Adenosine receptor agonists: synthesis and binding affinity of 2-(aryl)alkylthioadenosine derivatives

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Abstract
The synthesis of a new series of 2-(aryl)alkylthio derivatives of N-ethylcarboxamido adenosine (NECA) is described, in comparison with the corresponding derivatives of adenosine. Binding studies (A_1, A_{2A}, and A_3) and adenylyl cyclase activity (A_{2B}) at human adenosine receptor subtypes stably transfected in Chinese hamster ovary (CHO) cells showed that the 2-(aryl)alkylthioadenosine derivatives are more potent than the corresponding NECA derivatives at A_1 receptors, while the NECA derivatives possess highest affinity at both A_{2A} and A_3 receptors. Thus, the 2-(1-pentyl)thioadenosine (**7**) with a K_i A_1 = 91 nM, the 2-phenylethylthioNECA (**18**) with a K_i A_{2A} = 24 nM, and the 2-phenylmethylthioadenosine (**11**) with a K_i A_3 = 68 nM, could be useful tools to be modificated in order to find very potent and selective agonists for the human adenosine receptor subtypes.

Keywords: Adenosine receptors, adenosine receptor agonists, NECA derivatives, adenosine derivatives, (aryl)alkylthioadenosine derivatives

Introduction

Adenosine (Ado, **1**) is a naturally-occurring nucleoside which is reported to modulate a variety of physiological and pathophysiological processes through the interaction with at least for subtypes of a family of cell-surface G-protein-coupled receptors.[1-3] These four adenosine receptors (ARs), named A_1, A_{2A}, A_{2B}, and A_3, have widespread tissue distribution and are often co-expressed in the same cell type.[4]

The search for potent and selective A_{2A} adenosine receptor agonists has been a target of medicinal chemists, since it is now well known that the coronary vasodilation induced by Ado in different species is mediated by activation of A_{2A}AR and a compound capable of producing coronary vasodilation through activation of A_{2A}AR, but that is devoid of A_1- and A_3-agonist activity would have advantage over Ado for use in myocardial perfusion imaging studies. Other potential therapeutic applications of selective A_{2A}AR agonists are as anti-aggregatory, anti-inflammatory, anti-psychotic, and anti-Huntington's disease agents.[5]

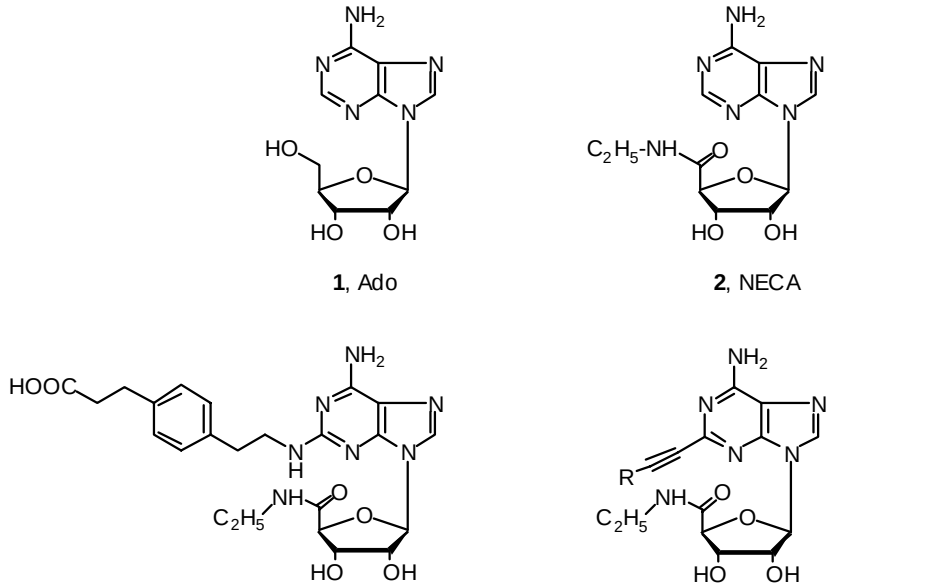


Figure 1

A series of 2-amino, 2-alkoxy, 2-alkythio-, 2-alkynyl-, and 2-alkenyl-derivatives of adenosine and N-ethylcarboxamidoadenosine (NECA, **2**)[6] have been synthesized and tested mainly on different models of rat A_1 and A_{2A} receptor subtypes. From these studies some ligands, such as CGS 21680 (**3**), 2-(1-hexyn-1-yl)-5'-N-ethylcarboxamidoadenosine (HENECA, **4**), and 2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-5'-N-ethylcarboxamidoadenosine (PHPNECA, **5**), showed to possess high A_{2A} affinity combined, in some cases, with good A_{2A} vs A_1 selectivity. More detailed characterization of these ligands at the four cloned human adenosine receptor subtypes revealed that none of the prototypical adenosine receptor agonists exhibits at the same time high affinity and selectivity for the human A_{2A}AR subtype. Both NECA and CGS 21680, which are available as radioligands for this subtype, have lower affinity at human than at rat receptor. The 2-alkynylNECA derivatives HENECA and PHPNECA showed high affinity also at human A_3 receptors. In particular, (*S*)-PHPNECA displayed K_is in the low nanomolar range at A_1, A_{2A}, and A_3 subtypes and an EC_{50} of 200 nM at human A_{2B} receptor.[7]

Further structure-activity relationship studies, carried out often only at A_1 and A_{2A} receptor subtypes, had anyhow defined important features of the recognition sites for ARs agonists. The

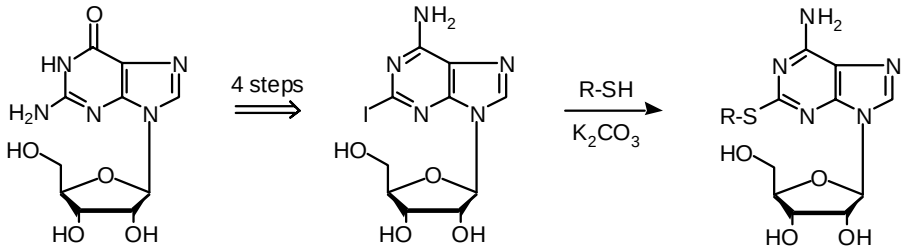
adenine ring could be substituted at the 2-position improving A_2AR selectivity and monosubstitution on the N^6-amino group was tolerated, particularly in the case of A_1AR . Substitution at both C2 and N^6 generally does not have additive effects on the A_2/A_1 affinity ratio. Combination of substitutions at the 2-position of adenosine and replacing of $-CH_2OH$ with CONHR usually increased A_2 vs A_1 selectivity. On the other hand, the effects on affinity at A_1 and A_2 receptors of concurrent N^6 and C5' modifications, leading to N^6-substituted N-alkyladenosine-5'-uronamides, resulted to be less than additive.[7]

On this basis the synthesis of a new series of 2-(aryl)alkylthio derivatives of NECA was designed to improve the A_{2A} affinity and selectivity of 2-(aryl)alkylthioadenosines which were reported to possess coronary vasodilating activity[8,9] and platelet aggregation inhibitory activity.[10,11]

Chemistry

The synthesis of several 2-(aryl)alkylthio derivatives of adenosine was previously accomplished by the opening-closure method, firstly reported by Kikugawa and Suehido in 1975.[12] Unfortunately, this procedure was unsuccessful in the case of 2-(aryl)alkylthio derivatives of NECA, owing to the basic condition and the high temperature used in the opening step.

Alternatively, a new synthesis has been performed by reacting 2-iodoadenosine (**6**)[12] and 2-iodoNECA (**14**)[14-16] with the appropriate mercaptans in dry DMF at 120 °C using potassium carbonate as a catalyst (Scheme 1 and Table 1).



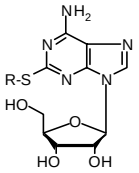
Scheme 1

Table 1. Preparation of compounds reported in Scheme 1

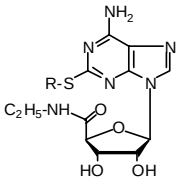
Compd no.	R	Time (h)	Chromatography solvent	Yield %	mp (°C)
7	$CH_2CH_2CH_2CH_2CH_3$	20	$CHCl_3$-CH_3OH-cC_6H_{12} (76:14:10)	32	177 dec lit 148-151
8	$CH_2CH_2CH_2CH_2OH$	16	$CHCl_3$-CH_3OH-cC_6H_{12} (72:18:10)	34	88-90
9	cC_5H_9	20	$CHCl_3$-CH_3OH-cC_6H_{12} (80:14:6)	38	213 dec lit 223-225
10	C_6H_5	20	$CHCl_3$-CH_3OH (88:12)	40	137-139 lit 125-126
11	$CH_2C_6H_5$	20	$CHCl_3$-CH_3OH-cC_6H_{12} (75:15:10)	63	158 dec lit 158
12	$CH_2CH_2C_6H_5$	16	$CHCl_3$-CH_3OH-cC_6H_{12} (77:13:10)	50	205 dec
13	$CH_2CH_2CH_2C_6H_5$	16	$CHCl_3$-CH_3OH-cC_6H_{12} (78:12:10)	37	92-95
15	$CH_2CH_2CH_2CH_2CH_3$	36	$CHCl_3$-CH_3OH-cC_6H_{12} (80:10:10)	11	> 270 dec
16	C_6H_5	6	$CHCl_3$-CH_3OH-cC_6H_{12} (80:10:10)	46	122-125
17	$CH_2C_6H_5$	6	$CHCl_3$-CH_3OH-cC_6H_{12} (80:10:10)	78	245 dec
18	$CH_2CH_2C_6H_5$	6	$CHCl_3$-CH_3OH-cC_6H_{12} (80:10:10)	57	97-99
19	$CH_2CH_2CH_2C_6H_5$	16	$CHCl_3$-CH_3OH (90:10)	31	185-188

Biological Results and Discussion

All the compounds were evaluated at the human recombinant adenosine receptors, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assay (A_{2B}). Receptor binding affinity was determined using [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) as radioligand for A_1 receptors, whereas [^{3}H]NECA was used for the A_{2A} and A_3 subtypes (K_i; nM). The relative potencies of these compounds for the A_{2B} subtype were measured by evaluating **TABLE 2.** Affinities of adenosine and NECA derivatives in radioligand binding assays at human A_1, A_{2A}, and A_3 adenosine receptors and effects on adenylyl cyclase activity at human A_{2B} adenosine receptor.



7-13



15-19

		K_i or EC_{50}, nM			
Cpd	R	K_i (A_1)[a]	K_i (A_{2A})[b]	EC_{50} (A_{2B})[c]	K_i (A_3)[d]
2 NECA		14 (16-28)	20 (12-35)	2,400 (1,900-3,000)	6.2 (3.5-11)
3 CGS 21680		289 (232-361)	27 (12-59)	89 μM (56-141)	67 (50-90)
4 HENECA		60 (50-72)	6.4 (3.8-11)	6,100 (4,000-9,300)	2.4 (2.0-2.9)
5 PHPNECA		2.7 (1.7-4.1)	3.1 (2.4-3.9)	1,100 (470-2,600)	0.42 (0.17-1.0)
7	CH_3-$(CH_2)_4$-	91 (83-99)	1,370 (993-1,890)	> 100 μM	365 (285-467)
15	CH_3-$(CH_2)_4$-	2,400 (2,150-2,690)	4,360 (3,840-4,950)	> 100 μM	1,490 (1,270-1,740)
8	HO-$(CH_2)_4$-	451 (427-477)	3,130 (2,920-3,360)	> 100 μM	1,030 (837-1,260)
9	cC_5H_9-	583 (498-682)	418 (318-685)	> 100 μM	843 (610-1,170)
10	C_6H_5-	611 (517-722)	990 (829-1,180)	> 100 μM	669 (563-794)
16	C_6H_5-	1,010 (937-1,100)	1,580 (602-4,170)	> 100 μM	182 (144-230)
11	C_6H_5-CH_2-	304 (260-354)	1,510 1,260-1,810)	> 100 μM	68 (54-85)
17	C_6H_5-CH_2-	480 (400-575)	615 (368-1,028)	> 100 μM	142 (115-175)
12	C_6H_5-$(CH_2)_2$-	99 (85-115)	85 (39-185)	> 100 μM	289 (191-438)
18	C_6H_5-$(CH_2)_2$-	189 (176-203)	24 (13-44)	> 100 μM	86 (42-173)
13	C_6H_5-$(CH_2)_3$-	264 (204-342)	1,640 (1,500-1.810)	> 100 μM	568 (463-696)
19	C_6H_5-$(CH_2)_3$-	466 (365-594)	1,020 (887-1,180)	> 100 μM	523 (353-774)

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

The receptor-stimulated adenylyl cyclase activity expressed as EC_{50}, nM. NECA (**2**), CGS 21680 (**3**), HENECA (**4**), and PHPNECA (**5**) were reported as reference compounds and the results are shown in Table 2.[17]

The reference compound NECA (**2)** showed high affinity at A_1, A_{2A}, and A_3 receptors and was slightly A_3 selective (K_i A_1 = 14 nM, K_i A_{2A} = 20 nM, and K_i A_3 = 6.2 nM). The potency for A_{2B} receptor, in the low micromolar range, characterized NECA as one of the most active nucleoside at this subtype (EC_{50} A_{2B} = 2,400 nM).

The introduction of a 4-(2-carboxyethyl)phenylethylamino group in 2-position of NECA resulted in a compound, CGS 21680 (**3**), which showed decreased affinity and potency at A_1, A_3, and A_{2B} adenosine receptor subtypes, while its affinity at the A_{2A} receptors was comparable to that of NECA, hence resulting A_{2A} selective [K_i A_1 = 290 nM, K_i A_{2A} = 27 nM, EC_{50} A_{2B} = 89 μM, and K_i A_3 = 67 nM]. On the other hand, the presence of alkynyl chains in 2-position of NECA (compounds **4** and **5**) resulted in improved A_{2A} affinity compared to NECA and CGS 21680 [HENECA (**4**) and PHPNECA(**5**), K_i A_{2A} = 6.4 nM, and K_i A_{2A} = 3.1 nM, respectively, *vs* NECA (**2**) and CGS 21680 (**3**), K_i A_{2A} = 20 nM, and K_i A_{2A} = 27 nM, respectively]. However, the increasing of A_{2A} affinity was characterized by a contemporary increase of the A_3 affinity, thus the two compounds were slightly A_3 selective as NECA itself. In particular, PHPNECA was found to be a very potent agonist at all adenosine receptor subtypes and one of the most potent A_{2B} agonist reported so far (**5**, K_i A_1 = 2.7 nM, K_i A_{2A} = 3.1 nM, EC_{50} A_{2B} = 1,100 nM, and K_i A_3 = 0.42 nM).[18]

The 2-(aryl)alkylthio derivatives of adenosine and NECA (compounds **7-13** and **15-19**, respectively) showed affinity at A_1, A_{2A}, and A_3 adenosine receptor subtypes ranging from low nM to low μM value. On the contrary, functional data demonstrated that they did not activate the A_{2B} receptors; in fact all the compounds possessed an EC_{50} A_{2B} > 100 μM.

Surprisingly, many compounds (**7**, **8**, **10**, **13**, **19**) showed slight selectivity for the A_1 receptor, a subtype that is known to be activated preferentially by N^6-substituted adenosine derivatives; the most potent was the 2-(1-pentyl)thioadenosine (**7**) with a K_i A_1 = 91 nM. The affinity of the corresponding NECA derivatives **15** decreased at all adenosine receptor subtypes, but these data are to be evaluated with caution because of problems of compound solubility. Substitution of the methyl of the 2-alkylthio chain in compound **7** with an hydroxyl group (compound **8**) decreased affinity at all receptors.

It is worthwhile to note that only three compounds (**9**, **12**, **18**) bound selectively the A_{2A} receptor subtype; thus, both in adenosine and in NECA series, the presence in 2-position of a phenylethylthio substituent was useful in order to improve A_{2A} affinity. In fact, the 2-phenylethylthioadenosine (**12**) and the 2-phenylethylthioNECA (**18**) were the most active A_{2A} agonists with a K_i A_{2A} = 85 nM and 24 nM, respectively. Once again, as in the case of CGS 21680, the presence of a particular substituent in the 2-position of NECA led to a compound which showed decreased affinity and potency at both A_1, A_3, and A_{2B} adenosine receptor subtypes, while maintaining a A_{2A} affinity comparable to that of NECA, hence resulting in A_{2A} selectivity.

On the other hand, the presence of a longer chain (phenylpropylthio) led to two derivatives, compounds **13** and **19**, which activated preferentially the A_1 receptors, while a shorter chain is better accommodated by the A_3 receptor subtype; in fact the 2-phenylmethylthioadenosine (**11**) and the 2-phenylmethylthioNECA (**17**) were A_3 selective and compounds **11** was the most active A_3 agonist among the two series with a K_i A_3 = 68 nM.

On the contrary, when the phenyl ring is directly linked to the sulfur atom the corresponding adenosine and NECA derivatives (compounds **10** and **16**, respectively) showed a different profile of activity; thus the 2-phenylthioadenosine was slightly A_1 selective, while the corresponding NECA derivatives **16** was A_3 selective.

In conclusion, although the preference for the different adenosine receptor subtypes seems do not clearly depend on the nature of the substituent present in the two position, in all cases the 2-(aryl)alkylthioadenosine derivatives are more potent than the corresponding NECA derivatives at A_1 receptors,[19] while the NECA derivatives possess higher affinity in comparison with adenosines at both A_{2A} and A_3 receptors, with the exception of compound **17** which is less active than the corresponding adenosine derivative **11** at A_3 receptor subtypes.

Conclusions

Biological data obtained with the two series of adenosine and NECA derivatives demonstrated that it is possible to modulate the activity at the A_1, A_{2A}, and A_3 adenosine receptor subtypes by introducing different (aryl)alkylthio substituents in the 2-position of adenosine and NECA. In fact the best compounds emerging from this study, 2-(1-pentyl)thioadenosine (**7**) with a K_i A_1 = 91 nM, 2-phenylethylthioNECA (**18**) with a K_i A_{2A} = 24 nM, and the 2-phenylmethylthioadenosine (**11**) with a K_i A_3 = 68 nM, could be useful tools to be modificated in order to find very potent and selective agonists for the human adenosine receptor subtypes.

Experimental Section

General Procedures. Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with Varian VXR 300 MHz spectrometer; ∂ in ppm, *J* in Hz. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses were determined on Carlo Erba model 1106 analyser and are within ± 0.4% of theoretical values.

Preparation of 2-(aryl)alkylthioadenosines (7-13)

A mixture of 2-iodoadenosine (**6**, 0.2 g, 0.51 mmol)[13] in 5 mL of dry DMF, 2.55 mmol of the appropriate mercaptan, and solid K_2CO_3 (150 mg, 1.05 mmol) was heated in a steel bomb at 120 °C for the time reported in Table 1. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents (Table 1) to give **7-13** as chromatographically pure white powders.

2-(1-Pentyl)thioadenosine (7). ^{1}H NMR (Me_2SO-d_6) ∂ 0.89 (t, 3H, *J* = 6.6 Hz, CH_2CH_3), 1.35 (m, 4H, $(CH_2)_2CH_3$), 1.65 (m, 2H, CH_2CH_2S), 3.04 (m, 2H, CH_2S), 3.60 (m, 2H, CH_2-5'), 3.92 (m, 1H, H-4'), 4.13 (m, 1H, H-3'), 4.62 (m, 1H, H-2'), 5.82 (d, 1H, *J* = 5.1 Hz, H-1'), 7.38 (bs, 2H, NH_2), 8.24 (s, 1H, H-8), ^{13}C NMR (Me_2SO-d_6) ∂ 14.62 (CH_3), 22.47 (CH_2CH_3), 29.52 ($CH_2CH_2CH_3$), 30.74 (CH_2CH_2S), 31.23 (CH_2S), 62.30 (C-5'), 71.16 (C-3'), 73.84 (C-2'), 86.09 (C-4'), 88.02 (C-1'), 117.60 (C-5), 139.51 (C-8), 150.80 (C-4), 156.19 (C-2), 164.47 (C-6). Anal. Calcd. for $C_{15}H_{23}N_5O_4S$ (369.4): C, 48.77; H, 6.28; N, 18.96. Found: C, 48.65; H, 6.15; N, 19.08.

2-(1-Butyl-4-hydroxy)thioadenosine (8). ^{1}H NMR (Me_2SO-d_6) ∂ 1.66 (m, 4H, $(CH_2)_2CH_2S$), 3.11 (m, 2H, CH_2S), 3.54 (m, 4H, CH_2-5' and CH_2OH), 3.92 (m, 1H, H-4'), 4.15 (m 1H, H-3'), 4.44 (m, 1H, CH_2OH), 4.62 (m, 1H, H-2'), 5.83 (d, *J* = 5.1 Hz, 1H, H-1'), 7.37 (bs, 2H, NH_2), 8.24 (s, 1H, H-8). Anal. Calcd. for $C_{14}H_{21}N_5O_5S$ (371.4): C, 45.27; H, 5.70; N, 18.86. Found: C, 45.08; H, 5.39; N, 18.98.

2-Cyclopentylthioadenosine (9). ^{1}H NMR (Me_2SO-d_6) ∂ 1.60 (m, 6H, cyclopentyl), 2.15 (m, 2H, cyclopentyl), 3.57 (m, 2H, CH_2-5'), 3.92 (m, 2H, H-4' and CHS), 4.12 (m 1H, H-3'), 4.64 (m, 1H, H-2'), 5.82 (d, *J* = 5.9 Hz, 1H, H-1'), 7.36 (bs, 2H, NH_2), 8.24 (s, 1H, H-8). Anal. Calcd. for $C_{15}H_{21}N_5O_4S$ (367.4): C, 49.03; H, 5.76; N, 19.06. Found: C, 48.81; H, 5.46; N, 19.30.

2-Phenylthioadenosine (10). ^{1}H NMR (Me_2SO-d_6) ∂ 3.32 (m, 2H, CH_2-5'), 3.84 (m, 2H, H-4' and H-3'), 4.55 (m, 1H, H-2'), 5.67 (d, *J* = 6.1 Hz, 1H, H-1'), 7.45 (m, 5H, H-Ph and NH_2), 7.60 (m, 2H, H-Ph), 8.24 (s, 1H, H-8). Anal. Calcd. for $C_{16}H_{17}N_5O_4S$ (375.4): C, 51.19; H, 4.56; N, 18.66. Found: C, 51.02; H, 4.44; N, 18.75.

2-Benzylthioadenosine (11). ^{1}H NMR (Me_2SO-d_6) ∂ 3.60 (m, 2H, CH_2-5'), 3.93 (m, 1H, H-4'), 4.15 (m, 1H, H-3'), 4.38 (s, 2H, CH_2S), 4.57 (m, 1H, H-2'), 5.88 (d, 1H, *J* = 6.1 Hz, H-1'), 7.30 (m, 3H, H-Ph), 7.46 (m, 4H, H-Ph and NH_2), 8.27 (s, 1H, H-8). Anal. Calcd. for $C_{17}H_{19}N_5O_4S$ (389.4): C, 52.43; H, 4.92; N, 17.98. Found: C, 52.34; H, 4.76; N, 18.15.

2-(1-Ethyl-2-phenyl)thioadenosine (12). ^{1}H NMR (Me_2SO-d_6) ∂ 2.99 (m, 2H, CH_2Ph), 3.32 (m, 2H, CH_2S), 3.61 (m, 2H, CH_2-5'), 3.96 (m, 1H, H-4'), 4.15 (m, 1H, H-3'), 4.62 (t, 1H, *J* = 5.5 Hz, H-2'), 5.92 (d, 1H, *J* = 6.1 Hz, H-1'), 7.30 (m, 5H, H-Ph), 7.45 (bs, 2H, NH_2), 8.29 (s, 1H, H-8), ^{13}C NMR (Me_2SO-d_6) ∂ 34.35 (CH_2S-), 61.46 (C-5'), 70.40 (C-3'), 73.50 (C-2'), 85.41 (C-4'), 87.26 (C-1'), 116.96 (C-5), 126.85 (C-Ph), 128.32 (C-Ph), 129.08 (C-Ph), 138.66 (C-8 and C-Ph), 150.10 (C-4), 155.53 (C-2), 163.19 (C-6). Anal. Calcd. for $C_{18}H_{21}N_5O_4S$ (403.5): C, 53.59; H, 5.25; N, 17.36. Found: C, 53.27; H, 5.18; N, 17.48.

2-(3-Phenyl-1-propyl)thioadenosine (13). ^{1}H NMR (Me_2SO-d_6) ∂ 1.98 (m, 2H, CH_2CH_2Ph), 2.74 (t, *J* = 7.1 Hz, 2H, CH_2Ph), 3.09 (m, 2H, CH_2S), 3.94 (m, 1H, H-4'), 4.16 (m, 1H, H-3'), 4.63 (m, 1H, H-2'), 5.84 (d, *J* = 5.9 Hz, 1H, H-1'), 7.25 (m, 5H, H-Ph), 7.39 (bs, 2H, NH_2), 8.25 (s, 1H, H-8). Anal. Calcd. for $C_{19}H_{23}N_5O_4S$ (417.5): C, 54.66; H, 5.55; N, 16.78. Found: C, 54.48; H, 5.37; N, 16.85.

Preparation of 5-(6-amino-2-(aryl)alkylthiopurin-9-yl)-3,4-dihydroxytetrahydro furan -2-carboxylic acid ethylamides (15-19)

A mixture of 5-(6-amino-2-iodopurin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carboxylic acid ethylamide (2-iodoNECA) (**14**, 0.15 g, 0.35 mmol)[14] in 5 mL of dry DMF, 1.75 mmol of the appropriate mercaptan, and solid K_2CO_3 (150 mg, 1.05 mmol) was heated in a steel bomb at 120 °C for the time reported in Table 1. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents (Table 1) to give **15-19** as chromatographically pure white powders.

5-(6-Amino-2-pentylsulfanylpurin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carboxylic acid ethylamide (15). ^{1}H NMR (Me_2SO-d_6) ∂ 0.89 (t, *J* = 6.9 Hz, 3H, $(CH_2)_4CH_3$), 1.04 (t, *J* = 7.0 Hz, 3H, CH_2CH_3), 1.32 (m, 4H, $(CH_2)_2CH_3$), 1.67 (m, 2H, CH_2CH_2S), 3.12 (m, 4H, CH_2CH_3 and CH_2S), 4.20 (m, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.72 (m, 1H, H-2'), 5.92 (d, *J* = 7.2 Hz, 1H, H-1'), 7.45 (bs, 2H, NH_2), 8.24 (t, *J* = 5.9 Hz, 1H, NH), 8.33 (s, 1H, H-8). Anal. Calcd. for $C_{17}H_{26}N_6O_4S$ (410.5): C, 49.74; H, 6.38; N, 20.47. Found: C, 49.54; H, 6.38; N, 20.47.

5-(6-Amino-2-phenylsulfanylpurin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carboxylic acid ethylamide (16). ^{1}H NMR (Me_2SO-d_6) ∂ 1.04 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 3.18 (m, 2H, CH_2CH_3), 4.12 (m, 1H, H-3'), 4.27 (s 1H, H-4'), 4.65 (m, 1H, H-2'), 5.82 (d, *J* = 7.2 Hz, 1H, H-1'), 7.45 (m, 5H, H-Ph and NH_2), 7.58 (m, 2H, H-Ph), 8.14 (t, *J* = 5.9 Hz, 1H, NH), 8.37 (s, 1H, H-8). Anal. Calcd. for $C_{18}H_{20}N_6O_4S$ (416.5): C, 51.91; H, 4.84; N, 20.18. Found: C, 51.63; H, 4.56; N, 20.54.

5-(6-Amino-2-benzylsulfanylpurin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carboxylic acid ethylamide (17). ^{1}H NMR (Me_2SO-d_6) ∂ 1.02 (t, *J* = 6.8 Hz, 3H, CH_2CH_3), 3.19 (m, 2H, CH_2CH_3), 4.18 (m, 1H, H-3'), 4.31 (s 1H, H-4'), 4.38 (s, 2H, CH_2-S), 4.64 (m, 1H, H-2'), 5.96 (d, *J* = 6.7 Hz, 1H, H-1'), 7.26 (m, 3H, H-Ph), 7.50 (m, 4H, H-Ph and NH_2), 8.27 (t, *J* = 5.4 Hz, 1H, NH), 8.36 (s, 1H, H-8). Anal. Calcd. for $C_{19}H_{22}N_6O_4S$ (430.5) C, 53.01; H, 5.15; N, 19.52. Found: C, 52.75; H, 4.95; N, 19.87.

5-(6-Amino-2-phenethylsulfanylpurin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carboxylic acid ethylamide (18). ^{1}H NMR (Me_2SO-d_6) ∂ 1.03 (t, *J* = 7.0 Hz, 3H, CH_2CH_3), 2.97 (m, 2H, CH_2Ph), 3.10-3.50 (m, 4H, CH_2CH_3 and CH_2S), 4.19 (m, 1H, H-3'), 4.32 (s, 1H, H-4'), 4.70 (m, 1H, H-2'), 5.98 (d, *J* = 7.0 Hz, 1H, H-1'), 7.39 (m, 5H, H-Ph), 7.50 (bs, 2H, NH_2), 8.26 (t, *J* = 5.8 Hz, 1H, NH), 8.36 (s, 1H, H-8), ^{13}C NMR (Me_2SO-d_6) ∂ 14.74 (CH_3), 31.87, 33.37, 35.59 (CH_2N, CH_2Ph and CH_2S), 72.47, 73.01 (C-2' and C-3'), 84.17 (C-4'), 86.95 (C-1'), 117.07 (C-5), 126.15 (C-Ph), 128.36 (C-Ph), 128.72 (C-Ph), 139.14, 140.58 (C-Ph and C-8), 150.34 (C-4), 155.64 (C-2), 163.48 (C-6), 169.13 (CO). Anal. Calcd. for $C_{20}H_{24}N_6O_4S$ (444.5): C, 54.04; H, 5.44; N, 18.91. Found: C, 53.79; H, 5.22; N, 19.17.

5-[6-Amino-2-(3-phenylpropylsulfanyl)purin-9-yl]-3,4-dihydroxytetrahydrofuran-2-carboxylic acid ethylamide (19). ^{1}H NMR (Me_2SO-d_6) ∂ 1.05 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 1.97 (m, 2H, CH_2CH_2Ph), 2.73 (m, 2H, CH_2Ph), 2.97-3.34 (m, 4H, CH_2CH_3 and CH_2S), 4.23 (m, 1H, H-3'), 4.32 (s, 1H, H-4'), 4.62 (m, 1H, H-2'), 5.94 (d, *J* = 7.2 Hz, 1H, H-1'), 7.28 (m, 5H, H-Ph), 7.45 (bs, 2H, NH_2), 8.25 (t, *J* = 5.7 Hz, 1H, NH), 8.34 (s, 1H, H-8). Anal. Calcd. for $C_{21}H_{26}N_6O_4S$ (458.5): C, 55.01; H, 5.72; N, 18.33. Found: C, 54.65; H, 5.49; N, 18.66.

Biological studies

Cloning of the human adenosine receptors, stable transfection of cells, cell culture, membrane preparation, radioligand binding, and adenylyl cyclase activity have been fully described elsewhere.[20] Briefly, all human subtypes were stably transfected into Chinese hamster ovary (CHO) cells in order to be able to study their pharmacological profile in an identical cellular background utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assays (A_{2B}).

Receptor binding affinity was determined using [^{3}H]CCPA as radioligand at A_1 receptors, whereas [^{3}H]NECA was used at A_{2A} and A_3 subtypes. The procedure was performed as described previously. Due to the lack of a suitable radioligand the relative potency of agonists at A_{2B} adenosine receptors was determined in adenylyl cyclase experiments. The procedure was carried out as described previously with minor modifications. [20]

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