCRs, if any. Thus, we proposed a novel strategy for compound optimization that combines an extensive SKR study with the classical SAR study in Chapter 2. This strategy is exemplified in Chapter 4. We demonstrated that compounds within one chemical class (chemotype) may have divergent kinetic properties, whilst diverse compounds may have similar kinetic characteristics ('kinotype').

Following this, we also investigated the mechanism of important pharmacological concepts, i.e., functional efficacy and GPCR allosterism, from a kinetic perspective (Chapters 5 and 6). These additional kinetic insights advanced our current understanding of these concepts.

In short, several case studies of drug-target residence time at the A₁R and A_{2A}R are presented in this thesis. In Chapter 7, this work is concluded with general remarks and future perspectives for this field of research. Hopefully, this thesis will contribute to stipulating the importance of kinetics-based drug design and discovery in the future.

References:

1. Arrowsmith J. Trial watch: Phase II failures: 2008-2010. *Nat Rev Drug Discov* 2011;10(5):328-329.
2. Copeland RA, Pompliano DL, Meek TD. Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov* 2006;5(9):730-739.
3. Swinney DC. 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* 2006;6(5):461-478.
4. Zhang R, Monsma F. The importance of drug-target residence time. *Curr Opin Drug Discov Devel* 2009;12(4):488-496.
5. Martis E, Radhakrishnan R, Badve R. High-throughput screening: the hits and leads of drug discovery-an overview. *Journal of Applied Pharmaceutical Science* 2011;1(1):2-10.
6. Copeland, RA. *Evaluation of enzyme inhibitors in drug discovery: a guide for medicinal chemists and pharmacologists*. New York: John Wiley & Sons; 2005. 153 p.
7. Swinney DC. The role of binding kinetics in therapeutically useful drug action. *Curr Opin Drug Discov Devel* 2009;12(1):31-39.
8. Zhang R, Monsma F. Binding kinetics and mechanism of action: toward the discovery and development of better and best in class drugs. *Expert Opin Drug Discov* 2010;5(11):1023-1029.
9. Nunez S, Venhorst J, Kruse CG. Target-drug interactions: first principles and their application to drug discovery. *Drug Discov Today* 2012;17(1-2):10-22.
10. Swinney DC. Biochemical mechanisms of drug action: what does it take for success? *Nat Rev Drug Discov* 2004;3(9):801-808.
11. Dierynck I, De Wit M, Gustin E, Keuleers I, Vandersmissen J, Hallenberger S, Hertogs K. Binding kinetics of darunavir to human immunodeficiency virus type 1 protease explain the potent

- antiviral activity and high genetic barrier. *J Virol* 2007;81(24):13845-13851.
12. Casarosa P, Kollak I, Kiechle T, Ostermann A, Schnapp A, Kiesling R, Pieper M, Sieger P, Gantner F. Functional and biochemical rationales for the 24-hour-long duration of action of olodaterol. *J Pharm Exp Ther* 2011;337(3):600-609.
 13. Tee AK, Koh MS, Gibson PG, Lasserson TJ, Wilson AJ, Irving LB. Long-acting beta₂-agonists versus theophylline for maintenance treatment of asthma. *Cochrane Database Syst Rev* 2007(3):CD001281.
 14. Watson C, Jenkinson S, Kazmierski W, Kenakin T. The CCR5 receptor-based mechanism of action of 873140, a potent allosteric noncompetitive HIV entry inhibitor. *Mol Pharmacol* 2005;67(4):1268-1282.
 15. Van Liefde I, Vauquelin G. Sartan-AT₁ receptor interactions: in vitro evidence for insurmountable antagonism and inverse agonism. *Mol Cell Endocrinol* 2009;302(2):237-243.
 16. Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Müller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. *Pharmacol Rev* 2011;63(1):1-34.
 17. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov* 2006;5(3):247-264.
 18. Dixon AK, Gubitzi AK, Sirinathsinghji DJ, Richardson PJ, Freeman TC. Tissue distribution of adenosine receptor mRNAs in the rat. *Br J Pharmacol* 1996;118(6):1461-1468.
 19. Chen JF, Eltzhig HK, Fredholm BB. Adenosine receptors as drug targets - what are the challenges? *Nat Rev Drug Discov* 2013;12(4):265-286.
 20. Albrecht-Kupper BE, Leineweber K, Nell PG. Partial adenosine A₁ receptor agonists for cardiovascular therapies. *Purinerg Signal* 2012;8:S91-S99.
 21. Stenberg D, Litonius E, Halldner L, Johansson B, Fredholm BB, Porkka-Heiskanen T. Sleep and its homeostatic regulation in mice lacking the adenosine A₁ receptor. *J Sleep Res* 2003;12(4):283-290.
 22. Maemoto T, Tada M, Mihara T, Ueyama N, Matsuoka H, Harada K, Yamaji T, Shirakawa K, Kuroda S, Akahane A, Iwashita A, Matsuoka N, Mutoh S. Pharmacological characterization of FR194921, a new potent, selective, and orally active antagonist for central adenosine A₁ receptors. *J Pharmacol Sci* 2004;96(1):42-52.
 23. Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. Structure and function of adenosine receptors and their genes. *N-S Arch Pharmacol* 2000;362(4-5):364-374.
 24. Kull B, Svenningsson P, Fredholm BB. Adenosine A_{2A} receptors are colocalized with and activate G_{oif} in rat striatum. *Mol Pharmacol* 2000;58(4):771-777.
 25. Corvol JC, Studler JM, Schonn JS, Girault JA, Herve D. G alpha(olf) is necessary for coupling D₁ and A_{2A} receptors to adenylyl cyclase in the striatum. *J Neurochem* 2001;76(5):1585-1588.
 26. Ohta A, Sitkovsky M. Role of G protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 2001;414(6866):916-920.
 27. Gao Z, Li Z, Baker SP, Lasley RD, Meyer S, Elzein E, Palle V, Zablocki JA, Blackburn B, Belardinelli L. Novel short-acting A_{2A} adenosine receptor agonists for coronary vasodilation: inverse relationship between affinity and duration of action of A_{2A} agonists. *J Pharmacol Exp Ther* 2001;298(1):209-218.
 28. Morelli M, Di Paolo T, Wardas J, Calon F, Xiao D, Schwarzschild MA. Role of adenosine A_{2A} receptors in parkinsonian motor impairment and l-DOPA-induced motor complications. *Prog Neurobiol* 2007;83(5):293-309.
 29. Hack SP, Christie MJ. Adaptations in adenosine signaling in drug dependence: therapeutic

- implications. *Crit Rev Neurobiol* 2003;15(3-4):235-274.
30. Ferre S, Diamond I, Goldberg SR, Yao L, Hourani SM, Huang ZL, Urade Y, Kitchen I. Adenosine A_{2A} receptors in ventral striatum, hypothalamus and nociceptive circuitry implications for drug addiction, sleep and pain. *Prog Neurobiol* 2007;83(5):332-347.
 31. Ghimire G, Hage FG, Heo J, Iskandrian AE. Regadenoson: a focused update. *J Nucl Cardiol* 2013;20(2):284-288.
 32. Hodgson RA, Bertorelli R, Varty GB, Lachowicz JE, Forlani A, Fredduzzi S, Cohen-Williams ME, Higgins GA, Impagnatiello F, Nicolussi E, Parra LE, Foster C, Zhai Y, Neustadt BR, Stamford AW, Parker EM, Reggiani A, Hunter JC. Characterization of the potent and highly selective A_{2A} receptor antagonists preladenant and SCH412348 [7-[2-[4-2,4-difluorophenyl]-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazol o[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine] in rodent models of movement disorders and depression. *J Pharmacol Exp Ther* 2009;330(1):294-303.
 33. Headrick JP, Hack B, Ashton KJ. Acute adenosinergic cardioprotection in ischemic-reperfused hearts. *Am J Physiol Heart Circ Physiol* 2003;285(5):H1797-1818.
 34. Reichelt ME, Willems L, Molina JG, Sun CX, Noble JC, Ashton KJ, Schnermann J, Blackburn MR, Headrick JP. Genetic deletion of the A_1 adenosine receptor limits myocardial ischemic tolerance. *Circ Res* 2005;96(3):363-367.
 35. Mustafa SJ, Morrison RR, Teng B, Pelleg A. Adenosine receptors and the heart: role in regulation of coronary blood flow and cardiac electrophysiology. *Handb Exp Pharmacol* 2009(193):161-188.
 36. Salamone JD. Preladenant, a novel adenosine A_{2A} receptor antagonist for the potential treatment of parkinsonism and other disorders. *IDrugs* 2010;13(10):723-731.

