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Review

Therapeutic potential of adenosine analogues and conjugates

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Abstract:
This review summarizes current knowledge of adenosine analogues and conjugates with promising therapeutic properties. Adenosine is a signaling molecule that triggers numerous physiological responses. It acts through the adenosine receptors (ARs), belonging to the family of G-protein-coupled receptors and widely distributed throughout the body. Moreover, adenosine is involved in key biochemical processes as a part of ATP, the universal energy currency. Thus, compounds that are analogues of adenosine and its conjugates have been extensively studied as potential therapeutics. Many inhibitors of ARs are in clinical trials as promising agents in treatment of inflammation, type 2 diabetes, arrhythmia and as vasodilators used in the myocardial perfusion imaging (MPI) stress test. Furthermore, adenosine analogues revealed high efficacy as enzyme inhibitors, tested for antitrypanosomal action and as bivalent ligands and adenosine-oligoarginine conjugates as inhibitors of protein kinases.

Key words:
adenosine, adenosine conjugates, adenosine receptors, inhibitors of protein kinases

Abbreviations: AdoMetDC – S-adenosylmethionine decarboxylase, AR – receptor for adenosine, β_2AR – β_2-adrenergic receptor, FFA – free fatty acids, GMC – glomerular mesangial cells, HAT – Human African Trypanosomiasis, IP – ischemic pre-conditioning, MPI – myocardial perfusion imaging, NEFA – nonesterified fatty acid, PK – protein kinase, T2D – type 2 diabetes, TG – triglycerides

Introduction

Adenosine (Fig. 1) is an endogenous purine nucleoside that plays an important role in the human body. It is present constitutively at a low level extracellularly (revised 1 μM), but its concentration increases under metabolic stress (e.g., hypoxia and ischemia) [56]. Adenosine acts through the adenosine receptor (AR) belonging to the family of G-protein-coupled receptors. So far, four subtypes of ARs (i.e., adenosine A_1, A_{2A}, A_{2B} and A_3 receptors) have been recognized [39]. The binding of adenosine to A_1 and A_3 receptors inhibits adenylyl cyclase activity, and binding to A_{2A} and A_{2B} subtypes causes an increase in cAMP concen-

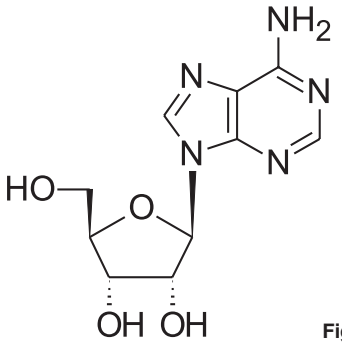


Fig. 1. Adenosine structure

Tab. 1. Distribution of adenosine receptor subtypes – high expression [39, 51, 76]

A_1	A_{2A}	A_{2B}	A_3
brain cortex, hippocampus, spinal cord, eye, adrenal gland, atria	stratium, nucleus accumbens, olfactory tubercle spleen, thymus, leukocytes, blood platelets	caecum, colon, bladder	testis (rat), mast cells (rat)

tration [42]. These membrane receptors are widely distributed throughout the body (Tab. 1). Numerous responses are triggered by AR activation, depending on the stimulated receptor subtype, the type and metabolic state of the tissue. The main role of adenosine is to maintain homeostasis. Equally important is its neuromodulatory action [23, 77]. Adenosine effectively suppresses pharmacoresistant seizures [62] and protects myocardium and the cerebrovascular system from ischemic damage [43]. An anti-inflammatory action was observed during acute lung injuries [99].

Besides being a ligand for the ARs, adenosine is involved in key biochemical processes. RNA requires purine nucleoside substrates for its synthesis. Moreover, adenosine is a part of ATP, the universal energy currency, and cAMP, a second messenger molecule [18].

Thus, adenosine has been an important target molecule for the development of new medicines against numerous diseases. This review describes the latest achievements in developing compounds containing the adenosine moiety that are considered potential drugs. The first group presented is composed of AR agonists with good selectivity and efficacy. The second group consists of adenosine analogues that can be used in the treatment of parasitic infections. Finally, the third noteworthy group includes binary drugs achieved by tethering adenosine to a ligand for a different domain or type of receptor.

Analogues of adenosine acting on adenosine receptors

Adenosine is a natural agonist for ARs, eliciting different physiological responses, as mentioned before. Unfortunately, the clinical utility of adenosine is restricted due to its short half-life in blood and non-selectivity towards different receptor subtypes. Regardless of these limitations, adenosine is used as an antiarrhythmic agent in paroxysmal supraventricular tachycardia. To ensure efficacy, adenosine must be injected as a rapid bolus administered directly into a vein [54].

Receptor selectivity is highly important to minimize side effects and achieve the desired effects. Therefore, numerous adenosine analogues have been synthesized. Most of them have relatively good affinity, but act as a ligand for more than one receptor subtype. In recent years, all four receptors were cloned, and adenosine receptor binding sites were modeled on the molecular level. As a result, new opportunities to search for more-selective and efficient agonists became possible.

A_1 receptor

The A_1 adenosine receptor is the best-characterized subtype of the four known ARs. Selective A_1AR agonists mediate antiarrhythmic, antinociceptive and neuro- and cardioprotective effects. Moreover, A_1AR agonists reduce lipolysis in adipose tissue [27, 37]. Agonists with low selectivity may additionally stimulate A_{2A} and A_{2B} receptors on blood vessels and cause lowering of blood pressure [88]. Unfortunately, full selective agonists can lead to severe cardiovascular side effects such as hypotension, bradycardia and side effects in other organs. Such compounds have limited clinical usefulness [69]. Thus, recent efforts have focused on developing ligands that will partially stimulate the A_1 receptor. So-called partial agonists help to prevent the desensitization of tissue and exert their effect at a greater level in tissues with higher density of receptors [101].

Antiarrhythmic agents

Significant progress was made in the development of the N^6-lipophilic substituted adenosine analogue, tecadenoson (CVT-510) (*N*-[3-(*R*)-tetrahydrofuranyl]-6-aminopurine riboside) (Fig. 2), with a half-life of ~30 min. Tecadenoson has been shown to be highly selective for A_1AR. Importantly, a significant lowering of blood pressure has not been observed [27]. It is a clinical candidate for the treatment of paroxysmal

supraventricular tachycardia and atrial fibrillation (phase III and II of clinical trials, respectively) [17, 82]. Data from phase III trials demonstrated the ability of tecadenoson to convert an abnormally rapid heart rhythm back to normal without significant side effects [31]. The mechanism of action involves activation of the inward rectifying potassium current and inhibition of the pacemaker current and the L type calcium currents [78].

The addition of a 5'-*N*-ethyl uronamide moiety as well as a cyclopentyl substituent at the N^6 position of adenosine created selodenoson, another potent agonist with much longer half-life of 150 min (DTI0009) (N^6-cyclopentyl-5'-(*N*-ethyl) carboxamidoadenosine) (Fig. 2). Selodenoson was designed to control heart rate without lowering blood pressure and decreasing heart function [6, 86]. The *iv* formulation for hospital use is currently in phase II clinical trials for the treatment of atrial fibrillation [44]. All six administered doses led to a decrease in ventricular rate in comparison with placebo [86]. The oral formulation has completed phase I clinical trials [25]. Moreover, the controlled-release formulation for oral administration has been patented [10]. This form is convenient for outpatient use for the chronic management of arrhythmia.

Antinociceptive agents

The stability of adenosine is poor due to its rapid degradation and metabolism by enzymes such as adenosine deaminase and purine nucleoside phosphorylase. It has been observed that 5'-chloro-5'-deoxyadenosine analogues are more stable in comparison to adenosine [4]. Based on these findings, a series of such derivatives have been synthesized. Two analogues, N^6-tetrahydrofuranyl-5'-chloro-5'-deoxyadenosine (Fig. 3) and 5'-chloro-5'-deoxy-(±)-ENBA (5'-chloro-5'-deoxy-N^6-(-*endo*-norborn-2-yl)adenosine) (Fig. 3) show high A_1AR affinity and selectivity (Tab. 2). 5'-Chloro-5'-deoxy-(±)-ENBA effectively reduced formalin-induced pain in mice [37]. However, it exerted antinociceptive activity at a higher concentration than did a less selective compound, (±)-ENBA (N^6-(-*endo*-norborn-2-yl)adenosine) (Fig. 3).

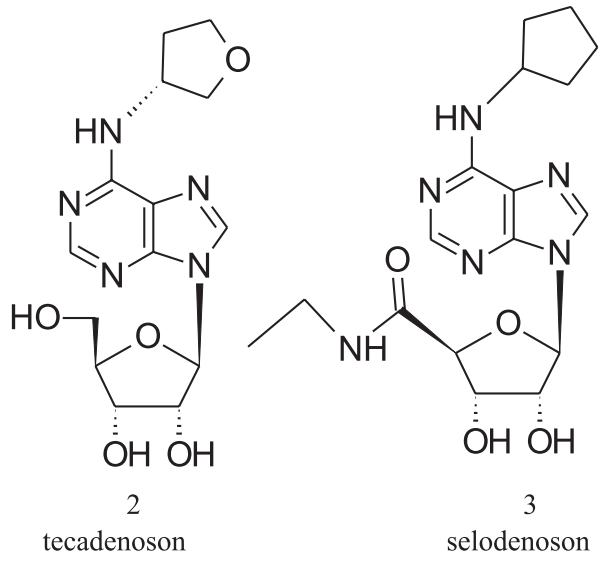
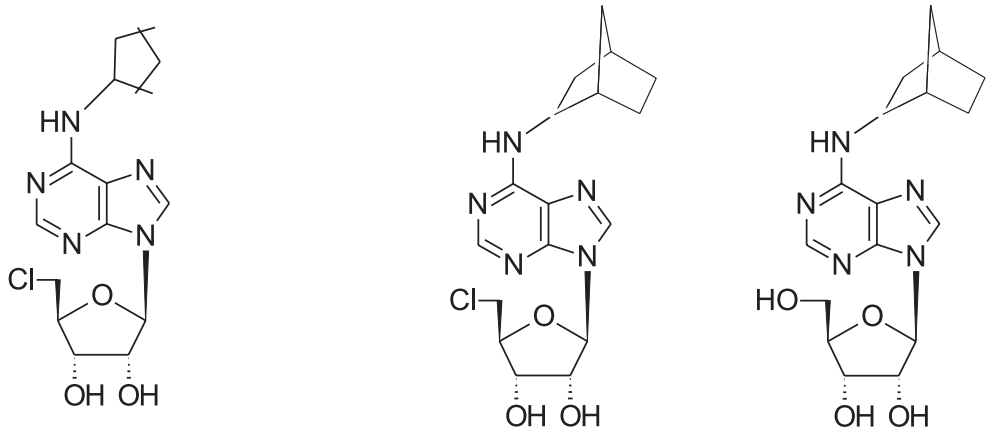


Fig. 2. A_1AR agonists under examination as antiarrhythmic agents

Antidiabetic agents

A_1AR agonists are promising agents in the treatment of type 2 diabetes (T2D) due to their antilipolytic properties. Stimulation of A_1 receptors reduces the formation and release of free fatty acids (FFA) from adipocytes, lowers levels of triglycerides (TG) and enhances insulin sensitivity [26]. Antilipolytic actions of adenosine were studied using A_1 receptor knockout mice. Administration of an adenosine analogue caused significant reduction of lipolysis in A_1R (+/+) in comparison to A_1R (–/–) mice [57]. Unfortunately, full A_1 receptor agonists cause cardiac side effects, such as slowing of heart rate. Thus, researchers have focused on developing partial agonist compounds. This approach facilitates obtaining tissue selectivity, so a greater effect is exerted in adipose tissue, which has a larger receptor reserve in comparison with cardiac tissue [64]. The compound GR79236 (*N*-(1*S*,2S)-2-hydroxycyclopentyladenosine) (Fig. 4) significantly lowers nonesterified fatty acid (NEFA) and TG levels [53]. However, as a full agonist it causes side effects. Thus, CVT-3619 (2-{6-[((1*R*,2*R*)-2-hydroxy-cyclopentyl)amino]purin-9-yl}(4*S*,5*S*,2*R*,3*R*)-5-[(2-fluorophenylthio)methyl]oxolane-3,4-diol) (Fig. 4), a modified adenosine with a 5'-arylsulfide, has been developed. CVT-3619 has properties of partial agonist that help to prevent tachyphylaxis. Preclinical studies have shown that it is a good antilipolytic agent. CVT-3619 efficiently reduces level of FFA, improves insulin sensitivity, and reduces elevated TG [32]. Data from phase I clinical trials demonstrated no significant changes in heart rate and blood pressure caused by orally administered CVT-3619 [24].



4

N^6-tetrahydrofuranyl-5'-chloro-5'-deoxyadenosine

5

5'-chloro-5'-deoxy-(±)-ENBA

6

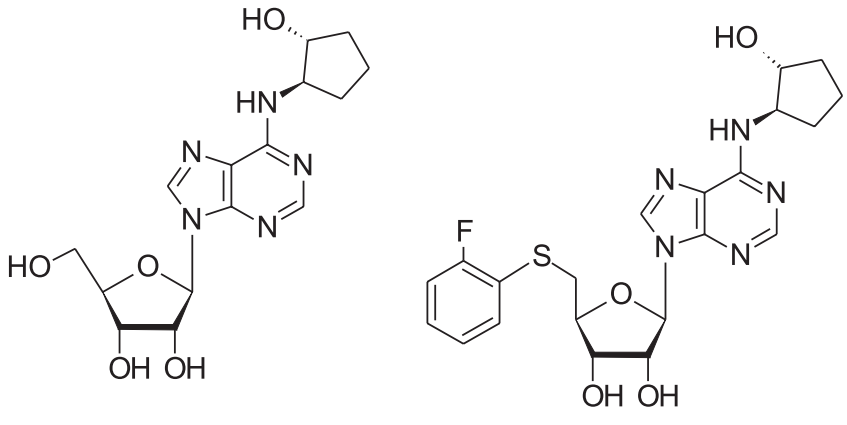
(±)-ENBA

Fig. 3. A_1AR agonists as antinociceptive agents

Tab. 2. Binding affinity of selective A_1 agonists at human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor subtypes [37]

Compound	K_i (nM)				Selectivity	
	A_1[a]	A_{2A}[b]	A_{2B}[c]	A_3[b]	A_{2A}/A_1	A_3/A_1
N^6-tetrahydrofuranyl-5'-chloro-5'-deoxyadenosine	0.59	837	3210	376	1470	637
5'-chlor 5'-chloro-(±)-ENBA	0.51	1340	2740	1290	2630	2530
(±)-ENBA	0.54	1270	4930	101	2350	187

[a] Displacement of [^{3}H]CCPA binding in CHO cells stably transfected with the human recombinant A_1 adenosine receptor. [b] Displacement of [^{3}H]NECA binding in CHO cells stably transfected with human recombinant A_1 or A_3 adenosine receptors. [c] K_i values were calculated from IC_{50} values determined by inhibition of NECA-stimulated adenylyl cyclase activity



7

GR79236

8

CVT-3619

Fig. 4. A_1AR agonists as antidiabetic agents

A_{2A} receptor

A_{2A} receptors are a predominant subtype in immune cells [74]. Moreover, they are expressed in brain, mainly in the striatum [38], but also in the cortex and hippocampus. A_{2A} receptors are also present on platelets and vasculature, including the cerebral endothelium and smooth muscle cells [21, 60]. The importance of A_{2A} receptors was studied using A_{2A}R-knockout mice. A_{2A}R (–/–) mice had increased blood pressure, heart rate and platelet aggregation. Furthermore, they reacted slower to acute pain stimulation [60]. Therefore, A_{2A}AR stimulation produces, *inter alia*, anti-inflammatory, immunosuppressive and hypotensive responses [68].

Immunomodulatory agents

Anti-inflammatory actions of adenosine A_{2A} receptor agonists are well described. A_{2A} receptors are present on inflammatory cells and their activation has an influence on neutrophil function. As a result, degranulation of neutrophils and superoxide production is reduced. Moreover, A_{2A} receptor agonists decrease production of tumor necrosis factor-α (TNF-α) by monocytes and macrophages and neutrophil-endothelial cell adherence. In addition, platelet and endothelial cell activation is decreased [71, 90].

Compounds with such properties have been developed as potential drugs in the treatment of asthma and chronic obstructive pulmonary disease [67]. To prevent unwanted cardiovascular effects, in particular hypotension, topical formulations such as dry powder for inhalation and vehicle gel have been assessed. It have been suggested that such forms have an advantage over orally administered agents. Few adenosine A_{2A} receptor agonists have been evaluated clinically. UK-432097 (Fig. 5) reached phase II clinical trials as an agent for the treatment of chronic obstructive pulmonary disease (dry powder for inhalation) [81]; sonedenoson (MRE0094) (2-[2-(4-chlorophenyl)ethoxy]adenosine) (Fig. 5) entered phase II clinical trials for induction of healing of diabetic foot ulcers (vehi-

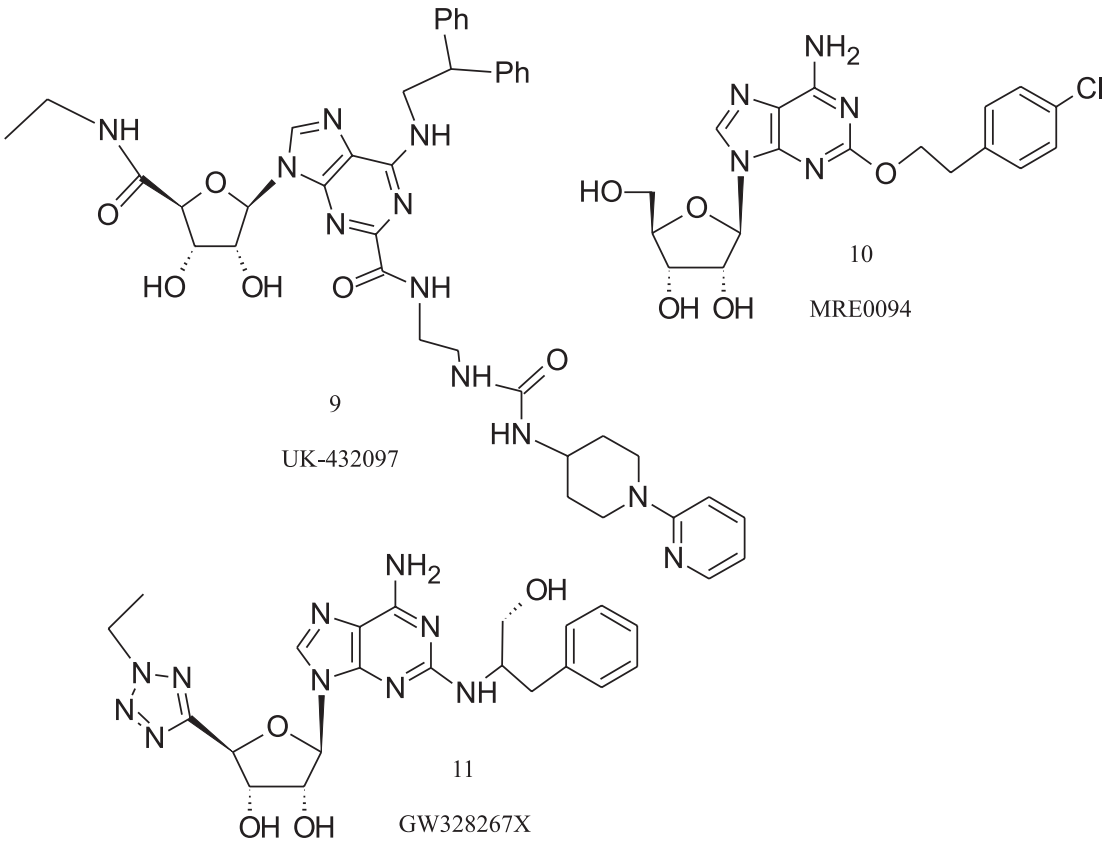
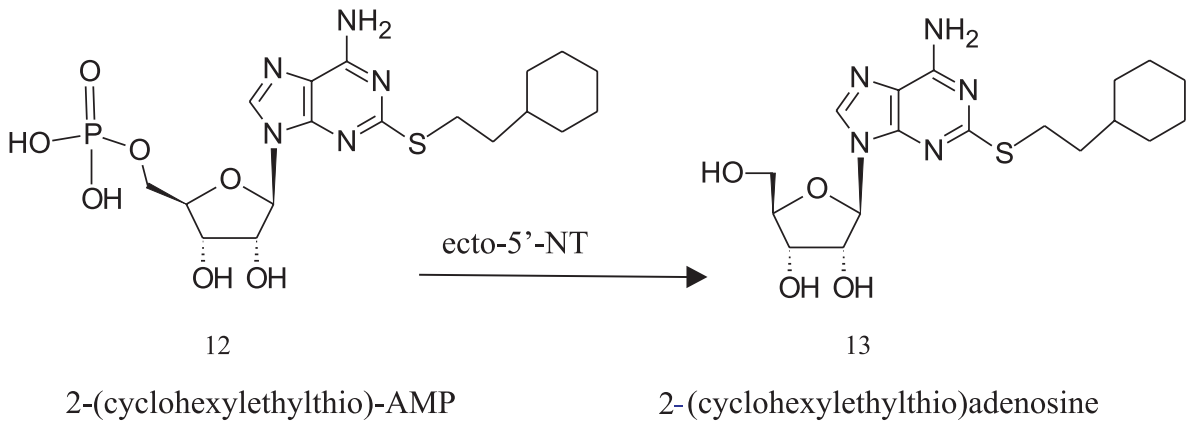


Fig. 5. A_{2A}AR agonists as immunomodulatory agents



12 2-(cyclohexylethylthio)-AMP

13 2-(cyclohexylethylthio)adenosine

Fig. 6. An A_{2A}AR agonist as a prodrug converted by ecto-5'-NT

cle gel) [83]. GW328267X in the inhaled form has been tested for allergic rhinitis [80] and asthma [66, 67] (Fig. 5). Unfortunately, all trials have been terminated because of unsatisfactory effects. Thus, there is a need to evaluate factors that contributed to low efficacy during the above clinical trials.

Recently, it has been proposed to develop A_{2A} adenosine receptor agonists as prodrugs by synthesizing inactive, phosphorylated forms of A_{2A} adenosine agonists [30]. Activation of such compounds requires the enzyme ecto-5'-nucleotidase (ecto-5'-NT, CD73, EC 3.1.3.5). The concentration of ecto-5'-NT is higher in inflamed tissues and facilitates conversion of phosphorylated prodrugs in pathological tissues. This approach may help to achieve desired anti-inflammatory actions and decrease unwanted side effects, such as hypotension. A compound with promising properties is 2-(cyclohexylethylthio)-AMP (Fig. 6), which is dephosphorylated at good rate to the 2-(cyclohexylethylthio)adenosine (Fig. 6). However, further studies are required to improve its affinity and selectivity to A_{2A}.

Vasodilators

Selective A_{2A} adenosine receptor agonists are a new class of coronary vasodilators used in stress perfusion imaging with radiotracers [47]. The myocardial perfusion imaging stress test is a noninvasive way to assess blood flow to the heart muscle to detect coronary artery disease. Regadenoson (Lexiscan, CVT-3416) (2-{4-[(methylamino)carbonyl]-1*H*-pyrazol-1-yl}adenosine) (Fig. 7) is the first FDA-approved selective A_{2A} receptor agonist that is currently in clinical use [8]. Regadenoson is administered intravenously as a fixed-dose bolus. Its efficacy is comparable to adenosine, which is the current agent of choice for pharmacologic stress testing. An important feature of regadenoson is its low affinity for the A_{2A}AR. A_{2A}AR is highly expressed in coronary arteries. Their stimulation by regadenoson allows maximal coronary vasodilation with minimal side effects connected with activation of receptors in other tissues. Two other selective A_{2A} adenosine receptor agonists have been tested as potential agents for MPI. Binodenoson (CorVue, MRE0470) (2-(cyclohexylmethylidenehydrazino-adenosine) (Fig. 7) has completed phase III clinical trials and apadenoson (Stedivaze, ATL146e) (Fig. 7) is currently in phase III clinical trials [16]. Positive results of completed clinical trials of binodenoson have been published. In comparison to adenosine, binodenoson showed fewer and less severe side effects (chest pain, shortness of breath and flushing). Moreover, there were no cases of atrioventricular block. The possibility to administer binodenoson as a single bolus dose is an advantage [93]. However, the FDA has not yet approved this compound as a drug. There are some inaccuracies regarding clinical trial results that need to be clarified [35]. There are no published results from phase III clinical trials of apadenoson, but phase I studies demonstrated good toleration in patients with asthma and chronic obstructive pulmonary disease [22].

In conclusion, selective A_{2A} adenosine receptor agonists are new promising drugs developed as vasodilators for the diagnosis of coronary artery disease used in the myocardial perfusion imaging stress test.

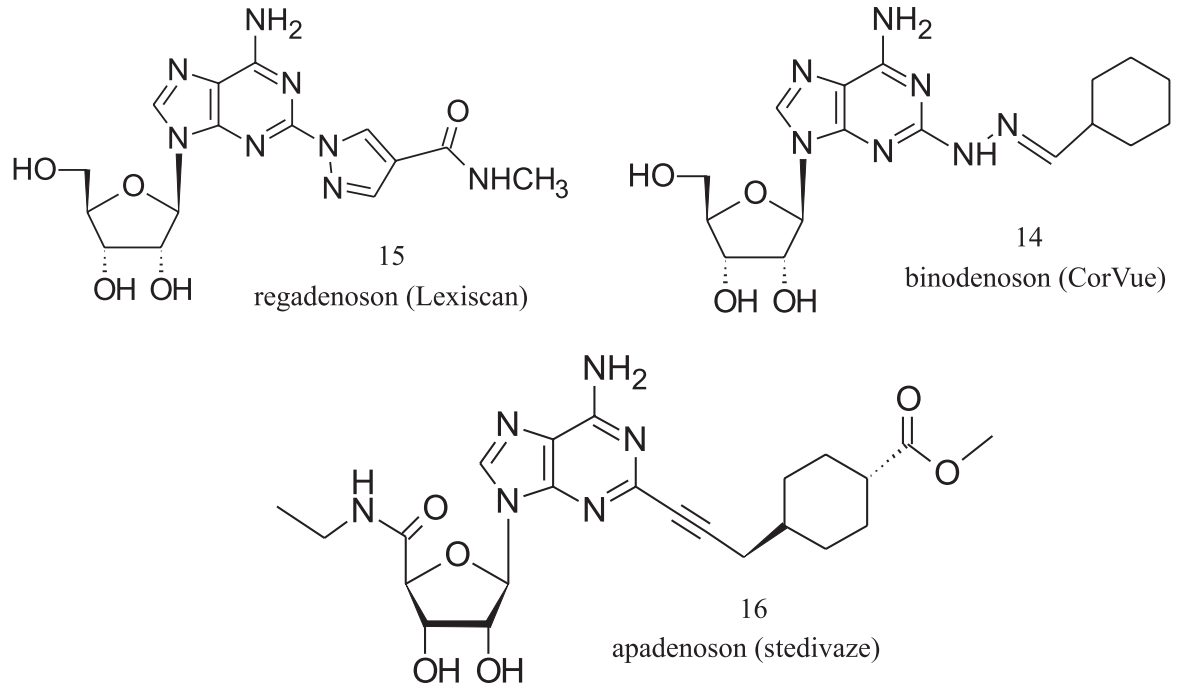


Fig. 7. A_{2A}AR agonists as vasodilators

Clinical studies have shown that they are a better alternative for adenosine. Additionally, future use may include other applications, such as cardiac perfusion, positron emission tomography, cardiac magnetic resonance imaging and myocardial contrast echocardiography [19].

A_{2B} receptor

A_{2B} is a low-affinity receptor subtype activated only in pathological conditions such as hypoxia or inflammation when adenosine concentration increases from nanomolar to micromolar levels [36]. The physiological effects of A_{2B}AR activation are poorly characterized due to difficulties with developing potent and selective ligands. However, studies on A_{2B}AR-knockout mice helped to establish the role of these receptors i.e., in hypoxia and ischemic pre-conditioning. The results showed that A_{2B}AR-deficient mice had increased vascular permeability and increased hypoxia-induced neutrophil infiltration, indicating a protective role of A_{2B}AR agonists in hypoxia-induced inflammation [7, 29]. Ischemic pre-conditioning (IP) is a process in which repeated brief episodes of sublethal ischemia are followed by reperfusion. In 1986, Murry and colleagues found that IP protects the myocardium from the effects of a prolonged ischemic insult [72]. Recent studies showed that IP induces A_{2B}AR expression in heart, lung and kidney. Replacing IP with an A_{2B}AR agonist protects these organs against injuries caused by prolonged ischemia [7]. Additionally, intestinal inflammation associated with gastrointestinal ischemia/reperfusion injury can be attenuated by stimulation of A_{2B} receptors [44]. A_{2B} receptor agonists also have therapeutic potential as drugs in the treatment of coronary artery disease by inhibiting platelet aggregation and vasodilative effects [7, 13]. Elevated levels of extracellular adenosine in the lung are observed in acute lung injury. Studies on murine models of ventilator-induced lung injury and on A_{2B}AR knockout mice suggested that stimulation of A_{2B} receptors during acute lung injury may be of therapeutic benefit [7]. However, the role of A_{2B}AR in chronic conditions, such as chronic obstructive pulmonary disease (COPD) and asthma, remains unclear. To elucidate the role of adenosine in chronic inflammation, mice deficient in adenosine deaminase, the enzyme responsible for deamination of adenosine to inosine, were studied. Mice lacking this enzyme have elevated levels of adenosine and experience adenosine-dependent pulmonary fibro-

sis. Treatment with A_{2B}AR antagonists resulted in attenuated inflammation [7, 91]. To confirm pathological role of A_{2B} receptors, mice deficient in adenosine deamination and A_{2B} receptors were examined. Interestingly, double knockout mice had enhanced pulmonary inflammation and airway destruction [7, 98].

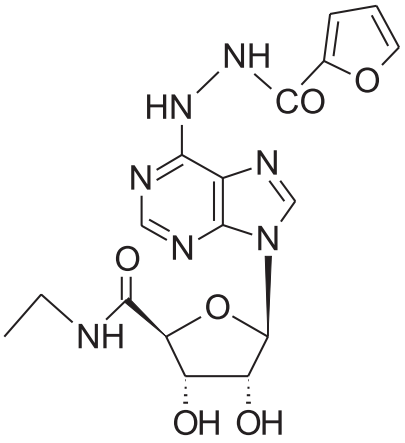
A_{2B} is the main subtype of adenosine receptors expressed in the gastrointestinal tract and is acted upon by extracellular adenosine during intestinal inflammation. It has been suggested that A_{2B}AR agonists play a protective role in such conditions as Crohn's disease or ulcerative colitis [7, 40]. Furthermore, there is evidence of role for A_{2B}AR in colonic motility. Agonists may be of therapeutic use in the treatment of constipation [20]. Contrasting results were demonstrated in studies on A_{2B}AR knockout mice in murine models of colitis. Instead of aggravating inflammation as expected, diarrhea induced by colitis and inflammatory cell infiltration were attenuated [7, 55]. Moreover, A_{2B}AR activation inhibits growth of glomerular mesangial cells (GMC). This effect can be clinically useful in protection against glomerular remodeling connected with glomerulosclerosis, renal disease and abnormal GMC growth associated with hypertension and diabetes [28]. Other studies have indicated that adenosine agonists of A_{2B}AR have a potential role in the treatment of erectile dysfunction [92] and stimulation of hair growth through fibroblast growth factor-7 gene expression in dermal papilla cells [46].

Recently, a few potent adenosine analogues that activate A_{2B} receptors have been synthesized. From a series of 2-N^6, 5'-substituted adenosine derivatives, MRS3997 (2-[3"-(6"-bromo-indolyl)ethyloxy]adenosine) (Fig. 8) revealed promising properties as a full agonist of A_{2A} and A_{2B}AR (A_{2A} EC_{50} = 39.7 nM, A_{2B} EC_{50} = 109 nM) and as a partial agonist of A_1 and A_3AR [5]. Of note is compound 18 (1-deoxy-1-{6-[*N*'- (furan-2-carbonyl) hydrazino]-9*H*-purin-9-yl}-*N*-ethyl-β-D-ribofuranuronamide) (Fig. 8). This fairly selective compound is a potent agonist, with an EC_{50} value in the nanomolar range (hA1, hA2A Ki 1000 nM; hA_{2B} EC_{50} = 82 nM; hA_3 Ki > 5000 nM) [12].

A_3 receptor

Selective A_3AR activation has cardioprotective [63] and cerebroprotective effects [94]. Agonists of A_3AR are currently involved in numerous clinical trials as anti-inflammatory, anti-tumor and hepatoprotective agents [49].

The first selective A_3 agonists were IB-MECA (N^6-(3-iodobenzyl-5'-*N*-methylcarboxamidoadenosine) (CF101) (Fig. 9) and its 2-chloro analogue (Cl-IB-MECA) (CF102) (Fig. 9). A_3ARs are overexpressed in patients with inflammatory diseases, such as rheu-



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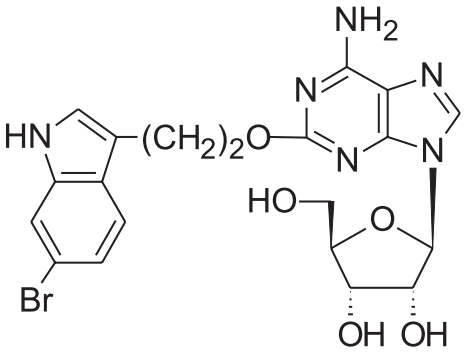
MRS3997



18

1-deoxy-1-{6-[*N*'-(furan-2-carbonyl) hydrazino]-9*H*-purin-9-yl}-*N*-ethyl-β-D-ribofuranuronamide

Fig. 8. Structures of the potent A_{2B} AR agonists

matoid arthritis, psoriasis and Crohn's disease [73]. Based on these findings, IB-MECA was proposed as a compound that can be used to treat immune-mediated inflammatory diseases. Data from a phase II clinical trial involving treatment of plaque-type psoriasis with oral IB-MECA demonstrated its safety, good toleration and efficacy. Due to satisfactory results, preparatory work for phase III trials has been initiated. A phase IIa study has been conducted in patients with rheumatoid arthritis who do not respond methotrexate therapy [3, 85]. IB-MECA administered twice daily for 12 weeks resulted in an improvement of disease signs and symptoms and was safe and well tolerated [87]. Furthermore, results from a phase II clinical trial that aimed to explore the safety and efficacy of IB-MECA in patients with moderate to severe

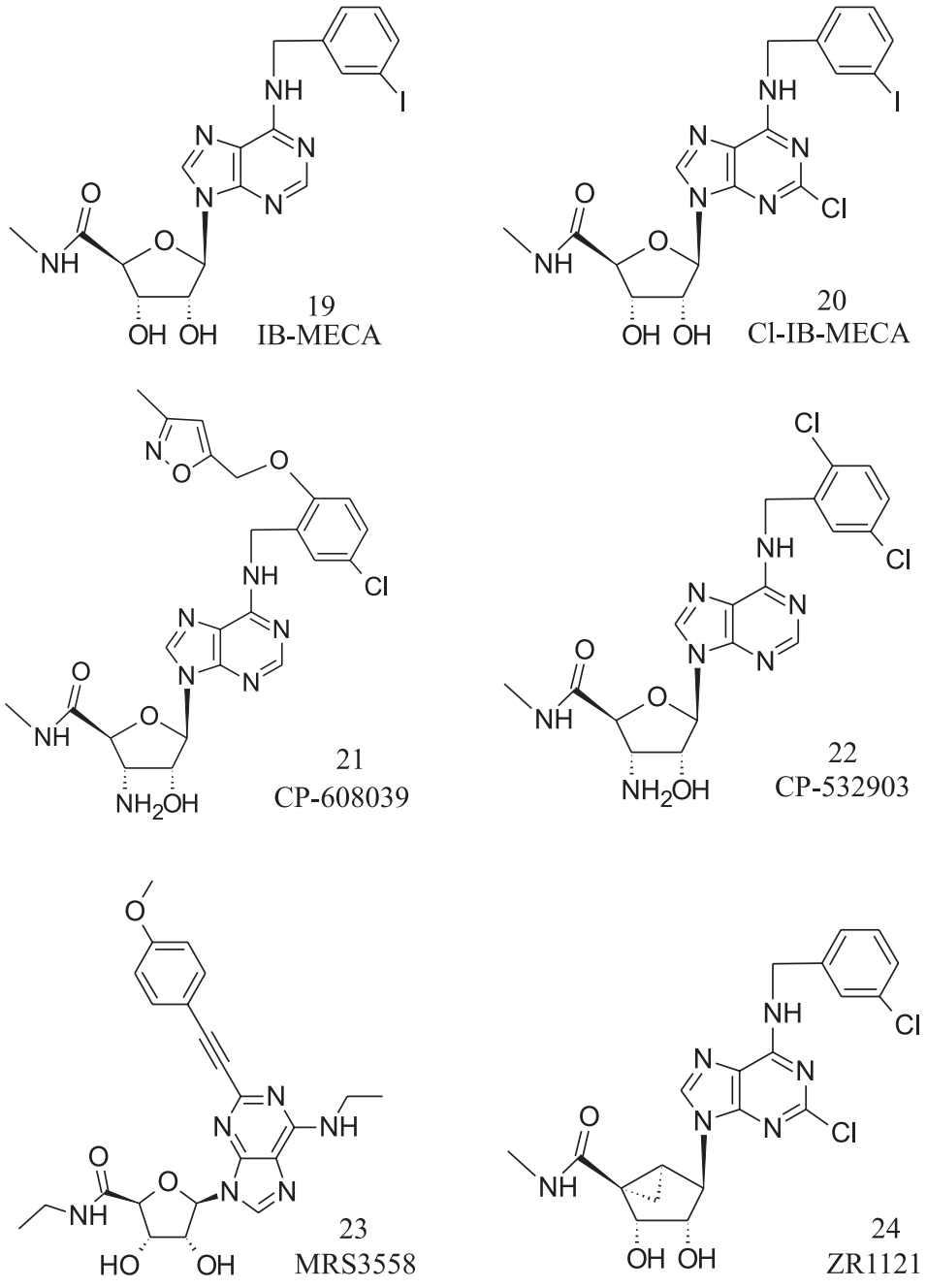


Fig. 9. Structures of the potent and selective A_3 AR agonists

dry eye syndrome demonstrated that IB-MECA given orally significantly improved corneal staining, tear break-up time and tear meniscus. Moreover, it was well tolerated [9]. Another finding from this study was a decrease in the intra ocular pressure. Based on this fact, preparation of a phase II clinical trial with orally administered IB-MECA for the treatment of glaucoma has been initiated [15].

Cl-IB-MECA is in phase I/II trials as an anti-cancer agent in patients with advanced hepatocellular carcinoma and in phase I/II trials in patients with chronic hepatitis C (Tab. 3) [1, 2]. The anti-tumor mechanism of action includes induction of apoptosis of hepatocellular carcinoma and deregulation of the Wnt and NF-κB signaling pathways [11]. In addition, preclinical studies have suggested that Cl-IB-MECA is active against HCV *via* inhibition of NS5, an RNA-dependent RNA polymerase [16]. Cl-IB-MECA is also a promising drug for the treatment of lung inflammation. Recent studies have shown this compound attenuates LPS-induced microvascular permeability. In pulmonary microvascular endothelial cells, Cl-IB-MECA reduced LPS-induced cytoskeletal remodeling and cell retraction [95]. IB-MECA and Cl-IB-MECA have good selectivity towards the adenosine A_3 receptor subtype in rats. However, selectivity against other subtypes is depressed when assessed on human receptors. The first selective agonist toward human A_3AR was CP-608039 ((2*S*,3*S*,4*R*,5*R*)-3-amino-5-{6-[5-chloro-2-(3-methylisoxazol-5-ylmethoxy)-benzylamino]purin-9-yl}-4-hydroxytetrahydrofuran-2-carboxylic acid methylamide) (Fig. 9) [2]. This N^6-substituted adenosine 5'-*N*-methyl uronamide, together with CP-532903 (N^6-(2,5-dichlorobenzyl)-3'-aminoadenosine-5'-*N*-methylcarboxamide) (Fig. 9), has been investigated as a cardioprotective agent [96]. Therapeutic activity of CP-532903 was investigated using an *in vivo* mouse model of infarction and on an isolated

Tab. 3. Status of clinical trials of adenosine receptor agonists [1–3, 6, 9, 15, 17, 67. 80–85, 89]

Compound	Condition	Status
tecadenoson	paroxysmal supraventricular tachycardia	phase III – completed
	atrial fibrillation	phase II – completed
selodenoson	atrial fibrillation – *iv* formulation	phase II – completed
	atrial fibrillation – oral formulation	phase I – completed
CVT-3619	cardiometabolic diseases	phase I – completed
UK-432097	chronic obstructive pulmonary disease (dry powder for inhalation)	phase II – terminated
sonedenoson	healing of diabetic foot ulcers (vehicle gel)	phase II – terminated
GW328267X	allergic rhinitis asthma	phase II – terminated
regadenoson	coronary artery disease (MPI studies)	in clinical use
binodenoson	coronary artery disease (MPI studies)	phase III – completed
apadenoson	coronary artery disease (MPI studies)	phase III – completed
IB-MECA	plaque psoriasis	phase II – completed
	osteoarthritis of the knee	phase II – entering
	ocular hypertension, glaucoma	phase II – entering
	keratoconjunctivitis sicca	phase II – completed
	rheumatoid arthritis	phase IIb – entering
Cl-IB-MECA	hepatocellular carcinoma	phase I/II – entering
	chronic hepatitis C	phase I/II – entering

heart model of global ischemia/reperfusion injury. The results confirmed the protective role of CP-532903 on ischemic myocardium by activation of the A_3 adenosine receptors. The mechanism involves opening sarcolemmal K_{ATP} channels [96]. CP-608039 revealed good human A_3 *vs.* A_1 receptor selectivity (1260-fold) but poor selectivity towards animal receptors. Satisfactory selectivity for human and animal receptors makes CP-532903 a useful agent for preclinical trials.

Other adenosine analogues include MRS3558 ((1'*R*,2'*R*,3'*S*,4'*R*,5'*S*)-4-(2-chloro-6-[(3-chlorophenylmethyl)amino]purin-9-yl)-1-(methylaminocarbonyl) bicyclo[3.1.0]hexane-2,3-diol) (Fig. 9) [48] and ZR1121 (Fig. 9) [100]. Both compounds have improved potency and selectivity and can be potential drugs acting through A_3 receptor (Tab. 4). A recent study showed that MRS3558 is more potent than IB-MECA in attenuating reperfusion lung injury [70].

Adenosine analogues as enzyme inhibitors

Antitrypanosomal agents

Human African Trypanosomiasis (HAT), also known as sleeping sickness, is caused by a parasitic protozoan *Trypanosoma brucei* that is transmitted by tsetse flies. Currently available drugs are either highly toxic or have to be administered intravenously, which limits their usefulness. Adenosine analogues are a new alternative for treatment of HAT, by inhibiting S-adenosylmethionine decarboxylase (AdoMetDC), an enzyme taking part in the rate-limiting step of polyamine biosynthesis [45]. Polyamines such as spermidine and spermine are essential for survival of eukaryotes [79]. AdoMetDC differs significantly from the human enzyme, making it an excellent target for antitrypanosomal agents. Trypanosomatid AdoMetDC is activated by heterodimer formation with a catalytically dead homolog, prozyme. This mechanism for controlling AdoMetDC activity is unique to trypanosomatidis [97].

In 2009, a new potent AdoMetDC inhibitor was reported [45]. The modification of MDL73811 (5'-{[(*Z*)-4-amino-2-butenyl]methylamino}-5'-deoxyadenosine), 5'-[(4-amino-2-butenyl)methylamino]-5'-deoxyadenosine (Fig. 10), which was the first effective inhibitor, gave an adenosine analogue, Genz-644131 (8-methyl-5'-{[(Z)-4-aminobut-2-enyl]-(methylamino)}adenosine) (Fig. 10), with improved properties in comparison to the parent compound [14]. The blood brain barrier penetration was increased as well as growth inhibition of *T. brucei*. However, it was not curative in the central nervous system stage of the disease. Nonetheless, Genz-644131 is a promising lead compound for the development of effective nontoxic trypanocides.

Adenosine conjugates

The development of bivalent ligands is a promising approach to design drugs that act simultaneously on two active sites on a receptor dimer or on two differ-

Tab. 4. Binding affinity of selective A_3 agonists at human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor subtypes

Compound	K_i (nM)				Selectivity
	A_1	A_{2A}	A_{2B}	A_3	A_1/A_3
IB-MECA	3.98	510	2040	0.215	18.5
Cl-IB-MECA	5.26	NI[a]	NI	0.637	8.26
ZR1121	558	4963	NI	0.748	746
MRS3558	260	2300	10000	0.29	896
CP-608,039	7300			5.8	1260
CP-532,903	4800			23	210

[a] NI Inhibition at 100 μM is less than 50% or no inhibition observed. Data for IB-MECA, Cl-IB-MECA, ZR1121 are from [101], MRS3558 from [74] and CP-608,039 and CP-532,903 are from [69]

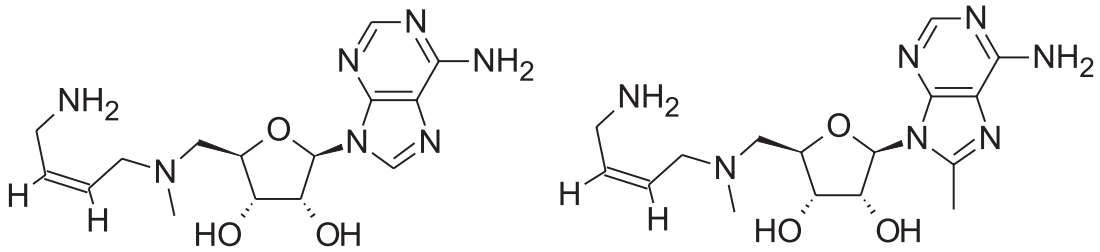


Fig. 10. Structures of MDL73811 (25) and Genz-644131 (26)

ent receptors. The bivalent ligand is composed of two pharmacophores linked by a tether [61]. The results of certain studies suggest that activity and affinity of such a moiety can be enhanced [59]. Examples of adenosine conjugates are inhibitors of protein kinases and linked agonists of the adenosine A_1 receptor and β_2-adrenergic receptor (β_2AR).

Inhibitors of protein kinases

Protein kinases (PKs) catalyze phosphorylation of substrate proteins, thus regulating their activity. These enzymes play a significant role in many processes, and in recent years have become an important target for drugs in the treatment of cancer and other diseases [33]. PKs have two active sites: the first binds ATP and the second binds substrate proteins (Fig. 11a) [75]. Therefore, efforts have been directed to find either competitive ATP inhibitors or compounds that block the binding of substrate protein. Both approaches have disadvantages, such as problems with selectivity and bioavailability [34]. To overcome these obstacles, researchers have proposed to develop dual inhibitors that connect ATP-like and peptide-like moieties.

Adenosine-oligoarginine conjugates have promising properties. Optimization of the linker between the adenosine derivative and arginine and the number of arginine moieties gave a few compounds with nanomolar to subnanomolar inhibitory concentrations of

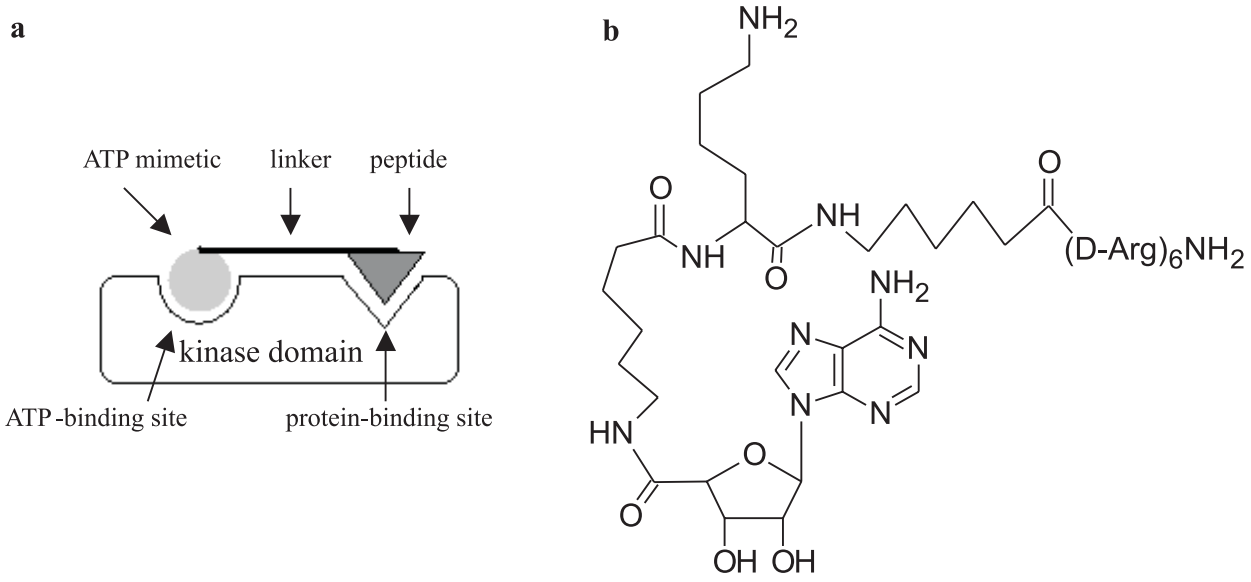


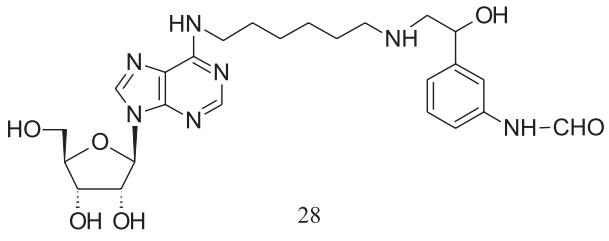
Fig. 11. Scheme of the protein kinase domain with a bound bisubstrate inhibitor (**a**) and structure of compound 27 (**b**)

basophilic PKs [58]. Compound 27 (Fig. 11b), with the best inhibitory potency, is an adenosine-4'-dehydroxymethyl-4'-carboxylic acid attached through linker to six D-arginine residues. The linker consists of two 6-aminohexanoic acid linkers separated with D-lysine. The IC_{50} values for inhibiting protein kinase A and B (isoform type γ) are 5.32 nM and 14.6 nM, respectively. Interestingly, when oligoarginine or adenosine were tested separately, they showed very weak or no inhibitory potency [65]. In conclusion, biligand inhibitors are of great interest as potential drugs against diseases connected with protein kinase activity, such as cancer, diabetes and Alzheimer's disease. Moreover, PK inhibitors may be useful as diagnostic tools in PK-related diseases [57].

Bivalent β_2-adrenergic and adenosine A_1 receptor ligands

β_2AR and adenosine A_1 receptors belong to the family of G protein-coupled receptors, and both of them affect the concentration of cAMP. Stimulation of β_2ARs inhibits adenylate cyclase, while activation of A_1ARs stimulates this enzyme. In some cell types, simultaneous activation of these receptors may have physiological consequences [52].

To examine β_2AR/A_1AR cross talk, bivalent ligands have been synthesized. The most active was compound 28 (*N*-(2-hydroxy-5-(1-hydroxy-2-(6-(N^6-adenosinyl) hexylamino)-ethyl)phenyl)formamide) (Fig. 12), which is based on adenosine and formoterol moieties linked through a hexyl spacer [52]. Data have shown that linking two different pharmacophores retained the activity at both receptors. In comparison to CPA and (–)-isoproterenol, selective agonists of A_1AR and β_2AR, respectively, affinities were slightly lower. The potency of the conjugate was in the nanomolar range (EC_{50} β_2AR = 6 nM). Three times this concentration was needed (20 nM) to obtain the same effect by (–)-isoproterenol. The synthesis of this type of bivalent conjugate is an interesting approach in the development of partial agonists that act through stimulation of two interacting receptors.



28

Fig. 12. The bivalent ligand of β_2AR and A_1AR

Conclusion

Adenosine is a nucleoside signaling molecule that takes part in numerous processes in the human body. Thus, the therapeutic potential of adenosine analogues and conjugates has been extensively studied in recent years. Many adenosine receptor agonists with antiarrhythmic, antidiabetic, immunomodulatory, anti-tumor, hepatoprotective and vasodilatory actions have revealed very promising properties and are in clinical trials. Regadenoson (Lexiscan), the selective A_{2A} receptor agonist, was approved by the FDA and is currently in clinical use as a coronary vasodilator used in stress perfusion imaging. In designing antitrypanosomal agents, the fact that human and parasitic enzymes differ significantly was manipulated. As a result, Genz-644131, a new potent AdoMetDC inhibitor, was synthesized. Bivalent ligands, such as adenosine-oligoarginine conjugates, are potent inhibitors of protein kinases. Moreover, bivalent β_2-adrenergic and adenosine A_1 receptor ligands were synthesized to understand the cross talk between these two receptors.

In summary, adenosine analogues and conjugates are of great importance and have multiple potential applications as drugs for treating cardiac, immune and other diseases.

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