Pharmacology & Therapeutics 198 (2019) 20–33

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

The adenosine A_{2B} G protein-coupled receptor: Recent advances and therapeutic implications

Elizabeth A. Vecchio [a,b], Paul J. White [a], Lauren T. May [a,*]

[a] Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia
[b] Heart Failure Pharmacology, Baker Heart & Diabetes Institute, Melbourne, VIC 3004, Australia

ARTICLE INFO

Keywords:
adenosine A_{2B} receptor
ischaemia-reperfusion injury
fibrosis
cancer
inflammation and immune response

ABSTRACT

The adenosine A_{2B} receptor (A_{2B}AR) is one of four adenosine receptor subtypes belonging to the Class A family of G protein-coupled receptors (GPCRs). Until recently, the A_{2B}AR remained poorly characterised, in part due to its relatively low affinity for the endogenous agonist adenosine and therefore presumed minor physiological significance. However, the substantial increase in extracellular adenosine concentration, the sensitisation of the receptor and the upregulation of A_{2B}AR expression under conditions of hypoxia and inflammation, suggest the A_{2B}AR as an exciting therapeutic target in a variety of pathological disease states. Here we discuss the pharmacology of the A_{2B}AR and outline its role in pathophysiology including ischaemia-reperfusion injury, fibrosis, inflammation and cancer.

Contents

Abbreviations: AC, adenylyl cyclase; ADA, adenosine deaminase; AR, adenosine receptor; A_1AR, adenosine A_1 receptor; A_{2A}AR, adenosine A_{2A} receptor; A_{2B}AR, adenosine A_{2B} receptor; A_3AR, adenosine A_3 receptor; αSMA, alpha-smooth muscle actin; BAY60-6583, 2-((6-amino-3,5-dicyano-4-(4-(cyclopropylmethoxy)phenyl)-2-pyridinyl)thio)acetamide; cAMP, cyclic adenosine monophosphate; COPD, chronic obstructive pulmonary disease; CTGF, connective tissue growth factor; CVT-6883 (GS-6201), 3-ethyl-1-propyl-8-[1-[[3-(trifluoromethyl)phenyl]methyl]pyrazol-4-yl]-7H-purine-2,6-dione; DAG, diacylglycerol; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; EPAC, exchange factor directly activated by cAMP; ERK1/2, extracellular signal-regulated protein kinase 1 and 2; Fra-1, Fos-related antigen-1; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinases; HEK, human embryonic kidney cells; HIF-1α, hypoxia-inducible factor 1α; IFN, interferon; IL, interleukin; IP_3, inositol triphosphate; IRI, ischaemia-reperfusion injury; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mRNA, messenger ribonucleic acid; MRS1754, N-(4-cyanophenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-7H-purin-8-yl)phenoxy]acetamide, NECA, 5'-N-ethylcarboxamidoadenosine; NF-κB, nuclear factor-kappa B; Nox4, NADPH oxidase 4; OSIP 333931, N-[2-[[2-phenyl-6-[4-(3-phenylpropyl)piperazine-1-carbonyl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl]amino]ethyl] acetamide; PDE1C, phosphodiesterase type 1C; Per2, circadian rhythm protein period 2; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PSB-1115, 4-(2,6-dioxo-1-propyl-3,7-dihydropurin-8-yl)benzenesulfonic acid; PSB-603, 8-[4-[4-(4-chlorophenyl)piperazin-1-yl]sulfonylphenyl]-1-propyl-3,7-dihydropurine-2,6-dione; SNARE, soluble N-ethylmaleimide-sensitive factor activating protein receptor; TGF-β1, transforming growth factor beta 1; TNF-α, tumour necrosis factor alpha; VCP746, 4-(5-amino-4-benzoyl-3-(3-(trifluoromethyl)phenyl)thiophen-2-yl)-N-(6-(9-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxylmethyl)tetrahydro-furan-2-yl)-9H-purin-6-ylamino)hexyl)benzamide; VEGF, vascular endothelial growth factor; ZM241385, 4-[2-[[7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl]amino]ethyl]phenol.

* Corresponding author at: Drug Discovery Biology and Department of Pharmacology, Monash Institute of Pharmaceutical Sciences, Monash University, 399 Royal Parade, Parkville, VIC 3052, Australia.
E-mail address: lauren.may@monash.edu (L.T. May).

https://doi.org/10.1016/j.pharmthera.2019.01.003
0163-7258/

1. Introduction

Adenosine is an endogenous purine nucleoside present both intracellularly and extracellularly in living cells. It is comprised of an adenine group attached to a ribose sugar by a glycosidic bond. Adenosine as both a precursor and metabolite of adenine nucleotides, provides the structural building block of adenosine triphosphate and thus plays a central role in the basic energy transfer of all living organisms (Fredholm, 2007; Layland, Carrick, Lee, Oldroyd, & Berry, 2014). Adenosine also acts as a ubiquitous extracellular signalling molecule to exert a plethora of physiological actions throughout the body. It is often described as a 'retaliatory metabolite' owing to the fact that adenosine reduces cellular work and restores energy balance in the very same cells within which it is produced (Newby, 1984; Shyrock & Belardinelli, 1997). Adenosine was first identified as a modulator of coronary vascular tone, heart rate and blood pressure in 1929 (Drury & Szent-Györgyi, 1929) and is still used as mainstay clinical therapy for patients with supraventricular tachycardia today (Eltzschig, 2009). Much of the early understanding of the physiological role of adenosine comes from the cardiovascular system, however it has since been recognised to have critical roles in nearly every organ system and tissue. This includes as a regulator of the; i) central nervous system (Dunwiddie & Masino, 2001; Fredholm, Chen, Masino, & Vaugeois, 2005), ii) inflammatory and immune response (Fredholm, 2007; Haskó, Linden, Cronstein, & Pacher, 2008), iii) endocrine system (Dong, Ginsberg, & Erlanger, 2001; Figler et al., 2011), and as an endogenous modulator of; i) pain (Sawynok, 2016; Zylka, 2011), ii) lung function (Wilson et al., 2009; Zhou, Schneider, & Blackburn, 2009) and iii) kidney function (Roberts, Cowan, Alexander, Robson, & Dwyer, 2014).

Despite being recognised as an endogenous regulator of the cardiovascular system in 1929 (Drury & Szent-Györgyi, 1929), it took some 40 years of scientific discovery before it was postulated that the actions of adenosine were due to occupancy of specific cell surface receptors (Sattin & Rall, 1970). Today it is recognised that adenosine mediates a myriad of physiological (and pathological) actions via activation of adenosine receptors, which belong to the superfamily of G protein-coupled receptors (GPCRs).

1.1. Classification of adenosine receptors

Adenosine is the endogenous agonist for 4 GPCRs; the adenosine A_1 receptor (A_1AR), the adenosine A_{2A} receptor (A_{2A}AR), the adenosine A_{2B} receptor (A_{2B}AR) and the adenosine A_3 receptor (A_3AR) (Fredholm et al., 2000). Adenosine receptors were originally classified based on the effects of receptor stimulation to either inhibit (A_1 or R_i) or stimulate (A_2 or R_a) the activity of adenylyl cyclase (AC) (van Calker, Müller, & Hamprecht, 1979; Londos, Cooper, & Wolff, 1980). Adenosine A_2 receptors were subdivided based on the capacity of low (0.5 μM) or high (>10 μM) concentrations of 2'-chloroadenosine, an adenosine analogue, to stimulate cAMP accumulation in the rat brain (Daly, Butts-Lamb, & Padgett, 1983) and were subsequently defined as the A_{2A}AR and A_{2B}AR respectively (Bruns, Lu, & Pugsley, 1986). The most recently discovered subtype, the A_3AR, was identified from its sequence homology to other adenosine receptors during molecular cloning from rat testis (Zhou et al., 1992). All four adenosine receptors have since been cloned in humans (and many other species) with the greatest similarity between the A_1AR and A_3ARs (49% sequence homology) and the A_{2A}AR and A_{2B}ARs (59% sequence identity) (Fredholm et al., 2000; Fredholm, IJzerman, Jacobson, Klotz, & Linden, 2001; Jacobson & Gao, 2006). Given the widening appreciation of the role of the A_{2B}AR in physiology and pathophysiology, the purpose of this review is to highlight the recent advances in the understanding of A_{2B}AR pharmacology and how this adenosine receptor subtype may be therapeutically targeted in disease.

2. Adenosine A_{2B} receptor: distribution, regulation, pharmacology

The A_{2B}AR is a GPCR of 332 amino acid length with a molecular weight of approximately 36 kDa (Stehle et al., 1992). At the time of its discovery, the A_{2B}AR was distinguished from the A_{2A}AR based on its ability to stimulate cAMP production at higher (>10 μM) 2'-chloroadenosine concentrations (Daly et al., 1983). The A_{2B}AR was subsequently classified as a low-affinity receptor due to its modest-to-negligible activation by adenosine and prototypical agonists (Beukers, Dulk den, van Tilburg, Brouwer, & IJzerman, 2000; Feoktistov & Biaggioni, 1997; Fredholm, Irenius, Kull, & Schulte, 2001). It was not until the development of subtype-selective ligands (in particular antagonists) that the A_{2B}AR was shown to couple to diverse intracellular signalling pathways and exert different physiological effects. Today, the A_{2B}AR is increasingly being recognised as an important target in numerous pathologies, including ischaemia-reperfusion injury (Eltzschig, Bonney, & Eckle, 2013; Hart, Jacobi, Schittenhelm, Henn, & Eltzschig, 2009), fibrosis (Dubey, Gillespie, & Jackson, 1998), inflammation and the immune response (Ham & Rees, 2008), sickle-cell anaemia (Zhang et al., 2011), diabetes (Merighi, Borea, & Gessi, 2015) and cancer (Cekic et al., 2011; Ma et al., 2010; Wei, Costanzi, Balasubramanian, Gao, & Jacobson, 2013).

2.1. Tissue distribution of adenosine A_{2B} receptors

Efforts to elucidate A_{2B}AR tissue expression were for a long time hampered by the lack of selective and useful radioligands or antibodies and as such, much of the early work on A_{2B}AR distribution relied on the expression data of the corresponding mRNA (Feoktistov & Biaggioni, 1997). The A_{2B}AR was first cloned from the rat hypothalamus and human brain in 1992 (Pierce, Furlong, Selbie, & Shine, 1992; Rivkees & Reppert, 1992) and was demonstrated to be present in the caecum, large intestine and bladder. Lower levels were also demonstrated in the brain, spinal cord, lung, vas deferens and pituitary (Stehle et al., 1992). Later it was shown to have a fairly ubiquitous distribution with A_{2B}AR mRNA detected at various levels, though generally low abundance, in all rat tissues studied (Dixon, Gubitz, Sirinathsinghji, Richardson, & Freeman, 1996). The more recent development of an A_{2B}AR-knockout/reporter gene-knock-in mouse provided a valuable tool that enabled the determination of A_{2B}AR tissue distribution *in vivo* (Yang et al., 2006). The A_{2B}AR was demonstrated to be extensively distributed throughout the vasculature of most organs including, heart, lung, brain, pancreas, retina, reproductive organs, liver, kidney and large intestine as well as patchy expression in smooth muscle and endothelial cells (St Hilaire, Carroll, Chen, & Ravid, 2009; Yang et al., 2006). Furthermore, many studies have shown the A_{2B}AR is widely expressed in many diverse cell types including taste cells, mast cells, neutrophils, macrophages, lymphocytes, neurons, glial cells, epithelial cells, myocardial cells and fibroblasts (Reviewed in: Feoktistov & Biaggioni, 1997; Sun & Huang, 2016). The functional expression of the A_{2B}AR in such a wide array of tissues and cell types, which can be further regulated by various environmental stimuli, highlights the physiological and pathological importance of this receptor subtype.

2.2. Regulation of adenosine A_{2B} receptor expression

A_{2B}AR expression is influenced by changes in the cellular microenvironment and is induced in response to inflammation, ischaemia-reperfusion injury and hypoxia (Fredholm, 2007; Ham & Rees, 2008; Haskó, Csóka, Németh, Vizi, & Pacher, 2009). Hypoxia directly increases the expression of the A_{2B}AR mRNA via a canonical hypoxia-inducible factor 1α (HIF-1α) binding site in the promoter (Feoktistov et al., 2004; Kong, Westerman, Faigle, Eltzschig, & Colgan, 2006). There is also a positive feedback mechanism whereby the A_{2B}AR increases HIF-1α through stabilisation of the circadian rhythm protein period 2 (Per2) (Eckle et al., 2012). Factors present in an inflammatory

environment such as the pro-inflammatory cytokines; tumour necrosis factor (TNF)-α (Kolachala et al., 2005), interferon (IFN)-γ (Xaus et al., 1999), interleukin (IL)-1β (Khoa, Montesinos, Williams, Kelly, & Cronstein, 2003), and the reactive oxygen species-generating enzyme Nox4 (St Hilaire, Koupenova, Carroll, Smith, & Ravid, 2008) also upregulate A_{2B}AR expression at the mRNA and protein level. In addition, the endogenous agonist adenosine has been demonstrated to recruit intracellular pools of A_{2B}AR to the plasma membrane on intestinal epithelial cells (Sitaraman et al., 2002; Lixin Wang et al., 2004) and may represent a more general mechanism of regulating A_{2B}AR expression at the cell-surface. Evidence for intracellular localisation of the A_{2B}AR has been further supported by fluorescence techniques which suggests the A_{2B}AR resides at the mitochondria rather than the sarcolemma in isolated cardiomyocytes (Grube et al., 2011).

2.3. Adenosine A_{2B} receptor binding partners/interacting proteins

The A_{2B}AR interacts with a number of proteins and binding partners that appear to have important roles in modulating receptor function (Sun & Huang, 2016). Adenosine deaminase (ADA), in addition to degrading extracellular adenosine, has been suggested to anchor to the cell surface and independent of enzymatic activity, increase the binding affinity of the A_{2B}AR for a non-selective adenosine receptor agonist NECA (Herrera et al., 2001). ADA was later shown to co-localise with the A_{2B}AR on dendritic cells and this co-localisation markedly increased the production of pro-inflammatory cytokines (Pacheco et al., 2005). Similarly, intracellular protein kinase C (PKC) increases the sensitivity of the A_{2B}AR and potentiates agonist activation of the pro-survival kinases, phosphoinositide 3-kinase (PI3K) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) which contributes to ischaemic preconditioning in the heart (Kuno et al., 2007). Co-expression with the A_{2A}AR appears to alter receptor pharmacology through heterodimeric A_{2A}AR-A_{2B}AR interactions (Hinz et al., 2018), and improves the cell-surface expression of the A_{2B}AR, presumably by providing the dominant forward-transport signal for export from the endoplasmic reticulum (Moriyama & Sitkovsky, 2010). Other proteins that have been implicated in the translocation of the A_{2B}AR to the plasma membrane include the scaffold-based regulatory proteins E3KARP and ezrin and the trafficking soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins (Sitaraman et al., 2002; Lixin Wang et al., 2004). These proteins may help to transport and stabilise the receptor in a signalling complex at the plasma membrane, possibly through interaction with a PDZ-binding motif on the C-terminal end of the A_{2B}AR (Sitaraman et al., 2002). The PDZ-binding domain of the A_{2B}AR also interacts with the cystic fibrosis transmembrane conductance regulator, leading to enhanced A_{2B}AR expression and agonist-mediated cAMP production which results in enhanced signalling of the innate lung defence system (Watson et al., 2011; Watson et al., 2016). In addition, the C-terminus of the A_{2B}AR is involved in complexing with the actin-filament-crosslinking protein, α-actinin-1, which stabilises the receptor's global and cell-surface expression (Sun et al., 2016). Functional interactions of the A_{2B}AR with phosphodiesterase type 1C (PDE1C) have recently been implicated in the cardioprotective actions of PDE1C inhibition and may be involved in the regulation and compartmentalisation of protective cAMP signalling within the heart (Hashimoto et al., 2018; Zhang, Knight, Chen, Mohan, & Yan, 2018). Like many GPCRs, the agonist-activated A_{2B}AR undergoes desensitisation to limit the duration and magnitude of receptor activation. This is evidenced by significantly reduced cAMP production following pre-treatment with the non-selective adenosine receptor agonist NECA in a variety of A_{2B}AR-endogenously expressing cells including; COS7 (Peters, Gies, Gelb, & Peterfreund, 1998), neuroblastoma-hybrid NG108-15 (Mundell & Kelly, 1998) and isolated rat pulmonary arterial smooth muscle cells (Haynes, Obiako, Babal, & Stevens, 1999). It is proposed that desensitisation of the A_{2B}AR occurs via recruitment of G protein-coupled receptor kinases (GRKs) and β-arrestins (Klaasse, IJzerman, Grip, & Beukers, 2007). GRKs phosphorylate the agonist bound receptor, which then promotes recruitment of β-arrestin1 and/or β-arrestin2 to sterically impede further G protein receptor engagement, thus acting to terminate G protein-signalling (See reviews: Pitcher, Freedman, & Lefkowitz, 1998; Lefkowitz, Rajagopal, & Whalen, 2006). Both GRK2 and GRK5 have been implicated in A_{2B}AR desensitisation (Mundell & Kelly, 1998; Nash et al., 2018). The subsequent internalisation and recycling of the A_{2B}AR via β-arrestins is dependent on a serine residue close to the COOH-terminus (Matharu, Mundell, Benovic, & Kelly, 2001) and requires the presence of the Type II PDZ-domain in the C-terminal tail of the receptor (Mundell et al., 2010). From changes in receptor trafficking and stabilisation, signalling pathways and desensitisation, it is clear these binding partners and protein complexes have the potential to alter A_{2B}AR pharmacology and contribute to the contrasting effects observed downstream of the A_{2B}AR.

2.4. Signal transduction pathways of adenosine A_{2B} receptors

The A_{2B}AR has been shown to activate adenylyl cyclase via interaction with G_s proteins in many different cell types (Feoktistov & Biaggioni, 1997). Further, it is now appreciated that additional intracellular signalling pathways are functionally coupled to the A_{2B}AR (Fig. 1). The A_{2B}AR has been proposed to interact with G_q proteins to activate phospholipase C (PLC) leading to increased PKC activation and elevations of inositol triphosphate (IP_3) and intracellular calcium (Linden, Thai, Figler, Jin, & Robeva, 1999). Ca^{2+} can also be mobilised via direct activation of calcium channels through a cholera toxin-sensitive mechanism likely downstream of Gβγ (Feoktistov, Murray, & Biaggioni, 1994) or indirectly via activation of PKA (Mogul, Adams, & Fox, 1993). The A_{2B}AR also stimulates ERK1/2, c-Jun N-terminal Kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) signalling (Feoktistov, Goldstein, & Biaggioni, 1999; Gao, Chen, Weber, & Linden, 1999; Schulte & Fredholm, 2003), however there is ongoing debate as to which G protein is upstream of these intracellular second messengers (Aherne, Kewley, & Eltzschig, 2011). In addition to G_s and G_q coupling, the A_{2B}AR is also proposed to interact with inhibitory $G_{i/o}$ proteins (Gao, Inoue, & Jacobson, 2018; Yang et al., 2011). A proportion of ERK1/2 phosphorylation (pERK1/2) was shown to be pertussis toxin-sensitive in endogenously expressing and A_{2B}AR-transfected HEK293 cells; where pertussis toxin selectively uncouples G_i and G_o proteins from receptor activation (Gao et al., 2018; Yang et al., 2011). In T24 bladder cancer cells, NECA-stimulated pERK1/2 was completely blocked by pertussis toxin and significantly enhanced in the presence of G_s-inhibitory cholera toxin, which highlights the interplay of multiple G protein-signalling pathways for the generation of ERK1/2 in this cell type (Gao et al., 2018). Preferential G protein-coupling and intracellular signalling appears to be largely cell background dependent, which may account for the plasticity of A_{2B}AR signalling and the differential actions of the A_{2B}AR.

2.5. Adenosine A_{2B} receptor ligands

The pharmacological characterisation of the A_{2B}AR had languished behind the other adenosine receptor subtypes due to the paucity of potent and selective A_{2B}AR ligands (Fredholm, IJzerman, et al., 2001). However, an improved understanding of the A_{2B}AR in recent years has followed with the development of new pharmacological tools, in particular high affinity antagonists.

2.5.1. Adenosine A_{2B} receptor antagonists

A_{2B}AR antagonists can be broadly divided into two classes, xanthines and non-xanthine derivatives. Xanthines are based on the structure of the classic non-selective adenosine receptor antagonists caffeine (Table 1), with theophylline and many of the current high affinity A_{2B}AR antagonists possessing this same core structure (Baraldi et al.,

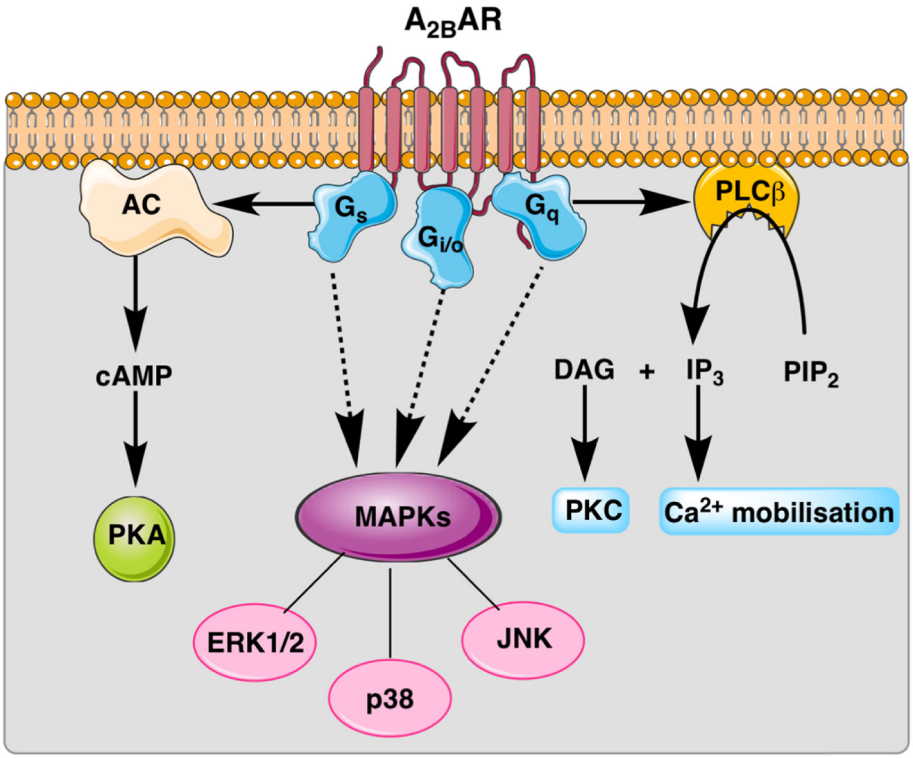


Fig. 1. General signalling pathways mediated by the A_{2B}AR. Figure created using the Servier Medical Art resource (https://smart.servier.com).

2006; Kalla & Zablocki, 2009). One of the first discovered derivatives was DPCPX; (Table 1) which showed good affinity at the A_{2B}AR but had higher affinity at the A_1AR (Ortore & Martinelli, 2010). Lengthening of the alkyl substituents in the 1,3-positions or 1,8-disubstitution generated the first high-affinity A_{2B}AR-selective antagonists, MRS1754 (Kim, Ji, Melman, Linden, & Jacobson, 2000) and PSB-1115 (Hayallah et al., 2002) (Table 1). Further modification generated PSB-603 (Table 1), which shows high affinity and selectivity not only in humans but also in rodents. It is commonly used as a pharmacological tool to study the A_{2B}AR due to its sub-nanomolar affinity (Borrmann et al., 2009). Modification of xanthines at the 8-position with certain aryl groups has given rise to high-affinity A_{2B}AR antagonists that have progressed as preclinical candidates. For example CVT-6883 (also known as GS-6201; Table 1) has been investigated for the treatment of asthma (Elzein et al., 2008). More recently, a fluorescently labelled A_{2B}AR antagonist, which combined an 8-substituted xanthine pharmacophore with a BODIPY derivative (PSB-12105) was developed as a novel tool to probe further probe A_{2B}AR pharmacology that has nanomolar affinity and significant subtype selectivity (Köse et al., 2018). Non-xanthine derivatives include ZM241385 (initially characterised as an A_{2A}AR-selective antagonist) and OSIP 339391 (Table 1; Ji & Jacobson, 1999; Stewart et al., 2004). Computational approaches using pharmacophore based virtual screening, recently identified a structurally unique pyrimidine derivative (Z1139491704) that has potent anti-sickling activity and low cytotoxicity and thus may represent a novel therapeutic agent for patients with sickle cell anaemia (Paz, de Jesus Pinheiro, do Espirito Santo, Villarreal, & Castilho, 2017).

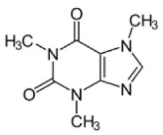
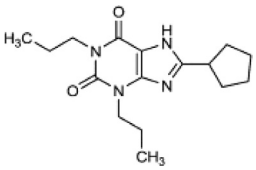
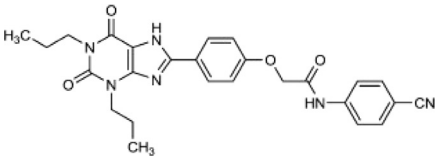
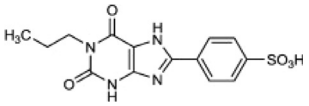
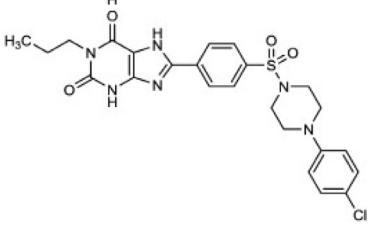
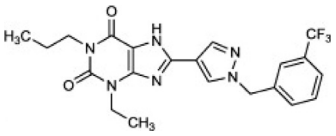
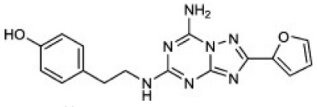
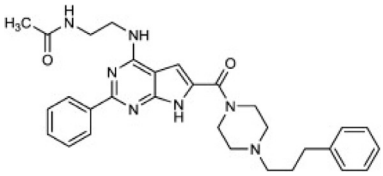
2.5.2. Adenosine A_{2B} receptor agonists

The goal of attaining subtype selectivity for A_{2B}AR agonists has been even more challenging than for antagonists, however progress has been made in recent years (Feoktistov & Biaggioni, 2011). Agonists can be classified as adenosine-like or non-adenosine ligands based on the presence or absence of a nucleoside-like core respectively (Baraldi, Tabrizi, Fruttarolo, & Romagnoli, 2009). Adenosine modifications focusing on the N^6 and C^2 positions of the purine heterocycle have generated agonists with increased potency for the A_{2B}AR and include the non-selective N^6-modified adenosine derivative, 5'-N-ethylcarboxamidoadenosine (NECA; Table 2) (de Zwart, Link, Kunzela, et al., 1998). Despite being non-selective across all adenosine receptor subtypes, NECA is still widely used as one of the most potent A_{2B}AR agonists (EC_{50} ≈150 nM) (Baraldi et al., 2009). Further modifications at the N^6 position yielded a novel series of NECA derivatives with higher potency but again low A_{2B}AR-selectivity (Baraldi et al., 2007). In pursuit of enhanced subtype-selectivity, drug discovery efforts shifted to the development of non-nucleoside agonists. A patent describing 2-aminopyridine-3,5-dicarbonitrile derivatives as adenosine receptor ligands identified 2-((6-amino-3,5-dicyano-4-(4-(cyclopropylmethoxy) phenyl)-2-pyridinyl)thio) acetamide (BAY60-6583; Table 2) as an A_{2B}AR agonist with potency in the low nanomolar range and high selectivity versus the other adenosine receptors (Rosentreter et al., 2001). BAY60-6583 has since been widely used in animal studies for the pharmacological characterisation of the role of the A_{2B}AR, however it has recently been identified as a partial agonist (Hinz, Lacher, Seibt, & Muller, 2014). A further series of 2-amino-4-phenyl-6-phenylsulfanylpyridine-3,5-dicarbonitriles identified agonists with enhanced A_{2B}AR activity, one of which behaved as a partial agonist (LUF5845) and another as a potent full agonist with an EC_{50} of 10 nM (LUF5835) (Beukers, Chang, Künzel, et al., 2004). None of the compounds were as selective for the A_{2B}AR as BAY 60-6583, however one derivative LUF5834 was proposed to be of particular interest thanks to its high potency at the A_{2B}AR (EC_{50}=12 nM) and significant selectivity versus the A_3AR making it a useful tool to distinguish the contributions of these two receptor subtypes in mast cells (Baraldi et al., 2009; Beukers et al., 2004).

2.5.3. Biased ligands

In recent years it has become evident that structurally-distinct ligands occupying the same GPCR in the same cellular background can generate different functional outcomes in a manner that cannot be explained by simple differences in stimulus-response coupling (Luttrell, Maudsley, & Bohn, 2015; Shonberg et al., 2014). The ability of a ligand to stabilise unique receptor conformations to selectively activate a

Table 1
Structure and binding affinity of A_{2B}AR antagonists.

Name	Chemical Structure	hA_{2B}AR Affinity pK_i (K_i nM)	References
Caffeine		4.69 - 4.98 (10,400-20,500)	(Bertarelli et al., 2006; Kim et al., 2002)
DPCPX		7.19 - 7.29 (51-64)	(Kim et al., 2002; Weyler et al., 2006)
MRS1754		8.84 (1.45)	(Ji, Kim, Ahern, Linden, & Jacobson, 2001)
PSB-1115		7.28 (53)	(Hayallah et al., 2002)
PSB-603		9.26 (0.55)	(Borrmann et al., 2009)
CVT-6883 (GS-6201)		7.66 (22)	(Elzein et al., 2008)
ZM241385		6.84 (145)	(Ji et al., 2001)
OSIP 339391		9.39 (0.41)	(Stewart et al., 2004)

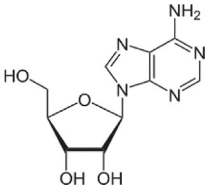
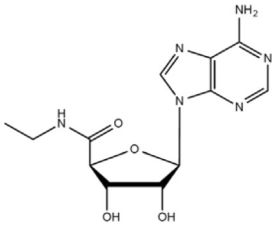
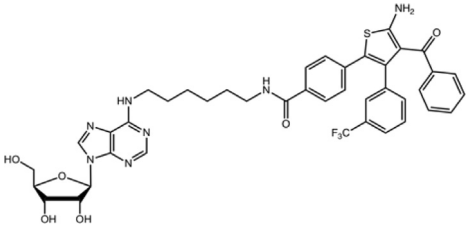
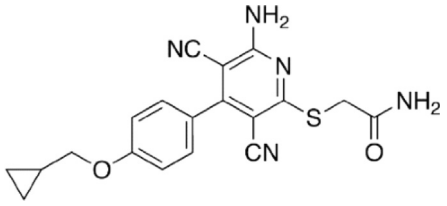
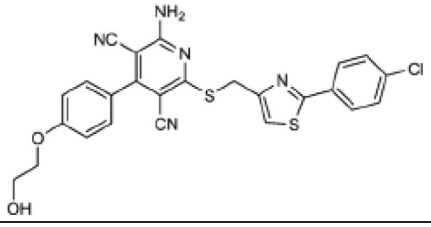
pK_i denotes the negative logarithm of the ligand equilibrium dissociation constant

subset of intracellular signalling pathways is termed 'functional selectivity' or 'biased agonism' (Kenakin & Christopoulos, 2013). Biased agonism has significant clinical potential, enabling the rational design of drugs that activate therapeutic signalling whilst eschewing on-target adverse effects (Violin, Crombie, Soergel, & Lark, 2014). Recent studies at the A_{2B}AR have identified that BAY60-6583 is a biased A_{2B}AR agonist with a unique signalling profile, even acting as an A_{2B}AR antagonist in low expressing MIN-6 mouse pancreatic β cells (Gao, Balasubramanian, Kiselev, Wei, & Jacobson, 2014). Further, compounds previously characterised as A_1AR agonists, capadenoson (Baltos et al., 2017; Table 2) and VCP746 (Vecchio et al., 2016; Table 2) were shown to stimulate A_{2B}AR-biased agonism. Of note, VCP746 had a higher affinity than NECA or BAY60-6583 and was a potent activator of all G protein signalling pathways relative to the reference ligand NECA (Vecchio, Chuo, et al., 2016). Capadenoson and VCP746 both have desirable activity profiles within cardiac cells which is proposed to be as consequence of their ability to stimulate potent A_{2B}AR-mediated cAMP accumulation (Baltos et al., 2017; Vecchio, Chuo, et al., 2016; Vecchio, White, & May, 2017). The continued understanding of beneficial signalling pathways in pathophysiology alongside agonist structure activity relationships should facilitate the future development of therapeutically efficacious biased A_{2B}AR ligands.

3. Role of adenosine A_{2B} receptors in pathophysiology

The A_{2B}AR is a low affinity receptor that until recently, was regarded as having minor physiological importance (Feoktistov & Biaggioni, 1997). However as illustrated below, the A_{2B}AR is upregulated and activated in numerous pathological conditions that are often associated with elevated adenosine levels. It provides protection against ischaemia-reperfusion injury, promotes cancer growth and metastasis, modulates the fibrotic response in a variety of organs and tissues and is an important regulator of the immune and inflammatory response.

Table 2
Structure and binding affinity of A_{2B}AR agonists.

Name	Chemical Structure	hA_{2B}AR Affinity pK_i (K_i nM)	References
Adenosine		4.62 (24,000)	(Yan, Burbiel, Maaß, & Müller, 2003)
NECA		6.48 (330)	(Linden et al., 1999)
VCP746		7.26 (55)	(Vecchio, Chuo, et al., 2016)
BAY 60-6583		6.67 (212)	(Hinz et al., 2014)
Capadenoson		5.49 (3240)	(Baltos et al., 2017)

pK_i denotes the negative logarithm of the ligand equilibrium dissociation constant

This section will review the literature for the role of the A_{2B}AR and outline some of the controversies that still remain regarding A_{2B}AR signalling in pathophysiology.

3.1. Ischaemia-reperfusion injury

During an ischaemic event the disruption or reduction in blood flow results in tissues being deprived of oxygen and nutrients, stimulating a series of biochemical and metabolic changes which contribute to cellular death. Therapy is aimed at restoring blood flow to the ischaemic area, however this unfortunately can elicit further tissue damage, collectively termed ischaemia-reperfusion injury (IRI) (Frank et al., 2012; Pantazi, Bejaoui, Folch-Puy, Adam, & Roselló-Catafau, 2016). Extracellular adenosine concentrations rapidly rise with ischaemic insult and this functions as an endogenous distress signal (Headrick, Hack, & Ashton, 2003; Headrick & Willis, 1988). Adenosine represents one of the most powerful and well-studied cytoprotective agents, particularly in the area of cardioprotection, with A_{2B}AR activation increasingly being implicated as an important modulator against IRI (Eltzschig, Rivera-Nieves, & Colgan, 2009; Sommerschild & Kirkebøen, 2000).

3.1.1. A_{2B}AR in myocardial ischaemia-reperfusion injury

Stimulation of adenosine receptors prior to ischaemia and at the time of reperfusion, has long been acknowledged to protect the myocardium from IRI in a variety of animal models (McIntosh & Lasley, 2012). It has provided an attractive therapeutic target for myocardial IRI that has been pursued in human clinical trials without fruition, in part due to the dose-limiting haemodynamic effects on heart rate and blood pressure (Kloner et al., 2006; Kopecky et al., 2003; Ross, Gibbons, Stone, Kloner, & Alexander, 2005). The A_{2B}AR has been identified as a novel modulator of myocardial cell survival by enhancing ischaemic tolerance (Chen, Eltzschig, & Fredholm, 2013; Eltzschig et al., 2013). The cardioprotective effects of the A_{2B}AR were first demonstrated after the A_{2B}AR-selective antagonist MRS1754 was shown to block the infarct-sparing action of the non-selective adenosine receptor agonist NECA in rabbit hearts (Philipp et al., 2006). This study also demonstrated ischaemic postconditioning, which involves brief cycles of reperfusion/occlusion at the end of the ischaemic phase, is cardioprotective via a PKC-A_{2B}AR-dependent pathway. Work from the same group suggested PKC phosphorylates the A_{2B}AR and raises the sensitivity of the heart to adenosine such that endogenous adenosine can promote protective Akt and ERK signalling via A_{2B}AR activation (Kuno et al., 2007, 2008). The cardioprotective actions of the A_{2B}AR were further demonstrated with

the A_{2B}AR-selective agonist, BAY60-6583, which was shown to reduce myocardial infarct size in isolated rabbit and rat hearts and intact mice when administered prior to, or at the onset of reperfusion (Grube et al., 2011; Kuno et al., 2007; Methner, Schmidt, Cohen, Downey, & Krieg, 2010; Xi et al., 2009). These effects were not observed in the hearts of A_{2B}AR knock out mice (Eckle et al., 2007). Most often, this A_{2B}AR-mediated reduction in infarct size was associated with concomitant stimulation of the A_{2A}AR (Busse et al., 2016; Lasley, Kristo, Keith, & Mentzer, 2006; Methner et al., 2010; Xi et al., 2009). A requirement for cooperative activation of adenosine receptors has also been observed for A_1AR-mediated cardioprotection (Urmaliya et al., 2009; Urmaliya, Pouton, Ledent, Short, & White, 2010; Zhan, McIntosh, & Lasley, 2011), suggesting both A_1AR and A_2AR subtypes are needed for the full effects of adenosine in protecting the heart against IRI.

A_{2B}AR-mediated cardioprotection has been suggested to involve a novel mechanism for improving oxygen-efficient metabolism through the stabilisation of the circadian rhythm protein Per2. Per2 mediates a metabolic switch that enhances myocardial glycolytic capacity, thereby providing enhanced ischaemic tolerance (Eckle et al., 2012). In addition to direct effects on the myocardium, A_{2B}AR reduction of IRI in the heart has been demonstrated to also involve modulation of the inflammatory response. *In vivo* studies using selective A_{2B}AR deletion on immune cells (Seo et al., 2015) or transplantation of wild-type bone marrow into A_{2B}AR knock out mice (Koeppen et al., 2012), demonstrated the A_{2B}AR dampens myocardial IRI via signalling on inflammatory cells. This appears to involve promotion of anti-inflammatory macrophage differentiation downstream of PI3K/Akt activation (Tian, Piras, Kron, French, & Yang, 2015) and inhibition of cardiomyocyte superoxide production, interestingly by a pertussis toxin sensitive $G_{i/o}$/ERK/PI3K pathway (Yang et al., 2011). The A_{2B}AR therefore represents an important modulator of adenosine-mediated cardioprotection against IRI. This is achieved via complex signalling on the myocardium and immune cells that possibly involves interplay with proteins such as Per2 and other adenosine receptor subtypes.

3.1.2. A_{2B}AR in ischaemia-reperfusion injury of other organs

The A_{2B}AR has also been implicated as an important modulator of IRI in other organs and tissues, though these effects have not been as widely studied as in the heart. Acute gastrointestinal IRI caused by surgery, organ transplantation, sepsis or haemorrhagic shock frequently results in bowel necrosis and is associated with a high mortality (Eltzschig et al., 2009). A protective role of the A_{2B}AR was initially suggested after mucosal scrapings following murine gastrointestinal IRI showed selective induction of A_{2B}AR expression (Hart et al., 2009). Pharmacological inhibition or targeted deletion of the A_{2B}AR enhanced intestinal inflammation and injury during ischaemia-reperfusion, whereas activation of the A_{2B}AR with the selective agonist BAY60-6583 was protective (Hart et al., 2009). The A_{2B}AR was further demonstrated to modulate intestinal barrier function (Yang et al., 2014) which may contribute to a protective role of the A_{2B}AR in gastrointestinal IRI. Similarly, using genetic and pharmacological manipulation, adenosine signalling was demonstrated to be protective against renal ischaemia via activation of the A_{2B}AR (Grenz et al., 2008). This study used A_{2B}AR bone marrow chimeric mice to elucidate that A_{2B}AR signalling on the renovasculature rather than haematopoietic cells was responsible for the renal protection conferred by ischaemic preconditioning (Grenz et al., 2008). Vascular A_{2B}AR activation has also been shown to promote endothelial barrier function to protect against hypoxia induced-vascular injury (Eckle, Faigle, Grenz, Laucher, & Thompson, 2008). Likewise, a model of acute mechanical vascular injury demonstrated a key role for the A_{2B}AR but this time showed bone marrow-derived A_{2B}ARs significantly contributed to tissue protection (Yang et al., 2008). In contrast, evidence in the lung suggests A_{2B}AR blockade significantly improves lung function and attenuates pro-inflammatory cytokine production following ischaemia-reperfusion, pointing at a detrimental effect of A_{2B}AR activation in lung IRI (Anvari et al., 2010; Huerter et al., 2016). Compared to peripheral organs, the role of the A_{2B}AR in cerebral IRI is not as well defined. Contrasting reports have suggested both A_{2B}AR agonism *in vivo* (Li et al., 2017) and antagonism *in vitro* (Fusco et al., 2018) provide a novel neuroprotective strategy for stroke. Collectively, these studies suggest a more widespread mechanism for A_{2B}AR-mediated protection from IRI throughout the body, with the exception perhaps of the lung and brain, though the precise signalling mechanisms still need to be elucidated.

3.2. Fibrosis

Fibrosis, characterised by the accumulation of extracellular matrix molecules that make up scar tissue, is a common feature of chronic tissue injury. Fibrosis underlies the pathology of many diseases including heart failure, idiopathic pulmonary fibrosis, scleroderma, hepatic cirrhosis and chronic kidney disease, which collectively represent a large disease burden with a huge unmet clinical need (Friedman, Sheppard, Duffield, & Violette, 2013). Adenosine, acting via the A_{2B}AR, has been identified as an important modulator of fibroblast homeostasis with both pro- and anti-fibrotic actions attributed to A_{2B}AR activation (Chan & Cronstein, 2009; Cronstein, 2011; Karmouty-Quintana, Xia, & Blackburn, 2013; Vecchio et al., 2017). As discussed in the following section, divergent signal transduction highlights both the complexity and ensuing challenges faced when targeting A_{2B}AR for the treatment of fibrosis.

3.2.1. Anti-fibrotic role of the A_{2B}AR

The A_{2B}AR was first proposed as a novel modulator of fibrosis within the heart some 20 years ago (Dubey et al., 1998; Dubey, Gillespie, Mi, & Jackson, 1997) and has subsequently been investigated in *in vitro* and *in vivo* models of cardiac fibrosis. Pharmacological targeting and genetic manipulation of the A_{2B}AR revealed that A_{2B}AR signal transduction inhibited proliferation and collagen synthesis in isolated cardiac fibroblasts (Chen et al., 2004; Dubey et al., 1998; Dubey, Gillespie, Zacharia, Mi, & Jackson, 2001). The expression of pro-fibrotic gene markers including collagen I and connective tissue growth factor (CTGF) was also inhibited in the presence of a novel A_{2B}AR agonist (Vecchio, Chuo, et al., 2016). In addition, activation of the A_{2B}AR attenuated endothelin-1- (Phosri et al., 2017), and angiotensin II- (Phosri, Bunrukchai, Parichatikanond, Sato, & Mangmool, 2018) stimulated expression of the myofibroblast marker α-smooth muscle actin (α-SMA), indicating the A_{2B}AR prevents conversion of fibroblasts to the pro-fibrogenic myofibroblast phenotype. The inhibitory activity on collagen synthesis and α-SMA expression is mediated by the second messenger cAMP and subsequent activation of an exchange protein directly activated by cAMP (EPAC) and PI3K dependent pathway (Fig. 2) (Epperson, Brunton, Ramirez-Sanchez, & Villarreal, 2009; Phosri et al., 2017; Villarreal, Epperson, Ramirez-Sanchez, Yamazaki, & Brunton, 2009). An anti-fibrotic role of the A_{2B}AR has been further supported by an *in vivo* study which demonstrated the selective A_{2B}AR antagonist MRS1754 was able to block an adenosine analogue (2-chloroadenosine)-mediated inhibition of collagen volume fraction and matrix metalloproteinase gene expression in a model of permanent coronary artery ligation (Wakeno et al., 2006). Additionally it is proposed that capadenoson, a partial A_1AR agonist that also stimulates A_{2B}AR cAMP signalling (Baltos et al., 2017), may reduce left ventricular interstitial fibrosis in dogs with advanced heart failure via an A_{2B}AR-dependent mechanism (Sabbah et al., 2013).

3.2.2. Pro-fibrotic role of the A_{2B}AR

In contrast to the majority of *in vitro* studies which identify an anti-fibrotic role for the A_{2B}AR, there are some *in vivo* models of cardiac fibrosis and remodelling that suggest a detrimental role of A_{2B}AR signalling. Blockade of the A_{2B}AR with a highly selective antagonist, GS-6201 significantly enhanced cardiac function and decreased fibrosis in the non-infarct and border zones in a model of myocardial IRI (Zhang

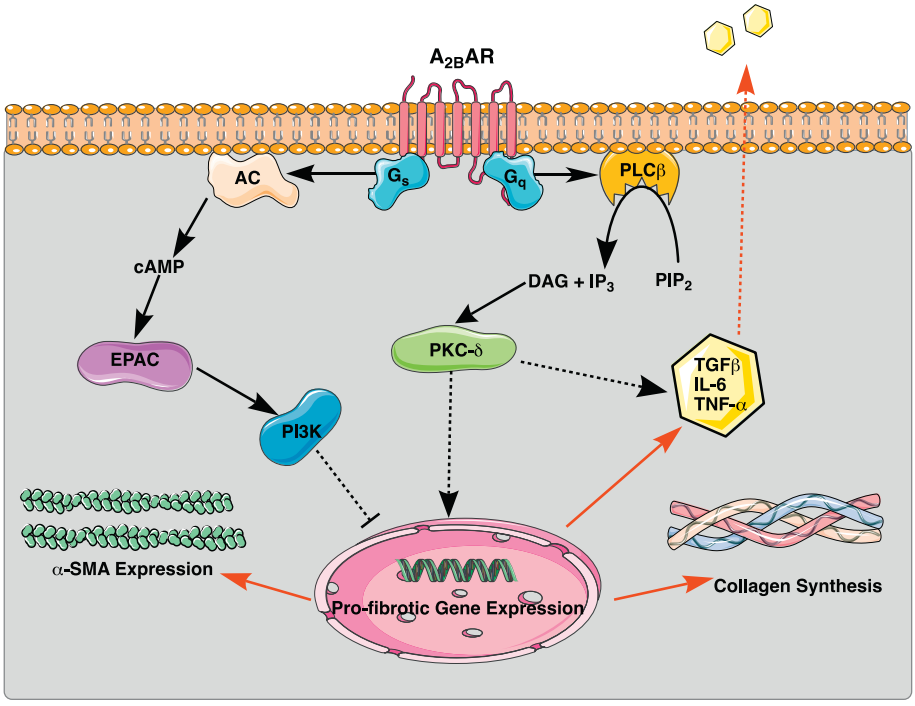


Fig. 2. Proposed A_{2B}AR-mediated signalling pathways involved in the regulation of fibrosis. Figure created using the Servier Medical Art resource (https://smart.servier.com).

et al., 2014). In agreement, a study in A_{2B}AR knock-out mice showed they had a similar infarct size but significantly reduced interstitial fibrosis and improved end diastolic pressure when compared to wild type mice 8 weeks after permanent left coronary ligation (Maas, Koupenova, Ravid, & Auchampach, 2008). Blockade of the A_{2B}AR inhibits pro-inflammatory leukocyte infiltration and caspase-1 activity (Toldo et al., 2012) and reduces secretion of transforming growth factor beta 1 (TGF-β1), TNF-α and IL-6, likely via a G_q/PKC-δ pathway in the heart, which suggests modulation of inflammatory mediators contributes to A_{2B}AR-mediated cardiac fibrosis (Feng, Song, Chen, Lu, & Zhang, 2009; Toldo et al., 2012; Zhang et al., 2014). A pro-fibrotic role of A_{2B}AR activation is also observed in other organ systems. In the lung, elevated adenosine levels are found in patients with asthma, chronic obstructive pulmonary disease (COPD) and idiopathic fibrosis and contributes to airway hyper-responsiveness, pulmonary inflammation, remodelling and fibrosis (Chan & Cronstein, 2009; Karmouty-Quintana et al., 2013). The A_{2B}AR has been shown to be critical for the action of adenosine in these chronic lung diseases (Sun et al., 2006). HIF-1α upregulation of the A_{2B}AR and subsequent receptor activation promotes the differentiation of human lung fibroblasts to myofibroblasts (Zhong, Belardinelli, Maa, & Zeng, 2005) and contributes to the progression of bleomycin-induced pulmonary fibrosis (Philip et al., 2017). Blockade with a highly selective A_{2B}AR antagonist CVT-6883 (also known as GS-6201) attenuates airway collagen deposition and pro-fibrotic gene expression and reduces myofibroblast number in ADA deficient and bleomycin-treated mice (Sun et al., 2006). Interestingly, genetic ablation of the A_{2B}AR had no effect on collagen levels or the extent of fibrosis in acute bleomycin-induced lung injury, but did significantly reduce fibrosis in a chronic bleomycin injury model (Zhou et al., 2011). It was further established that deletion of A_{2B}AR in myeloid cells protects mouse lung from chronic bleomycin exposure (Karmouty-Quintana et al., 2014), which suggests a pro-inflammatory role of the A_{2B}AR in the progression of pulmonary fibrosis. This is further supported by studies demonstrating the A_{2B}AR activates macrophages and stimulates IL-6 release from bronchial smooth muscle cells and lung fibroblasts (Zhong et al., 2004, 2005). Similarly, in the kidney, abrogation of A_{2B}AR signalling is beneficial in the setting of chronic kidney disease. In an ADA-deficient or angiotensin II-infusion mouse model of kidney disease, administration of either polyethylene glycol-modified ADA or the A_{2B}AR selective antagonist PSB-1115 attenuated renal dysfunction and fibrosis (Dai et al., 2011). A_{2B}AR mRNA expression was significantly enhanced in mice subjected to angiotensin II-infusion or unilateral ureteral obstruction and this was associated with elevated collagen content and expression of fibrotic gene markers in particular the pro-inflammatory cytokine IL-6 (Dai et al., 2011). Renal fibrosis in these mouse models could be reduced with either pharmacological or genetic abrogation of the A_{2B}AR (Dai et al., 2011). A_{2B}AR expression was also shown to be positively correlated with the degree of interstitial fibrosis in renal tissue in a mouse model of kidney IRI (Roberts, Lu, Dwyer, & Cowan, 2014) and in patients with chronic kidney disease (Zhang et al., 2013). A further study in a rat renal fibroblast cell line NRK-49F demonstrated activation of the A_{2B}AR induces a pro-fibrotic fibroblast phenotype associated with increased expression of the fibrotic markers α-SMA, fibronectin, TGF-β1 and CTGF (Wilkinson, Farrell, Morel, Law, & Murphy, 2016). Elevated adenosine concentrations and subsequent signalling via the A_{2B}AR have also been shown to contribute to dermal (Karmouty-Quintana et al., 2018) and penile (Wen et al., 2010) fibrosis.

3.3. Cancer

A growing body of evidence suggests the A_{2B}AR may provide a novel therapeutic target for the treatment of cancer, for which the A_{2B}AR appears to play an important pathological role. The A_{2B}AR is upregulated by HIF-1α (Kong et al., 2006), so is often highly expressed in the cells and tissues from the hypoxic microenvironment of many solid tumours including colon carcinomas (Ma et al., 2010), prostate cancer (Mousavi, Panjehpour, Izadpanahi, & Aghaei, 2015; Wei et al., 2013), oral squamous cell carcinomas (Kasama et al., 2015), lung adenocarcinoma (Li, Huang, & Peng, 2005; Ryzhov et al., 2008) and breast cancer (Mittal et al., 2016). The overexpression of the A_{2B}AR in different cancers relative to normal tissue, combined with the increase in adenosine concentrations in the tumour microenvironment, suggests a role in disease progression and highlights the therapeutic potential of A_{2B}AR antagonists as adjuvants in cancer therapy (Kazemi et al., 2017; Sepúlveda, Palomo, & Fuentes, 2016).

3.3.1. A_{2B}AR and cancer cell proliferation and tumour growth

One of the key roles of the A_{2B}AR in cancer pathophysiology is the promotion of cancer cell proliferation and tumour growth. Activation of the A_{2B}AR with the subtype-selective agonist BAY 60-6583 increased tumour growth in a mouse model of melanoma (Iannone, Miele, Maiolino, Pinto, & Morello, 2013). Conversely application of an A_{2B}AR-selective antagonist decreased proliferation of prostate cancer cell lines (Vecchio et al., 2016; Wei et al., 2013), and colon cancer cells (Ma et al., 2010) and reduced tumour volume of bladder cancer, breast cancer (Cekic et al., 2011) and melanoma in mice (Iannone et al., 2013). These results in could be recapitulated with genetic knockdown or knockout of the A_{2B}AR in various models of solid tumours (Cekic et al., 2011; Kasama et al., 2015; Ryzhov et al., 2008), confirming the role of this receptor subtype in cancer cell proliferation and growth. This likely involves G_s-mediated stimulation of cAMP and ERK1/2 phosphorylation as these pathways are known to be involved in A_{2B}AR-mediated proliferation of non-cancerous endothelial cells (Fang & Olah, 2007; Grant et al., 2001). In addition, increases in cAMP have been demonstrated to promote proliferation of various cells, including progression of prostate cancer (Flacke et al., 2013; Merkle & Hoffmann, 2011), which would support the role of A_{2B}AR-G_s signalling in mediating cancer cell growth in the general sense. A recent study with bladder urothelial carcinoma further suggests the ERK1/2, p38 and JNK MAPKs to be important in A_{2B}AR-mediated tumour progression (Fig. 3) (Zhou et al., 2017).

3.3.2. A_{2B}AR and metastasis

The A_{2B}AR also modulates the ability of cancer cells to metastasise and migrate which contributes to disease progression (Sepúlveda et al., 2016; Sun & Huang, 2016). The A_{2B}AR has higher expression in metastatic versus non-metastatic derived colorectal cancer cell lines (Futschik et al., 2002). It has also been shown to promote tumour-cell chemotaxis *in vitro* and enhance lung metastasis in *in vivo* models of breast cancer and melanoma resulting in poorer prognosis (Cekic et al., 2011; Mittal et al., 2016; Stagg et al., 2010). Recent data also shows that experimental and spontaneous lung metastasis could be suppressed by the use of an A_{2B}AR-selective antagonist or genetic knockdown with shRNA (Desmet et al., 2013; Mittal et al., 2016). The enhanced metastasis may involve A_{2B}AR-increased gene expression of a key metastatic transcription factor, Fos-related antigen-1 (Fra-1) (Desmet et al., 2013), and the suppression of Rap1 protein activity which has been associated with reduced cell adhesion and increased tumour cell migration through a PKA-dependent mechanism (Ntantie et al., 2013). Hence the A_{2B}AR appears to enhance tumour cell metastasis by promoting migration and induction of an invasive, metastatic phenotype.

3.3.3. A_{2B}AR and angiogenesis

Tumour growth is also enhanced by the A_{2B}AR via promotion of angiogenesis. The A_{2B}AR induces vascular endothelial growth factor (VEGF) (Feoktistov, 2002; Ryzhov et al., 2008; 2013) and IL-8 production in human endothelial and cancer cells (Merighi et al., 2007, 2009), which are essential for tumour angiogenesis. Stimulation of adenosine receptors has been shown to increase VEGF production five-fold in tumour-associated $CD45^+$ immune cells, an effect that is not observed in $CD45^+$ cells from A_{2B}AR knockout mice (Ryzhov et al., 2008). Unlike the effects on cancer cell proliferation, the induction of pro-angiogenic factors via the A_{2B}AR appear to be largely mediated through PLC downstream of G_q-coupling (Feoktistov, 2002). VEGF in particular appears to be stimulated by a mechanism involving the transcription factor JunB downstream of A_{2B}AR-mediated PLC-Rap1-MEK activation (Fig. 3) (Ryzhov et al., 2013).

In addition to direct effects on metastasis, proliferation and angiogenesis, the A_{2B}AR can have an indirect role on cancer progression via modulation of the immune system (discussed in Section 3.4). Together, this highlights the importance of the A_{2B}AR in cancer pathophysiology

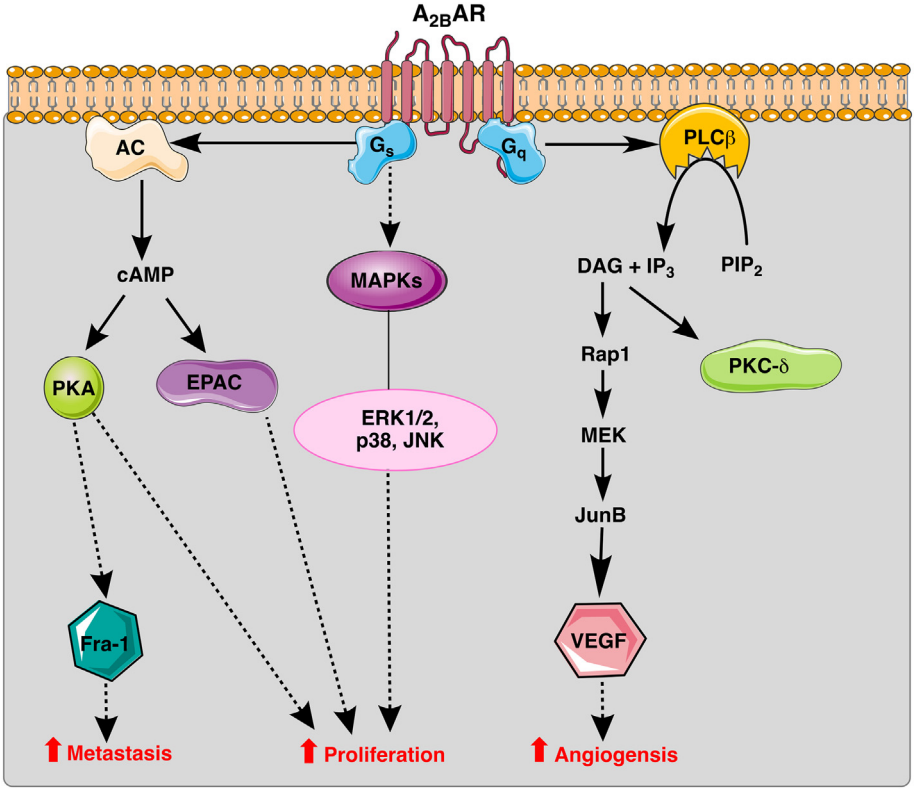


Fig. 3. Involvement of A_{2B}AR signalling in regulating cancer cell proliferation, metastasis and angiogenesis. Figure created using the Servier Medical Art resource (https:// smart.servier.com).

and reveals the therapeutic potential of A_{2B}AR antagonists in the treatment of cancer, particularly solid tumours.

3.4. Inflammation & the immune response

The A_{2B}AR is increasingly recognised as an important mediator of the immune response and the inflammatory cascade, which contributes to the pathophysiology of the above discussed ischaemia-reperfusion injury, fibrosis, cancer and other disease states such as asthma, inflammatory bowel disease and diabetes (Haskó et al., 2009; Sun & Huang, 2016). The A_{2B}AR is expressed on various cells of haematopoietic origin including macrophages, mast cells, lymphocytes, dendritic cells and neutrophils (Feoktistov & Biaggioni, 2011; Gessi et al., 2005; Mirabet et al., 1999; Novitskiy et al., 2008). As outlined previously, adenosine levels and A_{2B}AR expression are markedly upregulated during hypoxia and cell injury. Multiple mediators of the inflammatory response have also been demonstrated to increase A_{2B}AR expression in numerous immune cell types, including bacterial product lipopolysaccharide, pro-inflammatory cytokines TNF-α, IL-1β, and IFN-γ and the prostaglandin E2 (Reviewed in Haskó et al., 2009; Aherne et al., 2011). The expression and upregulation on immune cells therefore suggests the A_{2B}AR as an important therapeutic target in treating immune system dysfunction and inflammation.

3.4.1. A_{2B}AR anti-inflammatory signalling

The A_{2B}AR was suggested to dampen the immune response after an A_{2B}AR knockout mouse model showed increases in vasculature leukocyte adhesion and the augmentation of pro-inflammatory cytokines, TNF-α and IL-6, accompanied by decreases in the plasma level of the anti-inflammatory IL-10, compared to wild-type mice (Yang et al., 2006). An anti-inflammatory role for the A_{2B}AR was further demonstrated after stimulation of the A_{2B}AR was shown to increase the production of IL-10 from lipopolysaccharide-activated macrophages (Németh et al., 2005) and inhibit superoxide generation by neutrophils (van der Hoeven et al., 2011). In addition, studies have also shown that the A_{2B}AR limits leukocyte vascular permeability and neutrophil infiltration (Eckle et al., 2008; Haskó et al., 2009) and stimulates alternative macrophage activation (Csóka et al., 2012). This promotes an anti-inflammatory phenotype that protects against tissue injury and promotes tissue restitution (Haskó et al., 2009). The loss of A_{2B}AR on non-haematopoietic cells has also been shown to increase pro-inflammatory cytokines TNF-α, IL-1β and IL-6 and NF-κB activation, suggesting the A_{2B}AR had a role in preventing sepsis-induced mortality (Csóka et al., 2010). After myocardial infarction, expression of the A_{2B}AR was significantly upregulated on cells of the adaptive immune system, in particular T helper and cytotoxic T cells and suppressed secretion of the pro-inflammatory IFNγ (Borg et al., 2017). Likewise, the immunosuppressive actions of adenosine, acting via the A_{2B}AR, provide a protective role in a model of type I diabetes mellitus (Németh et al., 2007). However, these same A_{2B}AR-mediated anti-inflammatory effects may help tumour cells evade natural defences and promote tumour growth (Sepúlveda et al., 2016). For example, in addition to the variety of immunomodulatory actions discussed above, A_{2B}AR activation on dendritic cells induces expression of pro-angiogenic and immunosuppressant factors such as VEGF which promotes tumour growth and vascularisation (Novitskiy et al., 2008).

3.4.2. A_{2B}AR pro-inflammatory signalling

In addition to anti-inflammatory effects, a pro-inflammatory role of the A_{2B}AR has also been demonstrated. A_{2B}AR activation leads to increased IL-6 cytokine release by bronchial smooth muscle cells (Zhong et al., 2004), primary murine alveolar macrophages (Pedroza et al., 2011) and lung fibroblasts (Zhong et al., 2005). The A_{2B}AR via a PLC-PKC-p38 pathway, also stimulates IL-6 production in microglial cells, thus contributes to neuro-inflammation (Merighi et al., 2016). In mast cells, primarily through coupling to G_q, stimulation of the A_{2B}AR induces degranulation and release of pro-inflammatory cytokines IL-4, IL-8 and IL-13, which in turn promotes immunoglobulin E synthesis by B lymphocytes (Auchampach, Jin, Wan, Caughey, & Linden, 1997; Feoktistov et al., 1999; Ryzhov, Goldstein, Biaggioni, & Feoktistov, 2006). These actions contribute to chronic inflammatory lung pathology and rationalise the use of A_{2B}AR antagonists in the treatment of asthma and COPD (Wilson et al., 2009). Similarly, in intestinal epithelial cells, the A_{2B}AR increases IL-6 and keratinocyte-derived chemokine concentrations and contributes to murine colitis, suggesting A_{2B}AR blockade may be an effective strategy to treat inflammatory bowel disease (Kolachala et al., 2008; Sitaraman et al., 2001). Sustained elevated adenosine concentrations and subsequent activation of the A_{2B}AR on myeloid cells stimulate soluble IL-6 receptor signalling and nociceptor hyperexcitability, highlighting the role of the A_{2B}AR in promoting chronic pain through neuro-immune interaction. (Hu et al., 2016).

4. Emerging therapeutic opportunities and conclusions

The expanding knowledge of A_{2B}AR pharmacology and the continued efforts to uncover subtype selective ligands has repositioned the A_{2B}AR as a target with great biological importance. Alongside fibrosis, IRI, cancer and inflammation, the A_{2B}AR is currently being investigated for the treatment of diabetes (Figler et al., 2011; Merighi et al., 2015), pulmonary hypertension (Bessa-Gonçalves, Bragança, Martins-Dias, Correia-de-Sá, & Fontes-Sousa, 2018; Mertens et al., 2018), sickle cell anaemia (Paz et al., 2017; Zhang et al., 2011), and bone diseases (Daniele et al., 2017; Trincavelli et al., 2014). An A_{2B}AR antagonist has completed Phase I clinical trials for investigation in the prophylaxis and treatment of asthma (Kalla & Zablocki, 2009) and a dual A_{2A}AR/A_{2B}AR antagonist is currently entering Phase I clinical trial for patients with advanced malignancies (Vijayan, Young, Teng, & Smyth, 2017). While great progress has been made, complexities regarding how the A_{2B}AR can promote both beneficial and detrimental outcomes, sometimes even within the same tissue or organ, requires further elucidation. Most notably, there appears a temporal relationship between A_{2B}AR activation (or inactivation) and pathological outcome. Acute A_{2B}AR stimulation is largely protective in the setting of IRI as the receptor acts to dampen down inflammation and fibrosis in response to an acute insult. This is contrasted by the pro-inflammatory, pro-fibrotic role from the pathological overproduction of adenosine and prolonged A_{2B}AR activation in chronic models of disease such as sickle cell anaemia, asthma/COPD, inflammatory bowel disease, chronic kidney disease or within the microenvironment of tumours (Eisenstein, Patterson, & Ravid, 2015; Feoktistov & Biaggioni, 2011; Karmouty-Quintana et al., 2013). These opposing effects may reflect temporal changes in intracellular G protein/β-arrestin coupling or differential interplay of signalling mediators between the immune and tissue cells that change with disease progression (Aherne et al., 2011; Cohen, Yang, & Downey, 2010). It is hoped therefore that ever-developing genetic and pharmacological tools will delineate the differential A_{2B}AR effects and enable the therapeutic potential of the A_{2B}AR to be realised.

Conflict of interest statement

The authors declare that there are no conflicts of interest

Acknowledgement

This work was supported by Project Grant APP1084487 of the National Health and Medical Research Council of Australia (NHMRC). E.A. V is a recipient of the Australian Government Research Training Program Scholarship. L.T.M. is a recipient of an Australian Research Council Discovery Early Career Researcher Award (DE130100117).

References

Aherne, C. M., Kewley, E. M., & Eltzschig, H. K. (2011). The resurgence of A2B adenosine receptor signaling. *Biochimica et Biophysica Acta, Biomembranes 1808*, 1329–1339.

Anvari, F., Sharma, A. K., Fernandez, L. G., Hranjec, T., Ravid, K., Kron, I. L., et al. (2010). Tissue-derived proinflammatory effect of adenosine A2B receptor in lung ischemia–reperfusion injury. *The Journal of Thoracic and Cardiovascular Surgery 140*, 871–877.

Auchampach, J. A., Jin, X., Wan, T. C., Caughey, G. H., & Linden, J. (1997). Canine mast cell adenosine receptors: cloning and expression of the A3 receptor and evidence that degranulation is mediated by the A2B receptor. *Molecular Pharmacology 52*, 846–860.

Baltos, J. -A., Vecchio, E. A., Harris, M. A., Qin, C. X., Ritchie, R. H., Christopoulos, A., et al. (2017). Capadenoson, a clinically trialed partial adenosine A1 receptor agonist, can stimulate adenosine A2B receptor biased agonism. *Biochemical Pharmacology 135*, 79–89.

Baraldi, P. G., Preti, D., Tabrizi, M. A., Fruttarolo, F., Saponaro, G., Baraldi, S., et al. (2007). N6-[(Hetero)aryl/(cyclo)alkyl-carbamoyl-methoxy-phenyl]-(2-chloro)-5′-N-ethylcarboxamido-adenosines: The first example of adenosine-related structures with potent agonist activity at the human A2B adenosine receptor. *Bioorganic & Medicinal Chemistry 15*, 2514–2527.

Baraldi, P. G., Romagnoli, R., Preti, D., Fruttarolo, F., Carrion, M. D., & Tabrizi, M. A. (2006). Ligands for A2B adenosine receptor subtype. *Current Medicinal Chemistry 13*, 3467–3482.

Baraldi, P. G., Tabrizi, M. A., Fruttarolo, F., & Romagnoli, R. (2009). Recent improvements in the development of A2B adenosine receptor agonists. *Purinergic Signal 5*, 3–19.

Bertarelli, D. C. G., Diekmann, M., Hayallah, A. M., Rüsing, D., Iqbal, J., Preiss, B., et al. (2006). Characterization of human and rodent native and recombinant adenosine A2B receptors by radioligand binding studies. *Purinergic Signal 2*, 559–571.

Bessa-Gonçalves, M., Bragança, B., Martins-Dias, E., Correia-de-Sá, P., & Fontes-Sousa, A. P. (2018). Is the adenosine A2B 'biased' receptor a valuable target for the treatment of pulmonary arterial hypertension? *Drug Discovery Today 23*, 1285–1292.

Beukers, M. W., Chang, L. C. W., von Frijtag Drabbe Kunzel, J. K., Mulder-Krieger, T., Spanjersberg, R. F., Brussee, J., et al. (2004). New, non-adenosine, high-potency agonists for the human adenosine A2B receptor with an improved selectivity profile compared to the reference agonist N-Ethylcarboxamidoadenosine. *Journal of Medicinal Chemistry 47*, 3707–3709.

Beukers, M. W., Dulk den, H., van Tilburg, E. W., Brouwer, J., & IJzerman, A. P. (2000). Why are A2B receptors low-affinity adenosine receptors? Mutation of Asn273 to Tyr increases affinity of human A2B receptor for 2-(1-Hexynyl)adenosine. *Molecular Pharmacology 58*, 1349–1356.

Borg, N., Alter, C., Görldt, N., Jacoby, C., Ding, Z., Steckel, B., et al. (2017). CD73 on T cells orchestrates cardiac wound healing after myocardial infarction by purinergic metabolic reprogramming. *Circulation 136*, 297–313.

Borrmann, T., Hinz, S., Bertarelli, D. C. G., Li, W., Florin, N. C., Scheiff, A. B., et al. (2009). 1-Alkyl-8-(piperazine-1-sulfonyl)phenylxanthines: development and characterization of adenosine A2B receptor antagonists and a new radioligand with subnanomolar affinity and subtype specificity. *Journal of Medicinal Chemistry 52*, 3994–4006.

Bruns, R. F., Lu, G. H., & Pugsley, T. A. (1986). Characterization of the A2 adenosine receptor labeled by [3H]NECA in rat striatal membranes. *Molecular Pharmacology 29*, 331–346.

Busse, H., Bitzinger, D., Höcherl, K., Seyfried, T., Gruber, M., Graf, B. M., et al. (2016). Adenosine A2A and A2B receptor substantially attenuate ischemia/reperfusion injury in septic rat hearts. *Cardiovascular Drugs and Therapy*, 1–8.

van Calker, D., Müller, M., & Hamprecht, B. (1979). Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *Journal of Neurochemistry 33*, 999–1005.

Cekic, C., Sag, D., Li, Y., Theodorescu, D., Strieter, R. M., & Linden, J. (2011). Adenosine A2B receptor blockade slows growth of bladder and breast tumors. *Journal of Immunology 188*, 198–205.

Chan, E. S. L., & Cronstein, B. N. (2009). Adenosine in fibrosis. *Modern Rheumatology 20*, 114–122.

Chen, J. -F., Eltzschig, H. K., & Fredholm, B. B. (2013). Adenosine receptors as drug targets — what are the challenges? *Nature Reviews. Drug Discovery 12*, 265–286.

Chen, Y., Epperson, S., Makhsudova, L., Ito, B., Suarez, J., Dillmann, W., et al. (2004). Functional effects of enhancing or silencing adenosine A2b receptors in cardiac fibroblasts. *American Journal of Physiology. Heart and Circulatory Physiology 287*, H2478–H2486.

Cohen, M. V., Yang, X., & Downey, J. M. (2010). A2B adenosine receptors can change their spots. *British Journal of Pharmacology 159*, 1595–1597.

Cronstein, B. N. (2011). Adenosine receptors and fibrosis: a translational review. *F1000 Biol Rep 3*, 3–21.

Csóka, B., Németh, Z. H., Rosenberger, P., Eltzschig, H. K., Spolarics, Z., Pacher, P., et al. (2010). A2B adenosine receptors protect against sepsis-induced mortality by dampening excessive inflammation. *Journal of Immunology 185*, 542–550.

Csóka, B., Selmeczy, Z., Koscso, B., Németh, Z. H., Pacher, P., Murray, P. J., et al. (2012). Adenosine promotes alternative macrophage activation via A2A and A2B receptors. *The FASEB Journal 26*, 376–386.

Dai, Y., Zhang, W., Wen, J., Zhang, Y., Kellems, R. E., & Xia, Y. (2011). A2B Adenosine receptor-mediated induction of IL-6 promotes CKD. *Journal of American Society of Nephrology 22*, 890–901.

Daly, J. W., Butts-Lamb, P., & Padgett, W. (1983). Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. *Cellular and Molecular Neurobiology 3*, 69–80.

Daniele, S., Natali, L., Giacomelli, C., Campiglia, P., Novellino, E., Martini, C., et al. (2017). Osteogenesis is improved by low tumor necrosis factor alpha concentration through the modulation of Gs-coupled receptor signals. *Molecular and Cellular Biology 37*, 442–516.

van der Hoeven, D., Wan, T. C., Gizewski, E. T., Kreckler, L. M., Maas, J. E., Van Orman, J., et al. (2011). A role for the low-affinity A2B adenosine receptor in regulating superoxide generation by murine neutrophils. *The Journal of Pharmacology and Experimental Therapeutics 338*, 1004–1012.

Desmet, C. J., Gallenne, T., Prieur, A., Reyal, F., Visser, N. L., Wittner, B. S., et al. (2013). Identification of a pharmacologically tractable Fra-1/ADORA2B axis promoting breast cancer metastasis. *Proceedings of the National Academy of Sciences of the United States of America 110*, 5139–5144.

Dixon, A. K., Gubitz, A. K., Sirinathsinghji, D. J., Richardson, P. J., & Freeman, T. C. (1996). Tissue distribution of adenosine receptor mRNAs in the rat. *British Journal of Pharmacology 118*, 1461–1468.

Dong, Q., Ginsberg, H. N., & Erlanger, B. F. (2001). Overexpression of the A1 adenosine receptor in adipose tissue protects mice from obesity-related insulin resistance. *Diabetes, Obesity and Metabolism 3*, 360–366.

Drury, A. N., & Szent-Györgyi, A. (1929). The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *The Journal of Physiology 68*, 213–237.

Dubey, R. K., Gillespie, D. G., & Jackson, E. K. (1998). Adenosine inhibits collagen and protein synthesis in cardiac fibroblasts: role of A2B receptors. *Hypertension 31*, 943–948.

Dubey, R. K., Gillespie, D. G., Mi, Z., & Jackson, E. K. (1997). Exogenous and endogenous adenosine inhibits fetal calf serum–induced growth of rat cardiac fibroblasts: role of A2B receptors. *Circulation 96*, 2656–2666.

Dubey, R. K., Gillespie, D. G., Zacharia, L. C., Mi, Z., & Jackson, E. K. (2001). A2B receptors mediate the antimitogenic effects of adenosine in cardiac fibroblasts. *Hypertension 37*, 716–721.

Dunwiddie, T. V., & Masino, S. A. (2001). The role and regulation of adenosine in the central nervous system. *Annual Review of Neuroscience 24*, 31–55.

Eckle, T., Faigle, M., Grenz, A., Laucher, S., & Thompson, L. F. (2008). A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood 111*, 2024–2035.

Eckle, T., Hartmann, K., Bonney, S., Reithel, S., Mittelbronn, M., Walker, L. A., et al. (2012). Adora2b-elicited Per2 stabilization promotes a HIF- dependent metabolic switch crucial for myocardial adaptation to ischemia. *Nature Medicine 18*, 774–782.

Eckle, T., Krahn, T., Grenz, A., Kohler, D., Mittelbronn, M., Ledent, C., et al. (2007). Cardioprotection by ecto-5'-nucleotidase (CD73) and A2B adenosine receptors. *Circulation 115*, 1581–1590.

Eisenstein, A., Patterson, S., & Ravid, K. (2015). The many faces of the A2b adenosine receptor in cardiovascular and metabolic diseases. *Journal of Cellular Physiology 230*, 2891–2897.

Eltzschig, H. K. (2009). Adenosine: an old drug newly discovered. *Anesthesiology 111*, 904–915.

Eltzschig, H. K., Bonney, S. K., & Eckle, T. (2013). Attenuating myocardial ischemia by targeting A2B adenosine receptors. *Trends in Molecular Medicine 19*, 345–354.

Eltzschig, H. K., Rivera-Nieves, J., & Colgan, S. P. (2009). Targeting the A2B adenosine receptor during gastrointestinal ischemia and inflammation. *Expert Opinion on Therapeutic Targets 13*, 1267–1277.

Elzein, E., Kalla, R. V., Li, X., Perry, T., Gimbel, A., Zeng, D., et al. (2008). Discovery of a novel A2B adenosine receptor antagonist as a clinical candidate for chronic inflammatory airway diseases. *Journal of Medicinal Chemistry 51*, 2267–2278.

Epperson, S. A., Brunton, L. L., Ramirez-Sanchez, I., & Villarreal, F. (2009). Adenosine receptors and second messenger signaling pathways in rat cardiac fibroblasts. *American Journal of Physiology. Cell Physiology 296*, C1171–C1177.

Fang, Y., & Olah, M. E. (2007). Cyclic AMP-dependent, protein kinase A-independent activation of extracellular signal-regulated kinase 1/2 following adenosine receptor stimulation in human umbilical vein endothelial cells: role of exchange protein activated by cAMP 1 (Epac1). *The Journal of Pharmacology and Experimental Therapeutics 322*, 1189–1200.

Feng, W., Song, Y., Chen, C., Lu, Z. Z., & Zhang, Y. (2009). Stimulation of adenosine A2B receptors induces interleukin-6 secretion in cardiac fibroblasts via the PKC-δ-P38 signalling pathway. *British Journal of Pharmacology 159*, 1598–1607.

Feoktistov, I. (2002). Differential expression of adenosine receptors in human endothelial cells: role of A2B receptors in angiogenic factor regulation. *Circulation Research 90*, 531–538.

Feoktistov, I., & Biaggioni, I. (1997). Adenosine A2B receptors. *Pharmacological Reviews 49*, 381–402.

Feoktistov, I., & Biaggioni, I. (2011). Role of adenosine A2B receptors in inflammation. *Advances in Pharmacology 61*, 115–144.

Feoktistov, I., Goldstein, A. E., & Biaggioni, I. (1999). Role of p38 mitogen-activated protein kinase and extracellular signal-regulated protein kinase kinase in adenosine A2B receptor-mediated interleukin-8 production in human mast cells. *Molecular Pharmacology 55*, 726–734.

Feoktistov, I., Murray, J. J., & Biaggioni, I. (1994). Positive modulation of intracellular Ca2+ levels by adenosine A2b receptors, prostacyclin, and prostaglandin E1 via a cholera toxin-sensitive mechanism in human erythroleukemia cells. *Molecular Pharmacology 45*, 1160–1167.

Feoktistov, I., Ryzhov, S., Zhong, H., Goldstein, A. E., Matafonov, A., Zeng, D., et al. (2004). Hypoxia modulates adenosine receptors in human endothelial and smooth muscle cells toward an A2B angiogenic phenotype. *Hypertension 44*, 649–654.

Figler, R. A., Wang, G., Srinivasan, S., Jung, D. Y., Zhang, Z., Pankow, J. S., et al. (2011). Links between insulin resistance, adenosine A2B receptors, and inflammatory markers in mice and humans. *Diabetes 60*, 669–679.

Flacke, J. P., Flacke, H., Appukuttan, A., Palisaar, R. J., Noldus, J., Robinson, B. D., et al. (2013). Type 10 soluble adenylyl cyclase is overexpressed in prostate carcinoma and controls proliferation of prostate cancer cells. *The Journal of Biological Chemistry 288*, 3126–3135.

Frank, A., Bonney, M., Bonney, S., Weitzel, L., Koeppen, M., & Eckle, T. (2012). Myocardial ischemia reperfusion injury: from basic science to clinical bedside. *Seminars in Cardiothoracic and Vascular Anesthesia 16*, 123–132.

Fredholm, B. B. (2007). Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death and Differentiation 14*, 1315–1323.

Fredholm, B. B., Arslan, G., Halldner, L., Kull, B., Schulte, G., & Wasserman, W. (2000). Structure and function of adenosine receptors and their genes. *Naunyn-Schmiedeberg's Archives of Pharmacology 362*, 364–374.

Fredholm, B. B., Chen, J. -F., Masino, S. A., & Vaugeois, J. -M. (2005). Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. *Annual Review of Pharmacology and Toxicology 45*, 385–412.

Fredholm, B. B., IJzerman, A. P., Jacobson, K. A., Klotz, K. N., & Linden, J. (2001). International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacological Reviews 53*, 527–552.

Fredholm, B. B., Irenius, E., Kull, B., & Schulte, G. (2001). Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. *Biochemical Pharmacology 61*, 443–448.

Friedman, S. L., Sheppard, D., Duffield, J. S., & Violette, S. (2013). Therapy for fibrotic diseases: nearing the starting line. *Science Translational Medicine 5* 167sr1.

Fusco, I., Ugolini, F., Lana, D., Coppi, E., Dettori, I., Gaviano, L., et al. (2018). The selective antagonism of adenosine A2B receptors reduces the synaptic failure and neuronal death induced by oxygen and glucose deprivation in rat CA1 hippocampus in vitro. *Frontiers in Pharmacology 9*, 399.

Futschik, M., Jeffs, A., Pattison, S., Kasabov, N., Sullivan, M., Merrie, A., et al. (2002). Gene expression profiling of metastatic and nonmetastatic colorectal cancer cell lines. *Genome Letters 1*, 26–34.

Gao, Z. -G., Balasubramanian, R., Kiselev, E., Wei, Q., & Jacobson, K. A. (2014). Probing biased/partial agonism at the G protein-coupled A2B adenosine receptor. *Biochemical Pharmacology 90*, 297–306.

Gao, Z., Chen, T., Weber, M. J., & Linden, J. (1999). A2B adenosine and P2Y2 receptors stimulate mitogen-activated protein kinase in human embryonic kidney-293 cells: cross-talk between cyclic AMP and protein kinase C pathways. *The Journal of Biological Chemistry 274*, 5972–5980.

Gao, Z. -G., Inoue, A., & Jacobson, K. A. (2018). On the G protein-coupling selectivity of the native A2B adenosine receptor. *Biochemical Pharmacology 151*, 201–213.

Gessi, S., Varani, K., Merighi, S., Cattabriga, E., Pancaldi, C., Szabadkai, Y., et al. (2005). Expression, pharmacological profile, and functional coupling of A2B receptors in a recombinant system and in peripheral blood cells using a novel selective antagonist radioligand, [3H]MRE 2029-F20. *Molecular Pharmacology 67*, 2137–2147.

Grant, M. B., Davis, M. I., Caballero, S., Feoktistov, I., Biaggioni, I., & Belardinelli, L. (2001). Proliferation, migration, and ERK activation in human retinal endothelial cells through A2B adenosine receptor stimulation. *Investigative Ophthalmology and Visual Science 42*, 2068–2073.

Grenz, A., Osswald, H., Eckle, T., Yang, D., Zhang, H., Tran, Z. V., et al. (2008). The reno-vascular A2B adenosine receptor protects the kidney from ischemia. *PLoS Medicine 5*, e137.

Grube, K., Rüdebusch, J., Xu, Z., Böckenholt, T., Methner, C., Müller, T., et al. (2011). Evidence for an intracellular localization of the adenosine A2B receptor in rat cardiomyocytes. *Basic Research in Cardiology 106*, 385–396.

Ham, J., & Rees, D. A. (2008). The adenosine A2B receptor: its role in inflammation. *Endocrine, Metabolic & Immune Disorders Drug Targets 8*, 244–254.

Hart, M. L., Jacobi, B., Schittenhelm, J., Henn, M., & Eltzschig, H. K. (2009). Cutting edge: A2B adenosine receptor signaling provides potent protection during intestinal ischemia/reperfusion injury. *Journal of Immunology 182*, 3965–3968.

Hashimoto, T., Kim, G. E., Tunin, R. S., Adesiyun, T., Hsu, S., Nakagawa, R., et al. (2018). Acute enhancement of cardiac function by phosphodiesterase type 1 inhibition: A translational study in the dog and rabbit. *Circulation 138*, 1974–1987 Epub ahead of print.

Haskó, G., Csóka, B., Németh, Z. H., Vizi, E. S., & Pacher, P. (2009). A2B adenosine receptors in immunity and inflammation. *Trends in Immunology 30*, 263–270.

Haskó, G., Linden, J., Cronstein, B., & Pacher, P. (2008). Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nature Reviews. Drug Discovery 7*, 759–770.

Hayallah, A. M., Sandoval-Ramírez, J., Reith, U., Schobert, U., Preiss, B., Schumacher, B., et al. (2002). 1,8-disubstituted xanthine derivatives: synthesis of potent A2B-selective adenosine receptor antagonists. *Journal of Medicinal Chemistry 45*, 1500–1510.

Haynes, J., Obiako, B., Babal, P., & Stevens, T. (1999). 5-(N-ethylcarboxamido)adenosine desensitizes the A2b-adenosine receptor in lung circulation. *The American Journal of Physiology 276*, H1877–H1883.

Headrick, J. P., Hack, B., & Ashton, K. J. (2003). Acute adenosinergic cardioprotection in ischemic-reperfused hearts. *American Journal of Physiology. Heart and Circulatory Physiology 285*, H1797–H1818.

Headrick, J. P., & Willis, R. J. (1988). Mediation by adenosine of bradycardia in rat heart during graded global ischaemia. *Pfluegers Archiv 412*, 618–623.

Herrera, C., Casado, V., Ciruela, F., Schofield, P., Mallol, J., Lluis, C., et al. (2001). Adenosine A2B receptors behave as an alternative anchoring protein for cell surface adenosine deaminase in lymphocytes and cultured cells. *Molecular Pharmacology 59*, 127–134.

Hinz, S., Lacher, S. K., Seibt, B. F., & Muller, C. E. (2014). BAY60-6583 acts as a partial agonist at adenosine A2B receptors. *The Journal of Pharmacology and Experimental Therapeutics 349*, 427–436.

Hinz, S., Navarro, G., Borroto-Escuela, D., Seibt, B. F., Ammon, Y. -C., de Filippo, E., et al. (2018). Adenosine A2A receptor ligand recognition and signaling is blocked by A2B receptors. *Oncotarget 9*, 13593–13611.

Hu, X., Adebiyi, M. G., Luo, J., Sun, K., Le, T. -T. T., Zhang, Y., et al. (2016). Sustained elevated adenosine via ADORA2B promotes chronic pain through neuro-immune interaction. *Cell Reports 16*, 106–119.

Huerter, M. E., Sharma, A. K., Zhao, Y., Charles, E. J., Kron, I. L., & Laubach, V. E. (2016). Attenuation of pulmonary ischemia-reperfusion injury by adenosine A2B receptor antagonism. *The Annals of Thoracic Surgery 102*, 385–393.

Iannone, R., Miele, L., Maiolino, P., Pinto, A., & Morello, S. (2013). Blockade of A2B adenosine receptor reduces tumor growth and immune suppression mediated by myeloid-derived suppressor cells in a mouse model of melanoma. *Neoplasia 15*, 1400–1414.

Jacobson, K. A., & Gao, Z. -G. (2006). Adenosine receptors as therapeutic targets. *Nature Reviews. Drug Discovery 5*, 247–264.

Ji, X. D., & Jacobson, K. A. (1999). Use of the triazolotriazine [3H]ZM 241385 as a radioligand at recombinant human A2B adenosine receptors. *Drug Design and Discovery 16*, 217–226.

Ji, X. -D., Kim, Y. -C., Ahern, D. G., Linden, J., & Jacobson, K. A. (2001). [3 H] MRS 1754, a selective antagonist radioligand for A2B adenosine receptors. *Biochemical Pharmacology 61*, 657–663.

Kalla, R. V., & Zablocki, J. (2009). Progress in the discovery of selective, high affinity A2B adenosine receptor antagonists as clinical candidates. *Purinergic Signal 5*, 21–29.

Karmouty-Quintana, H., Molina, J. G., Philip, K., Bellocchi, C., Gudenkauf, B., Wu, M., et al. (2018). The antifibrotic effect of adenosine A2B receptor antagonism in a mouse model of dermal fibrosis. *Arthritis & Rhematology 70*, 1673–1684.

Karmouty-Quintana, H., Philip, K., Acero, L. F., Chen, N. Y., Weng, T., Molina, J. G., et al. (2014). Deletion of ADORA2B from myeloid cells dampens lung fibrosis and pulmonary hypertension. *The FASEB Journal 29*, 50–60.

Karmouty-Quintana, H., Xia, Y., & Blackburn, M. R. (2013). Adenosine signaling during acute and chronic disease states. *Journal of Molecular Medicine 91*, 173–181.

Kasama, H., Sakamoto, Y., Kasamatsu, A., Okamoto, A., Koyama, T., Minakawa, Y., et al. (2015). Adenosine A2B receptor promotes progression of human oral cancer. *BMC Cancer 15*, 1–12.

Kazemi, M. H., Raoofi Mohseni, S., Hojjat-Farsangi, M., Anvari, E., Ghalamfarsa, G., Mohammadi, H., et al. (2017). Adenosine and adenosine receptors in the immunopathogenesis and treatment of cancer. *Journal of Cellular Physiology 233*, 2032–2057.

Kenakin, T., & Christopoulos, A. (2013). Signalling bias in new drug discovery: detection, quantification and therapeutic impact. *Nature Reviews. Drug Discovery 12*, 205–216.

Khoa, N. D., Montesinos, M. C., Williams, A. J., Kelly, M., & Cronstein, B. N. (2003). Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. *Journal of Immunology 171*, 3991–3998.

Kim, Y. -C., Ji, X. -D., Melman, N., Linden, J., & Jacobson, K. A. (2000). Anilide derivatives of an 8-phenylxanthine carboxylic congener are highly potent and selective antagonists at human A2B adenosine receptors. *Journal of Medicinal Chemistry 43*, 1165–1172.

Kim, S. -A., Marshall, M. A., Melman, N., Kim, H. S., Müller, C. E., Linden, J., et al. (2002). structure−activity relationships at human and rat A2B adenosine receptors of xanthine derivatives substituted at the 1-, 3-, 7-, and 8-positions. *Journal of Medicinal Chemistry 45*, 2131–2138.

Klaasse, E. C., IJzerman, A. P., Grip, W. J., & Beukers, M. W. (2007). Internalization and desensitization of adenosine receptors. *Purinergic Signal 4*, 21–37.

Kloner, R. A., Forman, M. B., Gibbons, R. J., Ross, A. M., Alexander, R. W., & Stone, G. W. (2006). Impact of time to therapy and reperfusion modality on the efficacy of adenosine in acute myocardial infarction: the AMISTAD-2 trial. *European Heart Journal 27*, 2400–2405.

Koeppen, M., Harter, P. N., Bonney, S., Bonney, M., Reithel, S., Zachskorn, C., et al. (2012). Adora2b signaling on bone marrow derived cells dampens myocardial ischemia-reperfusion injury. *Anesthesiology 116*, 1245–1257.

Kolachala, V., Asamoah, V., Wang, L., Obertone, T. S., Ziegler, T. R., Merlin, D., et al. (2005). TNF-α upregulates adenosine 2b (A2b) receptor expression and signaling in intestinal epithelial cells: a basis for A2BR overexpression in colitis. *Cellular and Molecular Life Sciences 62*, 2647–2657.

Kolachala, V. L., Ruble, B. K., Vijay-Kumar, M., Wang, L., Mwangi, S., Figler, H. E., et al. (2008). Blockade of adenosine A2B receptors ameliorates murine colitis. *British Journal of Pharmacology 155*, 127–137.

Kong, T., Westerman, K. A., Faigle, M., Eltzschig, H. K., & Colgan, S. P. (2006). HIF-dependent induction of adenosine A2B receptor in hypoxia. *The FASEB Journal 20*, 2242–2250.

Kopecky, S. L., Aviles, R. J., Bell, M. R., Lobl, J. K., Tipping, D., Frommell, G., et al. (2003). A randomized, double-blinded, placebo-controlled, dose-ranging study measuring the effect of an adenosine agonist on infarct size reduction in patients undergoing primary percutaneous transluminal coronary angioplasty: the ADMIRE (AmP579 delivery for myocardial infarction REduction) study. *American Heart Journal 146*, 146–152.

Köse, M., Gollos, S., Karcz, T., Fiene, A., Heisig, F., Behrenswerth, A., et al. (2018). Fluorescent-labeled selective adenosine A2B receptor antagonist enables competition binding assay by flow cytometry. *Journal of Medicinal Chemistry 61*, 4301–4316.

Kuno, A., Critz, S. D., Cui, L., Solodushko, V., Yang, X. -M., Krahn, T., et al. (2007). Protein kinase C protects preconditioned rabbit hearts by increasing sensitivity of adenosine A2b-dependent signaling during early reperfusion. *Journal of Molecular and Cellular Cardiology 43*, 262–271.

Kuno, A., Solenkova, N. V., Solodushko, V., Dost, T., Liu, Y., Yang, X. M., et al. (2008). Infarct limitation by a protein kinase G activator at reperfusion in rabbit hearts is dependent on sensitizing the heart to A2B agonists by protein kinase C. *American Journal of Physiology. Heart and Circulatory Physiology 295*, H1288–H1295.

Lasley, R. D., Kristo, G., Keith, B. J., & Mentzer, R. M. (2006). The A2A/A2B receptor antagonist ZM-241385 blocks the cardioprotective effect of adenosine agonist pretreatment in in vivo rat myocardium. *American Journal of Physiology. Heart and Circulatory Physiology 292*, H426–H431.

Layland, J., Carrick, D., Lee, M., Oldroyd, K., & Berry, C. (2014). Adenosine: Physiology, pharmacology, and clinical applications. *JACC. Cardiovascular Interventions 7*, 581–591.

Lefkowitz, R. J., Rajagopal, K., & Whalen, E. J. (2006). New roles for b-arrestins in review cell signaling: not just for seven-transmembrane receptors. *Molecular Cell 24*, 643–652.

Li, Q., Han, X., Lan, X., Hong, X., Li, Q., Gao, Y., et al. (2017). Inhibition of tPA-induced hemorrhagic transformation involves adenosine A2b receptor activation after cerebral ischemia. *Neurobiology of Disease 108*, 173–182.

Li, S., Huang, S., & Peng, S. -B. (2005). Overexpression of G protein-coupled receptors in cancer cells: involvement in tumor progression. *International Journal of Oncology 27*, 1329–1339.

Linden, J., Thai, T., Figler, H., Jin, X., & Robeva, A. S. (1999). Characterization of human A2B adenosine receptors: radioligand binding, western blotting, and coupling to Gq in human embryonic kidney 293 cells and HMC-1 mast cells. *Molecular Pharmacology 56*, 705–713.

Londos, C., Cooper, D. M., & Wolff, J. (1980). Subclasses of external adenosine receptors. *Proceedings of the National Academy of Sciences of the United States of America 77*, 2551–2554.

Luttrell, L. M., Maudsley, S., & Bohn, L. M. (2015). Fulfilling the promise of 'biased' G protein-coupled receptor agonism. *Molecular Pharmacology 88*, 579–588.

Ma, D. -F., Kondo, T., Nakazawa, T., Nio, D. -F., Mochizuki, K., Kawasaki, T., et al. (2010). Hypoxia-inducible adenosine A2B receptor modulates proliferation of colon carcinoma cells. *Human Pathology 41*, 1550–1557.

Maas, J. E., Koupenova, M., Ravid, K., & Auchampach, J. A. (2008). Abstract 4831: The A2B adenosine receptor contributes to post-infarction heart failure. *Circulation 118*, S_946.

Matharu, A. -L., Mundell, S. J., Benovic, J. L., & Kelly, E. (2001). Rapid agonist-induced desensitization and internalization of the A2B adenosine receptor is mediated by a serine residue close to the COOH terminus. *The Journal of Biological Chemistry 276*, 30199–30207.

McIntosh, V. J., & Lasley, R. D. (2012). Adenosine receptor-mediated cardioprotection: are all 4 subtypes required or redundant? *Journal of Cardiovascular Pharmacology and Therapeutics 17*, 21–33.

Merighi, S., Bencivenni, S., Vincenzi, F., Varani, K., Borea, P. A., & Gessi, S. (2016). A2B adenosine receptors stimulate IL-6 production in primary murine microglia through p38 MAPK kinase pathway. *Pharmacological Research 117*, 9–19.

Merighi, S., Benini, A., Mirandola, P., Gessi, S., Varani, K., Simioni, C., et al. (2007). Caffeine inhibits adenosine-induced accumulation of hypoxia-inducible factor-1, vascular endothelial growth factor, and interleukin-8 expression in hypoxic human colon cancer cells. *Molecular Pharmacology 72*, 395–406.

Merighi, S., Borea, P. A., & Gessi, S. (2015). Adenosine receptors and diabetes: Focus on the A2B adenosine receptor subtype. *Pharmacological Research 99*, 229–236.

Merighi, S., Simioni, C., Gessi, S., Varani, K., Mirandola, P., Tabrizi, M. A., et al. (2009). Adenosine receptors modulate vascular endothelial growth factor and interleukin-8 expression in human melanoma cells treated with etoposideand doxorubicin. *Neoplasia 11*, 1064–1073.

Merkle, D., & Hoffmann, R. (2011). Roles of cAMP and cAMP-dependent protein kinase in the progression of prostate cancer: Cross-talk with the androgen receptor. *Cellular Signalling 23*, 507–515.

Mertens, T. C. J., Hanmandlu, A., Tu, L., Phan, C., Collum, S. D., Chen, N. -Y., et al. (2018). Switching-off Adora2b in vascular smooth muscle cells halts the development of pulmonary hypertension. *Frontiers in Physiology 9*, 555.

Methner, C., Schmidt, K., Cohen, M. V., Downey, J. M., & Krieg, T. (2010). Both A2a and A2b adenosine receptors at reperfusion are necessary to reduce infarct size in mouse hearts. *American Journal of Physiology. Heart and Circulatory Physiology 299*, H1262–H1264.

Mirabet, M., Herrera, C., Cordero, O. J., Mallol, J., Lluis, C., & Franco, R. (1999). Expression of A2B adenosine receptors in human lymphocytes: their role in T cell activation. *Journal of Cell Science 112*, 491–502.

Mittal, D., Sinha, D., Barkauskas, D., Young, A., Kalimutho, M., Stannard, K., et al. (2016). Adenosine 2B receptor expression on cancer cells promotes metastasis. *Cancer Research 76*, 4372–4382.

Mogul, D. J., Adams, M. E., & Fox, A. P. (1993). Differential activation of adenosine receptors decreases N-type but potentiates P-type Ca2+ current in hippocampal CA3 neurons. *Neuron 10*, 327–334.

Moriyama, K., & Sitkovsky, M. V. (2010). Adenosine A2A receptor is involved in cell surface expression of A2B receptor. *The Journal of Biological Chemistry 285*, 39271–39288.

Mousavi, S., Panjehpour, M., Izadpanahi, M. H., & Aghaei, M. (2015). Expression of adenosine receptor subclasses in malignant and adjacent normal human prostate tissues. *Prostate 75*, 735–747.

Mundell, S. J., & Kelly, E. (1998). Evidence for co-expression and desensitization of A2a and A2b adenosine receptors in NG108-15 cells. *Biochemical Pharmacology 55*, 595–603.

Mundell, S. J., Matharu, A. -L., Nisar, S., Palmer, T. M., Benovic, J. L., & Kelly, E. (2010). Deletion of the distal COOH-terminus of the A2B adenosine receptor switches internalization to an arrestin- and clathrin-independent pathway and inhibits recycling. *British Journal of Pharmacology 159*, 518–533.

Nash, C. A., Nelson, C. P., Mistry, R., Moeller-Olsen, C., Christofidou, E., Challiss, R. A. J., et al. (2018). Differential regulation of β2-adrenoceptor and adenosine A2B receptor signalling by GRK and arrestin proteins in arterial smooth muscle. *Cellular Signalling 51*, 86–98.

Németh, Z. H., Bleich, D., Csóka, B., Pacher, P., Mabley, J. G., Himer, L., et al. (2007). Adenosine receptor activation ameliorates type 1 diabetes. *The FASEB Journal 21*, 2379–2388.

Németh, Z. H., Lutz, C. S., Csoka, B., Deitch, E. A., Leibovich, S. J., Gause, W. C., et al. (2005). Adenosine augments IL-10 production by macrophages through an A2B receptor-mediated posttranscriptional mechanism. *Journal of Immunology 175*, 8260–8270.

Newby, A. C. (1984). Adenosine and the concept of 'retaliatory metabolites'. *Trends in Biochemical Sciences 9*, 42–44.

Novitskiy, S. V., Ryzhov, S., Zaynagetdinov, R., Goldstein, A. E., Huang, Y., Tikhomirov, O. Y., et al. (2008). Adenosine receptors in regulation of dendritic cell differentiation and function. *Blood 112*, 1822–1831.

Ntantie, E., Gonyo, P., Lorimer, E. L., Hauser, A. D., Schuld, N., McAllister, D., et al. (2013). An adenosine-mediated signaling pathway suppresses prenylation of the GTPase rap1b and promotes cell scattering. *Science Signaling 6*, ra39.

Ortore, G., & Martinelli, A. (2010). A2B receptor ligands: past, present and future trends. *Current Topics in Medicinal Chemistry 10*, 923–940.

Pacheco, R., Martinez-Navio, J. M., Lejeune, M., Climent, N., Oliva, H., Gatell, J. M., et al. (2005). CD26, adenosine deaminase, and adenosine receptors mediate costimulatory signals in the immunological synapse. *Proceedings of the National Academy of Sciences of the United States of America 102*, 9583–9588.

Pantazi, E., Bejaoui, M., Folch-Puy, E., Adam, R., & Roselló-Catafau, J. (2016). Advances in treatment strategies for ischemia reperfusion injury. *Expert Opinion on Pharmacotherapy 17*, 169–179.

Paz, O. S., de Jesus Pinheiro, M., do Espirito Santo, R. F., Villarreal, C. F., & Castilho, M. S. (2017). Nanomolar anti-sickling compounds identified by ligand-based pharmacophore approach. *European Journal of Medicinal Chemistry 136*, 487–496.

Pedroza, M., Schneider, D. J., Karmouty-Quintana, H., Coote, J., Shaw, S., Corrigan, R., et al. (2011). Interleukin-6 contributes to inflammation and remodeling in a model of adenosine mediated lung injury. *PLoS One 6*, e22667.

Peters, D. M., Gies, E. K., Gelb, C. R., & Peterfreund, R. A. (1998). Agonist-induced desensitization of A2B adenosine receptors. *Biochemical Pharmacology 55*, 873–882.

Philip, K., Mills, T. W., Davies, J., Chen, N. -Y., Karmouty-Quintana, H., Luo, F., et al. (2017). HIF1A up-regulates the ADORA2B receptor on alternatively activated macrophages and contributes to pulmonary fibrosis. *The FASEB Journal 31*, 4745–4758.

Philipp, S., Yang, X. -M., Cui, L., Davis, A. M., Downey, J. M., & Cohen, M. V. (2006). Postconditioning protects rabbit hearts through a protein kinase C-adenosine A2B receptor cascade. *Cardiovascular Research 70*, 308–314.

Phosri, S., Arieyawong, A., Bunrukchai, K., Parichatikanond, W., Nishimura, A., Nishida, M., et al. (2017). Stimulation of adenosine A2B receptor inhibits endothelin-1-induced cardiac fibroblast proliferation and α-smooth muscle actin synthesis through the cAMP/Epac/PI3K/Akt-signaling pathway. *Frontiers in Pharmacology 8*, 651.

Phosri, S., Bunrukchai, K., Parichatikanond, W., Sato, V. H., & Mangmool, S. (2018). Epac is required for exogenous and endogenous stimulation of adenosine A2B receptor for inhibition of angiotensin II-induced collagen synthesis and myofibroblast differentiation. *Purinergic Signal 14*, 141–156.

Pierce, K. D., Furlong, T. J., Selbie, L. A., & Shine, J. (1992). Molecular cloning and expression of an adenosine A2b receptor from human brain. *Biochemical and Biophysical Research Communications 187*, 86–93.

Pitcher, J. A., Freedman, N. J., & Lefkowitz, R. J. (1998). G protein-coupled receptor kinases. *Annual Review of Biochemistry 67*, 653–692.

Rivkees, S. A., & Reppert, S. M. (1992). RFL9 encodes an A2b-adenosine receptor. *Molecular Endocrinology 6*, 1598–1604.

Roberts, V. S., Cowan, P. J., Alexander, S. I., Robson, S. C., & Dwyer, K. M. (2014). The role of adenosine receptors A2A and A2B signaling in renal fibrosis. *Kidney International 86*, 685–692.

Roberts, V., Lu, B., Dwyer, K. M., & Cowan, P. J. (2014). Adenosine receptor expression in the development of renal fibrosis following ischemic injury. *Transplantation Proceedings 46*, 3257–3261.

Rosentreter, U., Henning, R., Bauser, M., Krämer, T., Vaupel, A., Hübsch, W., et al. (2001). Substituted 2-thio-3,5-dicyano-4-aryl-6-aminopyridines and the use thereof as adenosine reeceptor ligands. Bayer, Patent WO2001025210A2, 2001.

Ross, A. M., Gibbons, R. J., Stone, G. W., Kloner, R. A., & Alexander, R. W. (2005). A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). *Journal of the American College of Cardiology 45*, 1775–1780.

Ryzhov, S., Biktasova, A., Goldstein, A. E., Zhang, Q., Biaggioni, I., Dikov, M. M., et al. (2013). Role of JunB in adenosine A2B receptor-mediated vascular endothelial growth factor production. *Molecular Pharmacology 85*, 62–73.

Ryzhov, S., Goldstein, A. E., Biaggioni, I., & Feoktistov, I. (2006). Cross-talk between Gs- and Gq-coupled pathways in regulation of interleukin-4 by A2B adenosine receptors in human mast cells. *Molecular Pharmacology 70*, 727–735.

Ryzhov, S., Novitskiy, S. V., Zaynagetdinov, R., Goldstein, A. E., Carbone, D. P., Biaggioni, I., et al. (2008). Host A2B adenosine receptors promote carcinoma growth. *Neoplasia 10*, 987–995.

Sabbah, H. N., Gupta, R. C., Kohli, S., Wang, M., Rastogi, S., Zhang, K., et al. (2013). Chronic therapy with a partial adenosine A1-receptor agonist improves left ventricular function and remodeling in dogs with advanced heart failure. *Circulation. Heart Failure 6*, 563–571.

Sattin, A., & Rall, T. W. (1970). The effect of adenosine and adenine nucleotides on the cyclic adenosine 3', 5'-phosphate content of guinea pig cerebral cortex slices. *Molecular Pharmacology 6*, 13–23.

Sawynok, J. (2016). Adenosine receptor targets for pain. *Neuroscience 338*, 1–18.

Schulte, G., & Fredholm, B. B. (2003). The Gs-coupled adenosine A2B receptor recruits divergent pathways to regulate ERK1/2 and p38. *Experimental Cell Research 290*, 168–176.

Seo, S. W., Koeppen, M., Bonney, S., Gobel, M., Thayer, M., Harter, P. N., et al. (2015). Differential tissue-specific function of adora2b in cardioprotection. *Journal of Immunology 195*, 1732–1743.

Sepúlveda, C., Palomo, I., & Fuentes, E. (2016). Role of adenosine A2B receptor overexpression in tumor progression. *Life Sciences 166*, 92–99.
Shonberg, J., Lopez, L., Scammells, P. J., Christopoulos, A., Capuano, B., & Lane, J. R. (2014). Biased agonism at G protein-coupled receptors: the promise and the challenges-a medicinal chemistry perspective. *Medicinal Research Reviews 34*, 1286–1330.
Shyrock, J. C., & Belardinelli, L. (1997). Adenosine and adenosine receptors in the cardiovascular system: biochemistry, physiology, and pharmacology. *The American Journal of Cardiology 79*, 2–10.
Sitaraman, S. V., Merlin, D., Wang, L., Wong, M., Gewirtz, A. T., Si-Tahar, M., et al. (2001). Neutrophil-epithelial crosstalk at the intestinal lumenal surface mediated by reciprocal secretion of adenosine and IL-6. *The Journal of Clinical Investigation 107*, 861–869.
Sitaraman, S. V., Wang, L., Wong, M., Bruewer, M., Hobert, M., Yun, C. -H., et al. (2002). The adenosine 2B receptor is recruited to the plasma membrane and associates with E3KARP and ezrin upon agonist stimulation. *The Journal of Biological Chemistry 277*, 33188–33195.
Sommerschild, H. T., & Kirkebøen, K. A. (2000). Adenosine and cardioprotection during ischaemia and reperfusion–an overview. *Acta Anaesthesiologica Scandinavica 44*, 1038–1055.
St Hilaire, C., Carroll, S. H., Chen, H., & Ravid, K. (2009). Mechanisms of induction of adenosine receptor genes and its functional significance. *Journal of Cellular Physiology 218*, 35–44.
St Hilaire, C., Koupenova, M., Carroll, S. H., Smith, B. D., & Ravid, K. (2008). TNF-α upregulates the A2B adenosine receptor gene: The role of NAD(P)H oxidase 4. *Biochemical and Biophysical Research Communications 375*, 292–296.
Stagg, J., Divisekera, U., McLaughlin, N., Sharkey, J., Pommey, S., Denoyer, D., et al. (2010). Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. *Proceedings of the National Academy of Sciences of the United States of America 107*, 1547–1552.
Stehle, J. H., Rivkees, S. A., Lee, J. J., Weaver, D. R., Deeds, J. D., & Reppert, S. M. (1992). Molecular cloning and expression of the cDNA for a novel A2-adenosine receptor subtype. *Molecular Endocrinology 6*, 384–393.
Stewart, M., Steinig, A. G., Ma, C., Song, J. -P., McKibben, B., Castelhano, A. L., et al. (2004). [3H]OSIP339391, a selective, novel, and high affinity antagonist radioligand for adenosine A2B receptors. *Biochemical Pharmacology 68*, 305–312.
Sun, Y., Hu, W., Yu, X., Liu, Z., Tarran, R., Ravid, K., et al. (2016). Actinin-1 binds to the C-terminus of A2B adenosine receptor (A2BAR) and enhances A2BAR cell-surface expression. *The Biochemical Journal 473*, 2179–2186.
Sun, Y., & Huang, P. (2016). Adenosine A2B receptor: From cell biology to human diseases. *Frontiers in Chemistry 4*, 1329.
Sun, C. -X., Zhong, H., Mohsenin, A., Morschl, E., Chunn, J. L., Molina, J. G., et al. (2006). Role of A2B adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury. *The Journal of Clinical Investigation 116*, 2173–2182.
Tian, Y., Piras, B. A., Kron, I. L., French, B. A., & Yang, Z. (2015). Adenosine 2B receptor activation reduces myocardial reperfusion injury by promoting anti-inflammatory macrophages differentiation via PI3K/Akt pathway. *Oxidative Medicine and Cellular Longevity*, 1–8.
Toldo, S., Zhong, H., Mezzaroma, E., Van Tassell, B. W., Kannan, H., Zeng, D., et al. (2012). GS-6201, a selective blocker of the A2B adenosine receptor, attenuates cardiac remodeling after acute myocardial infarction in the mouse. *The Journal of Pharmacology and Experimental Therapeutics 343*, 587–595.
Trincavelli, M. L., Daniele, S., Giacomelli, C., Taliani, S., Da Settimo, F., Cosimelli, B., et al. (2014). Osteoblast differentiation and survival: A role for A2B adenosine receptor allosteric modulators. *Biochimica et Biophysica Acta, Molecular Cell Research 1843*, 2957–2966.
Urmaliya, V. B., Church, J. E., Coupar, I. M., Rose'Meyer, R. B., Pouton, C. W., & White, P. J. (2009). Cardioprotection induced by adenosine A1 receptor agonists in a cardiac cell ischemia model involves cooperative activation of adenosine A2A and A2B receptors by endogenous adenosine. *Journal of Cardiovascular Pharmacology 53*, 424–433.
Urmaliya, V. B., Pouton, C. W., Ledent, C., Short, J. L., & White, P. J. (2010). Cooperative cardioprotection through adenosine A1 and A2A receptor agonism in ischemia-reperfused isolated mouse heart. *Journal of Cardiovascular Pharmacology 56*, 379–388.
Vecchio, E. A., Chuo, C. H., Baltos, J. -A., Ford, L., Scammells, P. J., Wang, B. H., et al. (2016). The hybrid molecule, VCP746, is a potent adenosine A2B receptor agonist that stimulates anti-fibrotic signalling. *Biochemical Pharmacology 117*, 46–56.
Vecchio, E. A., Tan, C. Y. R., Gregory, K. J., Christopoulos, A., White, P. J., & May, L. T. (2016). Ligand-independent adenosine A2B receptor constitutive activity as a promoter of prostate cancer cell proliferation. *The Journal of Pharmacology and Experimental Therapeutics 357*, 36–44.
Vecchio, E. A., White, P. J., & May, L. T. (2017). Targeting Adenosine Receptors for the Treatment of Cardiac Fibrosis. *Frontiers in Pharmacology 8*, 781.
Vijayan, D., Young, A., Teng, M. W. L., & Smyth, M. J. (2017). Targeting immunosuppressive adenosine in cancer. *Nature Reviews. Cancer 17*, 709–724.
Villarreal, F., Epperson, S. A., Ramirez-Sanchez, I., Yamazaki, K. G., & Brunton, L. L. (2009). Regulation of cardiac fibroblast collagen synthesis by adenosine: roles for Epac and PI3K. *American Journal of Physiology. Cell Physiology 296*, C1178–C1184.
Violin, J. D., Crombie, A. L., Soergel, D. G., & Lark, M. W. (2014). Biased ligands at G-protein-coupledreceptors: promise and progress. *Trends in Pharmacological Sciences 35*, 308–316.
Wakeno, M., Minamino, T., Seguchi, O., Okazaki, H., Tsukamoto, O., Okada, K. I., et al. (2006). Long-term stimulation of adenosine A2B receptors begun after myocardial infarction prevents cardiac remodeling in rats. *Circulation 114*, 1923–1932.
Wang, L., Kolachala, V., Walia, B., Balasubramanian, S., Hall, R. A., Merlin, D., et al. (2004). Agonist-induced polarized trafficking and surface expression of the adenosine 2b receptor in intestinal epithelial cells: role of SNARE proteins. *American Journal of Physiology. Gastrointestinal and Liver Physiology 287*, G1100–G1107.
Watson, M. J., Lee, S. L., Marklew, A. J., Gilmore, R. C., Gentzsch, M., Sassano, M. F., et al. (2016). The cystic fibrosis transmembrane conductance regulator (CFTR) uses its C-terminus to regulate the A2B adenosine receptor. *Scientific Reports 6*, 1–13.
Watson, M. J., Worthington, E. N., Clunes, L. A., Rasmussen, J. E., Jones, L., & Tarran, R. (2011). Defective adenosine-stimulated cAMP production in cystic fibrosis airway epithelia: a novel role for CFTR in cell signaling. *The FASEB Journal 25*, 2996–3003.
Wei, Q., Costanzi, S., Balasubramanian, R., Gao, Z. -G., & Jacobson, K. A. (2013). A2B adenosine receptor blockade inhibits growth of prostate cancer cells. *Purinergic Signal 9*, 271–280.
Wen, J., Jiang, X., Dai, Y., Zhang, Y., Tang, Y., Sun, H., et al. (2010). Increased adenosine contributes to penile fibrosis, a dangerous feature of priapism, via A2B adenosine receptor signaling. *The FASEB Journal 24*, 740–749.
Weyler, S., Fülle, F., Diekmann, M., Schumacher, B., Hinz, S., Klotz, K. -N., et al. (2006). Improving potency, selectivity, and water solubility of adenosine a1 receptor antagonists: xanthines modified at position 3 and related pyrimido[1,2,3-cd]purinediones. *Chem Med Chem 1*, 891–902.
Wilkinson, P. F., Farrell, F. X., Morel, D., Law, W., & Murphy, S. (2016). Adenosine signaling increases proinflammatory and profibrotic mediators through activation of a functional adenosine 2B receptor in renal fibroblasts. *Annals of Clinical and Laboratory Science 46*, 339–345.
Wilson, C. N., Nadeem, A., Spina, D., Brown, R., Page, C. P., & Mustafa, S. J. (2009). Adenosine receptors and asthma. *Handbook of Experimental Pharmacology 193*, 329–362.
Xaus, J., Mirabet, M., Lloberas, J., Soler, C., Lluis, C., Franco, R., et al. (1999). IFN-gamma upregulates the A2B adenosine receptor expression in macrophages: a mechanism of macrophage deactivation. *Journal of Immunology 162*, 3607–3614.
Xi, J., McIntosh, R., Shen, X., Lee, S., Chanoit, G., Criswell, H., et al. (2009). Adenosine A2A and A2B receptors work in concert to induce a strong protection against reperfusion injury in rat hearts. *Journal of Molecular and Cellular Cardiology 47*, 684–690.
Yan, L., Burbiel, J. C., Maaß, A., & Müller, C. E. (2003). Adenosine receptor agonists: from basic medicinal chemistry to clinical development. *Expert Opinion on Emerging Drugs 8*, 537–576.
Yang, D., Koupenova, M., McCrann, D. J., Kopeikina, K. J., Kagan, H. M., Schreiber, B. M., et al. (2008). The A2b adenosine receptor protects against vascular injury. *Proceedings of the National Academy of Sciences of the United States of America 105*, 792–796.
Yang, Y., Qiu, Y., Wang, W., Xiao, W., Liang, H., Zhang, C., et al. (2014). Adenosine A2B receptor modulates intestinal barrier function under hypoxic and ischemia/reperfusion conditions. *International Journal of Clinical and Experimental Pathology 7*, 2006–2018.
Yang, X., Xin, W., Yang, X. -M., Kuno, A., Rich, T. C., Cohen, M. V., et al. (2011). A 2B adenosine receptors inhibit superoxide production from mitochondrial complex I in rabbit cardiomyocytes via a mechanism sensitive to pertussis toxin. *British Journal of Pharmacology 163*, 995–1006.
Yang, D., Zhang, Y., Nguyen, H. G., Koupenova, M., Chauhan, A. K., Makitalo, M., et al. (2006). The A2B adenosine receptor protects against inflammation and excessive vascular adhesion. *The Journal of Clinical Investigation 116*, 1913–1923.
Zhan, E., McIntosh, V. J., & Lasley, R. D. (2011). Adenosine A2A and A2B receptors are both required for adenosine A1 receptor-mediated cardioprotection. *American Journal of Physiology. Heart and Circulatory Physiology 301*, H1183–H1189.
Zhang, Y., Dai, Y., Wen, J., Zhang, W., Grenz, A., Sun, H., et al. (2011). Detrimental effects of adenosine signaling in sickle cell disease. *Nature Medicine 17*, 79–86.
Zhang, Y., Knight, W., Chen, S., Mohan, A., & Yan, C. (2018). A multi-protein complex with TRPC, PDE1C, and A2R plays a critical role in regulating cardiomyocyte cAMP and survival. *Circulation 118* Epub ahead of print.
Zhang, W., Zhang, Y., Wang, W., Dai, Y., Ning, C., Luo, R., et al. (2013). Elevated ecto-5'-nucleotidase-mediated increased renal adenosine signaling via A2B adenosine receptor contributes to chronic hypertension. *Circulation Research 112*, 1466–1478.
Zhang, H., Zhong, H., Everett, T. H., Wilson, E., Chang, R., Zeng, D., et al. (2014). Blockade of A2B adenosine receptor reduces left ventricular dysfunction and ventricular arrhythmias 1 week after myocardial infarction in the rat model. *Heart Rhythm 11*, 101–109.
Zhong, H., Belardinelli, L., Maa, T., Feoktistov, I., Biaggioni, I., & Zeng, D. (2004). A(2B) adenosine receptors increase cytokine release by bronchial smooth muscle cells. *American Journal of Respiratory Cell and Molecular Biology 30*, 118–125.
Zhong, H., Belardinelli, L., Maa, T., & Zeng, D. (2005). Synergy between A2B adenosine receptors and hypoxia in activating human lung fibroblasts. *American Journal of Respiratory Cell and Molecular Biology 32*, 2–8.
Zhou, Y., Chu, X., Deng, F., Tong, L., Tong, G., Yi, Y., et al. (2017). The adenosine A2b receptor promotes tumor progression of bladder urothelial carcinoma by enhancing MAPK signaling pathway. *Oncotarget 8*, 48755–48768.
Zhou, Q. Y., LI, C., Olah, M. E., Johnson, R. A., Stiles, G. L., & Civelli, O. (1992). Molecular cloning and characterization of an adenosine receptor: the A3 adenosine receptor. *Proceedings of the National Academy of Sciences of the United States of America 89*, 7432–7436.
Zhou, Y., Schneider, D. J., & Blackburn, M. R. (2009). Adenosine signaling and the regulation of chronic lung disease. *Pharmacology & Therapeutics 123*, 105–116.
Zhou, Y., Schneider, D. J., Morschl, E., Song, L., Pedroza, M., Karmouty-Quintana, H., et al. (2011). Distinct roles for the A2B adenosine receptor in acute and chronic stages of bleomycin-induced lung injury. *Journal of Immunology 186*, 1097–1106.
de Zwart, M., Link, R., von Frijtag Drabbe Kunzel, J. K., Cristalli, G., Jacobson, K. A., Townsend-Nicholson, A., et al. (1998). A functional screening of adenosine analogues at the adenosine A2B receptor: a search for potent agonists. *Nucleosides & Nucleotides 17*, 969–985.
Zylka, M. J. (2011). Pain-relieving prospects for adenosine receptors and ectonucleotidases. *Trends in Molecular Medicine 17*, 188–196.