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Structure–activity relationships of 2-chloro-*N*^{6}-substituted-4′-thioadenosine-5′-*N*,*N*-dialkyluronamides as human A_3 adenosine receptor antagonists

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Abstract

On the basis of potent and selective A_3 adenosine receptor (AR) antagonist, 2-chloro-*N*^{6}-(3-iodobenzyl)-4′-thioadeno-sine-5′-*N*,*N*-dimethyluronamide, structure–activity relationships were studied for a series of 5′-*N*,*N*-dialkyluronamide derivatives, synthesized from D-gulonic γ-lactone. From this study, it was revealed that removal of the hydrogen bond-donating ability of the 5′-uronamide was essential for the pure A_3AR antagonism. 5′-*N*,*N*-Dimethyluronamide derivatives exhibited higher binding affinity than larger 5′-*N*,*N*-dialkyl or 5′-*N*,*N*-cycloalkylamide derivatives, indicating that steric factors are crucial in binding to the human A_3AR. A *N*^{6}-(3-bromobenzyl) derivative **6c** (K_i = 9.32 nM) exhibited the highest binding affinity at the human A_3AR with very low binding affinities to other AR subtypes.

Keywords

4′-Thionucleoside; A_3 adenosine receptor antagonist; Conformational change; Radioligand binding

Adenosine (**1**) regulates many physiological functions through four subtypes (A_1, A_{2a}, A_{2b}, and A_3) of adenosine receptors (AR).[1] Among these subtypes, activation of the A_3AR inhibits adenylate cyclase to lower the level of cAMP.[1] Also, activation of the A_3AR stimulates phospholipase C (PLC) through the β, γ subunit of the G protein, to increase levels of inositol 1,4,5-tris-phosphate (IP_3). Control of A_3AR is thought to be relevant to experimental treatment modalities for a variety of disorders related to these signal

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transduction pathways, such as cancer, cerebral or cardiac ischemia, glaucoma, allergic conditions, and inflammation.[1,2]

On the basis of the structure of adenosine (**1**), worldwide efforts have been made to discover potent and selective human A_3AR agonists and antagonists.[2,3] Modification on the N^6- and/or 4′-hydroxymethyl group of **1** resulted in **2** (Cl-IB-MECA) as a potent and selective human A_3AR full agonist[4] (Chart 1). Bioisosteric replacement of the furanose oxygen of **2** with a sulfur atom produced another potent and selective human A_3AR full agonist **3** (LJ-529).[5] However, until recently it has been difficult to discover potent and selective human A_3AR full antagonists with a nucleoside skeleton because of the tendency of derivatives of adenosine (**1**) to act as full agonists. Thus, most of the potent and selective antagonists for the human A_3AR belong to the category of nonpurine heterocyclic compounds,[6–10] which act competitively with adenosine derivatives at the orthosteric binding site of the A_3AR. However, these nonpurine heterocyclic compounds showed weak binding affinity at the rat A_3AR[11,12] and were unsuitable for efficacy evaluation in small animal models and thus not optimal for further drug development. Therefore, it has been highly desirable to discover potent and selective A_3AR full antagonists of which the affinity and selectivity are independent of species. Since it was reported that antagonists with a nucleoside skeleton[13] could be species-independent, potent, and selective A_3AR full antagonists, we searched for novel nucleoside analogues, among which appending an additional methyl group to the 5′-uronamide nitrogen of compounds **2** and **3** converted these A_3AR full agonists into the potent and selective A_3AR full antagonists **4** and **5**.[14] 4′-Thionucleoside analogue **5** was found to be a more potent and selective antagonist than the corresponding 4′-oxonucleoside analogue **4**.[14] Thus, on the basis of these findings, we modified the N^6- and 4′-hydroxymethyl groups of 4′-thionucleoside analogue **5** in order to search for potent and selective A_3AR full antagonists. Herein, we report the structure–activity relationship (SAR) study of 2-chloro-N^6-substituted-4′-thioadenosine-5′-*N*,*N*-dialkylamides at the human A_3AR.

Synthesis of the key intermediates **11a**–**j** started from D-gulonic γ-lactone (**7**), as shown in Scheme 1.

D-Gulonic γ-lactone (**7**) was converted to the glycosyl donor **8**, using our previously published procedure.[5,15] Pummerer-type condensation of **8** with 2,6-dichloropurine in the presence of TMSOTf and Et_3N afforded the desired nucleoside **9**[5,15] as a single diastereomer.

Treatment of 2,6-dichloropurine derivative **9** with various alkyl or arylalkyl amines gave N^6-substituted analogues **10a**–**j**. Because of the difficulty in removing the isopropylidene group at the final step, the isopropylidene intermediates **10a**–**j** were converted to the TBS derivatives by treatment with 80% acetic acid followed by reprotection with TBSOTf. The resulting compounds were treated with sodium methoxide to give the key intermediates **11a**–**j**.

Conversion of **11a**–**j** to the target nucleosides **6a**–**r** is illustrated in Scheme 2. The primary hydroxyl groups of **11a**–**j** were oxidized by treatment with PDC in DMF to the carboxylic

acids **12a**–**j**, which were converted to various tertiary amides **6a**–**r**[16] by coupling the acids **12a**–**j** with various dialkyl or cycloalkyl amines in the presence of EDC and HOBt.

Binding assays were carried out using standard radioligands in Chinese hamster ovary (CHO) cells stably expressing a human AR subtype,[14,17] and binding affinities of the final nucleosides **6a**–**r** at the three subtypes of human ARs are shown in Table 1. In general, 5′-*N,N*-dimethylamide derivatives exhibited higher binding affinity than larger 5′-*N,N*-dialkyl or 5′-*N,N*-cycloalkylamide derivatives indicating that steric factors are crucial in binding to human A_3AR. The effects on the binding affinity of various N^6-substituted analogues also having a 5′-*N,N*-dimethyl group at the 5′-uronamide position were examined. The N^6-(3-halobenzyl) series generally showed better binding affinity at the human A_3AR than the N^6-dialkyl or N^6-cycloalkyl series. Within the N^6-(3-halobenzyl) series, the rank order of binding affinity at the human A_3AR was 3-bromobenzyl > 3-iodobenzyl > 3-chlorobenzyl > 3-fluorobenzyl. This result indicated that the human A_3AR prefers bromo as an optimal size of 3-halo substitution, and larger (I) or smaller (F) substitution reduced binding affinity. Among compounds tested, compound **6c** (K_i = 9.32 nM) exhibited the highest binding affinity at the human A_3AR with very low binding affinities to other AR subtypes.

The functional efficacy of compounds **5**[14] and **6c** showed that high binding affinity at human A_3AR was determined by inhibition of forskolin-stimulated cAMP production in AR-transfected CHO cells and measured at a concentration of 10 μM, in comparison to the maximal effect of a full agonist, *N*-ethyl-5′-*N*-ethylcarboxamidoadenosine (NECA), at 10 μM. In these functional assays, A_3AR agonism was absent in these compounds, indicating that these analogues are pure A_3AR antagonists, while the concentration–response curve for Cl-IB-MECA (**2**) indicated full agonism, as previously reported,[4] with an EC_{50} of 1.2 nM at the human A_3AR. To probe species differences, the affinity of *N,N*-5′-dimethylamide derivative **5** was also measured at the rat A_3AR. Although the affinity decreased with respect to the affinity at the human A_3AR, this compound showed moderate affinity (K_i = 321 ± 74 nM) at the rat A_3AR.[14]

Molecular modeling of the A_3AR indicated that the hydrogen bond-donating ability of the 5′-uronamide is responsible for the conformational change needed for receptor activation.[18,19] Because the synthesized 5′-*N,N*-dialkyl derivatives which removed the hydrogen bond-donating ability of the 5′-uronamide could not lead to the conformational change, resulting in the loss of ability to activate the A_3AR, the 5′-*N,N*-dialkyl series of compounds act as pure antagonists.

In summary, on the basis of potent and selective human A_3AR antagonism of the dimethyl derivative **5**, we carried out structure–activity relationship studies of 2-chloro-N^6-substituted-4′-thioadenosine-5′-*N,N*-dial-kylamides. From this study, we identified compound **6c** as a highly potent and selective human A_3AR antagonist, in which removal of the hydrogen bond-donating ability of the 5′-uronamide was essential for the pure A_3AR antagonism. 5′-*N,N*-dimethyluronamide derivatives generally exhibited higher binding affinity than larger 5′-*N,N*-dialkyl or 5′-*N,N*-dicycloalkylamide derivatives, indicating that steric factors in the 5′ region are important in binding of nucleosides to the human A_3AR.

The newly synthesized A_3AR antagonists studied here could be evaluated in models of a number of disorders related to the A_3AR, such as glaucoma, inflammation, and asthma.

Acknowledgments

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References and notes

1. Fredholm BB; IJzerman AP; Jacobson KA; Klotz KN; Linden J Pharmacol. Rev 2001, 53, 527. [PubMed: 11734617]
2. Jacobson KA; Gao Z-G Nat. Rev. Drug Discov 2006, 5, 247. [PubMed: 16518376]
3. Baraldi PG; Cacciari B; Romagnoli R; Merighi S; Varani K; Borea PA; Spalluto G Med. Res. Rev 2000, 20, 103. [PubMed: 10723024]
4. Kim HO; Ji X.-d.; Siddiqi SM; Olah ME; Stiles GL; Jacobson KA J. Med. Chem 1994, 37, 3614. [PubMed: 7932588]
5. (a)Jeong LS; Jin DZ; Kim HO; Shin DH; Moon HR; Gunaga P; Chun MW; Kim Y-C; Melman N; Gao Z-G; Jacobson KA J. Med. Chem 2003, 46, 3775; [PubMed: 12930138] (b)Jeong LS; Lee HW; Jacobson KA; Kim HO; Shin DH; Lee JA; Gao ZG; Lu C; Duong HT; Gunaga P; Lee SK; Jin DZ; Chun MW; Moon HR J. Med. Chem 2006, 49, 273. [PubMed: 16392812]
6. Kim YC; Ji XD; Jacobson KA J. Med. Chem 1996, 39, 4142. [PubMed: 8863790]
7. van Muijlwijk-Koezen JE; Timmerman H; Link R; van der Goot H; IJzerman AP J. Med. Chem 1998, 41, 3994. [PubMed: 9767637]
8. Baraldi PG; Cacciari B; Moro S; Spalluto G; Pastorin G; Da Ros T; Klotz K-N; Varani K; Gessi S; Borea PA J. Med. Chem 2002, 45, 770. [PubMed: 11831890]
9. Maconi A; Pastorin G; Da Ros T; Spalluto G; Gao ZG; Jacobson KA; Baraldi PG; Cacciari B; Varani K; Moro S; Borea PA J. Med. Chem 2002, 45, 3579. [PubMed: 12166930]
10. Okamura T; Kurogi Y; Hashimoto K; Sato S; Nishikawa H; Kiryu K; Nagao Y Bioorg. Med. Chem. Lett 2004, 14, 3775. [PubMed: 15203160]
11. Moro S; Spalluto G; Gao ZG; Jacobson KA Med. Res. Rev 2006, 26, 131. [PubMed: 16380972]
12. Yang H; Avila MY; Peterson-Yantorno K; Coca-Prados M; Stone RA; Jacobson KA; Civan MM Curr. Eye Res 2005, 30, 747. [PubMed: 16146920]
13. Gao ZG; Kim SK; Biadatti T; Chen W; Lee K; Barak D; Kim SG; Johnson CR; Jacobson KA J. Med. Chem 2002, 45, 4471. [PubMed: 12238926]
14. Gao Z-G; Joshi BV; Klutz A; Kim S-K; Lee HW; Kim HO; Jeong LS; Jacobson KA Bioorg. Med. Chem. Lett 2006, 16, 596. [PubMed: 16289820]
15. Jeong LS; Lee HW; Kim HO; Jung JY; Gao Z-G; Duong HT; Rao S; Jacobson KA; Shin DH; Lee JA; Gunaga P; Lee SK; Jin DZ; Chun MW Bioorg. Med. Chem 2006, 14, 4718. [PubMed: 16603368]
16. General procedure for the synthesis of **6a**–**r**: To a stirred solution of per mmol of 4′-hydroxymethyl analogues (**11a**–**j**) in dry DMF (10 mL) was added pyridinium dichromate (10.0 equiv), and the reaction mixture was stirred at rt for 20 h. After being poured into water (50 mL/mmol), the reaction mixture was stirred at rt for 1 h. The precipitate was filtered, and the filter cake was washed with excess water and dried under high vacuum to give brownish solid, which was used in the next step without further purification. To a solution of per mmol of acid derivatives (**12a**–**j**), EDC (1.5 equiv), HOBt (1.5 equiv), and appropriate amine (1.5 equiv) in CH_2Cl_2 (20 mL) was added DIPEA (3.0 equiv), and the mixture was stirred at rt for 12 h. The reaction mixture was evaporated, and the residue was purified by a silica gel column chromatography (hexane/EtOAc = 10:1–5:1) to give silyl amides as a white foam. To a stirred solution of per mmol of silyl amides in THF (5 mL) was added TBAF (2.5 equiv) and the reaction mixture was stirred at rt for 1 h. The solvent was evaporated and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/EtOAc/MeOH = 20:20:1) to give **6a**–**r**. Compound **6c**: yield 67%; white

solid; mp 180.4–182.0 °C; $[\alpha]_D^{20}$ − 16.5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 274 nm (pH 7); ^{1}H NMR (CD_3OD) δ 2.94 (s, 3 H, N–CH_3), 3.03 (s, 3 H, N–CH_3), 4.41 (d, 1H, *J* = 4.6 Hz, 4′-H), 4.57 (br dd, 1H, *J* = 4.6, 8.4 Hz, 3′-H), 4.64 (m, 1H, 2′-H), 4.73 (d, 2H, *J* = 5.7 Hz, N–CH_2), 5.96 (d, 1H, *J* = 5.4 Hz, 1′-H), 7.22–7.56 (m, 4H, aromatic H), 8.52 (s, 1H, H-8); ^{13}C NMR (CD_3OD) δ 36.6, 38.2, 44.6, 54.3, 64.6, 77.2, 80.7, 127.4, 128.8, 130.0, 131.1, 134.9, 141.4, 142.8, 143.1, 151.7, 155.8, 156.4, 172.8; FAB-MS *m*/*z* 528 (M^++H); Anal. ($C_{19}H_{20}BrClN_6O_3S$) C, H, N, S.

17. (a)Gao Z-G; Blaustein JB; Gross AS; Melman N; Jacobson KA Biochem. Pharmacol 2003, 65, 1675; [PubMed: 12754103] (b)Gao Z-G; Jeong LS; Moon HR; Kim HO; Choi WJ; Shin DH; Elhalem E; Comin MJ; Melman N; Mamedova L; Gross AS; Rodriguez JB; Jacobson KA Biochem. Pharmacol 2004, 67, 893. [PubMed: 15104242]
18. van Tilburg EW; von Frijtag Drabbe Künzel J; de Groote M; IJzerman AP J. Med. Chem 2002, 45, 420. [PubMed: 11784146]
19. Kim SK; Jacobson KA J. Chem. Inf. Model 2007, 47, 1225. [PubMed: 17338510]

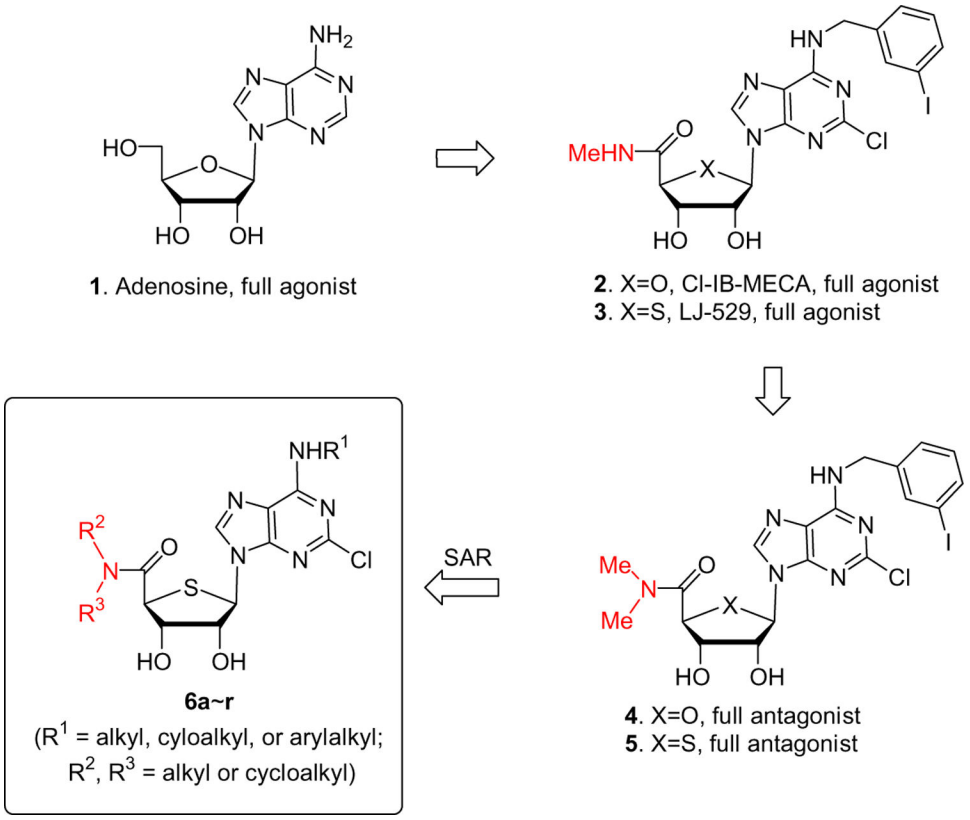
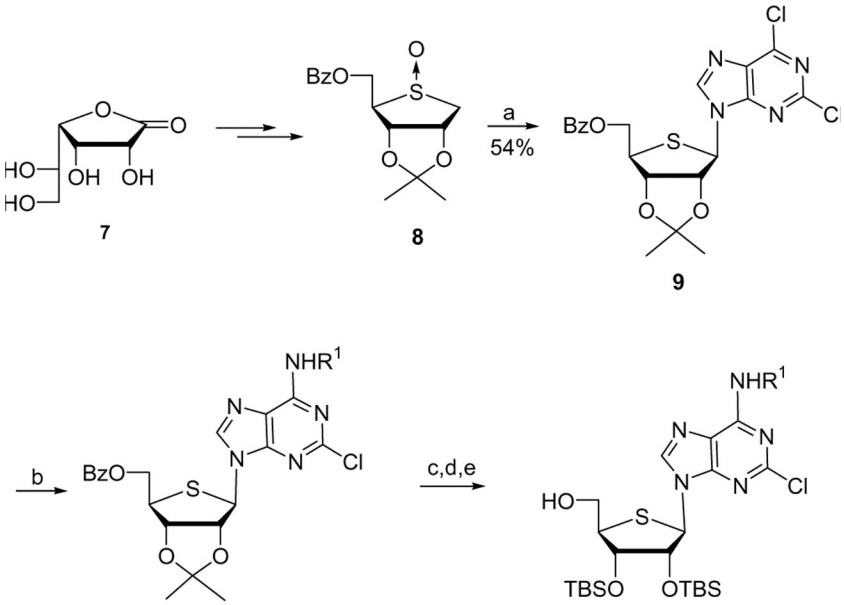


Chart 1.

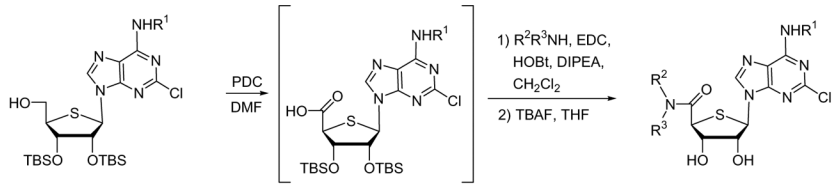


10a (R^1 = 3-iodobenzyl) (90%)
10b (R^1 = 3-fluorobenzyl) (91%)
10c (R^1 = 3-chlorobenzyl) (93%)
10d (R^1 = 3-bromobenzyl) (96%)
10e (R^1 = dimethyl) (88%)*
10f (R^1 = 2-methoxymethyl) (87%)
10g (R^1 = cyclopropyl) (84%)
10h (R^1 = cyclopropylmethyl) (93%)
10i (R^1 = cyclobutyl) (89%)
10j (R^1 = cyclopentyl) (85%)

11a (R^1 = 3-iodobenzyl) (80%)
11b (R^1 = 3-fluorobenzyl) (71%)
11c (R^1 = 3-chlorobenzyl) (85%)
11d (R^1 = 3-bromobenzyl) (80%)
11e (R^1 = dimethyl) (71%)*
11f (R^1 = 2-methoxymethyl) (69%)
11g (R^1 = cyclopropyl) (74%)
11h (R^1 = cyclopropylmethyl) (80%)
11i (R^1 = cyclobutyl) (83%)
11j (R^1 = cyclopentyl) (76%)

Scheme 1.

Reagents and conditions: (a) 2,6-dichloropurine, TMSOTf, Et_3N, CH_3CN–$ClCH_2CH_2Cl$, rt to 83 °C; (b) R^1NH_2, Et_3N, EtOH, rt; (c) 80% AcOH, 70 °C; (d) TBSOTf, pyridine, 50 °C; (e) NaOMe, MeOH. *N^6 $(CH_3)_2$.



11a (R^1 = 3-iodobenzyl)
11b (R^1 = 3-fluorobenzyl)
11c (R^1 = 3-chlorobenzyl)
11d (R^1 = 3-bromobenzyl)
11e (R^1 = dimethyl)*
11f (R^1 = 2-methoxymethyl)
11g (R^1 = cyclopropyl)
11h (R^1 = cyclopropylmethyl)
11i (R^1 = cyclobutyl)
11j (R^1 = cyclopentyl)

12a (R^1 = 3-iodobenzyl)
12b (R^1 = 3-fluorobenzyl)
12c (R^1 = 3-chlorobenzyl)
12d (R^1 = 3-bromobenzyl)
12e (R^1 = dimethyl)*
12f (R^1 = 2-methoxymethyl)
12g (R^1 = cyclopropyl)
12h (R^1 = cyclopropylmethyl)
12i (R^1 = cyclobutyl)
12j (R^1 = cyclopentyl)

5 (R^1 = 3-iodobenzyl, R^2 = R^3 = Me) (64%)
6a (R^1 = 3-fluorobenzyl, R^2 = R^3 = Me) (58%)
6b (R^1 = 3-chlorobenzyl, R^2 = R^3 = Me) (60%)
6c (R^1 = 3-bromobenzyl, R^2 = R^3 = Me) (67%)
6d (R^1 = dimethyl, R^2 = R^3 = Me) (51%)*
6e (R^1 = 2-methoxyethyl, R^2 = R^3 = Me (48%)
6f (R^1 = cyclopropyl, R^2 = R^3 = Me) (68%)
6g (R^1 = cyclopropylmethyl, R^2 = R^3 = Me) (59%)
6h (R^1 = cyclobutyl, R^2 = R^3 = Me) (66%)
6i (R^1 = cyclopentyl, R^2 = R^3 = Me) (66%)
6j (R^1 = 3-iodobenzyl, R^2 = Me, R^3 = propyl) (48%)
6k (R^1 = 3-iodobenzyl, R^2 = Me, R^3 = CH_2CH_2OH) (45%)
6l (R^1 = 3-iodobenzyl, R^2 = Et, R^3 = phenyl) (50%)
6m (R^1 = 3-iodobenzyl, R^2 = R^3 = piperidine) (63%)
6n (R^1 = 3-iodobenzyl, R^2 = R^3 = 4-methylpiperzine) (42%)
6o (R^1 = 3-iodobenzyl, R^2 = R^3 = azetidine) (55%)
6p (R^1 = 3-iodobenzyl, R^2 = R^3 = pyrrolidine) (61%)
6q (R^1 = 3-iodobenzyl, R^2 = R^3 = 4-hydroxypiperidine) (48%)
6r (R^1 = 3-iodobenzyl, R^2 = R^3 = thiomorpholine) (64%)

Scheme 2.
*N^6 $(CH_3)_2$.

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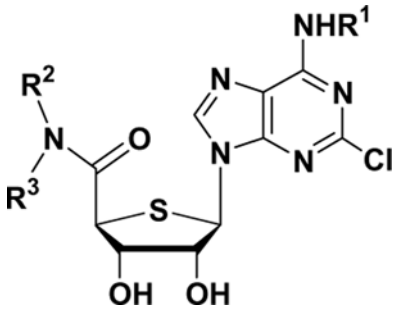
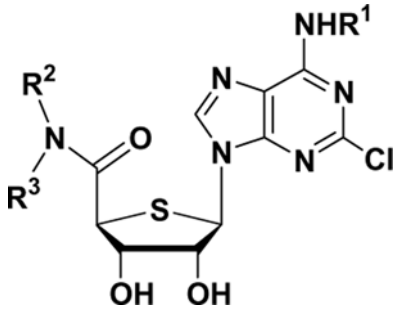
Table 1.

Potency of 2-chloro-4′-thioadenosine-5′-dialkylamide derivatives in binding at human A_1, A_{2A}, and A_3ARs expressed in CHO cells



Compound No. (R^1,R^2,R^3)	K_i (nM ± SEM) or % inhibition at 10 μM[a]		
	hA_1AR[b]	$hA_{2A}AR$[b]	hA_3AR[b]
2 [c]	222 ± 22	5360 ± 2470	1.4 ± 0.3
3 [c]	193 ± 46	223 ± 36	0.38 ± 0.07
4 [d]	5870 ± 930	>10,000	29.0 ± 4.9
5[d] (3-iodobenzyl, Me, Me)	6220 ± 640	>10,000	15.5 ± 3.1
6a (3-fluorobenzyl, Me, Me)	(*16* ± *4%*)	(*1%*)	121 ± 11
6b (3-chlorobenzyl, Me, Me)	(*10* ± *8%*)	(*−1%*)	21.3 ± 2.1
6c (3-bromobenzyl, Me, Me)	(*37* ± *2%*)	(*7%*)	9.32 ± 3.20
6d (Me_2, Me, Me)[e]	(*8* ± *2%*)	(*−2%*)	136
6e (2-methoxyethyl, Me, Me)	(*−7* ± *7%*)	(*1%*)	425
6f (cyclopropyl, Me, Me)	(*7* ± *16%*)	(*−8* ± *4%*)	109 ± 16
6g (cyclopropylmethyl, Me, Me)	404 ± 120	(*0* ± *6%*)	30.7 ± 9.4
6h (cyclobutyl, Me, Me)	345 ± 152	(*10%*)	115 ± 50
6i (cyclopentyl, Me, Me)	(*18* ± *21%*)	(*4* ± *27%*)	837
6j (3-iodobenzyl, Me, Propyl)	(*50* ± *1%*)	(*5%*)	727
6k (3-iodobenzyl, Me, CH_2CH_2OH)	237 ± 6	(*13%*)	126 ± 17
6l (3-iodobenzyl, Et, phenyl)	(*18* ± *5%*)	(*8%*)	398



Compound No. (R^1,R^2,R^3)	K_i (nM ± SEM) or % inhibition at 10 μM[a]		
	hA_1AR[b]	$hA_{2A}AR$[b]	hA_3AR[b]
6m (3-iodobenzyl, piperidine)	(*27* ± *5%*)	(*7%*)	565
6n (3-iodobenzyl, 4-methylpiperazine)	(*19* ± *5%*)	(*−7%*)	667
6o (3-iodobenzyl, azetidine)	(*8* ± *6%*)	(*12%*)	43.4 ± 2.6
6p (3-iodobenzyl, pyrrolidine)	(*20* ± *3%*)	(*14%*)	117 ± 31
6q (3-iodobenzyl, 4-hydroxypiperidine)	(*16* ± *7%*)	(*10%*)	1530
6r (3-iodobenzyl, thiomorpholine)	(*19* ± *9%*)	(*15%*)	867

[a] All experiments were done on CHO cells stably expressing one of three subtypes of human ARs. The binding affinity for A_1, A_{2A}, and A_3ARs was expressed as K_i values and was determined by using agonist radioligands ([^{3}H]CCPA), ([^{3}H]CGS21680), [^{125}I]I-AB-MECA, respectively. Values in parentheses are for weak binding, corresponding to an $IC_{50} \geqslant 10$ μM. Data are expressed as means ± standard error.

[b] K_i in binding, unless noted.

[c] K_i values from Ref. 5.

[d] K_i values from Ref. 14.

[e] N^6 $(CH_3)_2$.