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N^6-Cycloalkyl-2-substituted adenosine derivatives as selective, high affinity adenosine A_1 receptor agonists

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Abstract—A series of new selective, high affinity A_1-AdoR agonists is reported. Compound **23** that incorporated a carboxylic acid functionality in the 4-position of the pyrazole ring displayed K_{iL} value of 1 nM for the A_1-AdoR and >5000-fold selectivity over the A_3 and A_{2A}-AdoRs. In addition, compound **19** that incorporated a carboxamide functionality in the 4-position of the pyrazole ring displayed subnanomolar affinity for the A_1-AdoR (K_{iL} = 0.6 nM) and >600-fold selectivity over the A_3 and A_{2A}-AdoRs.

Adenosine is an endogenous purine nucleoside that modulates a variety of physiological functions as a result of its activation of specific G protein-coupled receptors defined as A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (AdoRs).[1] Physiological responses that are mediated by the A_1-AdoR include cardiac (negative inotropic, negative chronotropic, and negative dromotropic effects) and antilipolytic effects.[2] Therefore, A_1-AdoR agonists have received much attention as antiarrhythmic and antilipolytic agents. In general, selective A_1-AdoR agonists were obtained by monosubstitution of the N^6-position of adenosine (e.g., CPA, CHA)[3], whereas substitution at the C-2 position of adenosine yielded selective A_{2A}-AdoR agonists (e.g., CVT-3146 (Regadenoson), CGS21680).[4,5] However, the selectivity of AdoR agonists for the A_1 receptor was challenged by the discovery of the A_3-AdoR. Consequently, a wide range of N^6-substituted adenosine derivatives originally thought to be selective for the A_1-AdoR later turned out to be also active at the A_3-AdoR and that represented a new challenge to discover selective A_1-AdoR agonists.[6] Even though CCPA is considered to be one of the most selective A_1-AdoR agonists known to date, it displayed only 50-fold selectivity for the A_1-AdoR over the A_3-AdoR.[7] Hence, more selective A_1-AdoR agonists are needed. Simultaneous substitution at the N^6 and C-2 positions of adenosine has resulted in compounds with different activity and selectivity profiles and some of these disubstituted compounds have high affinity and selectivity for the A_1-AdoR.[8,9]

In previous communications, we have shown that introducing a methyl group into the N^6 position of CVT-3146 induces an increase in the affinity for the human A_3-AdoR and simultaneously decreases the affinity for the A_1 and A_{2A}-AdoRs, resulting in significant enhancement in A_3-AdoR selectivity.[10] During that study, we have also observed that increasing the size of the N^6 substituent from methyl to ethyl and propyl resulted in a decrease in the A_3-AdoR affinity and an increase in the A_1-AdoR affinity and selectivity. This prompted us to explore the effect of introducing substituents that are conducive to high A_1-AdoR binding affinity (e.g., cycloalkyls) into the N^6 position of our 2-pyrazolyl adenosine derivatives with the idea of enhancing A_1-AdoR binding affinity and selectivity. In addition to the N^6-cyclopentyl, N^6-norbornyl, and N^6-cyclohexyl substituents, we elected to incorporate the R-tetrahydrofuran (R-THF) group as our main N^6-substituent (R-THF is the N^6-substituent in CVT-510, our agent in phase III clinical trials as an antiarrhythmic agent, Fig. 1).

The synthesis of compounds **27**–**34** is outlined in Scheme 1. Treatment of commercially available 2′,3′,5′-tri-*O*-acetyl-2,6-dichloroadenosine with 3-(*R*)-aminotetrahydrofu-

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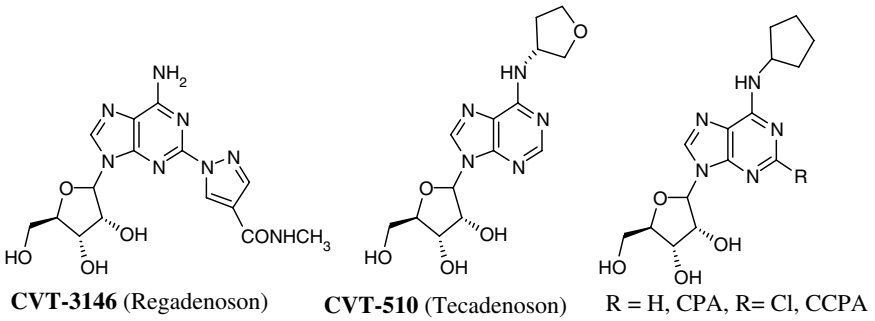
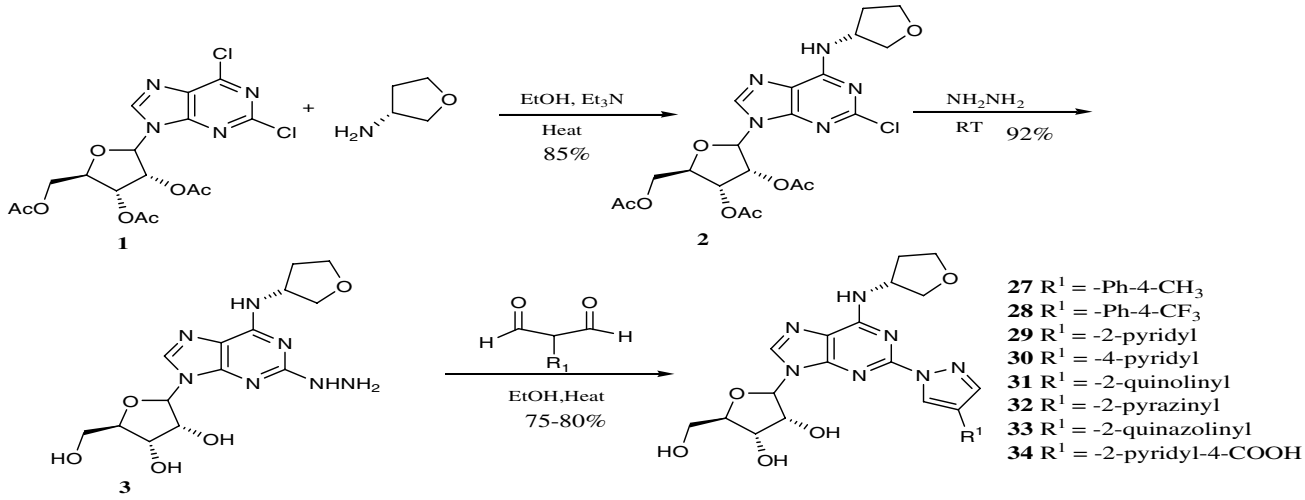


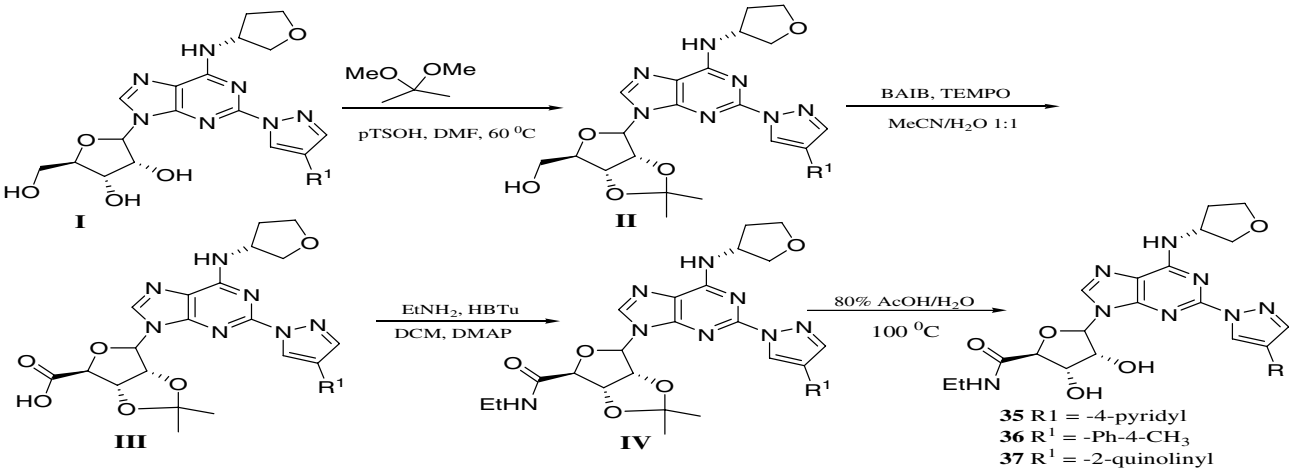
Figure 1. A_1 and A_{2A} adenosine receptor agonists.



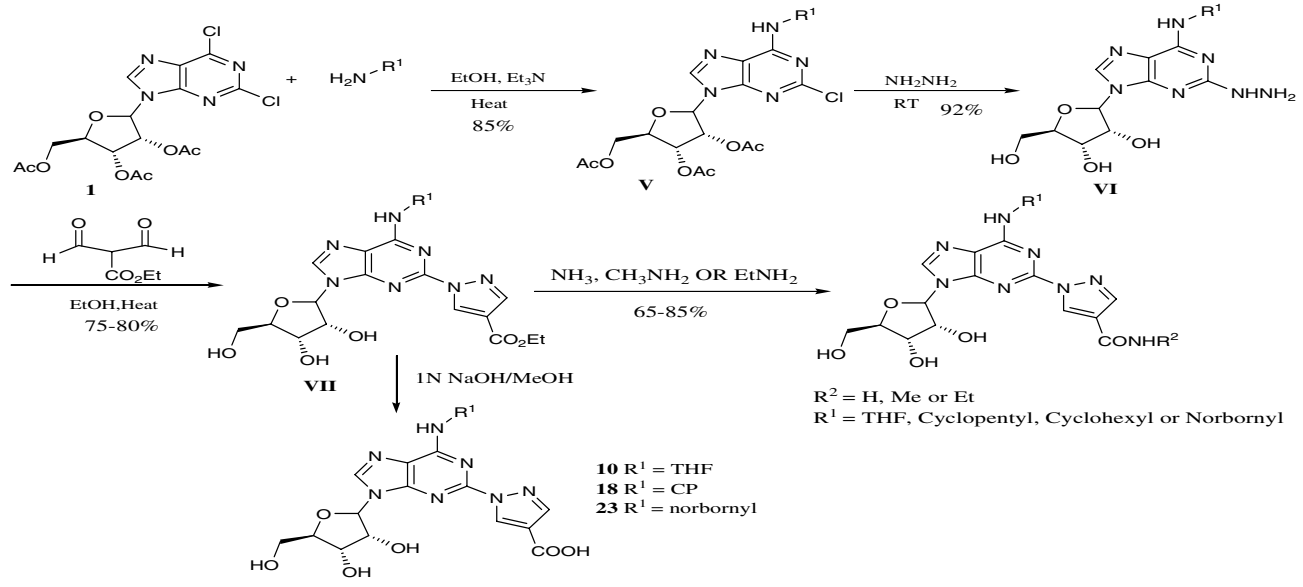
Scheme 1.

ran in EtOH using triethylamine as a base afforded **2** in 85% yield. Direct substitution of **2** with hydrazine and subsequent condensation of the resulting in 2-hydrazinoadenosine derivative **3** with the appropriate malonaldehyde in EtOH afforded the target compounds in 75–80% yields. The 5′-*N*-ethylcarboxamide analogs **35**–**37** were prepared from the acid[11] **III** and ethylamine hydrochloride using standard amino acid coupling (HBTu/HOBt) in DMF followed by removal of the isopropylidene group in 80% AcOH/H_2O (Scheme 2). As outlined in Scheme 3 condensation of 2-hydrazino-N^6-substituted adenosine derivative **VI** with ethyl 2,2-diformylacetate[12] in EtOH afforded the ester **VII**. Compounds **7**, **8**, **16**, **17**, **19**, **20**, and **26** were obtained by direct aminolysis of the ester **VII** with ammonia, methylamine or ethylamine. The pyrazole acid analogs **10**, **18**, and **23** were obtained via hydrolysis of ester **VII** using 1 N NaOH/MeOH. To enhance the solubility of the acid **6** in organic solvents (resulting from hydrolysis of ester **4**) the hydroxyl groups of **4** were protected with TBDMS group (Scheme 4). Hydrolysis of ester **5** and coupling of the resulting acid **6** with the appropriate amines afforded the target compounds **9**, **11**, and **13** after removal of the TBDMS group in 1 N NH_4F/MeOH.

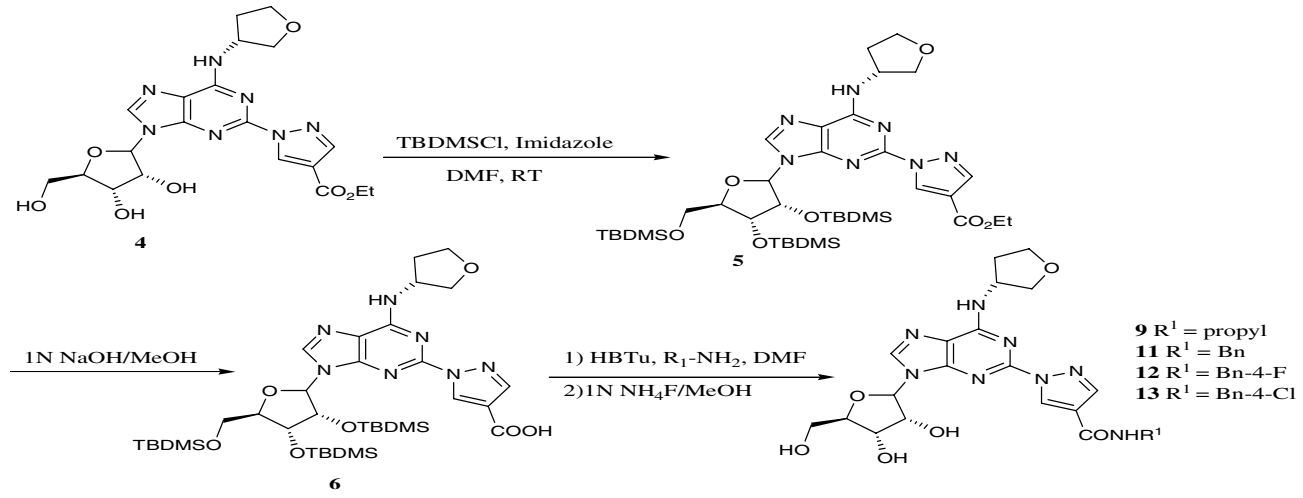
Binding affinities of compounds **4**–**23** for the G-protein coupled receptors ($K_{i\text{-High}}$, K_{iH}) and uncoupled receptor ($K_{i\text{-Low}}$, K_{iL}) were evaluated in radioligand binding assays and the results are shown in Tables 2 and 3. For the A_1-AdoR, K_{iL} was determined wherein GTPγS was added to uncouple the G-protein from the A_1-AdoR while for the A_{2A}, A_3, and A_{2B}-AdoRs K_{iH} were determined (in the absence of GTP). Agonists have been demonstrated to have 3- to 10-folds higher affinity for the G-protein coupled receptors than that for the uncoupled receptors.[13,14] For comparison purposes, selected data for N^6-methyl-2-pyrazolyl adenosine derivatives are also listed (**A**–**E**, Table 1). The A_1, A_{2A}, and A_3-AdoRs binding affinities of these compounds were previously reported.[10] Replacement of the N^6-methyl substituent in compound **A** (Table 1) with R-THF group as in **7** (Table 2) resulted in a significant enhancement in the A_1-AdoR binding affinity (K_{iL} = 23 nM) and 9-fold loss in A_3-AdoR binding affinity relative to **A** (K_{iH} = 710 nM). Increasing the length of the methylcarboxamide group in compound **7** to ethyl and propyl groups has minimum effect on the A_1-AdoR binding affinity and at the same time resulted in enhancement in the A_3-AdoR binding affinity (**8** and **9**). Negatively charged groups are known to diminish affinity at the adenosine receptors and several carboxylate-bearing



Scheme 2.

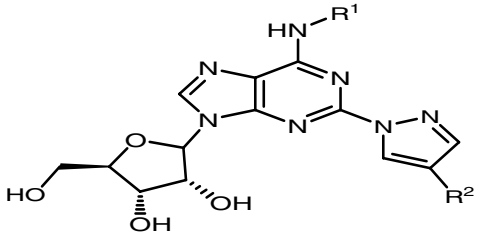


Scheme 3.



Scheme 4.

Table 1. A_1 and A_3-AdoRs binding affinities and selectivities of compounds **A**–**E**[10]

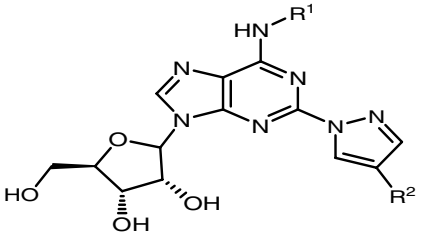


Compound[a]	R_1	R_2	K_{iL} A_1[b] (nM)	K_{iH} A_3[c] (nM)
A	CH_3	$CONHCH_3$	>6000	73
B	CH_3	–Ph-4-OCH_3	>4000	15
C	Et	–Ph-4-OCH_3	3700	41
D	CH_3	–2-Pyridyl	3800	2
E	Et	–2-Pyridyl	1300	107

[a] 95% confidence limits were generally ±15% of the mean value.
[b] Binding affinity for A_1-AdoR was determined using DDT membranes with [^{3}H]-CPX as the radioligand, ($K_{i\text{-Low}}$).
[c] Binding affinity for A_3-AdoR was determined using CHO-A_3 cells with [^{125}I]-AB-MECA as the radioligand ($K_{i\text{-High}}$).

A_1-AdoR agonists have been reported to have affinities about two orders of magnitude less than the corresponding hydrogen-bearing derivatives.[15] However, the acid analog **10** displayed A_1-AdoR binding affinity similar to **7** (K_{iL} = 24 nM) and at least 4-fold increased selectivity for the A_1-AdoR over A_3 and A_{2A}-AdoRs relative to **7**. Replacing the methylcarboxamide moiety in **7** with a benzylamide group yielded at least 10-fold enhancement in both A_1- and A_3-AdoRs binding affinity in comparison to **7** (**11**, K_{iL} A_1 = 2 nM, K_{iH} A_3 = 54 nM). Attempts to improve the A_1-AdoR selectivity of **11** over the A_3-AdoR by incorporating an electron-withdrawing group in the *p*-position of the phenyl ring of **11** led to a progressive enhancement in the A_3-AdoR binding affinity relative to **11** (**12** and **13**). Compound **15** that contains a carboxylic acid group in the *p*-position of the phenyl displayed high affinity (K_{iL} = 8 nM) and selectivity (>390-fold) for the A_1-AdoR over the A_3-AdoR. Exchanging the R-THF moiety in **7** with a cyclopentyl group as in **16** resulted in 11-fold improvement in the A_1-AdoR binding affinity (K_{iL} = 2 nM) and at least 20-fold increased selectivity for the A_1-AdoR over the A_3 and A_{2A}-AdoRs relative to **7**. Increasing the size of the methylcarboxamide group in **16** to ethyl as in **17**

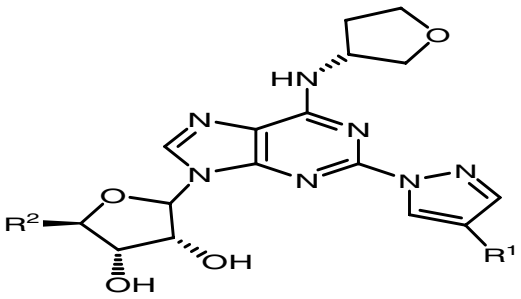
Table 2. A_1, A_3, A_{2B}, and A_{2A}-AdoRs binding affinities and selectivities



Compound	R_1	R_2	K_i[a] (nM)				
			K_{iL} (A_1)[b]	K_{iH} (A_3)[c]	K_{iH} (A_{2A})[d]	K_{iH} (A_{2B})[e]	A_3/A_1
4	(R)-THF	–$COOCH_2CH_3$	3	41	2410	>6000	13
7	(R)-THF	–$CONHCH_3$	23	710	726	>6000	30
8	(R)-THF	–$CONHCH_2CH_3$	10	132	1410	>6000	13
9	(R)-THF	–$CONH(CH_2)_2CH_3$	10	49	>5000	>6000	5
10	(R)-THF	–COOH	24	3210	>4000	>6000	133
11	(R)-THF	–CONHBn	2	54	>5000	>6000	27
12	(R)-THF	–CONHBn-4-F	43	15	>5000	>6000	0.34
13	(R)-THF	–CONHBn-4-Cl	27	4.0	>5000	>6000	0.15
14	(R)-THF	–CONHBn-4-CO_2Et	6	92	>5000	>6000	15
15	(R)-THF	–CONHBn-4-CO_2H	8	3160	>5000	>6000	395
16	Cyclopentyl	–$CONHCH_3$	2	1420	>5000	>6000	710
17	Cyclopentyl	–$CONHCH_2CH_3$	1	20	>5000	>6000	20
18	Cyclopentyl	–COOH	9	4120	>5000	>6000	468
19	Cyclopentyl	–$CONH_2$	0.6	380	>5000	>6000	633
20	Cyclopentyl	–$COOCH_2CH_3$	0.9	19	>5000	>6000	21
21	Cyclopentyl	H	34	105	>5000	>6000	3
22	Norbornyl	–$COOCH_2CH_3$	3	250	>5000	>6000	83
23	Norbornyl	–COOH	1	>5000	>5000	>6000	>5000
24	Norbornyl	H	0.4	1270	>5000	>6000	3175
25	Cyclohexyl	–$COOCH_2CH_3$	30	30	>5000	>6000	1
26	Cyclohexyl	–$CONHCH_3$	8	158	>5000	>6000	19

[a] 95% confidence limits were generally ±15% of the mean value.
[b] Binding affinity for A_1-AdoR was determined using DDT membranes with [^{3}H]-CPX as the radioligand ($K_{i\text{-Low}}$).
[c] Binding affinity for A_3-AdoR was determined using CHO-A_3 cells with [^{125}I]-AB-MECA as the radioligand ($K_{i\text{-High}}$).
[d] Binding affinity for A_{2A}-AdoR was determined using HEK-A_{2A} cells with [^{3}H]-ZM241385 as the radioligand ($K_{i\text{-High}}$).
[e] Binding affinity for A_{2B}-AdoR was determined using HEK-A_{2B} cells with ^{3}H-ZM241385 as the radioligand ($K_{i\text{-High}}$).

Table 3. A_1, A_3, A_{2B}, and A_{2A}-AdoRs binding affinities and selectivities of compounds **27**–**37**



Compound	R_1	R_2	K_i[a] (nM)				
			K_{iL} (A_1)[b]	K_{iH} (A_3)[c]	K_{iH} (A_{2A})[d]	K_{iH} (A_{2B})[e]	A_3/A_1
27	–Ph-4-CH_3	CH_2OH	74	94	2320	>6000	1
28	–Ph-4-CF_3	CH_2OH	31	15	>5000	>6000	0.5
29	–2-Pyridyl	CH_2OH	36	3	1180	>6000	0.08
30	–4-Pyridyl	CH_2OH	134	4	1480	>6000	0.03
31	–2-Quinolinyl	CH_2OH	39	26	239	>6000	0.6
32	–2-Pyrazinyl	CH_2OH	20	36	1170	>6000	2
33	–2-Quinazolinyl	CH_2OH	160	63	357	>6000	0.4
34	–2-Pyridyl-4-COOH	CH_2OH	64	77	>5000	>6000	1
35	–4-Pyridyl	–CONHEt	1060	NT	NT	>6000	
36	–Ph-4-CH_3	–CONHEt	121	0.7	>5000	>6000	0.005
37	–2-Quinolinyl	–CONHEt	89	0.57	>5000	>6000	0.006

NT, not tested.

[a] 95% confidence limits were generally ±15% of the mean value.

[b] Binding affinity for A_1-AdoR was determined using DDT membranes with [^{3}H]-CPX as the radioligand ($K_{i\text{-Low}}$).

[c] Binding affinity for A_3-AdoR was determined using CHO-A_3 cells with [^{125}I]-AB-MECA as the radioligand ($K_{i\text{-High}}$).

[d] Binding affinity for A_{2A}-AdoR was determined using HEK-A_{2A} cells with [^{3}H]-ZM241385 as the radioligand ($K_{i\text{-High}}$).

[e] Binding affinity for A_{2B}-AdoR was determined using HEK-A_{2B} cells with ^{3}H-ZM241385 as the radioligand ($K_{i\text{-High}}$).

resulted in 70-fold enhancement in A_3-AdoR binding affinity (K_{iH} = 20 nM) relative to **16** while maintaining the A_1-AdoR binding affinity (K_{iL} = 1 nM). This trend is similar to the one that was observed when the same structural changes were applied to compound **7**.

Taking into account the high A_1-AdoR binding affinity and selectivity of compound **7** relative to that of compounds **8**, **9**, **11** and also the high A_1-AdoR binding affinity and selectivity of compound **16** relative to that of **17**, we hypothesize that within this class of compounds steric factors at the 4-position of the pyrazole ring may play a crucial role in determining the binding affinity and selectivity for the A_1 and A_3-AdoRs. While smaller substituents seem to be conducive to high A_1-AdoR binding affinity, larger substituents may show preference in binding to the A_3-AdoR and are detrimental to A_1-AdoR binding affinity. To further test the validity of this hypothesis, the carboxamide analog **19** was synthesized. Compound **19** indeed displayed subnanomolar binding affinity (K_{iL} = 0.6) and substantial selectivity for the A_1-AdoR over both, the A_3 and A_{2A}-AdoRs providing additional support for the above hypothesis.

Encouraged by the high binding affinity and selectivity of the carboxylic acid analog **15**, we opted to investigate the effect of replacing the methylcarboxamide group in compounds **7** and **16** with a carboxylic acid functionality as in compounds **10** and **18**, respectively. Both the N^6-R-THF carboxylic acid analog **10** (K_{iL} A_1 = 24 nM, K_{iH} A_3 = 3120 nM) and the N^6-cyclopentyl carboxylic acid analog **18** (K_{iL} A_1 = 9 nM, K_{iH} A_3 = 4120 nM) displayed comparable high binding affinity and selectivity for the A_1-AdoR. While the cyclopentyl acid analog **18** showed comparable A_1-AdoR affinity and selectivity profile to the methyl carboxamide analog **16**, the THF acid analog **10** displayed slightly increased selectivity for the A_1-AdoR over both, the A_3 and the A_{2A}-AdoRs. However, the N^6-norbornyl acid analog **23** was the most selective compound in this study and displayed low nanomolar binding affinity for the A_1-AdoR (K_{iL} = 1 nM) and >5000-fold selectivity for the A_1-AdoR over the A_3 and A_{2A}-AdoRs. In addition, the N^6-norbornyl unsubstituted pyrazole analog **24** exhibited subnanomolar binding affinity (K_{iL} = 0.4 nM) and substantial selectivity for the A_1-AdoR over both, the A_3 and A_{2A}-AdoRs.

Replacement of the N^6-methyl group in compound **D** (Table 1) with N^6-R-THF resulted in **29** (K_{iH} = 3 nM) that displayed similar A_3-AdoR binding affinity to **D** and significant enhancement in the A_1-AdoR binding affinity (K_{iL} = 36 nM) in comparison to **D**. In general, compounds with aryl or heteroaryl in the 4-position of the pyrazole ring showed similar affinity for both A_1 and A_3-AdoRs (**27**, **28**, **31**, **32**, **34**). However, introducing a carboxamide group (which is known to enhance A_3-AdoR binding affinity) in the 5′-position of

compounds **27** and **31** indeed led to significant increase in the A_3-AdoR binding affinity (**36**, **37**, K_{iH} = 0.7 and 0.5 nM, respectively).

Most of the compounds were evaluated for their relative intrinsic activity in rat brain membranes.

The level of maximum stimulation of [^{35}S]GTPγS binding to G protein induced by the full agonist CPA was taken as 100%. The efficacy of all the tested compounds was comparable to that of CPA and hence all the compounds tested were considered to be full A_1-AdoR agonists (**15** and **18** showed 100% [^{35}S]GTPγS stimulation relative to CPA).

In summary, we have discovered analogs with high binding affinity and selectivity for the A_1-AdoR. Compound **23** that incorporated a carboxylic acid functionality in the 4-position of the pyrazole ring displayed K_{iL} value of 1 nM for the A_1-AdoR and >5000-fold selectivity over the A_3 and A_{2A}-AdoRs. In addition, compound **19** that incorporated a carboxamide functionality in the 4-position of the pyrazole ring displayed subnanomolar affinity for the A_1-AdoR (K_{iL} = 0.6 nM) and >600-fold selectivity over the A_3 and A_{2A}-AdoRs. A_1-AdoR agonists such as N^6-cyclohexyladenosine (CHA) have been shown to cause intense behavioral effects at low doses.[16–19] The locomotor depression elicited by peripherally administrered A_1-AdoR agonists is usually interpreted as a CNS effect. Our new compounds such as **23** contain a carboxylic acid group that would be mostly charged at physiological pH and is expected to diminish the diffusion of such compounds across the blood–brain barrier by analogy of carboxylate xanthine derivative BW-1433[20] and hence would serve as tools to aid in further delineating the central and peripheral effects of A_1-AdoR agonists. In addition, this new series of compounds may serve as leads to discover additional potent and selective A_1-AdoR agonists that may have potential use as therapeutic agents.

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