Recent improvements in the development of $A_{2B}$ adenosine receptor agonists

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Abstract Adenosine is known to exert most of its physiological functions by acting as local modulator at four receptor subtypes named $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ (ARs). Principally as a result of the difficulty in identifying potent and selective agonists, the $A_{2B}$ AR is the least extensively characterised of the adenosine receptors family. Despite these limitations, growing understanding of the physiological meaning of this target indicates promising therapeutic perspectives for specific ligands. As $A_{2B}$ AR signalling seems to be associated with pre/postconditioning cardioprotective and anti-inflammatory mechanisms, selective agonists may represent a new therapeutic group for patients suffering from coronary artery disease. Herein we present an overview of the recent advancements in identifying potent and selective $A_{2B}$ AR agonists reported in scientific and patent literature. These compounds can be classified into adenosine-like and nonadenosine ligands. Nucleoside-based agonists are the result of modifying adenosine by substitution at the $A^0$, $C^2$-positions of the purine heterocycle and/or at the 5'-position of the ribose moiety or combinations of these substitutions. Compounds 1-deoxy-1-[(6-N'-[(furan-2-carbonyl)-hydrazino]-9H-purin-9-yl]-N'-ethyl-β-D-ribofuranuronamide (19, $hA_1 K_i=1050$ nM, $hA_{2A} K_i=1550$ nM, $hA_{2B} EC_{50}=82$ nM, $hA_3 K_i>5$ μM) and its 2-chloro analogue 23 (h$A_1 K_i=3500$ nM, h$A_{2A} K_i=4950$ nM, h$A_{2B} EC_{50}=210$ nM, h$A_3 K_i>5$ μM) were confirmed to be potent and selective full agonists in a cyclic adenosine monophosphate (cAMP) functional assay in Chinese hamster ovary (CHO) cells expressing h$A_{2B}$ AR. Nonribose ligands are represented by conveniently substituted dicarbonitrilepyridines, among which 2-[6-amino-3,5-dicyano-4-(4-hydroxyphenyl)pyridin-2-ylsulfanyl]acetamide (BAY-60–6583, $hA_1$, $hA_{2A}$, $hA_3 EC_{50}>10$ μM; $hA_{2B} EC_{50}=3$ nM) is currently under preclinical-phase investigation for treating coronary artery disorders and atherosclerosis.

Keywords $A_{2B}$ adenosine receptor · $A_{2B}$ AR agonist · Atherosclerosis · Coronary artery disease · Cystic fibrosis · Impotence · Inflammation · Myocardial infarction · Septic shock

Abbreviations
ABOPX 3-(3,4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propyl-xanthine
ADP Adenosine Diphosphate
Ags Adenosine Receptors
ASMCs Arterial Smooth Muscle Cells
ATP Adenosine Triphosphate
BAY 2-[6-amino-3,5-dicyano-4-(4-hydroxyphenyl)pyridin-2-ylsulfanyl]acetamide
cAMP cyclic Adenosine Monophosphate
$[^{3}H]$CCPA $[^{3}H]$-2-chloro-$N^0$-cyclopentyladenosine
CFTR Cystic Fibrosis Transmembrane Conductance Regulator
CGS21680 2-[4-(2-carboxyethyl)phenylethyl]amino)-5'-N-ethoxycarbamidoadenosine
CHO cells Chinese Hamster Ovary cells
CNS Central Nervous System
COPD Chronic Obstructive Pulmonary Disease
DPCPX 1,3-dipropyl-8-cyclopentyl-xanthine
FAD Flavin Adenine Dinucleotide
GMCs Glomerular Mesangial Cells
$[^{3}H]$-CHA $[^{3}H]$-$N^0$-cyclohexyladenosine
HEK293 Human Embryonic Kidney cells
[125I]-AB-MECA [125I]N6-(4-amino-3-iodobenzyl)adenosine-5'-N-methyl-uronamide
[125I]IB-MECA [125I]N6-2-(4-amino-phenyl)ethyladenosine
APNEA 2-(4-amino-phenyl)-5-(1,3-dipropyl-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-1-methyl-1H-pyrazol-3-yl oxy]-acetamide
2029-F20 dipropyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)-1-methyl-1H-pyrazol-3-yl oxy]-acetamide
MRE N-benzo[1,3]dioxol-5-yl-2-[5-(1,3-dipropyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)-phenox y]-acetamide
MRS 1754 [N-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-phenoxy]y ace tamide
NAD Nicotinamide Adenine Dinucleotide
NECA 5'-N-ethylcarboxamidoadenosine
NO Nitric Oxide
OSIP339391 N-(2-(2-Phenyl-6-[4-(2,2,3,3-tetra tritrio-No Nitric Oxide
NECA 5'-N-ethylcarboxamidoadenosine
R-PIA N6-(R)-phenylisopropyladenosine
SAM S-Adenosyl-L-Methionine
TNFα Tumor Necrosis Factor α
ZM 241385 (4-(2-[1H]-[2-amin o-2-(2-furyl)][1,2,4]triazolo-[2,3-a][1,3,5]triazin-5-ylamino) ethyl)-phenol

Introduction

Adenosine is involved in important biochemical processes, such as energy transfer [adenosine triphosphate (ATP) and adenosine diphosphate (ADP)] and signal transduction [cyclic adenosine monophosphate (cAMP)] and it is a building block for some biologically significant molecules such as nicotinamide-adenine-dinucleotide (NAD), flavin-adenine-dinucleotide (FAD), S-adenosyl-L-methionine (SAM), DNA and RNA. The endogenous purine nucleoside (Ado, 1, Fig. 1) is ubiquitous in mammalian cell types and, in view of its function in regulating a wide number of physiopathological events (such as cytoprotective, anti-inflammatory, central nervous system neurotransmitters regulator, pain transmission and metabolism modulator agent [1–9]), there is wide-spread interest throughout the scientific community in the understanding of its molecular pharmacology and physiology.

Adenosine and ATP have been shown to induce signalling via P1 and P2 receptors, respectively. P1 [adenosine receptors (ARs)] receptors are divided into four subtypes, all belonging to the family of cell-membrane G-protein-coupled adenosine receptors named A1, A2A, A2B and A3, which have been cloned from many mammalian and some nonmammalian species [10–13].

A2B ARs have been generally defined as the “low-affinity ARs,” as their lower affinity for the endogenous ligand, adenosine, and for some typical agonists, such as 5'-N-ethylcarboxamidoadenosine (NECA, 2, Fig. 1), N6-(R)-phenylisopropyladenosine (R-PIA), and 2-[(4-(2-carboxyethyl)phenylethyl)amino)-5'-N-ethylcarboxamido adenosine (CGS21680), by contrast with other AR subtypes [14–15]. Under physiological conditions, intracellular adenosine reaches a concentration of 100 nM and thus is able to interact only with the high-affinity A1 and A2A AR subtypes. In hypoxic, ischaemic or inflammatory conditions, the intracellular levels of adenosine can grow to very high micromolar concentrations and, thanks to specific transports across cell membranes, the endogenous nucleoside can activate the low-affinity A2B and A3 AR subtypes. Activation of A2B AR implies stimulation of adenylate cyclase and activation of phospholipase C through the coupling to Gs and Gq/11 proteins, respectively. A2B ARs have been found on practically every cell in most species, and their sequences are highly similar across species. The human (h) A2B AR shares, for example, 86–87% amino acid sequence homology with the rat and mouse subtypes [14]. Determination of receptor-coding messenger RNA (mRNA) levels furnished important information about A2B AR tissue distribution. High concentrations of A2B ARs have been suggested in caecum, large intestine and urinary bladder, whereas a lower expression has been revealed in lung, blood vessels, eye, and mast cells. Adipose tissue, adrenal gland, brain, kidney, liver, ovary and pituitary gland are thought to have a very low concentration of A2B AR [12].

A2B ARs are known as the most poorly characterised of the adenosine P1 receptors from a pharmacological point of view, as their general low affinity towards prototypic ligands exercising specific high affinity and potency in activating each of the remaining AR subtypes. In particular, the scarcity of medicinal chemistry knowledge about the structural requirements necessary for potent and selective activation of the A2B AR subtype has created wide-ranging difficulty in detecting the physiological effects mediated by direct and selective A2B AR stimulation. Despite these limitations, growing and promising information in understanding the physiological meaning of these receptors has arisen from the exploitation of potency and selectivity of
ligands for A1, A2A and/or A3 ARs by employing a strategy of exclusion in a model in which more AR subtypes are coexpressed. The A2A AR-selective agonist CGS21680 has been reported, for example, as a useful tool for differentiation between A2A and A2B ARs [14]. Moreover, some potent and selective antagonists of the A2B AR have been employed to distinguish A2B AR-mediated effects. Until a few years ago, the characterisation of A2B ARs through radioligand binding studies using low-affinity and nonselective antagonists such as [3H]1,3-dipropyl-8-cyclopentyl-xanthine ([3H]DPCPX), [3H](4-(2-[7-amino-2-(2-furyl)-[1,2,4]triazolo-[2,3-a][1,3,5] triazin-5-ylamino] ethyl)-phenol ([3H]ZM 241385), [125I]3-(3,4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propyl-xanthine ([125I]ABOPX) [16]. A helpful advancement in the pharmacological characterisation of A2B ARs is supposed to be increased by the recent identification of the tritiated form of some new A2B AR antagonists with improved potency and selectivity, such as [3H][N-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-phenoxyl] acetamide ([3H]MRS 1754) [17], [3H]N-(2-(2-Phenyl-6-[4-(2,2,3,3-tetrahydro-3-phenylpropyl)-pipеразин-1-карбонил]-7Hпирроло[2,3-d]пirimidин-4-ylamino)-етил]acetamид ([3H]OSIP33931) [18] and [3H]N-benzo[1,3]dioxol-5-yl-2-

\[5-(1,3-dipropyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)-1-methyl-1H-pyrazol-3-yloxy]-acetamide ([3H]MRE 2029-F20) [19].

The contribution of genetic engineering and manipulation (for example, generation of A2B AR knockout mice and transgenic mice overexpressing this receptor), especially if combined with classic pharmacological investigations, have determined relevant progress in establishing the therapeutic potential of A2B AR ligands and, generally, the role of ARs, in a variety of diseases [20–23].

**A2B adenosine receptor physiology and pharmacology**

The A2B AR subtype has been recognised to regulate a wide range of physiopathological events. However, it is mainly involved in modulating cardiovascular functions and the genesis of inflammation processes. A role for A2B AR in regulating vascular tone, cardiac myocyte contractility, neurosecretion and neurotransmission, cell growth, gene expression, intestinal tone and secretion and mast-cell function has been suggested [14]. A2B AR activation is
known to induce angiogenesis [24–25], reduce vascular permeabilisation [26], increase release of inflammatory mediators from human and canine mast cells [24] and modulate neurohypophysial hormone output [27]. Adenosine, through A2B ARs, can exert long-term control over glycogen levels in primary cultures of mouse cortical astrocytes and might therefore play a significant role in pathophysiological processes involving long-term modulation of brain-energy metabolism [21]. There is evidence of a probable involvement of A2B ARs in the growth and development of some tumours, and A2B ARs have been proposed as targets to control cell growth and proliferation in a human breast cancer cell line [28].

ARs play a significant role in regulating ion transport in epithelial tissues through a variety of intracellular signalling pathways. Each of the four P1 receptors has distinctive roles in different epithelial tissue types. The A2B AR has been identified on both the mucosal and basolateral aspect of colonic epithelial cells. Activation at either site results in Cl− secretion via direct activation of the cAMP-activated Cl− channel cystic fibrosis transmembrane conductance regulator (CFTR) [29]. An analogous process of adenosine-mediated activation of Cl− secretion has been located at the lung epithelium [30]. The stimulated secretory response has been identified to be the result of A2B AR activation and was lost in a cell line derived from a cystic fibrosis patient with a defect in ion transport at CFTR, implicating this ion channel as the one responsible for the A2B AR-mediated Cl− secretion [31].

**Therapeutic potential of A2B adenosine receptor antagonists**

There are growing findings supporting adenosine as having a role in asthma and chronic obstructive pulmonary disease (COPD) [32]. Moreover, adenosine stimulates production of interleukin (IL)-4 and IL-13 in mast cells via A2B AR activation [33]. Treatment of asthma with selective A2B AR antagonists has so far been one of the most significant therapeutic options among AR ligands [34–38].

A2B AR antagonists are directed towards clinical use for treating diabetes, as these seem to antagonise the adenosine-induced hepatic glucose production determining reduction of blood glucose levels after oral administration [39]. A2B AR has been likewise reported to be involved in stimulating proliferation, differentiation and migration of retinal endothelial cells. Thus A2B AR antagonists may offer a way to inhibit retinal angiogenesis, providing a novel therapeutic approach for treating diseases associated with aberrant neovascularisation, such as diabetic retinopathy [40].

The opioid and adenosine systems seem to cooperate to some extent in modulating pain signalling. In particular, participation of A2B ARs in the analgesic effects mediated by caffeine in an acute animal model of nociception (hot-plate test) has been documented [41]. These findings support the potential therapeutic employment of specific A2B AR antagonists as valuable adjuvant drugs for opioid analgesia, with minimal side effects.

The purinergic regulation of epithelial transports and, above all, involvement of the A2B AR subtype in determining secretion stimulation, suggest the possibility of employing A2B AR-specific ligands as potential modulators of ion transport and the parallel flux of water, which can be considered a natural defence system working to “wash away” injuries in the setting of cellular damage or inflammation. Selective A2B AR ligands are under investigation for treating diarrhoea and cystic fibrosis [42–44].

**Therapeutic potential of A2B adenosine receptor agonists**

Ischaemic preconditioning (IPC) is a cardioprotective mechanism according to which brief and repeated episodes of sublethal ischaemia and reperfusion, before myocardial infarction, cause the heart to become resistant to infarction and result in attenuation of infarct size. Activation of cardiac A2B AR receptors at reperfusion showed to be protective in the rabbit, but because of the very low affinity of the receptors, endogenous cardiac adenosine is unable to elicit their signalling. Protein kinase C physiologically increases the heart’s sensitivity to adenosine so that endogenous adenosine can activate A2B AR-dependent signalling. 2-[6-amino-3,5-dicyano-4-(4-hydroxyphenyl)pyridin-2-ylsulfanyl]acetamide (BAY 60–6583), a highly selective A2B AR agonist [45] (Fig. 3), resulted in limited infarct size when given to rabbit-ischaemic hearts at reperfusion [46]. Postconditioning protects the heart with multiple brief reperfusion/ischaemia cycles immediately following the ischaemic insult. In rabbit hearts, binding of endogenous adenosine to A2B ARs in early reperfusion is a requirement for both IPC and postconditioning to limit infarction [47].

A pharmacological and gene-targeting approach performed with mice models to study the contributions of AR signalling to ischaemic preconditioning cardioprotection provided evidence that selective A2B AR agonists may offer important advantages in comparison with classical therapies of acute myocardial ischaemia [48]. Intravenous administration of nonselective adenosine is associated with side effects (bradycardia, hypotension, rapid receptor desensitisation), which could be circumvented by the use of specific A2B AR agonists [23].

Deletion of the gene encoding the A2B AR in the mouse (A2B AR-knockout mouse model) recently resulted in
increased production of proinflammatory cytokines, altered response to endotoxin exposure, increased leukocyte adhesion and increased leukocyte rolling on blood vessels [49]. Activation of A2B AR subtype would moreover increase production of the anti-inflammatory cytokine IL-10 [50].

The described association of A2B AR with pre/postconditioning cardioprotection, along with the documented strong anti-inflammatory role of A2B AR signalling [49], suggests that A2B AR agonists may represent a new group of therapeutics for patients suffering from coronary artery disease. Several reports contribute to strengthen this perspective, highlighting essential A2B AR-mediated cardiovascular effects. A NECA-mediated coronary vasodilation via the A2B AR subtype in isolated hearts from young (1–2 months) and mature (12–18 months) Wistar rats has been documented [51]. Adenosine-mediated vasorelaxation in mouse aorta is partially dependent on A2B AR [52], and A2B ARs mediate relaxation in human small resistance-like coronary arteries, which is independent of nitric oxide (NO) but partly coupled to potassium (K+) channel function [53].

Human aortic smooth muscle cells (SMCs) synthesise adenosine, which seems to protect against vasooocclusive disorders by inhibiting SMC proliferation and collagen synthesis via activation of A2B AR receptors [54]. Apoptosis of arterial smooth muscle cells (ASMCs) could play an important role in the pathogenesis of atherosclerosis and restenosis. Recent results indicate that adenosine-induced apoptosis of cultured human ASMCs is essentially mediated via A2B AR and involves a cAMP-dependent pathway [55]. These studies speculated that adenosine could play a dual role in the evolution of intimal thickening. Its action can be considered beneficial concerning control of intimal hyperplasia and thickening formation. In contrast, the same authors indicated that adenosine could contribute to the formation of the necrotic core in advanced atherosclerotic lesions, promoting—along with other concurrent factors—plaque rupture. These opposing effects suggest different therapeutic strategies based on the role of A2B AR stimulation-mediated effects in the pathogenesis of atherosclerosis and restenosis.

Glomerular mesangial cell (GMC) growth is inhibited by A2B AR activation coupled with inhibition of mitogen-activated protein kinase (MAPK) activity [56]. A2B AR function may therefore largely affect glomerular remodeling associated with GMC proliferation. Identification of pharmacological agents able to specifically activate A2B ARs has been purported to be of therapeutic importance in protecting against glomerular remodeling associated with glomerulosclerosis, renal disease and abnormal GMC growth associated with hypertension and diabetes.

Studies performed by the Shiseido Research Group indicate that adenosine, via A2B AR, might stimulate hair growth through fibroblast growth factor-7 gene expression upregulation in dermal papilla cells [57].

In the research field concerning vasculogenic erectile dysfunctions, there emerged key importance regarding purinergic transmission for initiating and maintaining penile erection [58]. Endothelial dysfunction of human corpus cavernosum may be correlated with the loss of adenosine A2B ARs activity, indicating a possible employment of specific A2B AR agonists as a new therapeutic approach to manage severe vasculogenic impotence resistant to common vasodilators [59].

As interaction of adenosine with A2B ARs inhibits production of the proinflammatory cytokine tumor necrosis factor (TNFα) by lipopolysaccharide-activated monocytes [49, 60], A2B AR agonists have been proposed for treating septic shock, confirming the broad anti-inflammatory potential of AR agonists in treating inflammatory disorders [61].

**A2B adenosine receptor agonists**

The lack of molecules endowed with selective and potent agonistic activity towards the hA2B ARs has limited the studies on this pharmacological target and consequently the evaluation of its therapeutic potential. Several ligands for A2B AR have been identified in recent years [62–64]. However, only very recently have some reports about important advancement in identifying A2B AR agonists with improved in vitro pharmacological profile been published.

Medicinal chemistry literature concerning the field of AR agonists is generally characterised by the absence of binding data related to the A2B AR subtype, caused by the substantial lack of a useful radiolabelled agonist. Therefore, for the time being, the selectivity profile of new potential A2B AR agonists can only be speculated in view of the ratio of binding parameters (Ki values for A1, A2A, A3 ARs) to functional parameters (EC50 for A2B, Tables 1, 2, 3, 4, 5, and 6).

The compounds of particular interest for the development of potent and selective A2B AR agonists can be classified into adenosine-like and nonadenosine-like ligands. Nucleoside-based agonists are the result of modifying the endogenous ligand, adenosine, by substitution at the N′-, C2-positions of the purine heterocycle and/or at the 5′-position of the ribose moiety. In particular, the most potent and subtype-selective ligands have been obtained by combining these substitutions (i.e., multiply substituted adenosines). This group can be subdivided into the following subclasses: N′-substituted adenosines, N′-substituted-5′-N-alkyl-carboxamidoadenosines, C2-substituted adenosines and C2-substituted-5′-N-alkyl-carboxamidoadenosines.

Nonadenosine derivatives so far reported are represented by conveniently substituted pyridine-3,5-dicarbonitrile derivatives.
Adenosine-like ligands

In the search for A2B AR agonists, de Zwart et al. reported a functional screening, based on adenylate cyclase stimulation, of known adenosine analogues variously modified at the 2-, 5-, 8-, N6 and 5′ positions (or combinations of these) [65]. This study indicated NECA (5′-N-carboxamidoadenosine, 2, Fig. 1) as the most potent ligand since then reported. More generally, after these first studies, the 5′-N-carboxamidoadenosines seemed more potent than the corresponding 4′-CH2OH derivatives, N6-substitution showed higher compatibility with A2B AR subtype compared with C2- and/or C8- substitutions and deazapurine derivatives resulted as inactive. Thus, N6-substituted-5′-N-carboxamidoadenosine derivatives were initially considered as the most promising tool for identifying A2B AR agonists. More recently, a series of carboxamido and thiocarboxamidoadenosines bearing several 5′-N-(cyclo)alkyl groups [66] have been synthesised and tested at the four AR subtypes. The replacement of the 5′-N-ethyl carboxamido function of NECA with other alkyl groups or the thiocarboxamido moiety led to a significant loss of A2B AR potency and, in some examples, to reduced intrinsic activity (data not shown).

N6-substitution

Some examples of N6-substituted adenosine derivatives endowed with satisfactory levels of A2B AR potency have been reported [62]. In particular, the introduction of (substituted) phenyl rings at the N6-position led to the identification of compounds such as the 4-I-phenyl and 4-aminosulfonylphenyl derivatives 3 and 4 (Fig. 1) displaying submicromolar potency in activating the A2B AR subtype [64]. N6-modification of NECA with substituted phenyl

| R | R' | hA1+ Kᵢ (nM) | hA2A+ Kᵢ (nM) | hA2B EC₅₀ (nM) | hA3+ Kᵢ (nM) |
|---|---|-------------|-------------|--------------|-------------|
| NECA | | 18.2±2.1 | 12.4±2.7 | 155±12 | 35.7±3.3 |
| 7 | H | 8.5±0.8 | >1000 (45%) | 7.3±0.6 | 38.4±3.7 |
| 8 | H | 2.3±0.2 | >1000 (48%) | 15.2±2.1 | 72.3±7.4 |
| 9 | H | 3.1±0.3 | >1000 (35%) | 12.3±1.4 | 34.2±3.7 |
| 10 | H | 3.5±0.4 | >1000 (26%) | 10.5±1.2 | 36.4±3.7 |
| 11 | H | 5.2±0.5 | >1000 (28%) | 30.2±2.8 | 85.2±8.3 |
| 12 | H | 4.0CH₃-Ph | 4.7±0.4 | >1000 (42%) | 32.4±3.3 | 25.3±2.6 |
| 13 | H | 3.4-OCH₂O-Ph | 8.4±0.9 | >1000 (5%) | 35.5±2.7 | 81.4±8.3 |
| 14 | H | 4-tert-Butyl-Ph | 18.6±2.1 | >1000 (1%) | 16.4±2.1 | 40.2±3.9 |
| 15 | H | 4-Pyridyl | 11.2±1.3 | >1000 (37%) | 32.3±2.4 | 42.3±4.7 |
| 16 | H | Benzyl | 20.4±2.1 | >1000 (49%) | 150±17 | 82.7±8.9 |
| 17 | Cl | Ph | 30.5±3.3 | >1000 (36%) | 42.6±4.2 | 107±10 |
| 18 | Cl | Benzyl | 22.6±2.4 | >1000 (49%) | 175±14 | 75.7±7.4 |

a Displacement of specific [1H]CHA binding at human A₁ receptors expressed in CHO cells. b Displacement of specific [1H]CGS21680 binding at human A₂A receptors expressed in CHO cells. In parentheses are indicated the percentage of displacement of the examined compounds (1 μM). c cAMP assay in CHO cells expressing human A₂B adenosine receptors EC₅₀ (nM). d Displacement of specific [125]IABMECA binding at human A₃ receptors expressed in CHO cells. Data are expressed as geometric means with 95% confidence limits.
groups yielded compounds endowed with similar activity as the corresponding adenosine analogues (N6-(4-chlorophenyl)NECA, EC50=0.73 μM) [67]. In a recent study of modelling and site-directed mutagenesis performed with the aim of defining the leading parameters affecting the interaction between A2A AR and its specific agonists [68], the N6-guanidino derivative of NECA (Fig. 1) was identified. Replacement of the 6-amino group of NECA with the guanidino moiety determined a threefold enhancement in A2B AR activation potency (EC50=54.5 nM versus 140 nM of NECA) and an increased selectivity versus A2A AR subtype (Ki=628 nM versus 2.2 nM), maintaining a high affinity at the A3 (Ki=5.1 nM) and A1 ARs (Ki=7.0 nM).

The disubstitution of the amino group at the 6-position of adenosine is not tolerated by A2B AR subtype (data not shown) [64].

A novel series of potent but low-selective A2B AR agonists structurally related to NECA has been recently reported by Baraldi et al. [69]. These compounds were designed modifying the N6-position of 5′-N-carboxamidoadenosine in analogy with the typical substitution pattern of some potent and selective A2B AR antagonists previously reported in the literature. Several A2B AR antagonists with high affinity and good selectivity have, in fact, been identified among structures based upon a xanthine core suitably substituted at the 1-, 3- and 8-positions [70]. In particular, Kim et al. [71] reported that a (substituted) phenylcarbamoyl-methoxy-phenyl chain at the 8-position of a series of 1,3-dipropyl-xanthines was able to specifically direct the antagonist activity to the A2B AR. Further evidence of the important role of the substituent at the 8-position as the structural selectivity element for the design of potent A2B AR antagonists was provided recently by our group [72].

Considering the previous structure activity relationship (SAR) studies, regarding NECA and adenosine derivatives indicating the N6 as a useful position for A2B AR binding-site recognition, a new series of N6-[(substituted)phenyl/ cycloalkyl/benzyl/heteroaryl-carbamoyl-methoxy-phenyl]-5′-N ethylcarboxamido-adenosine and 2-chloro-adenosine derivatives (7–18, Table 1) has been designed and synthesised. These molecules can be considered as molecular hybrids obtained by the introduction of an aryl-carbamoyl-methoxy-phenyl chain (supposed to grant A2B

Table 2  Binding affinities (hA1, hA2A, hA3) and functional parameters (hA2B) of the 6-(heteroaryl-carbonyl)-hydrazino-NECA derivatives 19–26 at the human adenosine receptors expressed in CHO cells [79, 80]

| R | R'   | hA1a Ki (nM) | hA2Aa Ki (nM) | hA2Bb EC50 (nM) | hA3d Ki (nM) |
|---|------|-------------|---------------|----------------|-------------|
| NECA |   | 18.3±2.5 | 12.5±2.8 | 160±20 | 34.6±3.3 |
| 19 | H 2-Furyl | 1050±132 | 1550±165 | 82±10 | > 5000 (23%) |
| 20 | H 5-Bromo-furan-2-yl | 780±34 | 1200±135 | 369±42 | > 5000 (13%) |
| 21 | H 5-Methyl-furan-2-yl | 700±25 | 1600±147 | 227±18 | > 5000 (15%) |
| 22 | H 5-Methyl-thiophen-2-yl | 1100±124 | 2100±185 | 273±12 | > 5000 (19%) |
| 23 | Cl 2-Furyl | 3500±275 | 4950±356 | 210±13 | > 5000 (26%) |
| 24 | Cl 5-Methyl-thiophen-2-yl | 2600±194 | 4100±390 | 175±20 | > 5000 (17%) |
| 25 | Cl Thiophen-3-yl | 933±76 | 3300±315 | 450±29 | > 5000 (18%) |
| 26 | Cl Thiophen-2-yl | 737±46 | 1700±180 | 200±20 | > 5000 (12%) |

a Displacement of specific [3H]CHA binding at human A1 receptors expressed in CHO cells. b Displacement of specific [3H]CGS21680 binding at human A2A receptors expressed in CHO cells. c cAMP assay in CHO cells expressing human A2B adenosine receptors EC50 (nM). d Displacement of specific [125I]ABMeca binding at human A3 receptors expressed in CHO cells. The percentages in the parentheses indicate the % of displacement of the new tested compounds in the binding experiments (5 μM). Data are expressed as geometric means with 95% confidence limits. The data are expressed as mean ± SEM.
AR selectivity as in the cited series of xanthine derivatives) at the \(N^6\)-position of the typical nucleoside nucleus responsible for AR activation. The key role of this position in the formation of the \(A_{2B}\) AR-ligand complex has been confirmed by a molecular modelling investigation performed with the human \(A_{2B}\) AR. The docking of known \(A_{2B}\) AR agonists highlighted, in fact, involvement of the exocyclic amino group at the 6-position of NECA in an important interaction with a residue of asparagine 254 belonging to the VI transmembrane receptor helix [73]. The 2-chloro atom was introduced, as the literature in the field of \(A_{2B}\) AR agonists indicates the 2-position as a second possible site of modification of the purine nucleus [74].

As described in Table 1, different kinds of substitutions have been considered at the nitrogen atom of the acetamide chain introduced at the \(N^6\)-position of NECA. All synthesised compounds were evaluated in radioligand-binding assays to define their affinities for human \(A_1\), \(A_{2A}\) and \(A_3\).

![Diagram](image-url)

### Table 3: Binding affinities (\(A_1\), \(A_{2A}\), \(A_3\)) and functional parameters (\(A_{2B}\)) of 2-substituted adenosine and NECA derivatives 27–38 at the adenosine receptors

| R′ | R | A₁ \(K_i\) nM | A₂ₐ \(K_i\) nM | A₂₈ \(EC_{50}\) μM | A₃ \(K_i\) nM |
|----|---|-----------|-----------|-------------|-----------|
| 2-Cl-Ado (27) [62,65] | Cl | 9.3 | 63 | 24 | 1,890 |
| (R,S)PHPAdo (28) [81] | -C≡C-CH(OH)Ph | 0.67 | (0.55–0.80) | (3.7–13) | (1.5–3.7) | 3.3 |
| (R)PHPAdo LUF5599 (29) [81] | -C≡C-CH(OH)Ph | 0.44 | (0.38–0.52) | (19–45) | (2.9–13) | 5.0 |
| (S)PHPAdo LUF 5600 (30) [81] | -C≡C-CH(OH)Ph | 0.67 | (0.47–0.96) | (1.1–3.0) | (0.71–1.2) | 1.4 |
| \(N^6\)-ethyl-(R,S)PHPAdo (31) [82] | -C≡C-CH(OH)Ph | 2.7 | 94.0 | 1.7 | 0.97 |
| (R,S)PHPNECA (32) [74] | -C≡C-CH(OH)Ph | 2.7 | (2.4–2.9) | (72–123) | (0.97–3.0) | (0.58–1.6) |
| (R)PHPNECA (33) [74] | -C≡C-CH(OH)Ph | 1.9 | (1.2–3.7) | (2.4–3.9) | (0.47–2.6) | (0.17–1.0) |
| (S)PHPNECA (34) [74] | -C≡C-CH(OH)Ph | 2.1 | (1.8–2.1) | (25–59) | (1.5–3.8) | (3.6–8.5) |
| \(N^6\)-ethyl-(R,S)PHPNECA (35) [74] | -C≡C-CH(OH)Ph | 15 | (8.0–29) | (48–170) | (1.3–3.2) | (1.3–4.4) |
| (R,S)-2-(3-hydroxy-1-pentynyl)NECA (36) [62] | -C≡C-CH(OH)Et | 4.1 | (8.0–29) | (48–170) | (1.3–3.2) | 1.0 |
| (R,S)-2-(4-hydroxy-1-pentynyl)NECA (37) [62] | -C≡C-CH₂(CH(OH))CH₃ | 40.0 | (26–62) | (10–20) | (3.4–4.9) |
| (R,S)PHPMECA (38) [74] | -C≡C-CH(OH)Ph | 14.0 | (8.7–22) | (1.7–5.4) | (3.5–7.2) | (1.0–2.7) |

\(\text{a Displacement of specific } [²³\text{H}]\text{CCPA binding in CHO cells stably transfected with human recombinant } A_1 \text{ adenosine receptor, expressed as } K_i \text{ (nM), unless noted.}

\(\text{b Displacement of specific } [²³\text{H}]\text{NECA binding in CHO cells stably transfected with human recombinant } A_{2A} \text{ adenosine receptor, expressed as } K_i \text{ (nM), unless noted.}

\(\text{c Measurement of receptor-stimulated adenylyl cyclase activity in CHO cells stably transfected with human recombinant } A_{2B} \text{ adenosine receptor, expressed as } EC_{50} \text{ (μM).}

\(\text{d Displacement of specific } [²³\text{H}]\text{PIA binding from rat brain membranes.}

\(\text{e Displacement of } [²⁵\text{I}]\text{APNEA binding in CHO cells stably transfected with the rat } A_{3}\text{-cDNA.}

\(\text{f Displacement of } [²³\text{I}]\text{CGS21680 from rat striatal membranes.}

\(\text{g Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{h Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{i Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{j Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{k Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{l Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{m Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{n Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{o Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{p Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{q Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{r Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{s Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

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\(\text{x Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{y Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}

\(\text{z Displacement of } [²³\text{I}]\text{APN from rat striatal membranes.}
The compounds were also evaluated in a functional assay, measuring their capacity to modulate cAMP levels in CHO cells expressing hA2B AR receptors. The compounds were shown to bind the adenosine A1 receptor (K_i-binding values ranging from 2.3 to 30.5 nM) and to activate the adenosine A2B AR (EC50 values ranging from 7.3 to 175 nM) in the low nanomolar range, displaying at the same time a considerable level of selectivity toward A2A AR subtypes (K_i > 1 μM) and a relevant capability to bind A3 ARs.

Substitution at the para-position of the phenyl ring with a halogen atom led to a two- to fourfold loss of A2B AR binding.

**Table 4** Binding affinities (hA1, hA2A, hA3) and functional parameters (hA2B) of 2-(hetero)arylethoxy-adenosine derivatives 39–47 at the human adenosine receptors [85, 86]

| R          | hA1, K_i nM | hA2A, K_i nM | hA2B, EC50 nM | hA3, K_i nM |
|------------|-------------|--------------|---------------|-------------|
| NECA       | 6.8±2.4     | 2.2±0.6      | 140±19        | 16.0±5.4    |
| 39         | 221±57      | 9.3±2.9      | 1440±70       | 54.2±14.3   |
| 40         | 141±51      | 16.1±7.0     | 1780±260      | 93.3±16.8   |
| 41         | 174±20      | 10.9±4.8     | 299±45        | 232±54      |
| MRS3534 (42)| 148±19      | 45.0±11.6    | 767           | 490±60      |
| 43         | 150±50      | 370±80       |               |             |
| 44         | 145±6       | 29.3±13.7    | 216±59        | 92.3±7.9    |
| MRS3997 (45)| 253±3       | 150±20       | 128±32        | 90±15       |
| MRS3854 (46)| 358±1       | 502±32       | 365±73        | 234±24      |
| 47         | 310±90      | 450±8        | 896           | 120±20      |

*a* Displacement of specific [3H]CCPA binding, membranes from CHO cells stably transfected with human recombinant A1 adenosine receptor, K_i (nM).  
*b* Displacement of specific [3H]CGS21680 binding, membranes from HEK-293 cells stably transfected with human recombinant A2A adenosine receptor, K_i (nM).  
*c* cAMP assay in CHO cells expressing human A2B adenosine receptors, EC50 (nM).  
*d* Displacement of specific [125I]ABMECA binding, membranes from CHO cells stably transfected with human recombinant A3 adenosine receptor, K_i (nM).

**Table 5** Binding affinities (hA1, hA2A, hA3), functional parameters (hA2B) and percentages of efficacy of the 2-amino-6-(1H-imidazol-2-ylmethylsulfanyl)-4-(substituted)phenyl pyridine-3,5-dicarbonitrile derivatives 48–52 as AR agonists and partial agonists [88]

| R          | hA1, K_i nM (efficacy, %) | hA2A, K_i nM (efficacy, %) | hA2B, EC50 nM (efficacy, %) | hA3, K_i nM (efficacy, %) |
|------------|--------------------------|---------------------------|-----------------------------|--------------------------|
| NECA       | 12 (9.6–15)              | 60±10                     | 104±15                      | 11±0.8                   |
| LUF 5833 (48)| 2.4±1.0(109)          | 28±4(55)                  | 19±7(81)                    | 171±109(84)              |
| LUF 5834(49)| 2.6±0.3(103)           | 28±4(55)                  | 12±2(74)                    | 538±210(23)             |
| LUF 5835(50)| 4.4±2.0(112)           | 21±2(80)                  | 10±3(92)                    | 104±49(95)              |
| LUF 5844(51)| 2.0±1.0(80)            | 105±22(49)                | 34±24(68)                   | 74±21(39)               |
| LUF 5845(52)| 7.0±0.8(46)            | 214±37(32)                | 9±3(33)                     | 24±7.6(73)            |

*a* Displacement of specific [3H]DPCPX binding at human A1 receptors expressed in CHO cells.  
*b* Displacement of specific [3H]ZM241385 binding at human A2A receptors expressed in HEK293 cells.  
*c* cAMP assay in CHO cells expressing human A2B adenosine receptors EC50 (nM).  
*d* Displacement of specific [125I]ABMECA binding at human A3 receptors expressed in HEK293 cells.
activity in comparison with the unsubstituted phenyl derivative 7 (EC$_{50}$ hA$_{2B}$=7.3 nM). The same behaviour has been observed by introducing functions with reverse electronic effects, such as the 4-methoxy group (12, EC$_{50}$ hA$_{2B}$=32.4 nM). Conversely, increasing the steric hindrance around the paraposition by introducing a tertiary-butyl led to obtaining a very potent agonist for the A$_{2B}$ AR (compound 14), with an EC$_{50}$ value comparable with that of the unsubstituted phenyl derivative 7. Replacement of the phenyl with the 4-pyridyl moiety resulted in a fourfold decrease in the potency (15, EC$_{50}$ hA$_{2B}$=32.3 nM). The presence of a chlorine atom at the 2-position had a slightly detrimental effect in terms of A$_{2B}$ AR activation, as emerged from the comparison of the biological data of the 2-chloro derivatives with the corresponding 2-unsubstituted compounds. Considering the binding and functional profile of NECA [69] (Table 1) and (S)-PHPNECA [74] ($K_i$ hA$_1$=2.1 nM; $K_i$ hA$_{2A}$=2.0 nM; EC$_{50}$ hA$_{2B}$=220 nM; $K_i$ hA$_3$=0.75 nM), which are among the most potent adenosine-like A$_{2B}$ AR agonists previously reported, these molecules represent a remarkable advance in the search for potent A$_{2B}$ AR agonists, albeit the selectivity profile must be undoubtedly improved. Most of the examined molecules, in fact, preferentially bound to the A$_1$ receptor, with $K_i$ binding values ranging from 2.3 to 30.5 nM. This experimental observation can be explained in light of the literature, indicating that A$_1$ AR selectivity is enhanced by monosubstitution of the exocyclic amino group at the 6-position of adenosine with bulky cycloalkyl or arylalkyl substituents [75]. A lower, but significant, affinity for the A$_3$ AR was observed. The most selective compounds versus A$_3$ AR subtype were the unsubstituted phenyl derivative (7) and the 4-halo-phenyl derivatives (8–11). The cAMP functional assay tested that the designed molecules behave as full A$_{2B}$ AR agonists. These compounds retain, to the best of our knowledge, the first report about adenosine-related structures capable of activating hA$_{2B}$ AR subtype in the very low nanomolar range.

In the search for nucleoside-based ligands for ARs, some N$^6$-carboxamido derivatives of adenosine-5’-N-ethyluronamide (NECA) have been synthesised and tested in binding and/or functional assays at the four known AR subtypes exerting a general behaviour as low-selective A$_1$ AR ligands [76]. Some N$^6$-(substituted-phenylcarbamoyl)-derivatives of NECA were instead found to have affinity at rat A$_3$ ARs in the low nanomolar range, with different degrees of selectivity versus A$_1$ and A$_{2A}$ ARs [77, 78]. These results indicated that small modifications of the chain at the 6-position of the purine nucleus can produce significant changes in the selectivity pattern of potential AR ligands. According to the principles of bioisosterism, the (hetero)aryl-urea function of the reported A$_3$ AR agonists has been recently replaced with the isomeric (hetero)aryl-carbonyl-hydrazino moiety, and the effect on binding and functional profile of the synthesised compounds has been evaluated [79, 80]. The coexisting effect of substitution at the 2-position of the purine with a chlorine atom has also been examined. Competitive-binding experiments were performed to evaluate the affinity of the synthesised compounds to hA$_1$, hA$_{2A}$ and hA$_3$ ARs expressed in CHO cells using as radioligands [$^3$H]-CHA, [$^3$H]-CGS 21680 and [$^{[125]}$I]-AB-MECA, respectively. The compounds were also evaluated in functional assays, measuring their capacity to modulate cAMP levels in CHO cells expressing hA$_{2B}$ ARs. Structures and biological data of a selection of the synthesised compounds are listed in Table 2.

The series has been developed introducing different (substituted) heteroaryl nuclei on the N$^6$-hydrazide chain. The new class of 1-deoxy-1-[6-[[hetero]aryl-carbonyl]-hydrazino]-9H-purin-9-yl]-N-ethyl-$\beta$-D-ribofuranuronamide and 1-deoxy-1-[2-chloro-6-[[((hetero)aryl-carbonyl)-hydrazino]-9H-purin-9-yl]-N-ethyl-$\beta$-D-ribofuranuronamide derivatives have been found to be the first examples of both potent and selective A$_{2B}$ AR agonists showing considerable potency in activating A$_{2B}$ ARs, with EC$_{50}$ values ranging from 82 to 450 nM. The most innovative finding rests in the analysis of the selectivity information emerging from the comparison between affinity and functional data related to the four AR subtypes. Of the examined molecules, the ones showing the capability to activate A$_{2B}$ ARs were inactive at the hA$_3$ AR ($K_i$>5,000 nM) and showed high nanomolar-micromolar affinity at the A$_1$ and A$_{2A}$ AR subtypes ($K_i$ varying from 700 to 5,000 nM). In particular, compound 1-deoxy-1-[6-[[furan-2-carbonyl]-hydrazino]-9H-purin-9-yl]-N-ethyl-$\beta$-D-ribofuranuronamide (19, hA$_1$,hA$_{2A}$ $K_i$>1,000 nM; hA$_{2B}$ EC$_{50}$=82 nM, hA$_3$ $K_i$>5,000 nM) was the most potent of the series, and it was confirmed to be a full

| Table 6 Potency of 2-amino-4-(substituted)phenyl pyridine-3,5-dicarbonitile derivatives 53–56 and BAY-60–6583 in activating ARs |
|------------------|------------------|------------------|------------------|------------------|
|                  | hA$_1$ CAMP assay EC$_{50}$ nM | hA$_{2A}$ CAMP assay EC$_{50}$ nM | hA$_{2B}$ CAMP assay EC$_{50}$ nM | hA$_3$ CAMP assay EC$_{50}$ nM |
| 53 [89]          | 0.2              | 236              | 0.1              | –                |
| 54 [89]          | 0.7              | 103              | 0.5              | –                |
| 55 [89]          | 0.4              | 142              | 0.3              | –                |
| 56 [89]          | 0.3              | 1200             | 1.4              | –                |
| BAY-60–6583 (58) [45, 46] | >10,000         | >10,000          | 3 nM             | >10,000          |

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agonist in a functional assay based on the measurement of its capacity to modulate cAMP levels in CHO cells expressing hA2B AR (Fig. 2). Nevertheless, both furan and thiophene rings were shown to exert similar favourable interactions for receptor activation [compare furan derivatives, 21 (hA2B EC50=227 nM) and 23, with the related thiophene derivatives 22 (hA2B EC50=273 nM) and 26]. The presence of the chlorine atom at the 2-position of the purine nucleus did not seem to affect the ability of the tested compounds to activate hA2B AR, as is clear from the comparison of chlorinated derivatives 23 (hA2B EC50=210 nM) and 24 (hA2B EC50=175 nM) with the corresponding nonchlorinated 19 (hA2B EC50=82 nM) and 22 (hA2B EC50=273 nM). The examined molecules can be considered valuable tools for the design and development of new and even more selective and potent ligands.

**C2-substitution**

The introduction of a bulky substituent at the 2-position of the adenine ring of NECA is known to induce A2A AR-selective agonistic activity. CGS21680 has, in fact, been considered one of the most potent A2A AR agonist and the ligand of choice to distinguish A2A A and A2B AR-mediated effects (K_i values from binding assays for hA1, hA2A and hA3 AR subtypes of 298, 27 and 67 nM respectively; EC50 value from stimulation of adenyl cyclase activity through A2B AR of 88.8 μM) [11]. The first attempts to substitute the 2-position of adenosine indicated that A2B AR did not tolerate well such structural modulation [65]. An appreciable improvement of A2B AR affinity has, however, been recognised, introducing a 2-chloro atom (2-ClAdo, 27, Table 3, EC50 from measurement of receptor-stimulated adenylate cyclase activity in CHO, stably transfected with hA2B AR of 24 μM) or 2-alkynyl chains. The racemic 2-

![Fig. 2 Dose-response curve of NECA and compound 19 on cAMP assays in hA2B AR CHO cells](image)

phenylhydroxypropyladenosine [(R,S)-PHPAdo, 28] [15] had been found to exert selectivity at A2B AR comparable with that of NECA (EC50=2.4 μM). The (R) diastereomer of PHPAdo (LUF 5599, 29, EC50=6.2 μM) was almost sevenfold less potent than the (S) optical isomer (LUF 5600, 30, EC50=0.92 μM). The introduction of small alkyl chains at the N6 position of (R,S)-PHPAdo was shown to be tolerated for interaction with A2B AR, whereas large groups abolished A2B AR potency (data not shown) [81]. In particular, for N6-ethyl-(R,S)-PHPAdo (31), an EC50 from adenyl cyclase assay of 1.7 μM has been reported [82]. Substitution of the 2-position of NECA with alkynyl chains results in an increase of A2B AR affinity, as demonstrated by the compound named 2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine (PHPNECA, 32) displaying agonistic activity in a functional assay at this AR subtype, with an EC50 value of 1.1 μM [83]. The racemic (R,S)-PHPNECA resulted in a twofold greater potency than the optically pure (R)-PHPNECA (33, hA2B EC50=2.4 μM) and a five fold smaller potency than the (S) diastereomer (34, hA2B EC50=0.22 μM). (S)-PHPNECA was, therefore, 11-fold more active than (R)-PHPNECA. The combination of the N6- and 5′ substitutions as in compound N6-ethyl-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine 35 (hA2B EC50=2.0 μM) led to decreased affinity for A2B AR [74]. Displacement of the phenyl ring of the alkynyl group had no

![Fig. 3 2-Amino-4-(substituted)phenyl pyridine-3,5-dicarbonitrile derivatives: novel A3B AR agonists of particular interests for their potential therapeutic applications](image)
effect on binding, as demonstrated by derivative (R,S)-2-(3-hydroxy-1-penteny) NECA 36, which was as potent as (R,S)-PHPNECA in activating A2B AR subtype (hA2B EC50=1.3 μM). The presence of a hydroxyl group in α to the triple bond appeared to be important for activity. The (R,S)-2-(3-hydroxy-1-penteny) NECA 37, bearing a hydroxyl group in β to the triple bond, was, in fact, 12-fold less potent than (R,S)-PHPNECA (hA2B EC50=13.3 μM) [62]. The 5′-methylcarboxamido analogue of (R,S)-PHPNECA ((R,S)-PHPMECA, 38) was 4.5-fold less active than the parent compound (hA2B EC50=5.0 μM) [74].

A computational molecular docking of an heterogeneous set of 46 known adenosine-like AR agonists, based on the molecular models of the 3D structure of the four known AR subtypes, was recently performed [84]. Comparison between the putative ligand-receptor complexes for each receptor subtype suggested a general agonist-binding mode, along with possible explanations for the differences in agonist activities and AR selectivities. Some interesting estimations about the binding mode of agonists at the A2B AR subtype have been highlighted, with particular attention to PHPNECA and its 2-hydroxypropynyl-substituted congeners. Specifically, the supposed orientations of ligands inside the AR binding sites suggested that, in general, the A2A and A2B AR subtypes have a smaller volume of the putative hydrophobic pocket surrounding the 5′-N-alkyl substituent than the A1 and A3 ARs. That gave a possible explanation for the decreased affinity characterising carboxamido derivatives in which sterically demanding alky groups were introduced at the 5′-N-position. Comparative analysis of the different binding modes of optical isomers of the 2-hydroxypropynyl-substituted agonists led to a critical involvement of the orientation of the hydroxyl group, which resulted in affecting the capability to establish key H-bond interactions with the binding site. Specifically, the proposed binding mode of PHPNECA gave a rational explanation for the higher affinity of the (S)-PHPNECA in comparison with its (R) diastereomer, demonstrating that the hydroxyl group of the (S)-phenylhydroxypropynyl fragment could be hydrogen bonded to a cysteine residue located in the second extracellular loop. On the contrary, the hydroxyl group of the (R)-phenylhydroxypropynyl chain seems to be surrounded by hydrophobic residues of Leu and Ala, thus resulting in unfavourable ligand-receptor interactions.

In a recent study by Jacobson et al. [85], a wide series of 2-substituted adenosine derivatives was evaluated for their affinity and efficacy through radioligand binding and cAMP functional assays in intact CHO cells at the four AR subtypes. This study included different 2-(cyclo)alkoxy, 2-(substituted/hetero)aryloxy, 2-phenethylamino and 2-phenethylsulfanyl substitutions of adenosine. Most of these compounds were found to be extremely weak at the A2B AR; nevertheless, 2-(phenylethoxy)adenosine 39 (Table 4, EC50=3.49 μM), 2-[2-(2-naphthyl)ethoxy]adenosine 40 (EC50=1.44 μM) and 2-[2-(2-thienyl)ethoxy]adenosine 41 (EC50=1.78 μM) have been reported to be moderately potent A2B AR agonists. Based on the findings that among these molecules, specific 2-(2-aryloxy)ether derivatives also displayed significant activity at the A2B AR subtype and that 2-ethers were more potent than the corresponding amines or thioethers, the same authors subsequently reported on a structure–activity relationship study of 2,N5,5′-substituted adenosine derivatives, which led to the identification of compounds with enhanced potency at the A2B AR and reduced potency at the other AR subtypes [86]. In particular, 2-(3-indolyl)ethoxy) adenosines substituted at the 5′ or 6′ positions of the 2-indole moiety with halogens or a hydroxyl function exerted micromolar potency in activating A2B AR (EC50 values from cAMP functional assay ranging from 0.128 to 1 μM), with slightly improved selectivity versus the other AR subtypes in comparison with previously reported reference compounds [NECA, (S)-PHPNECA and 6-guanidino-NECA]. Structures and corresponding potency/affinity data of a selected series of these compounds at the four known AR subtypes are reported in Table 4. Compound 2-(3′-indolylethoxy)adenosine 42 was found to be a rather potent agonist at the hA2B AR (EC50=299 nM), although the selectivity profile was not so satisfactory (Ki values at A1, A2A, A3 ARs of 148, 45, 232 nM, respectively). Substitution of the indole moiety with other (hetero)aryl nuclei, such as phenyl, naphthyl, thiophene, pyrrole, benzoimidazole or benzotriazole, did not succeed in enhancing A2B AR potency. Elongation or branching of the 2-alkyl spacer proved to weaken the affinity against all ARs. 2-Indolyl derivative decreased markedly A2B AR potency compared with 3-indolyl analogues, revealing that altered connectivity failed to improve the binding profile of the series (data not shown). The 5′-N-ethylcarboxamido analogue of 42 was synthesised, considering that replacement of the 4′-hydroxymethyl group with a 5′-N-ethylcarboxamido function is generally known to favour A2B AR interaction. Unexpectedly, in the 2-(3-indolyl)ethoxyadenosine series, this structural modification generated a threefold loss of potency (EC50 hA2B=989 nM), hypothetically due to an unfavourable change in the conformation of the ribose ring in the ligand-binding site. Similar results were achieved introducing at the N′-position of 42 an ethyl group (EC50 hA2B=3,270 nM). Considering the potency of compound 42, the authors replaced its 6-amino group with a N′-guanidino moiety detecting decreased potency at the A2B AR (hA2B=40% of activation at 10 μM), along with reduced selectivity versus A1 (Ki hA1=73.6 nM) and A3 (Ki hA3=90 nM) AR subtypes. The best results in terms of A2B AR potency and selectivity were achieved by substitution of the indole nucleus of compound 42 with halogens (compounds 43-46, Table 4).
In particular, the 6-bromo derivative 45 exerted higher potency (EC_{50} hA_{2B}=128 nM) and an improved binding profile in comparison with NECA and (S)-PHP-NECA in activating A_{2B} AR, (hA_{1} K_{i} / hA_{2B} EC_{50}=1.97, hA_{2A} K_{i} / hA_{2B} EC_{50}=1.17, hA_{3} K_{i} / hA_{2B} EC_{50}=0.7). Activation curves of this compound denoted a behaviour as partial agonist at the hA_{1} and hA_{3} ARs and as full agonist at A_{2A} and A_{2B} AR subtypes (data not shown). A molecular modeling investigation performed by docking compound 2-(3″- (6″-bromo-indolyl)ethyloxy)adenosine 45 in the rhodopsin-based molecular model of the human A_{2B} AR gave rational explanations for the experimental pharmacological results, indicating that all the interactions previously proposed for adenosine could be strengthened by favourable interactions of the 2-(6-bromo-indol-3-yl)ethyloxy chain with a distal region of agonist-receptor-binding site. Moreover, the 2-oxygen atom seemed to be involved in H bonding, with a residue of Asn, whereas the NH of the indole ring seemed in proximity of the OH of a residue of Ser, with which a hydrogen bond, even not fully detected, cannot be excluded.

Nonadenosine agonists

Based on some patent claims concerning the synthesis of a series of substituted 2-amino-4-phenyl-6-phenylsulfanylpyridine-3,5-dicarbonitriles as agonists for ARs [45, 87], IJzerman et al. reported a series of five 2-amino-6-(1H-imidazol-2-ylmethylsulfanyl)-4-(substituted)phenyl pyridine-3,5-dicarbonitrile derivatives displaying high-potency agonistic activity for the hA_{2B} AR with somewhat significant selectivity versus the hA_{3} AR subtype [88]. The ability of such compounds to activate the human A_{2B} AR has been determined through cAMP assay in CHO cells stably expressing this receptor. For comparison, affinity for the hA_{1}, hA_{2A}, and hA_{3} ARs stably expressed on CHO cells (A_{1}) or HEK293 cells (A_{2A}, A_{3}) was determined in radioligand binding studies with [3H]DPCPX, [3H]ZM241385 and [125I]I-ABMECA as radioligands, respectively (Table 5). All the reported compounds interacted with the hA_{2B} AR, with EC_{50} ranging from 9 to 34 nM. Percentages of efficacy in modulation (inhibition for A_{1} and A_{3}, stimulation for A_{2A} and A_{2B} ARs) of the cAMP production functional assay reported in Table 5 highlighted that both the nature and the position of the substituent at the 4-phenyl ring considerably affect the intrinsic efficacy of the examined molecules, among which A_{2B} AR partial and full agonists have been identified. 2-Amino-4-(3-hydroxyphenyl)-6-(1H-imidazol-2-ylmethylsulfanyl)pyridine-3,5-dicarbonitrile (50, LUF5835) displayed the highest efficacy of the series, 92% compared with the reference agonist NECA, combined with a low EC_{50} of 10 nM. The 4-p-methoxy-phenyl derivative 52

![Fig. 4 Schematic overview of the most important structural modifications of adenosine and nonadenosine derivatives for a potent and/or selective activation of the A_{2B} AR](image-url)
(LUF5845) behaved as a potent (EC$_{50}$=9 nM) partial agonist (efficacy of 33% compared with NECA) of the hA$_{2B}$ AR. The authors proved that the effect on cAMP production was mediated by interaction of the reported compounds with the hA$_{2B}$ AR, establishing that the potent AR antagonist CGS15943 was able to cause a dose-dependent decrease of the cAMP production induced by NECA and by the examined structures. The 4-p-OH-phenyl derivative (49, LUF5834) is of particular interest, thanks to its high potency at the hA$_{2B}$ AR (EC$_{50}$=12 nM) associated with a significant selectivity versus the hA$_{3}$ AR subtype (K$_{i}$=538 nM, efficacy 74%). This ligand can be considered a useful tool for distinguishing the relative contributions of the A$_{2B}$ and A$_{3}$ ARs to mast-cell-mediated activation of angiogenesis, a process that seems to be regulated by a combined action of A$_{2B}$ and A$_{3}$ AR subtypes [88]. The 3-methoxyphenyl derivative 51 and the 4-methoxyphenyl derivative 52 also showed appreciable selectivity for A$_{2B}$ versus A$_{2A}$ ARs (3- and 24-fold, respectively) but reduced selectivity for A$_{2B}$ versus A$_{3}$ ARs (2.1- and 2.6-fold, respectively) in comparison with the corresponding 3/4-hydroxyphenyl analogues 50 (K$_{i}$ A$_{2A}$/EC$_{50}$ A$_{2B}$=2.1; K$_{i}$ A$_{3}$/EC$_{50}$ A$_{2B}$=10.4) and 49 (K$_{i}$ A$_{2A}$/EC$_{50}$ A$_{2B}$=2.3; K$_{i}$ A$_{3}$/EC$_{50}$ A$_{2B}$=45).

A recent patent application [89] claimed the possible employment of 2-amino-6-[[{2-[(substituted)phenylamino]-1,3-thiazol-4-yl}methyl]thio]-4-[(substituted)phenyl-pyridine-3,5-dicarbonitrile derivatives of general formula I and II (compounds 53–56, Fig. 3, Table 6) as dual A$_{1}$/A$_{2B}$ AR agonists for treating diseases such as dyslipidemia, metabolic syndrome and diabetes, metabolic syndrome and diabetes in connection with hypertension and diseases of the cardiovascular system. Moreover, new experimental evidence points to compound 53 as the representative dual A$_{1}$/A$_{2B}$ AR agonist (EC$_{50}$ values of 0.2, 0.1 and 236 nM for A$_{1}$, A$_{2B}$ and A$_{2A}$ hAR subtypes, respectively), also potentially useful for treating and/or preventing hypertension, hypertonya, restenosis and thrombosis [90].

Compounds 2-[6-amino-3,5-dicyano-4-(4-hydroxyphenyl) pyridin-2-ylsulfanyl]acetamide (57) and 2-[6-amino-3,5- dicyano-4-[4-(cyclopropylmethoxy)phenyl]pyridin-2-ylsulfanyl]acetamide (BAY-60–683, 58) have been examined as A$_{2B}$ AR agonists for their potential in treating disorders of the coronary arteries and atherosclerosis [45], as well as in the production of pharmaceuticals for prophylaxis and/or treatment of ischaemia-reperfusion injury [91]. Other possible clinical developments seem related to limitation of reperfusion cellular damage in mammals, especially in humans, following, for example, myocardial infarction, coronary artery bypass grafting and open heart surgery. In particular, compound BAY-60–683 is under preclinical-phase investigation for treating angina pectoris. BAY-60–683, characterised with CHO cells expressing recombinant human A$_{1}$, A$_{2A}$ or A$_{2B}$ ARs, showed EC$_{50}$ values for receptor activation >10,000 nM for both A$_{1}$ and A$_{2A}$ AR and 3 nM for A$_{2B}$ AR subtypes. Moreover, it showed no agonistic activity in the adenosine A$_{3}$ergic assay up to a concentration of 10 μM [46]. In a rabbit model of myocardial ischaemic injury, this compound (100 mcg/kg i.v.) reduced the infarction area when administered to ischaemic rabbit hearts just prior to reperfusion, thus mimicking the effects of postconditioning procedure, which consisted of four cycles of 30-s reperfusion/30-s occlusion following ischaemia. Furthermore, the addition of nonspecific and A$_{2B}$ AR-selective antagonists (MRS 1754 [71]) blocked protection from postconditioning. Together, these data demonstrate that protection from postconditioning involves A$_{2B}$ ARs [92].

The possible use of substituted 2-thio-3,5-dicyano-4-phenyl-6-aminopyridines (with particular attention to compound 59) for the production of a medicament for the prophylaxis and/or treatment of nausea and vomiting is under investigation [93].

Conclusions

With this review, we provide an overview of the latest advancements in the research field concerning the identification of agonists for A$_{2B}$ AR, with particular attention to the past 2 years. The lack of agonists endowed with satisfactory levels of A$_{2B}$ AR potency and selectivity has hampered the pharmacological characterisation of this potential therapeutic target. Important progresses in the field has been newly attained, thanks to the identification of both nucleoside-like (1-deoxy-1-{6-[N’-(furan-2-carbonyl)-hydrazino]-9H-purin-9-yl].N-ethyl-β-D-ribofuranurona- mide, 19) and nonadenosine (BAY-60–683) molecules with undoubtedly improved in vitro pharmacological profile. A schematic overview of the most effective substitutions of adenosine and nonadenosine derivatives is furnished in Fig. 4. In particular, the gain in A$_{2B}$ AR selectivity promoted in these new agonists would provide useful pharmacological probes for exploring the role of in vivo receptor activation, and thus a more complete insight of the prospective employment of A$_{2B}$ AR ligands in clinical therapy might be offered.

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