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Structural Sweet Spot for A_1 Adenosine Receptor Activation by Truncated (N)-Methanocarba Nucleosides: Receptor Docking and Potent Anticonvulsant Activity

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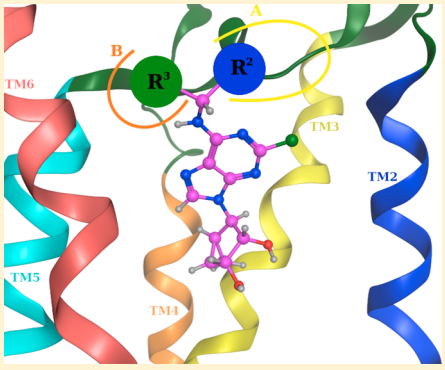
Supporting Information

ABSTRACT: A_1 adenosine receptor (AR) agonists display antiischemic and antiepileptic neuroprotective activity, but peripheral cardiovascular side effects impeded their development. SAR study of N^6-cycloalkylmethyl 4′-truncated (N)-methanocarba-adenosines identified **10** (MRS5474, N^6-dicyclopropylmethyl, K_i = 47.9 nM) as a moderately A_1AR-selective full agonist. Two stereochemically defined N^6-methynyl group substituents displayed narrow SAR; groups larger than cyclobutyl greatly reduced AR affinity, and those larger or smaller than cyclopropyl reduced A_1AR selectivity. Nucleoside docking to A_1AR homology model characterized distinct hydrophobic cyclopropyl subpockets, the larger "A" forming contacts with Thr270 (7.35), Tyr271 (7.36), Ile274 (7.39), and carbon chains of glutamates (EL2) and the smaller subpocket "B" forming contacts between TM6 and TM7. **10** suppressed minimal clonic seizures (6 Hz mouse model) without typical rotarod impairment of A_1AR agonists. Truncated nucleosides, an appealing preclinical approach, have more druglike physicochemical properties than other A_1AR agonists. Thus, we identified highly restricted regions for substitution around N^6 suitable for an A_1AR agonist with anticonvulsant activity.



Extracellular adenosine acts through four subtypes of G-protein-coupled adenosine receptors (ARs), i.e., at A_1-, A_{2A}-, A_{2B}-, and A_3AR subtypes.[1] Endogenous adenosine begins to activate the A_1AR at low concentrations (~10 nM) to induce cytoprotective and anti-ischemic functions. Full or partial agonists of the A_1AR are being considered for treatment of various conditions, including seizures, stroke, diabetes, cardioprotection, and cardiac arrhythmias.[2−4] A_1AR agonists are highly neuroprotective in ischemic[5,6] and epileptic[7−9] models. A_1AR agonists are also being explored for antidepressant,[10] antianxiety,[11] and other neuropsychiatric effects. A_1AR agonists are also useful for pain,[12] due to their presynaptic action to decrease the release of excitatory neurotransmitters in the brain.[13] However, peripheral cardiovascular side effects have prevented the introduction of A_1AR agonists for treating disorders of the central nervous system (CNS).[14]

Structural modification of adenosine to achieve selectivity in activating one or more AR subtypes has centered on the adenine C2- and N^6-positions and on the ribose moiety.[1,15] Modifications of the ribose moiety, such as 5′-amides and alternate carbocyclic ring systems, are especially useful for enhancing AR affinity. For example, replacement of the ribose tetrahydrofuryl ring with a methanocarba ([3.1.0]-bicyclohexane) ring system can enforce a North (N)-envelope conformation, depending on the fusion position of the cyclopropane ring. This replacement, which is highly preferred in AR binding over the isomer of the opposite South (*S*) conformation, maintains or enhances affinity at A_1- and A_3ARs with respect to the ribosides, but decreases affinity at A_{2A}AR.[16] Another widely explored modification, truncation at the 4′-carbon, i.e. removal of the 5′-group, is generally tolerated in A_3AR binding for various chemical series, including 4′-thio (e.g., **1a**, Chart 1) and (N)-methanocarba analogues (e.g., **2a**).[17,18] However, the observed effects of this truncation on the relative efficacy of nucleoside derivatives to activate ARs are variable for the different AR subtypes and chemotypes. For example, truncation in the 4′-thio adenosine series tends to produce nucleosides that antagonize the A_3AR and in some cases may additionally agonize the A_{2A}AR, such as **1b**.[19] Truncation in the (N)-methanocarba-adenosine series results in nucleosides that typically lose 2 orders of magnitude of affinity at the A_1- and A_{2A}AR, depending on the N^6-substituent and display a wide range of efficacies at the A_1- and A_3AR, e.g. A_3AR antagonist **2a**.[18,20] Focusing on the neuroprotective A_1AR, we scanned a wide range of N^6-substituents known to enhance

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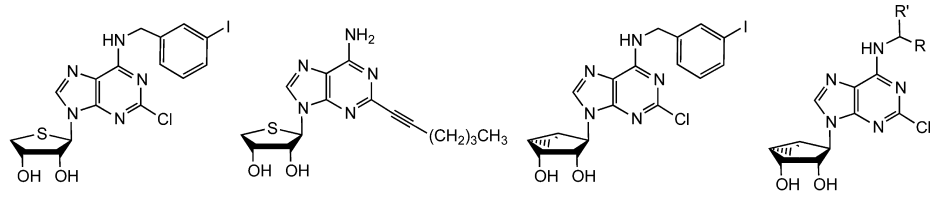
Chart 1. Structures of Representative 4′-Truncated Adenosine Analogues and Their Receptor Binding Affinities in the Series of 4′-Thioribosides (1a,b) and Ring-Constrained (N)-Methanocarba Nucleosides (2a,b)[a]



1a LJ1251
A_1: 2490; A_{2A}: 341; A_3: 4.16

1b
A_1: >10,000; A_{2A}: 7.19; A_3: 11.8

2a MRS5127
A_1: 3040; A_{2A}: 1080; A_3: 1.44

2b R,R' = H, alkyl, cycloalkyl

[a] K_i values in nM in binding to the hARs are indicated.[17,18,20,44]

A_1AR affinity[21] and concluded that only certain N^6-cycloalkylmethyl and dicycloalkylmethyl groups (e.g., **2b**) maintain selectivity and agonist efficacy at this AR subtype.[20]

A goal of this study was to characterize the structure−activity relationship (SAR) of an expanded set of closely related and stereochemically defined N^6-cycloalkylmethyl and dicycloalkylmethyl 4′-truncated (N)-methanocarba adenosine derivatives as human (h) A_1AR agonists. Both binding and efficacy studies were performed on the novel derivatives, and selected nucleosides (including ribosides for comparison) were examined in in vivo anticonvulsant models.

Finally, receptor homology modeling and ligand docking were used to gain insight into the structural basis for AR recognition and activation in this series of closely related N^6-derivatives. The modeling was based on an agonist-bound A_{2A}AR X-ray structure recently reported by Xu et al.[22] The interaction of ligands with the G_i-coupled A_1AR was compared to the G_i-coupled A_3AR, at which the affinity in this nucleoside series was also dramatically modulated. Pharmacological properties have been related to specific binding site interactions, especially in a small and sterically restricted region of the hydrophobic pocket, where the N^6-group has been proposed to reside. The loss of interactions in the ribose 5′-region was compensated by structural characteristics of a few N^6-substituents in the 4′-truncated analogues. Thus, the present study had three objectives: to more clearly define the narrow structural limits for binding and full activation of the A_1AR within the N^6-cycloalkylmethyl series, to correlate these findings with molecular modeling based on an X-ray structure of the A_{2A}AR, and to characterize anticonvulsant activity known to be associated with the A_1AR in the brain. Because the requirements for A_1AR selectivity and full agonist efficacy in this series were found to be highly restricted, we identified a structural sweet spot within the SAR of 4′-truncated nucleosides.

RESULTS

Chemical Synthesis. In an effort to increase A_1AR affinity and selectivity, we explored a set of N^6-substitutions of 4′-truncated (N)-methanocarba adenosines (Table 1), initially containing 2-chloro, that expanded upon our previous communication on an overlapping series of N^6-modified truncated nucleosides.[20] The present series included substitution of a N^6-methynyl group that was either chiral or achiral, e.g. **7**, **12**−**21**, including the N^6-dicyclopropylmethyl derivative **10** (associated previously with moderate A_1AR selectivity) and the N^6-dicyclopentylmethyl **11** derivative. In many cases, a N^6-cyclopropylmethyl group was further substituted on the α-methynyl carbon with an acyclic (**12**−**15**) or cycloalkyl group (**16**−**19**) or a phenyl ring (**20**, **21**). The stereochemistry at the methynyl carbon was clearly defined. A few previously reported N^6-unsubstituted (**3**) and N^6-alkyl/cycloalkyl derivatives (**4**−**6**, **8**, and **9**)[20] were included for comparison in the biological assays. This fine-tuning at the N^6-position was followed by several modifications at the C2-position, incorporating 2-H, 2-iodo, and 2-hydrazino substitutions (**37**, **40**). Additionally, 2-pyrazolyl substitutions (**42**, **43**) were patterned after a set of A_1AR selective riboside agonists reported by Elzein et al.[23]

The synthetic route to the truncated 2-chloro derivatives involved nucleophilic displacement by the appropriate amine of a 6-chloroadenine group in an 2′,3′-isopropylidene-protected precursor **22a** (Scheme 1). A 2-chloro substitution of the adenine ring has been shown to increase affinity at either or both A_1AR and A_3AR, and in some cases, to alter AR efficacy.[24] Finally, while maintaining constant the most effective N^6-substituent dicyclopropylmethyl, substitution of the C2-position was varied by the synthetic routes shown in Scheme 1 (2-iodo, **37b**) and Scheme 2 (2-H, **37a**; 2-hydrazino, **40**; 2-pyrazolo, **42**, **43**). The attempted synthesis of the 2-hydrazino derivative **40** by acid treatment to remove the isopropylidene group of the protected intermediate **39** resulted in decomposition. However, its preparation by hydrazine treatment of the unprotected nucleoside **10** was successful. Condensation of hydrazine compound **39** with methyl 2-formyl-3-oxopropionate under reflux conditions[23] gave the pyrazole derivative **41**, which underwent an acid hydrolysis in the presence of Dowex 50 to provide compound **42**. Hydrolysis of the methyl ester of compound **42** in the presence of 1 N NaOH afforded acid derivative **43**.

Pharmacological Evaluation. Binding affinity at three hAR subtypes was measured in assays using standard agonist radioligands (**47**, [^{3}H]*R*-PIA; **48**, [^{3}H]CGS21680; **49**, [^{125}I]I-AB-MECA) and membrane preparations from Chinese hamster ovary (CHO) cells (A_1AR and A_3AR) or human embryonic kidney (HEK) 293 cells (A_{2A}AR) stably expressing a hAR subtype (Table 1).[18] Because activity within the class of (N)-methanocarba nucleosides was previously noted to be very weak or absent at the hA_{2B}AR,[16,25] we did not include this receptor in the initial pharmacological screen. Known potent A_1AR agonists, i.e., ribosides (**44a**, CPA; **44b**, CCPA; **45**, NECA; **46**, ADAC), were included for comparison.[20]

In the series of 2-chloro derivatives, the A_1AR affinity ($K_i \geq$ 50 nM) of 5′-truncated (N)-methanocarba adenosine derivatives (**7**−**21**) was often greater than the affinity at the A_3AR (K_i typically ≥500 nM). The N^6-dicyclopropylmethyl derivative **10** was the most potent in binding to the A_1AR with a K_i value of 47.9 nM and 10-fold selectivity compared to the A_3AR.[20]

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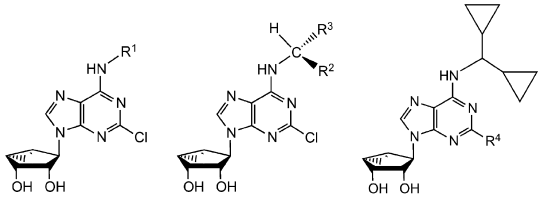
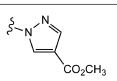
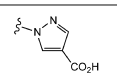
Table 1. In Vitro Potency of a Series of Truncated (N)-Methanocarba Adenosine Derivatives in Binding to Three Subtypes of hARs and Relative Efficacy at hA_1AR

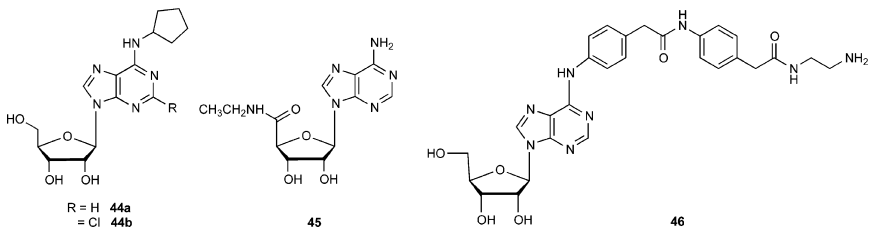


3–7 8–21 37, 40, 42, 43

Compound (configuration of Cα)	R^1 =		Affinity K_i, nM or *(% inhibition)*[a]			% Inhibition, cyclic AMP[d]
			A_1	A_{2A}	A_3	A_1
3[b]	H		350±90	3140±450	160±42	68.1±4.4
4[b]	CH_2CH_3		930±110	*(11%)*	6.6±1.6	ND
5[b]			68.4±8.9	4410±1090	8.9±1.9	81.0±21.1
6[b,c]	 (R,S)		86.8±23.7	*(41%)*	110±17	45.5±4.8
7			780±100	*(45±3%)*	670±10	-9.0±4.1
	R^2 =	R^3 =				
8[b,e]	CH_3	CH_3	72.2±16.4	*(39%)*	12±1	50.5±6.4
9[b,e]	C_2H_5	C_2H_5	78.8±15.6	3700±300	52±14	28.6±3.8
10[b,e]			47.9±10.5	3950±410	470±15	94.3±5.3
11			*(34±3%)*	*(13±3%)*	*(48±2%)*	ND
12 (*R*)		C_2H_5	68.1±5.0	3610±500	150±2	37.3±4.3
13 (*S*)	C_2H_5		150±10	4910±430	780±70	57.9±1.1
14 (*R*)			76.0±8.0	1570±180	780±80	33.8±2.3
15 (*S*)			150±50	3650±370	1720±140	20.8±6.1

Compound (configuration of Cα)	R^2 =	R^3 =	Affinity K_i, nM or *(% inhibition)*[a]			% Inhibition, cyclic AMP[d]
			A_1	A_{2A}	A_3	A_1
16 (*R*)			270±60	5470±300	2930±480	33.4±5.2
17 (*S*)			120±40	6450±720	2790±720	18.1±6.8
18 (*R*)			490±90	4840±400	1760±210	18.4±2.1
19 (*S*)			170±20	2550±170	550±50	24.0±5.2
20 (*R*)	Ph		3000±440	*(46±4%)*	790±100	-7.4±6.0
21 (*S*)		Ph	*(50±5%)*	*(36±4%)*	2200±470	-2.4±3.7
	R^4 =					
37a	H		56.1±11.0	*(38±5%)*	17.0±2.0	ND
37b	I		488±95	2230±640	182±14	ND
40	$NHNH_2$		210±80	*(43±7%)*	70.0±35.0	ND
42			730±50	4110±750	490±150	ND
43			1080±230	*(17±5%)*	640±150	ND
44a	j		1.8±0.5[f]	794[h]	72±12[e]	100±3
44b	j		0.83[g]	2270[h]	38±6[e]	ND
45	j		6.8±2.4[f]	20[h]	35±12[e]	100±15
46	j		10.4±3.8[i]	370±100[i]	12.4±4.1[i]	94±26[i]

[a]Using CHO or HEK293 (A_{2A} only) cells stably expressing a hAR (Supporting Information); affinity was expressed as K_i value (n = 3–5) or percent inhibition of radioligand binding at 10 μM. The radioligands used were [^{3}H]-N^6-*R*-phenylisopropyladenosine **47**, [^{3}H]-2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine **48**, or [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide **49**, respectively, unless noted. [b]Compounds **3**–**6** and **8**–**10** were prepared previously.[44] [c]**6** is a diastereomeric mixture. [d]Maximal efficacy (at 10 μM) in an A_1AR functional assay unless noted was determined by inhibition of forskolin-stimulated cyclic AMP production in AR-transfected CHO cells, expressed as percent inhibition (mean ± standard error, n = 3–5) in comparison to effect (100%) of full agonist **44a** at 10 μM. ND, not determined [e]Tosh et al.[20] or Gao et al.[21] [f]Gao et al.[59] [g]Klotz et al.[25] [h]Müller and Jacobson[60] [i]Klutz et al.[61] Functional assay in guanine nucleotide binding. [j]Structures as shown:



R = H **44a**
= Cl **44b**

45 **46**

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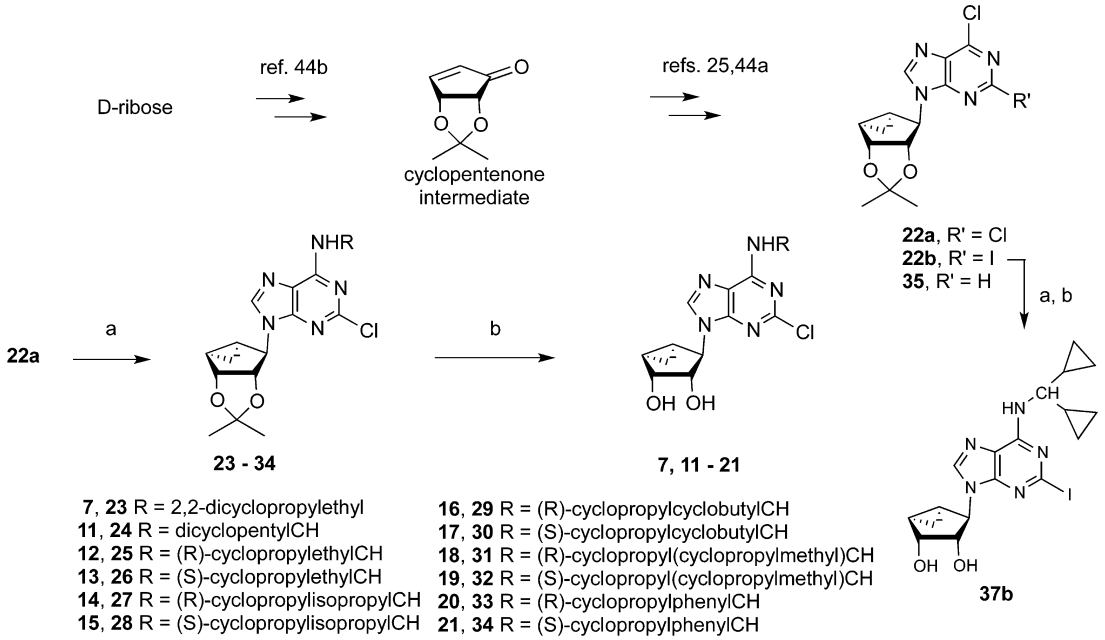
Scheme 1. Synthesis of N^6-Substituted 4′-Truncated Derivatives in the Ring-Constrained (N)-Methanocarba Adenosine Series[a]



[a]Intermediate **22a** was prepared as described.[44] Reagents and conditions: (a) RNH_2, Et_3N, MeOH, rt; (b) Dowex 50, $MeOH/H_2O$, rt. Compound **24** was used for the following step without isolation.

Nevertheless, cycloalkyl derivative **7** was nonselective, and a cyclopropyl/ethyl derivative **12** displayed considerable affinity (K_i = 150 nM) in A_3AR binding. In comparing pairs of pure diastereoisomers differing only in the chirality of the α-methynyl carbon of the N^6-group, compounds **12**, **17**, **19**, and **20** were more potent in A_1AR affinity than the opposite isomers, i.e., **13**, **16**, **18**, and **21**, respectively. N^6-Cyclopropyl/isopropylmethyl diastereoisomers **14** and **15** differed by only 2-fold in A_1AR binding affinity. The novel N^6-(*S*)-((cyclopropylcyclobutyl)methyl) derivative **17** was 23-fold selective for human A_1AR (K_i = 120 nM) in comparison to A_3AR and 53-fold in comparison to A_{2A}AR. Other potent analogues in hA_1AR binding were (*R*)-cyclopropyl/ethyl **12** (K_i = 68 nM) and (*R*)-cyclopropyl/isopropyl **14** (K_i = 76 nM) derivatives. Compounds **14** and **15**, closely related in structure to **10** except for lacking a bond to close one of the cyclopropyl rings, were also moderately selective for the A_1AR. Analogues containing a phenyl (**20**, **21**) group on the N^6-methynyl substituent bound weakly at the three ARs, and a dicyclopentyl analogue (**11**) inhibited less than 50% binding at 10 μM.

By introducing subtle structural changes, we attempted to indirectly characterize the environment of the receptor binding site surrounding each substituent on the chiral or prochiral N^6-methynyl carbon atom. A graphical comparison of the most potent truncated adenosine derivatives arranged by rank order of hA_1AR binding affinity, and illustrating hA_1AR/hA_3AR selectivity, is shown in Figure 1. We delineated separate binding preferences for the N^6-methynyl substituents, i.e., R^2 and R^3 in Table 1, and the conformational effects of these groups. Compounds **10**, **12**, **14**, **13**, **17**, and **15** displayed similar A_1AR affinities in the range of 50−150 nM, but differed in degree of hA_1AR/hA_3AR selectivity. Dimethyl analogue **8** was an A_3AR ligand (K_i = 12 nM) with moderate selectivity in comparison to A_1AR; diethyl analogue **9** showed comparable affinity at A_1 and A_3ARs. When R^3 was enlarged from a 3-membered to a 4-membered ring (**17**), A_1AR affinity was better preserved than when the same change occurred at R^2 (**16**). Reducing the rigidity of R^3 better preserved the A_1AR selectivity than reducing the rigidity of R^2 (cf. **12**−**15**). Introduction of a methylene group that separates a cyclopropyl substituent from the N^6-methynyl atom (**18** and **19**) did not maintain A_1AR affinity. Thus, the optimal R^3 substituent, i.e., cyclopropyl, could be reduced in size or rigidity to ethyl or isopropyl with only a minor reduction of A_1AR affinity. Also, an enlargement of R^3 to cyclobutyl reduced affinity by only 2.5-fold. However, when the R^2 substituent was made smaller than the cyclopropyl ring, a more substantial loss of A_1AR affinity occurred. Therefore, these two subpockets surrounding the N^6-substituent had slightly different steric requirements. A greater than one bond deviation or C-atom alteration from the size of cyclopropyl either dramatically reduced the AR affinity or the A_1AR selectivity.

Replacement of 2-Cl on compound **10** with 2-H in **37a** was tolerated at the A_1AR, but affinity at the A_3AR increased 28-fold. Substitution of 2-chloro of **10** with iodo greatly reduced affinity and selectivity in **37b**. Efforts to combine the N^6-dicyclopropylmethyl group with large C2 substituents based on the C2-pyrazolyl derivatives, known to be compatible with A_1AR affinity, produced only weak nonselective ligands **42** and **43**. The 2-hydrazino derivative **40** was also nonselective, and **10** remained the optimal A_1AR-selective structure.

Functional data determined at a single concentration (10 μM) in an assay of adenylate cyclase (A_1AR-induced inhibition of cyclic AMP production in CHO cells stably expressing the receptor[26]) are reported in Table 1. The potent and selective agonist **44a** was used as the standard full agonist, and the nonselective AR agonist 5′-*N*-ethylcarboxamidoadenosine **45** was also a full agonist in this assay. Most of the N^6-cycloalkylmethyl analogues were partial agonists of the A_1AR. However, a concentration−response analysis for **10** and **45** in A_1AR-mediated inhibition of cyclic AMP indicated that EC_{50} values were 40.7 ± 19.7 and 10.2 ± 3.3 nM, respectively, which

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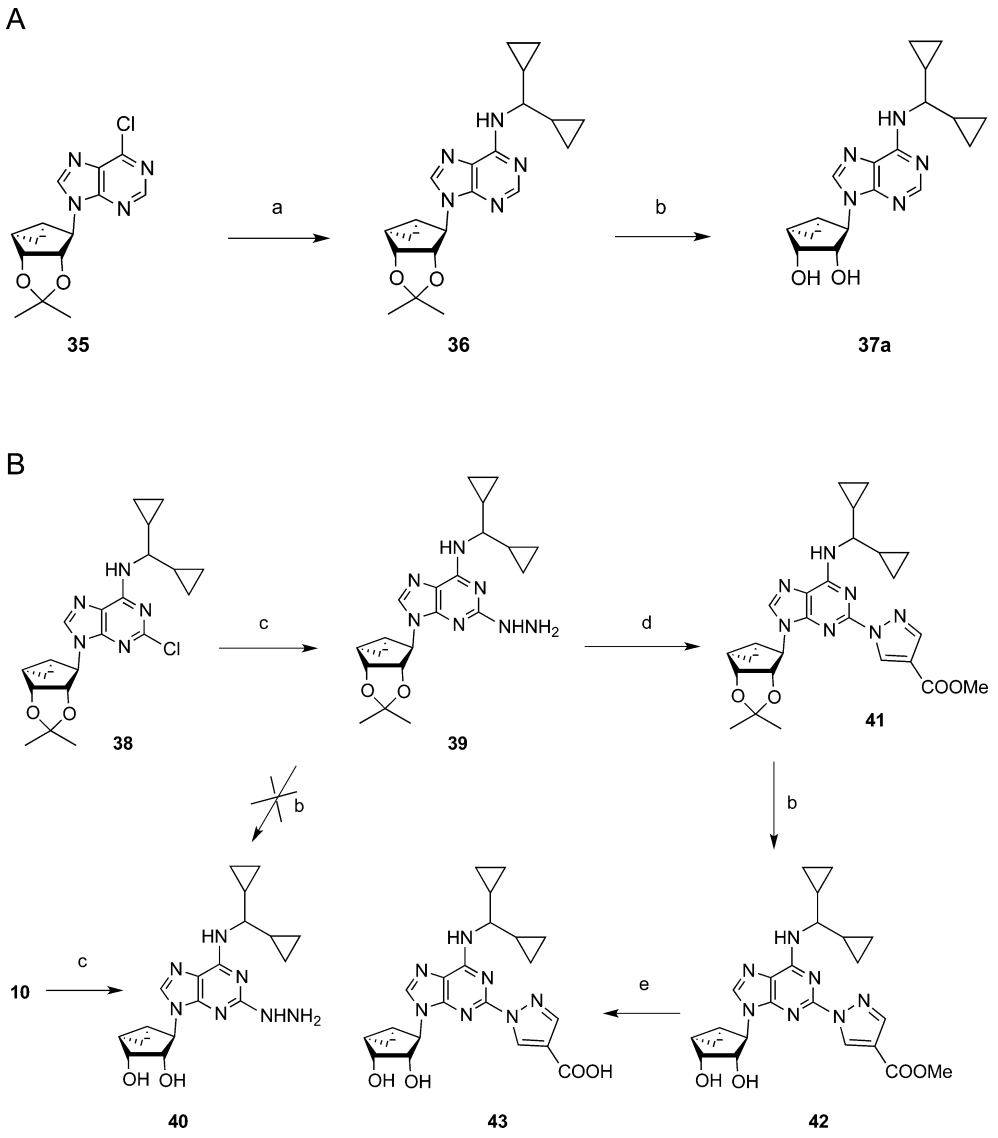
Scheme 2. Substitution of the C2-Position of Truncated Nucleosides Containing N^6-Dicyclopropylmethyl Substitution, i.e., the Most Effective for Preservation of hA_1AR Binding Affinity, Selectivity, and Efficacy[a]



[a]Reagents and conditions: (a) RNH_2, DIPEA, *i*-PrOH, reflux; (b) Dowex 50, MeOH/H_2O, rt; (c) hydrazine, reflux; (d) methoxycarbonyl malondialdehyde, EtOH, reflux; (e) 1 N NaOH, MeOH, rt.

was in close agreement with their A_1AR binding affinities. Compound **13** was of intermediate maximal efficacy (57.9% of full agonist) at the A_1AR based on results at a single saturating concentration. Other truncated analogues that proved to be of low efficacy in activation of the A_1AR, but still within the range of 30−40%, were **12**, **14**, and **16** (for which affinity at the A_1AR exceeded other AR subtypes). Curiously, a phenyl/cyclopropyl analogue **20** did not activate the A_1AR at 10 μM, a concentration that exceeded its K_i value by 13-fold, suggesting possible antagonism. Compound **10** was tested for functional activity in stimulation of adenylate cyclase through the $hA_{2B}AR$ expressed in CHO cells and was found to be nearly inactive (13.7 ± 4.4% of activity of full agonist **45** at 10 μM). Thus, the A_1AR selectivity of **10** was maintained within the entire AR family.

Molecular Modeling. The binding at ARs of the newly synthesized truncated (N)-methanocarba nucleosides was also evaluated through molecular modeling studies. Previously reported homology models of the hA_1- and hA_3ARs,[20] built using a recently reported agonist-bound $A_{2A}AR$ crystallographic structure (PDB ID: 3QAK) as a template,[22] were used to perform docking simulations of each of the compounds **8**−**21**. In the $A_{2A}AR$ crystal structure, the agonist ligand contained a bulky N^6-substituent, which opened EL3 to allow binding of other N^6-substituted nucleosides. The thermostabilized agonist-bound $A_{2A}AR$ structure of Lebon et al.,[27] in which EL3 is closer to EL2 and which contains mutations in the transmembrane (TM) region, including the ribose binding site, was not used as a template. In our previous study, the docking pose of compound **10** at the hA_1AR was compared with that of 5′-*N*-ethylcarboxamidoadenosine **45**.[20] Here we give a more detailed description of the binding mode of this new series of truncated (N)-methanocarba nucleosides in terms of their affinity and selectivity profiles.

A docking pose of compound **10** inside the hA_1AR binding site (Figure 2, panel A) featured most of the main receptor−

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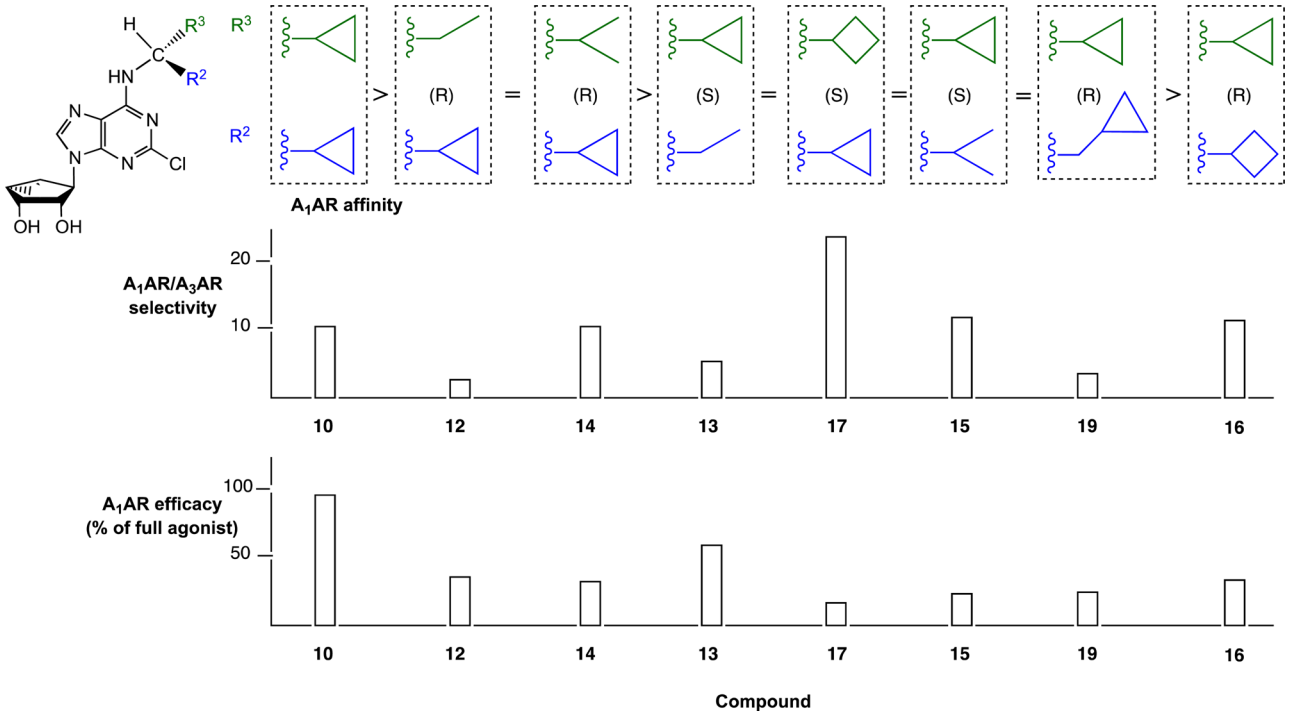

Figure 1. Graphical comparison of the most potent truncated adenosine derivatives arranged by rank order of hA_1AR binding affinity (ranging from 48 to 270 nM). The lower plots represent the hA_1AR selectivity in comparison to the hA_3AR and maximal efficacy at the hA_1AR (data in Table 1).

ligand interactions observed in the agonist-bound $hA_{2A}AR$ crystal structures.[22,27] These interactions, involving both the adenine core and the ribose ring, were noted in the docking poses of this new series of truncated (N)-methanocarba nucleosides at both the hA_1- and hA_3ARs. In particular, the 3′- and 2′-hydroxyl groups formed H-bonds with residues (using a GPCR numbering convention[28]) at positions 7.42 (Thr277 in hA_1AR and Ser271 in hA_3AR) and 7.43 (His278 in hA_1AR and His272 in hA_3AR), respectively. The side chain of asparagine 6.55 (Asn254 in hA_1AR and Asn250 in hA_3AR) strongly interacted with these compounds through two H-bonds involving the 6-amino group and the N^7 atom of the adenine ring. Moreover, the adenine core was anchored inside the binding site by a $\pi-\pi$ stacking interaction with a phenylalanine in EL2 (Phe171 in hA_1AR and Phe168 in hA_3AR) and strong hydrophobic contacts with leucine 6.51 (Leu250 in hA_1AR and Leu246 in hA_3AR) and isoleucine 7.39 (Ile274 in hA_1AR and Ile268 in hA_3AR).

Two key interactions observed for the crystallographic poses of **45**-like agonists (5′-*N*-ethylcarboxamido derivatives) at the $hA_{2A}AR$ were necessarily missing in these truncated derivatives. In the $hA_{2A}AR$ X-ray structures, Thr88 (3.36) and His250 (6.52) coordinated, through H-bonding interactions, the 5′-CO-NH-alkyl groups of the cocrystallized agonists **45** and 6-(2,2-diphenylethylamino)-9-((2*R*,3*R*,4*S*,5*S*)-5-(ethylcarbamoyl)-3,4-dihydroxytetrahydrofuran-2-yl)-*N*-(2-(3-(1-(pyridin-2-yl)piperidin-4-yl)ureido)ethyl)-9*H*-purine-2-carboxamide (**50**, UK-432097).[22,27] The threonine at position 3.36 is conserved among all four AR subtypes, while the histidine at position 6.52 is conserved in hA_{2A}-, hA_{2B}-, and hA_1ARs, but is substituted with a serine in the hA_3AR. Therefore, these conserved residues were predicted to also be important in agonist binding at the hA_1AR. The difference at position 6.52 could additionally be related to the different behavior of the truncated ring-constrained nucleosides at A_1- and A_3ARs, as previously hypothesized.[20]

Moreover, the hydrophilic region of the receptor associated with ribose binding is key to the activation process, which likely involves essential residues of TM3, TM6, and TM7 throughout the AR family, as observed in the $A_{2A}AR$.[22,27] Thus, the loss of the 5′-substituent is expected to affect AR efficacy, which it clearly does at the A_3AR, as observed for other previously reported truncated (N)-methanocarba nucleosides.[31] However, the effect at the A_1AR is highly variable with relative maximal efficacies of this series ranging from low to high (80−100% in compounds **5** and **10**).

The structural basis for the full agonism of **10**, in contrast to closely related compounds that have greatly reduced efficacy at this subtype, is difficult to identify but might be related to some interactions formed at the entrance of the binding site, i.e., the N^6-binding region, that are crucial in orienting and stabilizing the compound inside the cavity.

A detailed analysis of ligand interactions with the upper region of the binding site has helped to clarify the affinity and selectivity profiles of this new series of truncated (N)-methanocarba derivatives. As shown in Figure 2 (panel B), the orientation of the N^6-dicyclopropylmethyl substituent of compound **10** allowed us to identify two distinct upper subpockets in the hA_1AR. The larger of the two subpocket, designated "A", corresponds to the main entrance of the binding site and accommodated one cyclopropyl ring to form hydrophobic contacts with Thr270 (7.35), Tyr271 (7.36), and Ile274 (7.39) and the carbon chains of Glu170 (EL2) and Glu172 (EL2). The other cyclopropyl ring of **10** perfectly fits a smaller side subpocket, designated "B", which was located between TM6 and TM7 and delimited by Leu253 (6.54), Thr257 (6.58), Thr270 (7.35), and at the bottom by Leu250 (6.51). Subpocket A corresponds also to the region that

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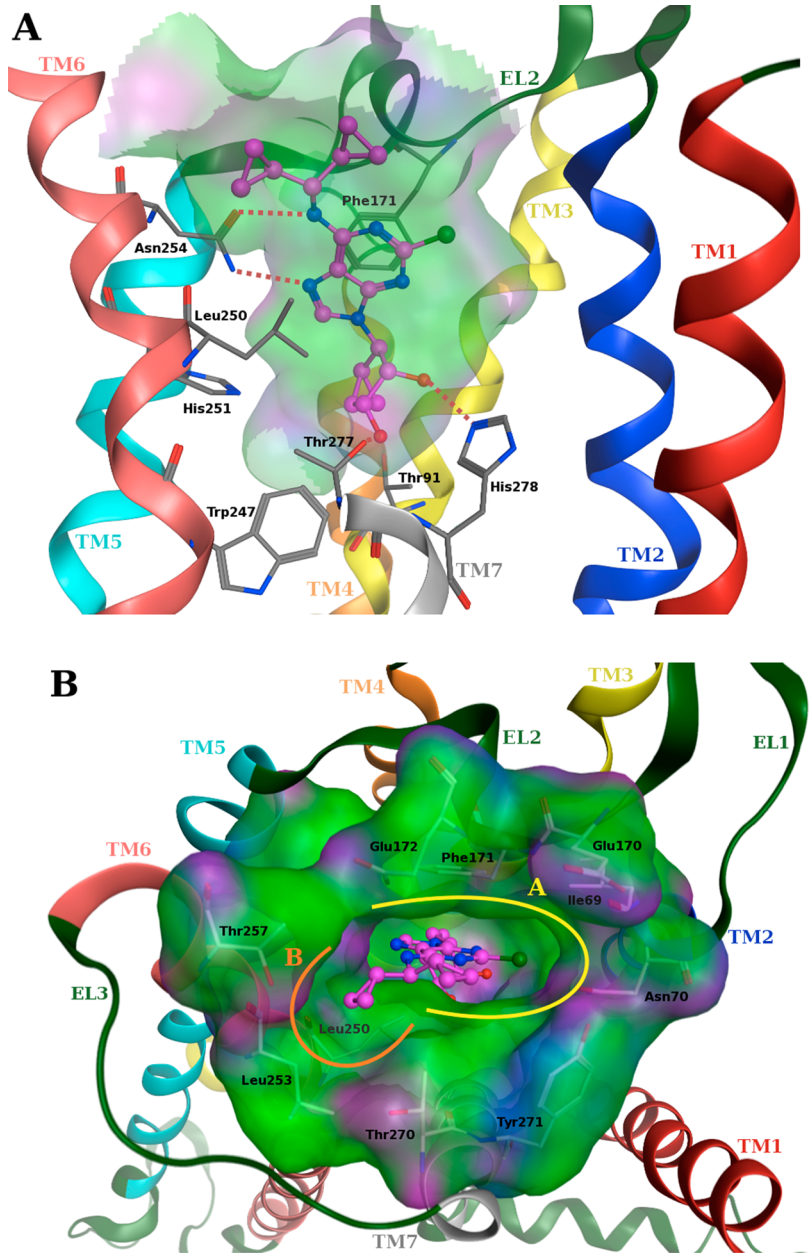

Figure 2. (A) Side view and (B) top view of the docking pose of compound **10** (in magenta) inside the binding site of the hA_1AR model. Side chains of some amino acids important for ligand recognition and H-bonding interactions are highlighted. Hydrogen atoms are not displayed. The Connolly surface of some amino acids surrounding the binding site is displayed. Surface color indicates hydrophobic regions (green), mildly polar regions (blue) and H-bonding regions (magenta). The boundaries of the two identified subpockets are highlighted (larger subpocket A in yellow and smaller subpocket B in orange). R^2 (Table 1) is predicted to occupy subpocket A, and R^3 is predicted to occupy subpocket B.

accomodates the extended N^6 and C2 groups of 50 in the $A_{2A}AR$ crystal structure.

With respect to this N^6-region, docking results showed some differences between hA_1- and hA_3ARs in the binding of these new truncated (N)-methanocarba derivatives at ARs that could explain their different affinity profiles at these receptors. In fact, the orientation and the interactions of the N^6-substituents were particular for each receptor subtype, mainly due to differences between the residues present in the upper region of the binding site. In particular, three residues delimiting subpocket B in the hA_1AR are substituted in the hA_3AR with amino acids bearing bulkier side chains, namely Ile249 (6.54), Ile253 (6.58), and Leu264 (7.35). While at the $hA_{2A}AR$, these residues are substituted with Ile252 (6.54), Thr256 (6.58), and Met270 (7.35), respectively, giving rise to again a different scenario. Consequently, at the hA_3AR there was no side pocket B between TM6 and TM7 able to accommodate a cyclopropyl ring, and this determined a different orientation of the N^6-substituent inside the cavity (Figure 3). In fact, the upper region of the hA_3AR binding site was overall more hydrophobic as compared to that of the hA_1AR, but its shape was more suitable to accommodate unbranched hydrophobic substituents. Due to the more difficult fit of the branched N^6-group in this region, the position of compound **10** was slightly shifted within the hA_3AR binding site and so it established weaker interactions with the key residues deeper in the cavity. This finding can explain the low affinity for the hA_3AR of the present set of (N)-methanocarba nucleosides bearing α-branched N^6-

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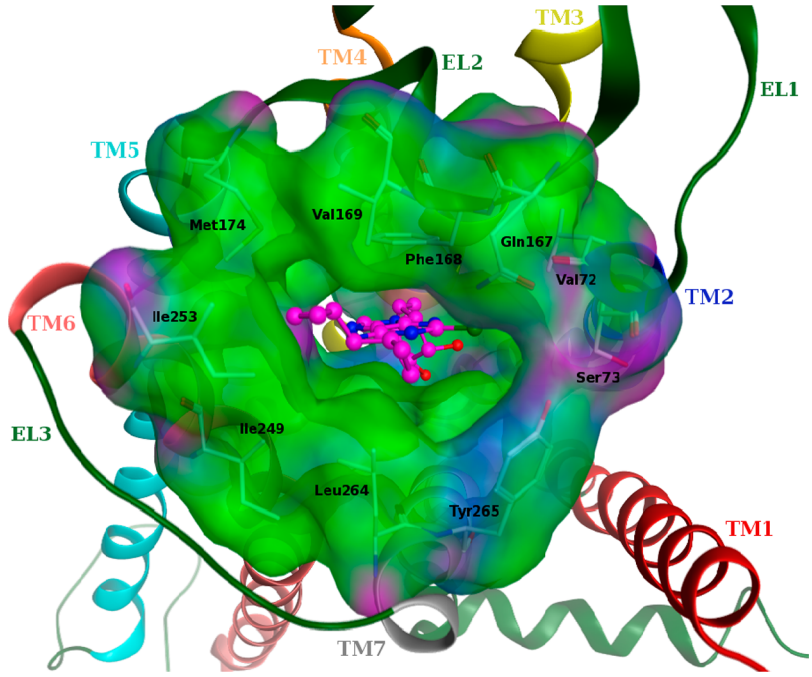

Figure 3. Top view of the docking pose of compound **10** (in magenta) inside the binding site of the hA_3AR model. Side chains of amino acids at the entrance of the binding site are highlighted and their Connolly surface is displayed. Surface color indicates hydrophobic regions (green), mildly polar regions (blue), and hydrogen-bonding regions (magenta). Hydrogen atoms are not displayed.

substituents, with affinity generally decreasing with an increase in volume of the groups on the branches. This view is also consistent with the enhanced hA_3AR affinity associated with reported truncated (N)-methanocarba analogs having unbranched hydrophobic substituents, such as benzyl, at the N^6-position (also compare compounds **5** and **6**).[20]

Overall, increasing or decreasing the size of the groups on the α-carbon from cyclopropyl decreased hA_1AR affinity. Thus, the steric hindrance of these larger groups disfavored the precise conformational arrangement necessary to occupy the two subpockets. Pocket A, although larger in its opening to the extracellular side of the receptor, did not tolerate deviation from R^2 = cyclopropyl, while pocket B could accommodate R^3 = cyclobutyl or ethyl with some cost in affinity.

Moreover, docking results and conformational analysis of the dihedral N^6-Cα bond angles showed that the preferred binding conformation at the hA_1AR for this series of truncated (N)-methanocarba nucleosides placed the hydrogen atom on the α-carbon pointing toward TM7, even though an alternative conformation, with the hydrogen oriented toward TM5, was also found (Figure S1, Supporting Information). Therefore, in its preferred conformation, the R^3 group on the Cα occupied the smaller subpocket B, while the R^2 group was located in the larger subpocket A. Depending on the groups attached to the α-carbon and their steric complementarity with the two subpockets, two opposite diastereoisomers bound with different strength to the receptor binding site and so possessed different binding affinity. In fact, if a small group (e.g., ethyl) with low complementarity with the larger subpocket A was present on the Cα, then the most potent diastereoisomer was the one that can more easily accommodate that group in subpocket B (compound **12**, ethyl group in R^3, as compared to compound **13**, ethyl group in R^2). On the other hand, if an extended group (e.g., cyclopropylmethyl) was present on the Cα, then the isomer able to locate this group in the larger subpocket A possesses higher affinity at the receptor (e.g., **19**, cyclopropylmethyl group in R^2, as compared to **18**, cyclopropylmethyl group in R^3).

Anticonvulsant Testing. Three A_1AR agonists were examined in models of electrically and chemically induced seizures (Table 2).[29] The anticonvulsant activity of 2-chloro-

Table 2. Anticonvulsant Activity in Mice of A_1AR Agonists

compd[a]	behavioral toxicity TD_{50}, mg/kg	6 Hz model ED_{50}, mg/kg	MES model[b] (dose, mg/kg)	scMET model[b] (dose, mg/kg)
10	>30[d]	2.74[c]	1/4 (3)	no protection (3)
44b	0.84[c]	0.12[c]	1/4 (1)	1/4 (1)
46	0.14[e]	0.03[c]	4/8 (2)	5/8 (1)

[a]Administered ip. [b]Qualitative results, expressed as number of animals protected from convulsions/total number tested. [c]Measured at 1 h (time of peak of effect) postinjection, dose range for **10** was 0.75−10 mg/kg. [d]No rotarod toxicity at 30 mg/kg. [e]Measured at 4 h (time of peak of effect) post injection.

N^6-cyclopentyladenosine (**44b**, CCPA) was studied previously,[7,9] and **44b** was included in the present study as a potent reference A_1AR agonist that does not distinguish between central and peripheral action. N^6-[4-[[[4-[[[(2-Aminoethyl)-amino]carbonyl]methyl]anilino]carbonyl]methyl]phenyl]-adenosine (**46**, ADAC) is a potent A_1AR agonist that has been shown to be neuroprotective in various models.[6,30] Full agonist **10** displayed efficacy in a seizure model in mice without the toxicity observed in the active dose range (tested up to 30 mg/kg, ip). Other A_1AR agonists began to show toxicity, i.e., the side effects evident in the rotarod performance test (i.e., inability to remain on the rotarod) overlapping the active dose range.

The 6 Hz minimal clonic seizure model is an acute electroshock seizure test that produces a seizure with a limbic

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Table 3. Summary of the Characteristics of the Two Pockets in A_1AR Surrounding Substituents of the N^6-Methynyl Carbon Atom, Based on Observed SAR and Predictions from Molecular Modeling (see Table 1 for definition of R^2 and R^3)

characteristic	pocket A	pocket B
size	larger (extends upward, but has narrow dimensions near N^6-methynyl atom)	smaller
location	TM7−EL2	between TM6 and TM7
contact residues[a]	Thr270 (7.35), Tyr271 (7.36), Ile274 (7.39), Glu170 (EL2), Glu172 (EL2)	Leu253 (6.54), Thr257 (6.58), Thr270 (7.35), Leu250 (6.51)
correspondence to N^6-group of **47**[b]	site of $PhCH_2$ binding	site of CH_3 binding
present in A_3AR?	yes	no[c]
correspondence to R (assuming most favorable docking with N^6−C-H toward TM7)	R^2	R^3
preferred group	*c*-Pr	*c*-Pr
accommodates group smaller than *c*-Pr? (Et)	no (exact steric complementarity is important)	yes
accommodates group less rigid than *c*-Pr? (*i*-Pr)	no	yes
accommodates group larger than *c*-Pr? (*c*-Bu)	no[d]	yes (only *c*-Bu)

[a]The residue numbering convention of Ballesteros and Weinstein is used.[28] [b]**47**, (*R*)-N^6-phenylisopropyladenosine. [c]Consistent with lower affinity of N^6-α-branched analogues at the A_3AR. [d]No clear explanation from modeling, except that the dimensions near the N^6-methynyl atom might be sterically restrictive. A larger group is tolerated, with intermediate affinity, only if there is an α-CH_2 spacer (**19**).

phenotype and displays a unique pharmacological profile to established antiepileptic drugs. In particular, the 6 Hz seizure is uniquely sensitive to the antiepileptic drug levetiracetam and partially resistant to the Na^+ channel blockers.[31] Three A_1AR agonists, the two reference compounds **44b** and **46** and the truncated derivative **10**, displayed efficacy in the 6 Hz model. The activities of **10** and **44b** were quantified at 1 h postadministration of the compound, which was also equal to the time of peak effect (TPE). Complete dose−response curves using five different doses each of **10**, **44b**, and **46** indicated ED_{50} values of 2.74, 0.12, and 0.03 mg/kg, respectively. Compound **10** protected in four out of eight mice at 1.5 mg/kg. Compared to the activity in the 6 Hz model, these compounds had no or minimal effect in the traditional seizure models, the maximal electroshock (MES) model, or the subcutaneous metrazol (pentylenetetrazol) model (scMET) at the same dosing range.

In the minimal behavioral toxicity test using the rotarod, the three compounds showed significant differences. The toxicity of **44b** was observed roughly in the same dose range (0.84 vs 0.12 mg/kg) as its protection in the 6 Hz model and at the same time point (1 h postinjection). Animals became lethargic and were unable to stay on the rotarod following ip drug administration, as exhibited with other A_1AR agonists.[32] For compound **10**, no toxicity (zero out of eight mice) was observed at all doses tested up to 30 mg/kg, the highest dose tested, which was nearly completely protective (seven out of eight animals) in the 6 Hz model. Therefore, the therapeutic window for **10** appeared to be superior to that of **44b**.

Compound **10** was also tested in the corneal kindled mouse model to examine its effect on focal seizures. In a qualitative test, an EC_{50} (±SE) of 1.79 ± 0.71 mg/kg (n = 8) was determined at 1 h postinjection. This unique response profile of compound **10** makes it as an attractive candidate to treat drug-resistant epilepsy.

DISCUSSION

Our previous closely related communication[20] demonstrated that the affinity of truncated ring-constrained analogues, in comparison to ribosides, was less well preserved at the A_1AR than at the A_3AR. However, certain analogues, most notably the dicyclopropylmethyl analogue **10**, were relatively well preserved in binding and activation of the A_1AR. In this study, we built new analogues on the previous observation that **10** had 10-fold A_1AR selectivity in comparison to the A_3AR and 74-fold of selectivity vs A_{2A}AR. Moreover, this analogue was a full agonist at the hA_1AR. The fine-tuning of the structure of **10** now indicates that even minor adjustments of the structure cause a loss of potency, selectivity, or efficacy at the hA_1AR.

Our binding results at the hA_1AR showed that only Cl was preferred at the adenine C2-position, and N^6-substituents other than dicyclopropylmethyl displayed inferior pharmacological profiles. Separate substituents (R^2 and R^3) of the Cα methynyl group had distinct and very narrow SAR requirements. Groups that were much larger or smaller than cyclopropyl were not compatible with A_1AR affinity, selectivity, and efficacy. Therefore, we describe the compound with optimal pharmacological properties, compound **10**, as a structural sweet spot for potent and selective activation of the A_1AR.

Molecular docking studies of these truncated (N)-methanocarba nucleosides at the hA_1AR highlighted how a precise complementarity in the N^6-region was needed to determine a good affinity and selectivity profile and to compensate for the missing anchoring effect of the ribose 5′-region. In fact, a detailed modeling analysis of the upper part of the hA_1AR binding site predicted two different subpockets, A and B, able to accommodate the N^6-substituents of these derivatives, and it seemed that an optimal occupancy of both subpockets was required for enhanced affinity at this receptor subtype. In particular, the smaller subpocket B can readily accommodate up to a cyclobutyl group, while subpocket A can fit substituents that are extended in the direction of EL2. The steric restriction of the subpockets can be the reason for the null affinity at hA_1AR of compound **11** bearing two cyclopentyl groups, as they are too bulky to fit in either pocket. On the other hand, smaller groups on the α-carbon of the N^6-substituent, such as methyl or diethyl (compounds **8** and **9**, respectively), even though they can occupy the pockets, possess lower complementarity as compared to the cyclopropyl group and consequently have no selectivity and lower affinity at the A_1AR.

The identification of these two subpockets and the binding conformation proposed here are also in agreement with the binding data of others known A_1AR agonists.[21] For example, the moderately selective A_1AR agonist (*R*)-(−)-N^6-(2-phenylisopropyl)adenosine (**47**) is more potent than the corresponding opposite isomer (*S*-PIA). A hypothetical binding

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mode for these adenosine derivatives, similar to the one proposed for our new rigid, truncated nucleosides, would readily place the phenylmethyl group of the R isomer in the larger subpocket A and the methyl group in the smaller subpocket B. Thus, there is a close correspondence of our new findings to the previously explored preference for N^6-Cα-branched *R* isomers in comparison to the corresponding *S* isomers.[33] A summary of the characteristics of the two pockets surrounding substituents of the N^6-methynyl carbon atom is provided in Table 3.

Complementary anchoring of the N^6-substituent inside the two subpockets seems important in orienting and stabilizing the compound inside the cavity. It could also help in keeping the adenine-methanocarba moiety in an efficacious active conformation, able to form strong H-bonds and hydrophobic interactions with residues in TM6, TM7, and EL2, while compensating for the missing interactions due to the lack of a 5′-substituent.

In in vivo testing, compound **10** was the only A_1AR agonist examined here that displayed a clear separation of anticonvulsant activity and toxicity. For example, a prototypical A_1AR agonist **44b** displayed toxicity in the rotarod assay (two out of eight animals) at a dose of 0.1 mg/kg. At a dose of 0.5 mg/kg **44b**, three out of eight animals displayed toxicity. Another known A_1AR agonist, **46**, was more potent than **44b** in both in vivo anticonvulsant activity and toxicity, but it also failed to demonstrate a separation of the two activities.

The unique response profile of compound **10** (inactive in MES and scMET, active in 6 Hz and corneal kindling models) and its novel mechanism of action through the A_1AR make it a potential candidate to treat drug-resistant epilepsy. Traditional antiepileptic drugs (AEDs) carbamazepine, lamotrigine, phenytoin and topiramate are Na^+ channel blockers, which have strong efficacy in the MES model.[34] They are either inactive or only partially efficacious in the 6 Hz model. On the other hand, newer AEDs having different mechanisms of action, such as levetiracetam and retigabine, are potent and efficacious in the 6 Hz model, which makes the 6 Hz model a model for identifying compounds that potentially target drug-resistant epilepsy.[31] The kindling models are useful in searching for drugs to treat complex partial seizures, because kindled seizures not only provide an experimental model of focal seizures but also a means of testing drugs to stop seizure spread and generalization from a focus.[35] The corneal kindled mouse model demonstrates a pharmacological profile consistent with the traditional hippocampal kindled rat model, while it requires no implantation surgery and less compound quantity for testing.[36] The unique activities of compound **10** in the 6 Hz and corneal kindled mouse model and its overcoming the limitations of other A_1AR agonists (i.e., clear separation of anticonvulsant activity and toxicity) make it an attractive AED candidate for additional testing.

Many of the efforts to develop A_1AR agonists for peripheral applications, such as treating cardiac arrhythmias, have tried to limit central nervous system (CNS) penetration to avoid centrally mediated side effects. Conversely, other envisioned applications of A_1 agonists, i.e., for neurodegenerative and neurological disorders, depend on brain entry. A_1AR agonists have distinct neuroprotective and antinociceptive properties,[5,6,12] and activation of the A_1AR by endogenous adenosine mediates the protective effects of fractalkine/CX3CL1.[37] However, the clinical development of previous generations of such agents has been limited by side effects, including cardiovascular effects. In previous studies of the activity of A_1AR agonists in the CNS, only a small fraction of a peripherally administered agent crossed the blood−brain barrier (BBB).[38] However, a similar attempt to alter the biodistribution by removing the 2′-hydroxyl group of **44a** did not enhance brain uptake.[35]

The peripheral side effects of exogenously administered A_1AR agonists that do not distinguish between the brain and periphery have impeded the development of such agents for the treatment of epileptic seizures. Recently, it was proposed that the antiseizure effect of endogenous adenosine could be boosted indirectly by inhibiting formation of the neurabin-RGS4 complex for "fine-tuning adenosine receptor function in the nervous system".[39] Other approaches to solve this problem involved the design of ligands that favor the CNS over the periphery, such as N^6-[*R*-(2-benzothiazolyl)thio-2-propyl]-2-chloroadenosine (NNC-21-0136, K_d = 1.16 nM at rat A_1AR), which showed efficacy in in vivo stroke models and were reduced in their accompanying cardiovascular side effects.[5] Prodrug approaches and localized adenosine delivery have also been explored.[12,40]

The physicochemical properties of nucleosides that act as AR agonists often lead to limited in vivo bioavailability and reduced passage across the BBB. The cLog P of compound **10** is 1.41 (more favorable than cLog P of 0.14 for A_1AR-selective riboside and prototypical agonist **44a**), with the optimal for small molecular pharmaceutical substances being typically 2−3.[41] Also, the total polar surface area (tPSA) for **10** and **44a** are calculated to be 92.8 and 122 Å^2, respectively. Most druglike small molecules have a PSA smaller than 120 Å^2; thus, **10** is also preferred by this criterion. Compound **10** has fewer hydroxyl groups than **44a**, which would favor bioavailability in brain, and the molecular weight of **10** (376 Da) is comfortably within the most desirable range for pharmaceuticals.[41] Therefore, by several criteria **10** is more druglike than **44a**.

We speculate on the basis for the apparent lack of peripheral side effects of compound **10** up to 30 mg/kg. Both **44b** and **10** evidently enter the bloodstream and pass the BBB into the brain. Although **44b** was ∼58-fold more potent than **10** in binding at the A_1AR, **10** had better physicochemical properties (cLogP, tPSA, H-bond donors, molecular weight), so it might penetrate the BBB better than **44b**. The bioavailability in the brain of peripherally administered **10**, i.e. whether its altered physicochemical properties may facilitate its passage across the BBB, is undetermined. Normally a drug travels from the bloodstream into brain, such that when the A_1AR in brain is activated, the A_1AR in heart should be activated within the same dose range as with **44b**. For **10**, the fact that no adverse effects were observed even at 30 mg/kg (ten times the ED_{50}) suggests that the free concentration of **10** in plasma might be reduced relative to standard A_1AR agonists, possibly due to plasma protein binding. If the free drug concentration in blood would be lower, its ability to cross the BBB may remain unaffected or even increased. This phenomenon has been observed for a number of CNS drugs.[42] This hypothesis could be tested by pharmacokinetic and plasma protein binding assays.

A_1AR agonists hold interest therapeutically for their cardio- and neuroprotective, antiarrhythmic, antiseizure, antilipolytic, antiglaucoma, and anxiolytic actions. Some of the novel derivatives were partial A_1AR agonists, which are of interest for both cerebroprotective and cardiovascular application, depending on levels of endogenous adenosine and on receptor

reserve.[3,4,43] It is conceivable that the expanded range of physical properties in the present series of truncated derivatives would offer pharmacokinetic advantages. Therefore, this approach is appealing for preclinical development. This hypothesis will have to be evaluated in further in vivo studies.

EXPERIMENTAL PROCEDURES

Chemical Synthesis. *General Methods.* All reagents and solvents (regular and anhydrous) were of analytical grade, obtained from commercial suppliers and used without further purification. All amines were purchased from Asiba Pharmatech (Edison, NJ), except 2,2-dicyclopropylethylamine, which was obtained from Ryan Scientific, Inc. (Mount Pleasant, SC), and dicyclopropylmethylamine, which was obtained from J&W PharmLab (Levittown, PA). Compounds **22a** and **35** were synthesized as reported.[44] Reactions were conducted under an atmosphere of nitrogen whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates with a fluorescence indicator which were visualized (a) under UV light, (b) by dipping in a mixture of anisaldehyde (2.5 mL)/concentrated H_2SO_4 (5 mL)/methanol (425 mL), or (c) by dipping the plate in a solution of ninhydrin (0.3 g in 100 mL EtOH, containing AcOH, 1.3 mL) followed by heating. Silica gel column chromatography was performed with silica gel (SiO_2, 200–400 mesh, 60 Å) using moderate air pressure. Evaporation of solvents was carried out under reduced pressure at a temperature below 50 °C. After column chromatography, appropriate fractions were pooled, evaporated, and dried at high vacuum for at least 12 h to give the desired products in high purity. ^{1}H NMR spectra were recorded with a Bruker 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane or using deuterated solvent as the internal standard (δH: $CDCl_3$ 7.26 ppm). ESI-high resolution mass spectroscopic (HRMS) measurements were performed on a proteomics-optimized Q-TOF-2 (Micromass-Waters) using external calibration with polyalanine. Observed mass accuracies are those expected on the basis of the known performance of the instrument as well as the trends in masses of standard compounds observed at intervals during the series of measurements. Reported masses are observed masses uncorrected for this time-dependent drift in mass accuracy. TLC analysis was carried out on glass sheets precoated with silica gel F_{254} (0.2 mm) from Sigma-Aldrich (St. Louis, MO). The purity of final nucleoside derivatives was checked using a Hewlett–Packard 1100 HPLC equipped with a Zorbax SB-Aq 5 μm analytical column (50 × 4.6 mm; Agilent Technologies Inc., Palo Alto, CA). The mobile phase was a linear gradient solvent system of 5 mM TBAP (tetrabutylammonium dihydrogenphosphate)–CH_3CN from 80:20 to 0:100 in 13 min; the flow rate was 0.5 mL/min. Peaks were detected by UV absorption with a diode array detector at 230, 254, and 280 nm. All derivatives tested for biological activity showed >95% purity by HPLC analysis (detection at 254 nm).

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-(2,2-dicyclopropylethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**7**).* Dowex 50 resin (8 mg) was added to a solution of compound **23** (12 mg, 0.027 mmol) in methanol (0.5 mL) and water (0.5 mL) and the mixture stirred at room temperature overnight. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 35:1) to give the desired compound **7** (8.2 mg, 74%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 4.90 (d, J = 6.8 Hz, 1H), 3.72 (d, J = 6.4 Hz, 2H), 2.01–1.96 (m, 1H), 1.71–1.64 (m, 1H), 1.34–1.30 (m 2H), 0.78–0.72 (m, 2H), 0.51–0.42 (m, 5H), 0.28–0.17 (m, 4H); HRMS calcd for $C_{19}H_{25}ClN_5O_2$ (M + H)$^+$ 390.1697, found 390.1708.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-(dicyclopentylmethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**11**).* Dicyclopentylmethylamine (1.0 mg, 0.024 mmol) and triethylamine (0.1 mL, 0.16 mmol) were added to a solution of compound **22a** (4.2 mg, 0.012 mmol) in methanol (0.8 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was roughly purified by flash silica gel column chromatography. The resulting compound **24** was dissolved with methanol (0.6 mL) and water (0.3 mL). Dowex 50 (4 mg) was added to the solution and stirring continued at room temperature overnight. After complete reaction of the starting material, the reaction mixture was filtered and the filtrate was evaporated under vacuum. The residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the desired compound **11** (3.4 mg, 64%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.17 (s, 1H), 4.80 (s, 1H), 4.71 (t, J = 6.0 Hz, 1H), 4.40 (t, J = 6.8 Hz, 1H), 3.91 (d, J = 6.4 Hz, 1H), 2.20–1.18 (m, 1H), 1.98–1.96 (m, 1H), 1.81–1.74 (m, 4H), 1.69–1.53 (m, 10H), 1.42–1.29 (m 4H), 0.92–0.90 (m, 1H), 0.81–0.74 (m, 1H); HRMS calcd for $C_{19}H_{25}ClN_5O_2$ (M + H)$^+$ 390.1697, found 390.1708.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-1-cyclopropylpropylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**12**).* Dowex 50 resin (5 mg) was added to a solution of compound **25** (10 mg, 0.024 mmol) in methanol (0.5 mL) and water (0.5 mL) and the mixture stirred at room temperature overnight. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the desired compound **12** (6.1 mg, 69%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.8 Hz, 1H), 3.66 (br s, 1H), 2.00–1.97 (m, 1H), 1.84–1.65 (m, 3H), 1.34–1.28 (m, 2H), 1.01 (t, J = 7.6 Hz, 3H), 0.80–0.74 (m, 1H), 0.60–0.58 (m, 1H), 0.45–0.42 (m, 2H), 0.36–0.32 (m, 1H); HRMS calcd for $C_{17}H_{23}ClN_5O_2$ (M + H)$^+$ 364.1540, found 364.1538.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-1-cyclopropylpropylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**13**).* Dowex 50 resin (3 mg) was added to a solution of compound **26** (7 mg, 0.017 mmol) in methanol (0.3 mL) and water (0.3 mL) and the mixture stirred at room temperature overnight. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the desired compound **13** (4.3 mg, 68%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.8 Hz, 1H), 3.67 (br s, 1H), 2.02–1.98 (m, 1H), 1.86–1.59 (m, 3H), 1.32–1.24 (m, 2H), 1.01 (t, J = 7.6 Hz, 3H), 0.78–0.76 (m, 1H), 0.59–0.57 (m, 1H), 0.45–0.42 (m, 2H), 0.34–0.32 (m, 1H); HRMS calcd for $C_{17}H_{23}ClN_5O_2$ (M + H)$^+$ 364.1540, found 364.1535.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-1-cyclopropyl-2-methylpropylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**14**).* Dowex 50 resin (7 mg) was added to a solution of compound **27** (10.71 mg, 0.025 mmol) in methanol (0.6 mL) and water (0.5 mL) and the mixture stirred at room temperature overnight. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 35:1) to give the desired compound **14** (7.82 mg, 82%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.17 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.4 Hz, 1H), 3.62 (t, J = 7.2 Hz, 1H), 2.07–1.97 (m, 2H), 1.88–1.64 (m, 1H), 1.36–1.31 (m, 1H), 1.08–1.05 (m, 8H), 0.80–0.74 (m, 1H), 0.66–0.61 (m, 1H), 0.45–0.36 (m, 2H); HRMS calcd for $C_{18}H_{25}ClN_5O_2$ (M + H)$^+$ 378.1697, found 378.1691.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-1-cyclopropyl-2-methyl-propylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**15**).* Dowex 50 resin (5 mg) was added to a solution of compound **28** (6.8 mg, 0.016 mmol) in methanol (0.5 mL) and water (0.5 mL) and the mixture stirred at room temperature overnight. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 35:1) to give the desired compound **15** (4.8 mg, 81%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.17 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.4 Hz, 1H), 3.63 (t, J = 7.2 Hz, 1H), 2.09–1.96 (m, 2H), 1.86–1.64 (m, 1H), 1.34–1.30 (m, 1H), 1.08–1.05 (m, 8H), 0.80–0.74 (m, 1H), 0.65–0.61 (m, 1H), 0.45–0.36 (m, 2H); HRMS calcd for $C_{18}H_{25}ClN_5O_2$ (M + H)$^+$ 378.1697, found 378.1694.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-cyclopropylcyclobutylmethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**16**).* Dowex 50

resin (4 mg) was added to a solution of compound **29** (5.9 mg, 0.013 mmol) in methanol (0.4 mL) and water (0.4 mL) and the mixture stirred at room temperature for 7 h. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the desired compound **16** (3.9 mg, 74%) as an oil: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.4 Hz, 1H), 3.73 (br s, 1H), 2.69−2.64 (m, 1H), 2.10−2.07 (m, 1H), 2.01−1.91 (m, 4H), 1.89−1.80 (m, 1H), 1.69−1.65 (m, 1H), 1.34−1.30 (m, 3H), 0.95−0.91 (m, 1H), 0.78−0.74 (m, 1H), 0.56−0.53 (m, 1H), 0.40−0.36 (m, 2H); HRMS calcd for $C_{19}H_{25}ClN_5O_2$ $(M + H)^+$ 390.1697, found 390.1711.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-cyclopropylcyclobutylmethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**17**).* Dowex 50 resin (6 mg) was added to a solution of compound **30** (7.95 mg, 0.018 mmol) in methanol (0.6 mL) and water (0.4 mL) and the mixture stirred at room temperature for 7 h. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the desired compound **17** (5.3 mg, 74%) as an oil: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.4 Hz, 1H), 3.73 (br s, 1H), 2.69−2.62 (m, 1H), 2.12−2.07 (m, 1H), 2.03−1.88 (m, 4H), 1.89−1.80 (m, 1H), 1.69−1.65 (m, 1H), 1.34−1.31 (m, 3H), 0.95−0.91 (m, 1H), 0.78−0.76 (m, 1H), 0.56−0.53 (m, 1H), 0.40−0.36 (m, 2H); HRMS calcd for $C_{19}H_{25}ClN_5O_2$ $(M + H)^+$ 390.1697, found 390.1697.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-1,2-dicyclopropylethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**18**).* Dowex 50 resin (3 mg) was added to a solution of compound **31** (4.94 mg, 0.018 mmol) in methanol (0.3 mL) and water (0.3 mL) and the mixture stirred at room temperature for 7 h. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 35:1) to give the desired compound **18** (3.4 mg, 78%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.8 Hz, 1H), 3.80 (br s, 1H), 2.02−1.97 (m, 1H), 1.70−1.61 (m, 2H), 1.34−1.30 (m, 3H), 1.10−1.08 (m, 1H), 0.91−0.76 (m, 2H), 0.62−0.58 (m, 1H), 0.47−0.36 (m, 4H), 0.17−0.07 (m, 2H); HRMS calcd for $C_{19}H_{25}ClN_5O_2$ $(M + H)^+$ 390.1697, found 390.1691.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-1,2-dicyclopropylethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**19**).* Dowex 50 resin (5 mg) was added to a solution of compound **32** (7.6 mg, 0.019 mmol) in methanol (0.5 mL) and water (0.5 mL) and the mixture stirred at room temperature for 7 h. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 35:1) to give the desired compound **19** (5.2 mg, 76%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 4.79 (s, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.89 (d, J = 6.8 Hz, 1H), 3.80 (br s, 1H), 2.00−1.97 (m, 1H), 1.69−1.61 (m, 2H), 1.34−1.30 (m, 3H), 1.10−1.08 (m, 1H), 0.89−0.75 (m, 2H), 0.60−0.59 (m, 1H), 0.47−0.36 (m, 4H), 0.17−0.07 (m, 2H); HRMS calcd for $C_{19}H_{25}ClN_5O_2$ $(M + H)^+$ 390.1697, found 390.1697.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-cyclopropylphenylmethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**20**).* Dowex 50 resin (5 mg) was added to a solution of compound **33** (6.6 mg, 0.014 mmol) in methanol (0.6 mL) and water (0.4 mL) and the mixture stirred at room temperature for 7 h. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the desired compound **20** (4.8 mg, 80%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.16 (s, 1H), 7.48 (d, J = 7.6 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.26 (t, J = 7.6 Hz, 1H), 4.78 (s, 2H), 4.69 (t, J = 5.6 Hz, 1H), 3.88 (t, J = 7.2 Hz, 1H), 2.01−1.95 (m, 1H), 1.68−1.63 (m, 1H), 1.42−1.30 (m, 2H), 0.79−0.73 (m, 1H), 0.65−0.62 (m, 2H), 0.54−0.51 (m, 2H); HRMS calcd for $C_{21}H_{23}ClN_5O_2$ $(M + H)^+$ 412.1540, found 412.1533.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-cyclopropylphenylmethylamino)-9H-purin-9-yl)bicyclo[3.1.0]hexane-2,3-diol (**21**).* Dowex 50 resin (7 mg) was added to a solution of compound **34** (8.6 mg, 0.019 mmol) in methanol (0.8 mL) and water (0.4 mL) and the mixture stirred at room temperature for 7 h. The reaction mixture was filtered on a Celite bed, the filtrate was evaporated under vacuum, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the desired compound **21** (6.2 mg, 79%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.17 (s, 1H), 7.48 (d, J = 7.6 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 7.24 (t, J = 7.2 Hz, 1H), 4.78 (s, 2H), 4.71 (t, J = 5.2 Hz, 1H), 3.88 (t, J = 7.6 Hz, 1H), 2.02−1.95 (m, 1H), 1.68−1.65 (m, 1H), 1.43−1.30 (m, 2H), 0.79−0.73 (m, 1H), 0.65−0.60 (m, 2H), 0.54−0.47 (m, 2H); HRMS calcd for $C_{21}H_{23}ClN_5O_2$ $(M + H)^+$ 412.1540, found 412.1544.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-(2,2-dicyclopropylethylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**23**).* 2,2-Dicyclopropylethylamine (38 mg, 0.30 mmol) and triethylamine (0.12 mL, 0.84 mmol) were added to a solution of compound **22a** (20.91 mg, 0.061 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **23** (22 mg, 84%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.14 (s, 1H), 5.36 (t, J = 6.0 Hz, 1H), 4.97 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.73 (d, J = 6.4 Hz, 2H), 2.09−2.03 (m, 1H), 1.76−1.71 (m, 1H), 1.52 (s, 3H), 1.25 (s, 3H), 0.95−0.90 (m, 2H), 0.75−0.72 (m, 2H), 0.51−0.42 (m, 5H), 0.28−0.17 (m, 4H); HRMS calcd for $C_{22}H_{29}ClN_5O_2$ $(M + H)^+$ 430.2010, found 430.2013.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-1-cyclopropylpropylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**25**).* (*R*)-1-Cyclopropylpropylamine hydrochloride (16.16 mg, 0.11 mmol) and triethylamine (0.11 mL, 0.82 mmol) were added to a solution of compound **22a** (20.33 mg, 0.05 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **25** (18.7 mg, 78%) as a foamy syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (s, 1H), 5.36 (t, J = 6.4 Hz, 1H), 4.96 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.66 (br s, 1H), 2.07−2.04 (m, 1H), 1.86−1.79 (m, 1H), 1.77−1.70 (m, 2H), 1.52 (s, 3H), 1.25 (s, 3H), 1.03−0.99 (m, 4H), 0.95−0.90 (m, 2H), 0.62−0.56 (m, 1H), 0.45−0.41 (m, 2H), 0.36−0.33 (m, 1H); HRMS calcd for $C_{20}H_{27}ClN_5O_2$ $(M + H)^+$ 404.1853, found 404.1855.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-1-cyclopropylpropylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**26**).* (*S*)-1-Cyclopropylpropylamine hydrochloride (32.8 mg, 0.11 mmol) and triethylamine (0.11 mL, 0.82 mmol) were added to a solution of compound **22a** (20.43 mg, 0.05 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **26** (16.6 mg, 80%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (s, 1H), 5.36 (t, J = 6.4 Hz, 1H), 4.96 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.67 (br s, 1H), 2.09−2.03 (m, 1H), 1.88−1.79 (m, 1H), 1.77−1.72 (m, 2H), 1.52 (s, 3H), 1.25 (s, 3H), 1.03−0.99 (m, 4H), 0.96−0.89 (m, 2H), 0.61−0.56 (m, 1H), 0.47−0.41 (m, 2H), 0.36−0.30 (m, 1H); HRMS calcd for $C_{20}H_{27}ClN_5O_2$ $(M + H)^+$ 404.1853, found 404.1854.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-1-cyclopropyl-2-methylpropylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**27**).* (*R*)-1-Cyclopropyl-2-methylpropylamine hydrochloride (18.22 mg, 0.12 mmol) and triethylamine (0.11 mL, 0.85 mmol) were added to a solution of compound **22a** (20.78 mg, 0.06 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **27** (19 mg, 75%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 5.36 (t, J = 6.0 Hz, 1H), 4.97 (s, 1H), 4.70 (d, J = 6.4 Hz, 1H), 3.62 (t, J = 7.2 Hz, 1H), 2.08−2.03 (m, 1H), 1.76−1.72 (m, 1H), 1.52 (s, 3H), 1.25 (m, 4H), 1.08−1.05 (m, 7H), 0.96−0.88 (m, 2H), 0.65−0.61 (m, 1H), 0.45−0.36 (m, 2H); HRMS calcd for $C_{21}H_{29}ClN_5O_2$ $(M + H)^+$ 418.2010, found 418.2004.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-1-cyclopropyl-2-methylpropylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**28**).* (*S*)-1-Cyclopropyl-2-methylpropylamine hydrochloride (16.5 mg, 0.12 mmol) and triethylamine (0.12 mL, 0.84 mmol) were added to a solution of compound **22a** (20.85 mg, 0.06 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **28** (19.8 mg, 78%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 5.36 (t, J = 6.0 Hz, 1H), 4.97 (s, 1H), 4.71 (d, J = 6.4 Hz, 1H), 3.61 (t, J = 7.2 Hz, 1H), 2.08−2.04 (m, 1H), 1.76−1.71 (m, 1H), 1.51 (s, 3H), 1.25 (m, 4H), 1.08−1.05 (m, 7H), 0.96−0.88 (m, 2H), 0.65−0.61 (m, 1H), 0.45−0.36 (m, 2H); HRMS calcd for $C_{21}H_{29}ClN_5O_2$ $(M + H)^+$ 418.2010, found 418.2017.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-cyclopropylcyclobutylmethylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**29**).* (*R*)-Cyclopropylcyclobutylmethylamine hydrochloride (28.3 mg, 0.17 mmol) and triethylamine (0.11 mL, 0.81 mmol) were added to a solution of compound **22a** (19.92 mg, 0.05 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **29** (20.8 mg, 83%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 5.36 (t, J = 6.0 Hz, 1H), 4.96 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.85 (br s, 1H), 2.72−2.67 (m, 1H), 2.10−2.89 (m, 6H), 1.87−1.71 (m, 2H), 1.52 (s, 3H), 1.25 (s, 3H), 0.96−0.89 (m, 3H), 0.58−0.53 (m, 1H), 0.39−0.36 (m, 3H); HRMS calcd for $C_{22}H_{29}ClN_5O_2$ $(M + H)^+$ 430.2010, found 430.2018.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-cyclopropylcyclobutylmethylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**30**).* (*S*)-Cyclopropylcyclobutylmethylamine hydrochloride (35.8 mg, 0.22 mmol) and triethylamine (0.14 mL, 1.03 mmol) were added to a solution of compound **22a** (25.2 mg, 0.73 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **30** (26 mg, 82%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (s, 1H), 5.36 (t, J = 6.0 Hz, 1H), 4.96 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.84 (br s, 1H), 2.71−2.67 (m, 1H), 2.09−2.89 (m, 6H), 1.87−1.71 (m, 2H), 1.51 (s, 3H), 1.25 (s, 3H), 0.95−0.88 (m, 3H), 0.58−0.54 (m, 1H), 0.37−0.36 (m, 3H); HRMS calcd for $C_{22}H_{29}ClN_5O_2$ $(M + H)^+$ 430.2010, found 430.2008.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-1,2-dicyclopropylethylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**31**).* (*R*)-1,2-Dicyclopropylethylamine hydrochloride (28.4 mg, 0.17 mmol) and triethylamine (0.11 mL, 0.82 mmol) were added to a solution of compound **22a** (20.0 mg, 0.05 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **31** (18.8 mg, 75%) as a foamy syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (s, 1H), 5.36 (t, J = 6.4 Hz, 1H), 4.96 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.80 (br s, 1H), 2.06−2.03 (m, 1H), 1.73−1.57 (m, 3H), 1.51 (s, 3H), 1.25 (s, 3H), 1.09−1.06 (m, 1H), 0.94−0.85 (m, 3H), 0.60−0.58 (m, 1H), 0.44−0.35 (m, 5H), 0.17−0.16 (m, 1H), 0.09−0.07 (m, 1H); HRMS calcd for $C_{22}H_{29}ClN_5O_2$ $(M + H)^+$ 430.2010, found 430.2025.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-1,2-dicyclopropylethylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]hexane (**32**).* (*S*)-1,2-Dicyclopropylethylamine hydrochloride (31.5 mg, 0.19 mmol) and triethylamine (0.12 mL, 0.91 mmol) were added to a solution of compound **22a** (22.21 mg, 0.05 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1) to give the desired product **32** (21.5 mg, 77%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (s, 1H), 5.36 (t, J = 6.4 Hz, 1H), 4.96 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.80 (br s, 1H), 2.07−2.03 (m, 1H), 1.74−1.58 (m, 3H), 1.51 (s, 3H), 1.25 (s, 3H), 1.09−1.05 (m, 1H), 0.95−0.84 (m, 3H), 0.60−0.58 (m, 1H), 0.44−0.35 (m, 5H), 0.17−0.15 (m, 1H), 0.09−0.07 (m, 1H); HRMS calcd for $C_{22}H_{29}ClN_5O_2$ $(M + H)^+$ 430.2010, found 430.2002.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((R)-N-cyclopropyl-N-phenylmethylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]-hexane (**33**).* (*R*)-*N*-Cyclopropyl-*N*-phenylmethylamine hydrochloride (22 mg, 0.12 mmol) and triethylamine (0.11 mL, 0.84 mmol) were added to a solution of compound **22a** (20.68 mg, 0.06 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:2) to give the desired product **33** (22.15 mg, 81%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 7.48 (d, J = 7.6 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 2.24 (t, J = 7.2 Hz, 1H), 5.35 (t, J = 6.0 Hz, 1H), 4.95 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.60 (br s, 1H), 2.06−2.03 (m, 1H), 1.74−1.71 (m, 1H), 1.51 (s, 3H), 1.43−1.37 (m, 1H), 1.24 (s, 3H), 0.94−0.89 (m, 2H), 0.65−0.63 (m, 2H), 0.53−0.46 (m, 2H); HRMS calcd for $C_{24}H_{27}ClN_5O_2$ $(M + H)^+$ 452.1853, found 452.1858.

*(1R,2R,3S,4R,5S)-4-(2-Chloro-6-((S)-N-cyclopropyl-N-phenylmethylamino)-9H-purin-9-yl)-2,3-O-(isopropylidene)bicyclo[3.1.0]-hexane (**34**).* (*S*)-*N*-Cyclopropyl-*N*-phenylmethylamine hydrochloride (32.8 mg, 0.17 mmol) and triethylamine (0.11 mL, 0.84 mmol) were added to a solution of compound **22a** (20.43 mg, 0.06 mmol) in methanol (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuum and the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:2) to give the desired product **34** (22.1 mg, 82%) as a syrup: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 7.48 (d, J = 7.2 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 2.24 (t, J = 7.2 Hz, 1H), 5.35 (t, J = 6.8 Hz, 1H), 4.95 (s, 1H), 4.69 (d, J = 7.2 Hz, 1H), 3.60 (br s, 1H), 2.06−2.03 (m, 1H), 1.75−1.71 (m, 1H), 1.51 (s, 3H), 1.42−1.37 (m, 1H), 1.24 (s, 3H), 0.96−0.88 (m, 2H), 0.66−0.62 (m, 2H), 0.54−0.43 (m, 2H); HRMS calcd for $C_{24}H_{27}ClN_5O_2$ $(M + H)^+$ 452.1853, found 452.1856.

Molecular Modeling. *hA_1AR and hA_3AR Homology Models.* Previously reported molecular models of the hA_1AR and hA_3AR, built using the alignment and the homology modeling tools implemented in the program Molecular Operating Environment (MOE),[45] were used in this study. Both models were built using as template the crystal structure of the human $A_{2A}AR$ cocrystallized with the agonist **50** (PDB ID: 3QAK),[22] as described by Tosh et al.[20] In particular, hA_1AR and hA_3AR sequences were retrieved from the UniProt database[46] and aligned against the sequence of the $A_{2A}AR$ template, taking into account the highly conserved residues in each TM domain. Then, homology models were built using the automated Homology Modeling protocol implemented in the MOE suite.

Molecular Docking of Truncated Methanocarba Derivatives in the hA_1AR and hA_3AR Models. Compounds structures were built using the builder tool implemented in the MOE suite[48] and subjected to energy minimization using the MMFF94x force field, until a rms gradient of 0.05. The minimized conformations of each compound were used as a starting point for the docking study. The flexible docking of the ligands in the hA_1AR and hA_3AR models was performed by means of the Glide[47] package part of the Schrödinger suite.[48]

The docking site was defined with key residues in the binding pocket of the hA_1AR and hA_3AR models, namely Phe(EL2), Asn(6.55), Trp(6.48) and His(7.43), and a 20 Å × 20 Å × 20 Å box was centered on those residues. Docking of the ligands was performed in the rigid binding site of the models with Glide using the XP (extra precision) procedure.

The top scoring docking conformations were retained and subjected to the receptor sampling by means of the Refinement module in Prime.[49] The Prime side chain sampling was performed on all the residues within a 6 Å of the ligand. The refined model for each ligand was chosen as the final binding conformation.

In Vivo Testing. *Animals and Test Substances Used for Seizure Testing.* Adult male CF No. 1 albino mice (26−30 g, 6 Hz; 18−25 g all other tests) were obtained from Charles River, Portage, MI. Animals were housed in an Association for Assessment and

Accreditation of Laboratory Animal Care, International (AAALAC)-accredited temperature and humidity controlled facility and maintained on a standard 12 h:12 h light−dark cycle (lights on at 0600) with free access to standard laboratory chow (Prolab RMH 3000) and water ad libitum. All animal experiments were performed in accordance with the guidelines set by National Institutes of Health and the University of Utah Institutional Animal Care and Use Committee (IACUC) committee. All animals were allowed free access to both food and water except when they were removed from their cages for the experimental procedure. Except for the kindling studies, animals were used once. All animals were euthanized in accordance with the Institute of Laboratory Resources policies on the humane care of laboratory animals.

Anticonvulsant Tests. In vivo anticonvulsant activity was established by both electrical and chemoconvulsant seizure tests that have been described previously.[50−52] The electrical tests used were the maximal electroshock (MES) seizure test, the 6 Hz minimal clonic seizure test, and the corneal kindled mouse test. The chemical test was the sc metrazol seizure tests. TPE is deduced from data generated in initial qualitative test procedures. Five groups of four animals each are administered the test compound, and each group is tested at one of five different time intervals: 0.25, 0.5, 1, 2, and 4 h. The time point at which the compound produces the most activity/toxicity was chosen as TPE.

MES Test and 6 Hz Test. For the MES and 6 Hz tests, a drop of anesthetic/electrolyte solution (0.5% tetracaine hydrochloride in 0.9% saline) was applied to the eyes of each animal prior to placement of the corneal electrodes. The electrical stimulus in the MES test was 50 mA, 60 Hz, for mice. Abolition of the hind leg tonic extensor component of the seizure was used as the end point.

The ability of the test substance to prevent seizures in mice induced by 6 Hz corneal stimulation (32 mA, 3 s duration) was evaluated at a convulsive current that evokes a seizure in 97% of the population tested (CC_{97}). Six hertz seizures are characterized by a minimal clonic phase that is followed by stereotyped, automatiztic behaviors described originally as being similar to the aura of human patients with partial seizures.[53,54] Animals not displaying this behavior were considered protected.

Corneal-Kindled Mouse Model of Partial Seizures. Mice were kindled according to the methods described by Matagne and Klitgaard.[55] Briefly, mice were stimulated twice daily with a corneal stimulation of 3 mA for 3 s for an average of 12 days. Prior to each stimulation, a drop of 0.9% saline containing 0.5% tetracaine hydrochloride (Sigma-Aldrich, St. Louis, MO) was applied to the cornea to ensure local anesthesia and good electrical conductivity. Stimulations were at least 4 h apart. Animals were considered kindled when they displayed five consecutive stage 5 seizures according to the Racine scale.[56] At the completion of the kindling acquisition, mice were permitted at least a 3-day stimulation-free period prior to any drug testing. Mice were stimulated once the day before drug testing to ensure they had achieved and maintained a kindled state. On the day of the drug study, corneal kindled mice ($n = 4$ or 8) received a single ip dose of test compound. Mice were challenged with the corneal kindling stimulus of 3 mA for 3 s at TPE after test compound administration. Mice were scored as protected (seizure score of ±3) or not protected (seizure score ≥ 4) based on the Racine scoring.[56]

Minimal Behavioral Toxicity Tests. Minimal behavioral toxicity was identified in mice by the rotarod performance test.[57] When a mouse is placed on a 1-in. knurled rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for long periods of time. The animal was considered toxic if it fell off this rotating rod three times during a 1-min period.

Determination of Median Effective (ED_{50}) or Behavioral Toxic Dose (TD_{50}). All quantitative in vivo anticonvulsant/toxicity studies were conducted at the previously determined TPE. Groups of at least eight mice were tested with various doses of the candidate drug until at least two points were established between the limits of 100% protection or minimal toxicity and 0% protection or minimal toxicity. The dose of drug required to produce the desired end point in 50% of animals (ED_{50} or TD_{50}) in each test, the 95% confidence interval, the slope of the regression line, and the SEM of the slope were then calculated by a computer program based on the method described by Finney.[58]

Compound Administration. The test compound was administered at a concentration that permitted optimal accuracy of dosing without the volume contributing excessively to total body fluid. Thus, test compounds are administered to mice in a volume of 0.01 mL/g of body weight. Compound **10** or other AR agonist was initially dissolved in DMSO (50 mg/mL) as a stock solution. To prepare the formulation for testing, an appropriate amount of stock solution was first diluted in DMSO to achieve 10% DMSO (v/v) in the final volume. Then, 30% PEG400 (J. T. Baker) was gradually added to the aqueous DMSO solution to make the final formulation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures for compounds **37** and **39**−**43**, spectroscopic characterization, in vitro bioassay procedures, anticonvulsant data, and a modeling figure. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADAC, N^6-[4-[[[4-[[[(2-aminoethyl)amino]carbonyl]methyl]-anilino]carbonyl]methyl]phenyl]adenosine; AR, adenosine receptor; cyclic AMP, adenosine 3′,5′-cyclic phosphate; BBB, blood−brain barrier; CCPA, 2-chloro-N^6-cyclopentyladenosine; CHO, Chinese hamster ovary; GPCR, G-protein-coupled receptor; HEK, human embryonic kidney; ip, intraperitoneal; MES, maximal electroshock; MRS5127, (1′*S*,2′*R*,3′*S*,4′*R*,5′*S*)-4′-[2-chloro-6-(3-iodobenzylamino)purine]-2′,3′-*O*-dihydroxybicyclo[3.1.0]hexane; NECA, 5′-*N*-ethylcarboxamidoadenosine; PIA, N^6-phenylisopropyladenosine; scMET, subcutaneous metrazol model; TM, transmembrane domain; TPE, time of peak effect; tPSA, total polar surface area.

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