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REVIEW

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 N^6-, 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]-9*H*-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** (hA_1 K_i=3500 nM, hA_{2A} K_i= 4950 nM, hA_{2B} EC_{50}=210 nM, hA_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 hA_{2B} AR. Nonribose ligands are represented by conveniently substituted dicarbonitrilepyridines, among which 2-[6-amino-3, 5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]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
Ars	Adenosine Receptors
ASMCs	Arterial Smooth Muscle Cells
ATP	Adenosine Triphosphate
BAY 60–6583	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^6-cyclopentyladenosine
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CGS21680	2-([4-(2-carboxyethyl)phenylethyl]amino)-5′-*N*-ethylcarboxamidoadenosine
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^6-cyclohexyladenosine

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HEK293 cells	Human Embryonic Kidney cells
[^{125}I]-AB-MECA	[^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyl-uronamide
[^{125}I] APNEA	[^{125}I]N^6–2-(4-amino-phenyl)ethyladenosine
IB-MECA	N^6-(3-iodo-benzyl) adenosine-5′-*N*-methyluronamide
IL	Interleukin
IPC	Ischemic Preconditioning
MAPK	Mitogen-Activated Protein Kinase
MRE 2029-F20	N-benzo[1,3]dioxol-5-yl-2-[5-(1,3-dipropyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide
MRS 1754	[*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide
NAD	Nicotinamide Adenine Dinucleotide
NECA	5′-*N*-ethylcarboxamidoadenosine
NO	Nitric Oxide
OSIP339391	N-(2-(2-Phenyl-6-[4-(2,2,3,3-tetratritrio-3-phenylpropyl)-piperazine-1-carbonyl]-7*H*pyrrolo[2,3-d]pyrimidin-4-ylamino)-ethyl)-acetamide
PHPAdo	2-phenylhydroxypropynyladenosine
PHPNECA	2-phenylhydroxypropynyl-5′-*N*-ethylcarboxamidoadenosine
R-PIA	N^6-(*R*)-phenylisopropyladenosine
SAM	S-Adenosyl-L-Methionine
TNFα	Tumor Necrosis Factor α
ZM 241385	(4-(2-[7-amino-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 A_1, A_{2A}, A_{2B} and A_3, which have been cloned from many mammalian and some nonmammalian species [10–13].

A_{2B} 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), N^6-(*R*)-phenylisopropyladenosine (R-PIA), and 2-([4-(2-carboxyethyl)phenylethyl]amino)-5′-N-ethylcarboxamidoadenosine (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 A_1 and A_{2A} 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 A_{2B} and A_3 AR subtypes. Activation of A_{2B} AR implies stimulation of adenylate cyclase and activation of phospholipase C through the coupling to Gs and Gq/11 proteins, respectively. A_{2B} ARs have been found on practically every cell in most species, and their sequences are highly similar across species. The human (h) A_{2B} 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 A_{2B} AR tissue distribution. High concentrations of A_{2B} 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 A_{2B} AR [12].

A_{2B} 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 exerting 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 A_{2B} AR subtype has created wide-ranging difficulty in detecting the physiological effects mediated by direct and selective A_{2B} 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

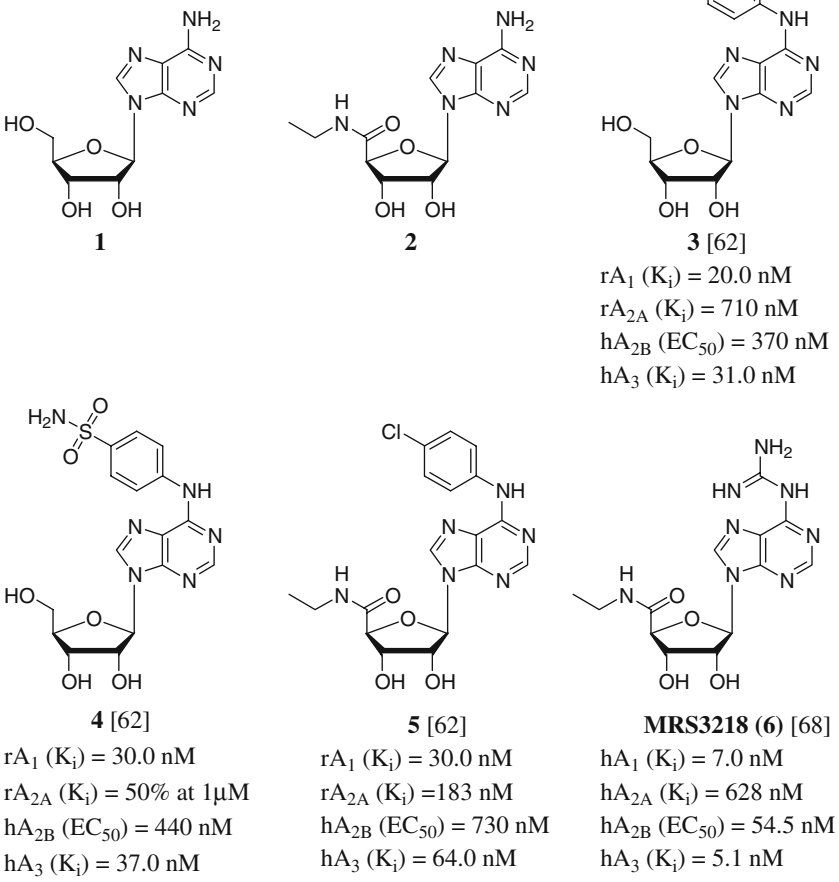

Fig. 1 Adenosine (**1**), NECA (**2**), N^6-substituted-adenosine (**3**, **4**) and NECA (**5**, **6**) derivatives as nonselective A_{2B} AR agonists

ligands for A_1, A_{2A} and/or A_3 ARs by employing a strategy of exclusion in a model in which more AR subtypes are coexpressed. The A_{2A} AR-selective agonist CGS21680 has been reported, for example, as a useful tool for differentiation between A_{2A} and A_{2B} ARs [14]. Moreover, some potent and selective antagonists of the A_{2B} AR have been employed to distinguish A_{2B} AR-mediated effects. Until a few years ago, the characterisation of A_{2B} ARs through radioligand binding studies using low-affinity and nonselective antagonists such as [^{3}H]1,3-dipropyl-8-cyclopentyl-xanthine ([^{3}H]DPCPX), [^{3}H](4-(2-[7-amino-2-(2-furyl)-[1,2,4]triazolo-[2,3-a][1,3,5] triazin-5-ylamino] ethyl)-phenol ([^{3}H]ZM 241385), [^{125}I]3-(3,4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propyl-xanthine ([^{125}I]ABOPX) [16]. A helpful advancement in the pharmacological characterisation of A_{2B} ARs is supposed to be increased by the recent identification of the tritiated form of some new A_{2B} AR antagonists with improved potency and selectivity, such as [^{3}H][*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy] acetamide ([^{3}H]MRS 1754) [17], [^{3}H]N-(2-(2-Phenyl-6-[4-(2,2,3,3-tetratritrio-3-phenylpropyl)-piperazine-1-carbonyl]-7*H*pyrrolo[2,3-d]pyrimidin-4-ylamino)-ethyl)-acetamide ([^{3}H]OSIP339391) [18] and [^{3}H]N-benzo[1,3]dioxol-5-yl-2-[5-(1,3-dipropyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide ([^{3}H]MRE 2029-F20) [19].

The contribution of genetic engineering and manipulation (for example, generation of A_{2B} 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 A_{2B} AR ligands and, generally, the role of ARs, in a variety of diseases [20–23].

A_{2B} adenosine receptor physiology and pharmacology

The A_{2B} 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 A_{2B} 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]. A_{2B} 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 A_{2B} 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 A_{2B} ARs in the growth and development of some tumours , and A_{2B} 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 A_{2B} 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 A_{2B} 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 A_{2B} AR-mediated Cl-secretion [31].

Therapeutic potential of A_{2B} 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 A_{2B} AR activation [33]. Treatment of asthma with selective A_{2B} AR antagonists has so far been one of the most significant therapeutic options among AR ligands [34–38].

A_{2B}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]. A_{2B} AR has been likewise reported to be involved in stimulating proliferation, differentiation and migration of retinal endothelial cells. Thus A_{2B} 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 A_{2B} ARs in the analgaesic 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 A_{2B} AR antagonists as valuable adjuvant drugs for opioid analgaesia, with minimal side effects.

The purinergic regulation of epithelial transports and, above all, involvement of the A_{2B} AR subtype in determining secretion stimulation, suggest the possibility of employing A_{2B} 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 A_{2B} AR ligands are under investigation for treating diarrhoea and cystic fibrosis [42–44].

Therapeutic potential of A_{2B} 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 A_{2B} 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 A_{2B} AR-dependent signalling. 2-[6-amino-3,5-dicyano-4-(4-hydroxyphenyl) pyridin-2-ylsulfanyl]acetamide (BAY 60–6583), a highly selective A_{2B} 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 A_{2B} 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 A_{2B} 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 A_{2B} AR agonists [23].

Deletion of the gene encoding the A_{2B} AR in the mouse (A_{2B} 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 A_{2B} AR subtype would moreover increase production of the anti-inflammatory cytokine IL-10 [50].

The described association of A_{2B} AR with pre/postconditioning cardioprotection, along with the documented strong anti-inflammatory role of A_{2B} AR signalling [49], suggests that A_{2B} 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 A_{2B} AR-mediated cardiovascular effects. A NECA-mediated coronary vasodilation via the A_{2B} 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 A_{2B} AR [52], and A_{2B} 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 vasoocclusive disorders by inhibiting SMC proliferation and collagen synthesis via activation of A_{2B} 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 A_{2B} 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 A_{2B} AR stimulation-mediated effects in the pathogenesis of atherosclerosis and restenosis.

Glomerular mesangial cell (GMC) growth is inhibited by A_{2B} AR activation coupled with inhibition of mitogen-activated protein kinase (MAPK) activity [56]. A_{2B} AR function may therefore largely affect glomerular remodeling associated with GMC proliferation. Identification of pharmacological agents able to specifically activate A_{2B} 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 A_{2B} 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 A_{2B} ARs activity, indicating a possible employment of specific A_{2B} AR agonists as anew therapeutic approach to manage severe vasculogenic impotence resistant to common vasodilators [59].

As interaction of adenosine with A_{2B} ARs inhibits production of the proinflammatory cytokine tumor necrosis factor (TNFα) by lipopolysaccharide-activated monocytes [49, 60], A_{2B} AR agonists have been proposed for treating septic shock, confirming the broad anti-inflammatory potential of AR agonists in treating inflammatory disorders [61].

A_{2B} adenosine receptor agonists

The lack of molecules endowed with selective and potent agonistic activity towards the hA_{2B} ARs has limited the studies on this pharmacological target and consequently the evaluation of its therapeutic potential. Several ligands for A_{2B} AR have been identified in recent years [62–64]. However, only very recently have some reports about important advancement in identifying A_{2B} 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 A_{2B} AR subtype, caused by the substantial lack of a useful radiolabelled agonist. Therefore, for the time being, the selectivity profile of new potential A_{2B} AR agonists can only be speculated in view of the ratio of binding parameters (K_i values for A_1, A_{2A}, A_3 ARs) to functional parameters (EC_{50} for A_{2B}, Tables 1, 2, 3, 4, 5, and 6).

The compounds of particular interest for the development of potent and selective A_{2B} 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^6-, C^2-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^6-substituted adenosines, N^6-substituted-5′-*N*-alkyl-carboxamidoadenosines, C^2-substituted adenosines and C^2-substituted-5′-*N*-alkyl-carboxamidoadenosines.

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

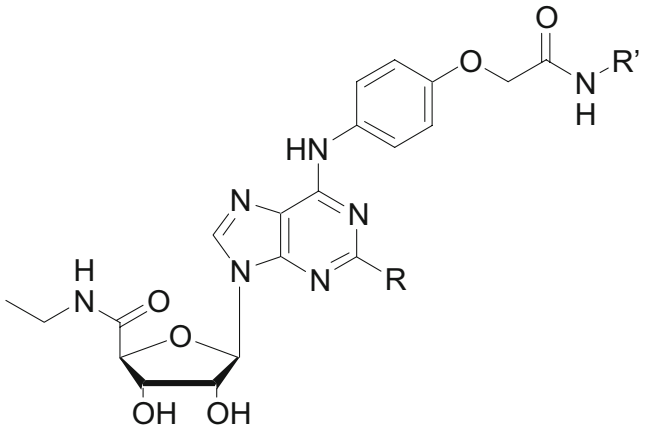

Table 1 Binding affinities (hA_1, hA_{2A}, hA_3) and functional parameters (hA_{2B}) of the N^6-(hetero)aryl-carbamoyl-methoxy-phenyl)-(2-chloro)-NECA derivatives **7**–**18** at the human adenosine receptors expressed in CHO cells [69]

	R	R′	hA_1[a] K_i (nM)	hA_{2A}[b] K_i (nM)	hA_{2B}[c] EC_{50} (nM)	hA_3[d] K_i (nM)
NECA			18.2±2.1	12.4±2.7	155±12	35.7±3.3
7	H	Ph	8.5±0.8	>1000 (45%)	7.3±0.6	38.4±3.7
8	H	4-F-Ph	2.3±0.2	>1000 (48%)	15.2±2.1	72.3±7.4
9	H	4-Cl-Ph	3.1±0.3	>1000 (35%)	12.3±1.4	34.2±3.7
10	H	4-Br-Ph	3.5±0.4	>1000 (26%)	10.5±1.2	36.4±3.7
11	H	4-I-Ph	5.2±0.5	>1000 (28%)	30.2±2.8	85.2±8.3
12	H	4-OCH_3-Ph	4.7±0.4	>1000 (42%)	32.4±3.3	25.3±2.6
13	H	3,4-OCH_2O-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 [^{3}H]CHA binding at human A_1 receptors expressed in CHO cells. [b] Displacement of specific [^{3}H]CGS21680 binding at human A_{2A} 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 A_{2B} adenosine receptors EC_{50} (nM). [d] Displacement of specific [^{125}I]ABMECA binding at human A_3 receptors expressed in CHO cells. Data are expressed as geometric means with 95% confidence limits.

Adenosine-like ligands

In the search for A_{2B} 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-, N^6 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′-CH_2OH derivatives, N^6-substitution showed higher compatibility with A_{2B} AR subtype compared with C^2- and/or C^8- substitutions and deazapurine derivatives resulted as inactive. Thus, N^6-substituted-5′-*N*-carboxamidoadenosine derivatives were initially considered as the most promising tool for identifying A_{2B} 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 A_{2B} AR potency and, in some examples, to reduced intrinsic activity (data not shown).

N^6-substitution

Some examples of N^6-substituted adenosine derivatives endowed with satisfactory levels of A_{2B} AR potency have been reported [62]. In particular, the introduction of (substituted) phenyl rings at the N^6-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 A_{2B} AR subtype [64]. N^6-modification of NECA with substituted phenyl

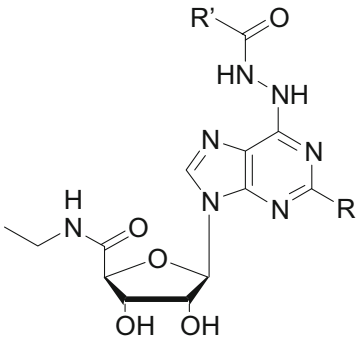

Table 2 Binding affinities (hA_1, hA_{2A}, hA_3) and functional parameters (hA_{2B}) of the 6-(heteroaryl-carbonyl)-hydrazino-NECA derivatives **19**–**26** at the human adenosine receptors expressed in CHO cells [79, 80]

	R	R′	hA_1[a] K_i (nM)	hA_{2A}[b] K_i (nM)	hA_{2B}[c] EC_{50} (nM)	hA_3[d] K_i (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 [^{3}H]CHA binding at human A_1 receptors expressed in CHO cells. [b] Displacement of specific [^{3}H]CGS21680 binding at human A_{2A} receptors expressed in CHO cells. [c] cAMP assay in CHO cells expressing human A_{2B} adenosine receptors EC_{50} (nM). [d] Displacement of specific [^{125}I]ABMECA binding at human A_3 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.

groups yielded compounds endowed with similar activity as the corresponding adenosine analogues (N^6-(4-chlorophenyl) NECA, **5**, EC_{50}=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 A_{2A} AR and its specific agonists [68], the N^6-guanidino derivative **6** of NECA (Fig. 1) was identified. Replacement of the 6-amino group of NECA with the guanidino moiety determined a threefold enhancement in A_{2B} AR activation potency (EC_{50}=54.5 nM versus 140 nM of NECA) and an increased selectivity versus A_{2A} AR subtype (K_i=628 nM versus 2.2 nM), maintaining a high affinity at the A_3 (K_i=5.1 nM) and A_1ARs (K_i=7.0 nM).

The disubstitution of the amino group at the 6-position of adenosine is not tolerated by A_{2B} AR subtype (data not shown) [64].

A novel series of potent but low-selective A_{2B} AR agonists structurally related to NECA has been recently reported by Baraldi et al. [69]. These compounds were designed modifying the N^6-position of 5′-*N*-carboxamidoadenosine in analogy with the typical substitution pattern of some potent and selective A_{2B} AR antagonists previously reported in the literature. Several A_{2B} 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 A_{2B} AR. Further evidence of the important role of the substituent at the 8-position as the structural selectivity element for the design of potent A_{2B} AR antagonists was provided recently by our group [72].

Considering the previous structure activity relationship (SAR) studies, regarding NECA and adenosine derivatives indicating the N^6 as a useful position for A_{2B} AR binding-site recognition, a new series of N^6-[(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 A_{2B}

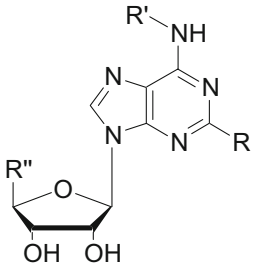

Table 3 Binding affinities (A_1, A_{2A}, A_3) and functional parameters (A_{2B}) of 2-substituted adenosine and NECA derivatives **27**–**38** at the adenosine receptors

	R	R′	R″	A_1[a] K_i nM	A_{2A}[b] K_i nM	A_{2B}[c] EC_{50} μM	A_3[d] K_i nM
2-Cl-Ado (27) [62,65]	Cl	H	CH_2OH	9.3[e]	63.0[f]	24.0	1,890[g]
(*R*,*S*)PHPAdo (28) [81]	-C≡C-CH(OH)Ph	H	CH_2OH	0.67 (0.55–0.80)	7.0 (3.7–13)	2.4 (1.5–3.7)	3.3 (2.3–4.8)
(*R*)PHPAdo LUF5599 (29) [81]	-C≡C-CH(OH)Ph	H	CH_2OH	0.44 (0.38–0.52)	29.0 (19–45)	6.2 (2.9–13)	5.0 (3.2–7.7)
(*S*)PHPAdo LUF 5600 (30) [81]	-C≡C-CH(OH)Ph	H	CH_2OH	0.67 (0.47–0.96)	1.8 (1.1–3.0)	0.92 (0.71–1.2)	1.4 (0.78–2.4)
N^6-ethyl-(*R*,*S*)PHPAdo (31) [82]	-C≡C-CH(OH)Ph	Et	CH_2OH	2.7 (2.4–2.9)	94.0 (72–123)	1.7 (0.97–3.0)	0.97 (0.58–1.6)
(*R*,*S*)PHPNECA (32) [74]	-C≡C-CH(OH)Ph	H	CONHEt	2.7 (1.2–3.7)	3.1 (2.4–3.9)	1.1 (0.47–2.6)	0.42 (0.17–1.0)
(*R*)PHPNECA (33) [74]	-C≡C-CH(OH)Ph	H	CONHEt	1.9 (1.8–2.1)	39.0 (25–59)	2.4 (1.5–3.8)	5.5 (3.6–8.5)
(*S*)PHPNECA (34) [74]	-C≡C-CH(OH)Ph	H	CONHEt	2.1 (1.2–3.7)	2.0 (1.2–3.5)	0.22 (0.22–0.23)	0.75 (0.52–1.1)
N^6-ethyl-(*R*,*S*)PHPNECA (35) [74]	-C≡C-CH(OH)Ph	Et	CONHEt	15 (8.0–29)	90 (48–170)	2.0 (1.3–3.2)	2.1 (1.3–4.4)
(*R*,*S*)-2-(3-hydroxy-1-pentynyl) NECA (36) [62]	-C≡C-CH(OH)Et	H	CONHEt	4.1	3.1	1.3	1.0
(*R*,*S*)-2-(4-hydroxy-1-pentynyl) NECA (37) [62]	-C≡C-CH_2CH(OH)CH_3	H	CONHEt	40.0 (26–62)	14.0 (10–20)	13.3	4.1 (3.4–4.9)
(*R*,*S*)PHPMECA (38) [74]	-C≡C-CH(OH)Ph	H	$CONHCH_3$	14.0 (8.7–22)	3.1 (1.7–5.4)	5.0 (3.5–7.2)	1.7 (1.0–2.7)

[a] Displacement of specific [^{3}H]CCPA binding in CHO cells stably transfected with human recombinant A_1 adenosine receptor, expressed as K_i (nM), unless noted. [b] Displacement of specific [^{3}H]NECA binding in CHO cells stably transfected with human recombinant A_{2A} adenosine receptor, expressed as K_i (nM), unless noted. [c] Measurement of receptor-stimulated adenylyl cyclase activity in CHO cells stably transfected with human recombinant A_{2B} adenosine receptor, expressed as EC_{50} (μM). [d] Displacement of specific [^{3}H]NECA binding in CHO cells stably transfected with human recombinant A_3 adenosine receptor, expressed as K_i (nM), unless noted. [e] Displacement of [^{3}H]PIA binding from rat brain membranes. [f] Displacement of [^{3}H]CGS21680 from rat striatal membranes. [g] Displacement of [^{125}I]APNEA binding in CHO cells stably transfected with the rat A_3-cDNA.

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

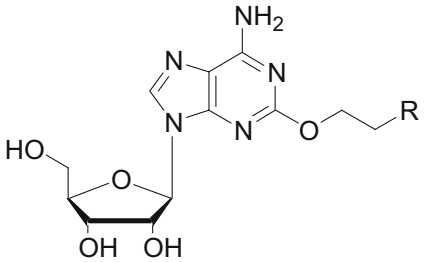

Table 4 Binding affinities (hA_1, hA_{2A}, hA_3) and functional parameters (hA_{2B}) of 2-(hetero)arylethyloxy-adenosine derivatives **39**–**47** at the human adenosine receptors [85, 86]

	R	hA_1[a] K_i nM	hA_{2A}[b] K_i nM	hA_{2B}[c] EC_{50} nM	hA_3[d] K_i nM
NECA		6.8±2.4	2.2±0.6	140±19	16.0±5.4
39	Ph	221±57	9.3±2.9	3490±1490	54.2±14.3
40	2-naphthyl	141±51	16.1±7.0	1440±70	130±8
41	2-thienyl	174±20	10.9±4.8	1780±260	93.3±16.8
MRS3534 (42)	3-indolyl	148±19	45.0±11.6	299±45	232±54
43	3-(5-F-indolyl)	150±50	370±80	767	490±60
44	3-(6-Cl-indolyl)	145±6	29.3±13.7	216±59	92.3±7.9
MRS3997 (45)	3-(6-Br-indolyl)	253±3	150±20	128±32	90±15
MRS3854 (46)	3-(5-Br-indolyl)	358±1	502±32	365±73	234±24
47	3-(5-OH-indolyl)	310±90	450±8	896	120±20

[a] Displacement of specific [^{3}H]CCPA binding, membranes from CHO cells stably transfected with human recombinant A_1 adenosine receptor, K_i (nM). [b] Displacement of specific [^{3}H]CGS21680 binding, membranes from HEK-293 cells stably transfected with human recombinant A_{2A} adenosine receptor, K_i (nM). [c] cAMP assay in CHO cells expressing human A_{2B} adenosine receptors, EC_{50} (nM). [d] Displacement of specific [^{125}I] ABMECA binding, membranes from CHO cells stably transfected with human recombinant A_3 adenosine receptor, K_i (nM).

ARs. The compounds were also evaluated in a functional assay, measuring their capacity to modulate cAMP levels in CHO cells expressing hA_{2B} AR receptors. The compounds were shown to bind the adenosine A_1 receptor (K_i-binding values ranging from 2.3 to 30.5 nM) and to activate the adenosine A_{2B} AR (EC_{50} values ranging from 7.3 to 175 nM) in the low nanomolar range, displaying at the same time a considerable level of selectivity toward A_{2A} AR subtypes (K_i>1 μM) and a relevant capability to bind A_3 ARs.

Substitution at the paraposition of the phenyl ring with a halogen atom led to a two- to fourfold loss of A_{2B} AR

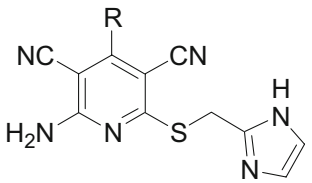

Table 5 Binding affinities (hA_1, hA_{2A}, hA_3), functional parameters (hA_{2B}) and percentages of efficacy of the 2-amino-6-(1*H*-imidazol-2-ylmethylsulfanyl)-4-(substituted)phenyl pyridine-3,5-dicarbonitrile derivatives **48**–**52** as AR agonists and partial agonists [88]

	R	hA_1[a] K_i nM (efficacy, %)	hA_{2A}[b] K_i nM (efficacy, %)	hA_{2B}[c] EC_{50} nM (efficacy, %)	hA_3[d] K_i nM (efficacy, %)
NECA		12 (9.6–15)	60±10	104±15	11±0.8
LUF 5833 (48)	Phenyl	2.4±1.0(109)	28±4(55)	19±7(81)	171±109(84)
LUF 5834(49)	p-OH-phenyl	2.6±0.3(103)	28±4(55)	12±2(74)	538±210(23)
LUF 5835(50)	m-OH-phenyl	4.4±2.0(112)	21±2(80)	10±3(92)	104±49(95)
LUF 5844(51)	m-OCH_3-phenyl	2.0±1.0(80)	105±22(49)	34±24(68)	74±21(39)
LUF 5845(52)	p-OCH_3-phenyl	7.0±0.8(46)	214±37(32)	9±3(33)	24±7.6(73)

[a] Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells. [b] Displacement of specific [^{3}H]ZM241385 binding at human A_{2A} receptors expressed in HEK293 cells. [c] cAMP assay in CHO cells expressing human A_{2B} adenosine receptors EC_{50} (nM). [d] Displacement of specific [^{125}I]ABMECA binding at human A_3 receptors expressed in HEK293 cells.

Table 6 Potency of 2-amino-4-(substituted)phenyl pyridine-3,5-dicarbonitrile 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

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 *tert*-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 represent, 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. Competition-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]-9*H*-purin-9-yl]-N-ethyl-β-D-ribofuranuronamide and 1-deoxy-1-[2-chloro-6-[((hetero)aryl-carbonyl)-hydrazino]-9*H*-purin-9-yl]-N-ethyl-β-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-[*N*′-(furan-2-carbonyl)-hydrazino]-9*H*-purin-9-yl}-N-ethyl-β-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

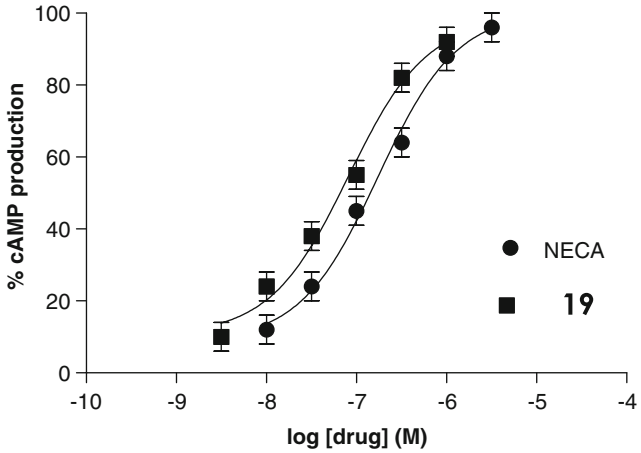

Fig. 2 Dose-response curve of NECA and compound **19** on cAMP assays in hA_{2B} AR CHO cells

agonist in a functional assay based on the measurement of its capacity to modulate cAMP levels in CHO cells expressing hA_{2B} AR (Fig. 2). Nevertheless, both furan and thiophene rings were shown to exert similar favourable interactions for receptor activation [compare furan derivatives, **21** (hA_{2B} EC_{50}=227 nM) and **23**, with the related thiophene derivatives **22** (hA_{2B} EC_{50}=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 hA_{2B} AR, as is clear from the comparison of chlorinated derivatives **23** (hA_{2B} EC_{50}=210 nM) and **24** (hA_{2B} EC_{50}= 175 nM) with the corresponding nonchlorinated **19** (hA_{2B} EC_{50}=82 nM) and **22** (hA_{2B} EC_{50}=273 nM). The examined molecules can be considered valuable tools for the design and development of new and even more selective and potent ligands.

C^2-substitution

The introduction of a bulky substituent at the 2-position of the adenine ring of NECA is known to induce A_{2A} AR-selective agonistic activity. CGS21680 has, in fact, been considered one of the most potent A_{2A} AR agonist and the ligand of choice to distinguish A_{2A}- and A_{2B} AR-mediated effects (K_i values from binding assays for hA_1, hA_{2A} and hA_3 AR subtypes of 298, 27 and 67 nM respectively; EC_{50} value from stimulation of adenylyl cyclase activity through A_{2B} AR of 88.8 μM) [11]. The first attempts to substitute the 2-position of adenosine indicated that A_{2B} AR did not tolerate well such structural modulation [65]. An appreciable improvement of A_{2B} AR affinity has, however, bgeem recognised, introducing a 2-chloro atom (2-ClAdo, **27**, Table 3, EC_{50} from measurement of receptor-stimulated adenylate cyclase activity in CHO, stably transfected with hA_{2B} AR of 24 μM) or 2-alkynyl chains. The racemic 2-phenylhydroxypropynyladenosine [(*R*,*S*)-PHPAdo, **28**] [15] had been found to exert activity at A_{2B} AR comparable with that of NECA (EC_{50}=2.4 μM). The (*R*) diastereomer of PHPAdo (LUF 5599, **29**, EC_{50}=6.2 μM) was almost sevenfold less potent than the (*S*) optical isomer (LUF 5600, **30**, EC_{50}=0.92 μM). The introduction of small alkyl chains at the N^6 position of (*R*,*S*)-PHPAdo was shown to be tolerated for interaction with A_{2B} AR, whereas large groups abolished A_{2B} AR potency (data not shown) [81]. In particular, for N^6-ethyl-(*R*,*S*)-PHPAdo (**31**), an EC_{50} from adenylyl 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 A_{2B} 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 EC_{50} value of 1.1 μM [83]. The racemic (*R*,*S*)-PHPNECA resulted in a twofold greater potency than the optically pure (*R*)-PHPNECA (**33**, hA_{2B} EC_{50}=2.4 μM) and a five fold smaller potency than the (*S*) diastereomer (**34**, hA_{2B} EC_{50}= 0.22 μM). (*S*)-PHPNECA was, therefore, 11-fold more active than (R)-PHPNECA. The combination of the N^6- and 5′ substitutions as in compound N^6-ethyl-2-phenylhydroxypropynyl-5′-*N*-ethylcarboxamidoadenosine **35** (hA_{2B} EC_{50}=2.0 μM) led to decreased affinity for A_{2B} AR [74]. Displacement of the phenyl ring of the alkynyl group had no

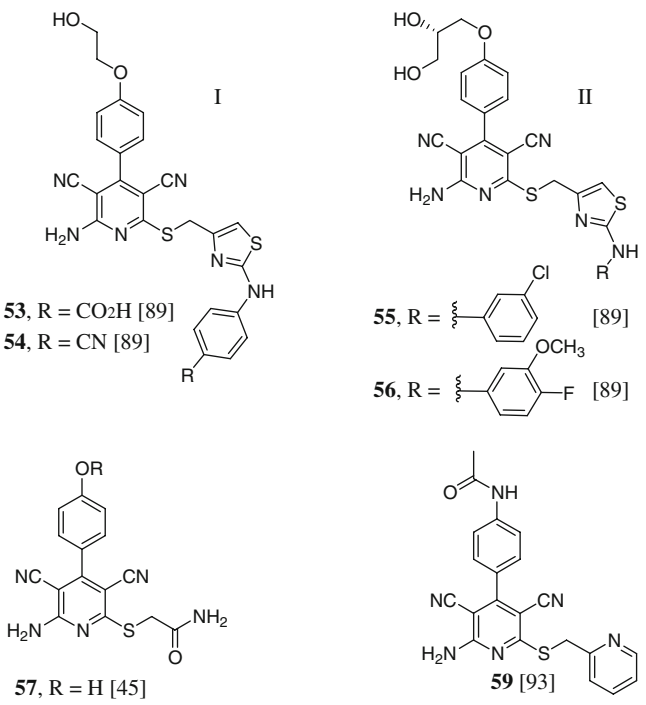

Fig. 3 2-Amino-4-(substituted)phenyl pyridine-3,5-dicarbonitrile derivatives: novel A_{2B} AR agonists of particular interests for their potential therapeutic applications

effect on binding, as demonstrated by derivative (*R*,*S*)-2-(3-hydroxy-1-pentynyl) NECA **36**, which was as potent as (*R*,*S*)-PHPNECA in activating A_{2B} AR subtype (hA_{2B} EC_{50}= 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-(4-hydroxy-1-pentynyl) NECA **37**, bearing a hydroxyl group in β to the triple bond, was, in fact, 12-fold less potent than (*R*,*S*)-PHPNECA (hA_{2B} EC_{50}=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 (hA_{2B} EC_{50}=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 A_{2B} 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 A_{2A} and A_{2B} AR subtypes have a smaller volume of the putative hydrophobic pocket surrounding the 5′-*N*-alkyl substituent than the A_1 and A_3 ARs. That gave a possible explanation for the decreased affinity characterising carboxamido derivatives in which sterically demanding alkyl 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 suppose 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)arylalkyloxy, 2-phenethylamino and 2-phenethylsulfanyl substitutions of adenosine. Most of these compounds were found to be extremely weak at the A_{2B} AR; nevertheless, 2-(phenylethyloxy)adenosine **39** (Table 4, EC_{50}=3.49 μM), 2-[2-(2-naphthyl)ethyloxy]adenosine **40** (EC_{50}=1.44 μM) and 2-[2-(2-thienyl)ethyloxy] adenosine **41** (EC_{50}=1.78 μM) have been reported to be moderately potent A_{2B}AR agonists. Based on the findings that among these molecules, specific 2-(2-aryethyloxy)ether derivatives also displayed significant activity at the A_{2B} 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,N^6,5′-substituted adenosine derivatives, which led to the identification of compounds with enhanced potency at the A_{2B} AR and reduced potency at the other AR subtypes [86]. In particular, 2-(3-indolyl)ethyloxy) adenosines substituted at the 5″ or 6″ positions of the 2-indole moiety with halogens or a hydroxyl function exerted micromolar potency in activating A_{2B} AR (EC_{50} 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″-indolylethyloxy)adenosine **42** was found to be a rather potent agonist at the hA_{2B} AR (EC_{50}=299 nM), although the selectivity profile was not so satisfactory (K_i values at A_1, A_{2A}, A_3 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 A_{2B} AR potency. Elongation or branching of the 2-alkyl spacer proved to weaken the affinity against all ARs. 2-Indolyl derivative decreased markedly A_{2B} 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*-ethyluronamido analogue of **42** was synthesised, considering that replacement of the 4′-hydroxymethyl group with a 5′-*N*-ethylcarboxamido function is generally known to favour A_{2B} AR interaction. Unexpectedly, in the 2-(3-indolyl)ethyloxyadenosine series, this structural modification generated a threefold loss of potency (EC_{50} hA_{2B}=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^6-position of **42** an ethyl group (EC_{50} hA_{2B}= 3,270 nM). Considering the potency of compound **42**, the authors replaced its 6-amino group with a N^6-guanidino moiety detecting decreased potency at the A_{2B} AR (hA_{2B}= 40% of activation at 10 μM), along with reduced selectivity versus A_1 (K_i hA_1=73.6 nM) and A_3 (K_i hA_3=90 nM) AR subtypes. The best results in terms of A_{2B} 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 strengthen by favourable interactions of the 2-(6-bromoindol-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-(1*H*-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 [^{3}H]DPCPX, [^{3}H]ZM241385 and [^{125}I]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-(1*H*-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**

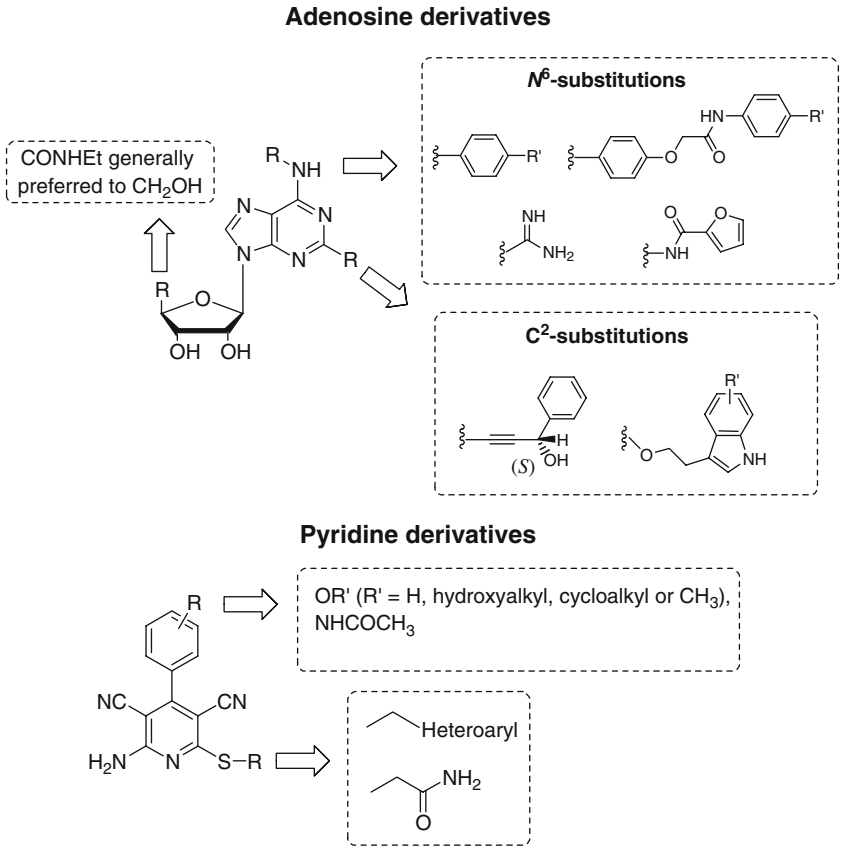

Fig. 4 Schematic overview of the most important structural modifications of adenosine and nonadenosine derivatives for a potent and/or selective activation of the A_{2B} AR

(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 hypertonia 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-60–6583, **58**) have been examined as A_{2B} AR agonists for their potential in treating disorders of the coronary arteries and atherosclerosis [45], as well 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-60–6583 is under preclinical-phase investigation for treating angina pectoris. BAY-60–6583, 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-Gα16 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]-9*H*-purin-9-yl}-N-ethyl-β-D-ribofuranuronamide, **19**) and nonadenosine (BAY-60–6583) 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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