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Structure–Affinity Relationships of the Affinity of 2-Pyrazolyl Adenosine Analogues for the Adenosine A_{2A} Receptor

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Abstract—The structure–affinity relationships of two novel 2-substituted adenosine series containing a substituted pyrazole attached at the N-1 or C-4 position for the adenosine (ADO) A_{2A} receptor are described. Compounds in the 2-(*N*-1-pyrazolyl) adenosine series **IV** provided the highest affinity for the ADO A_{2A} receptor as compared to the 2-(C-4-pyrazolyl) series **V**. The main structural differences between the two series point to the N-1 nitrogen of series **IV** imparting more favorable binding interactions with the receptor than those of series **V**.

Adenosine is an endogenous ligand that has affinity for all four adenosine receptor subtypes—A_1, A_{2A}, A_{2B}, and A_3.[1] Multiple physiological responses are mediated via local adenosine production that is dependent on the concentration of adenosine produced and the proximity to the various adenosine receptor subtypes, since adenosine has an extremely short half-life in plasma.[2] Structurally modified adenosine analogues provide the ability to introduce added plasma stability and selective affinity for a specific receptor subtype.[2] Ongoing studies toward therapeutic intervention with a modified adenosine analogue that selectively activates one receptor subtype with specific physiological responses are as follows: Ado A_1 receptor- negative dromotropic effect (i.e., supraventricular antiarrhythmic agents),[3,4] Ado A_{2A} receptor—coronary vasodilatation (i.e., pharmacological stress agent)[5–7] and anti-inflammatory properties,[8] Ado A_{2B} receptor—angiogenesis,[9] and Ado A_3 receptor—cardioprotection.[10] We describe our initial efforts in preparing adenosine analogues with high affinity towards the Ado A_{2A} receptor. Further pharmacological characterization including selectivity and pharmaceutical use as coronary vasodilators of selected Ado A_{2A} agonists of this communication have been described elsewhere.[6]

Potent and selective A_{2A} agonists that contain a lipophilic substituent at the 2-position linked through three types of functional groups have been described: a pi system, a polar atom—nitrogen or oxygen, or both a pi system and polar atom as illustrated in Figure 1.[1] The 2-hexynyl **1a**[11] and 2-*trans*-hexenyl **1b**[12] adenosine analogues previously described are both potent and selective A_{2A} agonists (**Ia** $K_i = 2.2$ nM, **Ib** $K_i = 14$ nM from ref 12) that are representatives of the pi system class. An example of a 2-substituted adenosine analogue with a polar linkage is found in 2-[2-*p*-methoxyphenethyl)amino derivative **II** that has an IC_{50} of 23 nM at the ADO A_{2A} receptor with a 40-fold selectivity over the A_1 receptor with respect to affinity (rat membrane).[13] The 2-cyclohexylmethylidene hydrazinoadenosine analogue **III** (WRC-0470, $K_i = 20$ nM) represents a compound with a linkage containing both a pi system and polar atom, and its side chain is shown in the *Z*-conformation (see below).[14] Structural elements of compounds **Ib**, **II**, and **III** are mimicked by design in the *N*-pyrazole and C-pyazole class exemplified by general structures **IV** and **V** in Figure 1, respectively.[15] Briefly, the *N*-pyazole class can be construed as a constrained mimetic of the Z-hydrazone derivitive **III** (WRC-0470), and both classes can be perceived as constrained double bond mimetics by virtue of their substitution pattern. Elements of design are discussed below, including the similarities of compound **II** with members of the *N*-

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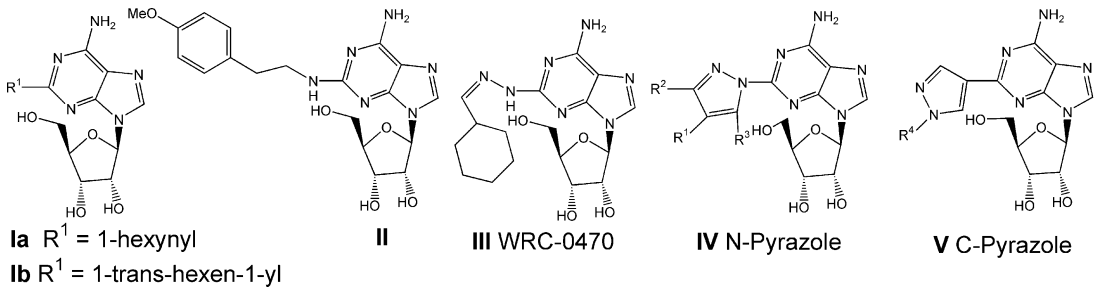
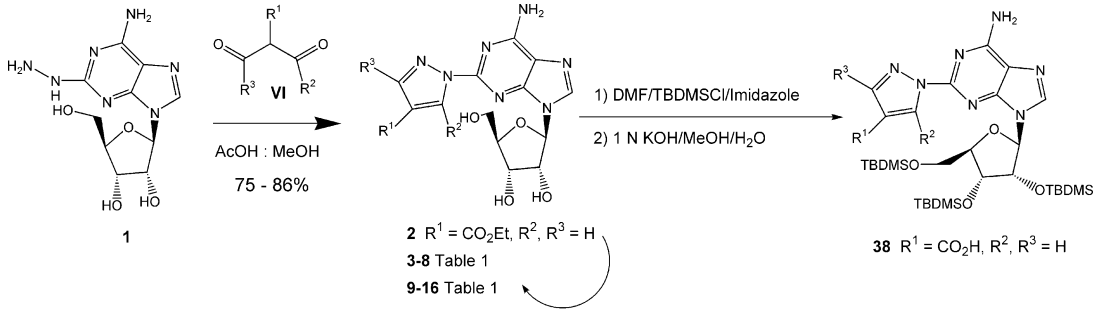


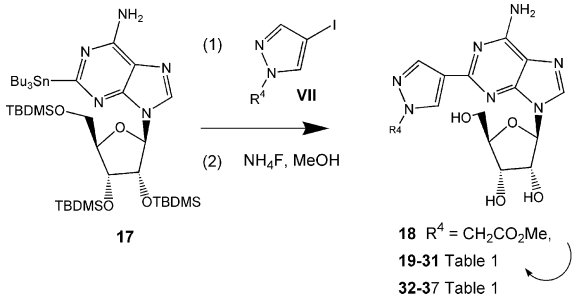
Figure 1. Compounds **I**–**III** are known selective ADO A_{2A} receptor agonists and general structures **IV** and **V** represent the pyrazole series described herein that incorporate elements of compounds **I**–**III**.



Scheme 1.

pyrazole class (**3**–**5**). In general, our findings show that despite a similar substitution pattern on the 5-membered ring heterocycle between series **IV** and **V**, the compounds of *N*-pyrazole class **IV** tolerated a larger variance of substitution including lipophilic and hydrophilic substituents that retained affinity for the ADO A_{2A} receptor. The compounds of C-pyrazole class tolerated only selected lipophilic substituents that retained affinity for the ADO A_{2A} receptor.

The *N*-pyrazole class was prepared by condensation of 1,3-dicarbonyl compounds (e.g., ethoxycarbonylmalondialdehyde[16]) with the known hydrazine **1** (Scheme 1) to afford compounds **2**–**8** which are shown in Table 1.[17] The ester **2** was converted directly to amides **9**–**13** by aminolysis with ammonia, methylamine, dimethylamine, ethylamine, and propylamine, respectively (Table 1). Amides **14**–**16** (Table 1) were prepared from the acid of ester 2 obtained through basic hydrolysis (1 N KOH/MeOH/H_2O) followed by standard peptide coupling (HBTU/HOBT in dichloromethane) with the 2′, 3′, and 5′ hydroxyl groups protected as TBDMS throughout the process (not shown).[18]

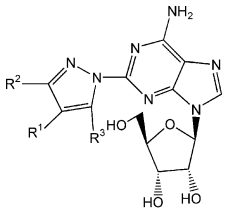


Scheme 2.

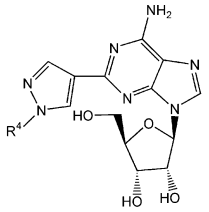
The preparation of the C-pyrazole class **V** starts with known 2-stannyl 2′,3′,5′-*tris*-*t*-butyldimethylsilyl protected adenosine analogue **17** utilizing a Stille coupling [tetrakis(triphenylphosphine palladium(0) and cuprous iodide][19,20] with appropriately substituted *N*-alkyl-4-iodopyrazoles **VII** to afford compounds **18**–**31** of Table 1 after deprotection with ammonium fluoride (Scheme 2).[21] The various *N*-alkyl substituted 4-iodopyrazole analogues **VII** were prepared by alkylating commercially available 4-iodopyrazole with the corresponding alkyl halide in DMF using sodium hydride as the base. Amides **33**, **34**, **35**, and **36** were prepared directly from ester **18** through aminolysis with 40% aqueous methylamine, 30% aqueous ethylamine, *n*-propylamine and *N*,*N*-dimethylamine, respectively (Table 1). Compound **37** was prepared by reduction of the ester of **18** with sodium borohydride in ethanol.

Our goal was to discover novel A_{2A} agonists with good affinity towards the A_{2A} receptor through the structural modification of adenosine by the addition of a 2-pyrazolyl substituent as represented by series **IV** and **V** (Fig. 1). First, we will describe the SAR of the *N*-pyrazole class **IV**, a series that contains a linkage with both a pi system and a polar atom. to the 2 position of adenosine. Compounds **6**–**8** suggest that simple lipophilic 3,4,5 tri- and 3,5 di-substituted *N*-pyrazolyl 2-substituted adenosine derivatives are not well tolerated by the Ado A_{2A} receptor. For this reason, we therefore focused on 4-monosubstituted N-pyrazolyl derivatives, and in addition, they are more favorable mimetics of the extended *trans*-hexenyl system of compound **Ib** (Fig. 1). A comparison of ethyl ester **2**, and ethyl amide **12**, suggest that both ester and amide links at the 4-position of the *N*-pyrazolyl can provide favorable affinity. In general, the 4-substituted amide derivatives **9**–**16** that con-

Table 1. Binding affinity of *N*-pyrazole **IV** and C-pyrazole **V** for the ADO A_{2A} receptor[a]



IV N-Pyrazole



V C-Pyrazole

Compd	R^1	R^2	R^3	R^4	K_i (uM) Rat[a]	K_i (uM) Pig[a]
2	$-CO_2Et$	H	H		0.06±0.01	1.28±0.43
3	–Ph-4-Cl	H	H		0.39±0.09	1.12±0.20
4	–Ph-4-OMe	H	H		0.19±0.08	0.56±0.10
5	4-Me-Ph	H	H		0.19 ±0.05	0.49±0.16
6	–*n*-Propyl	Me	Me		—	>100
7	–*n*-Butyl	Me	Me		—	>100
8	H	Ph	Me		—	>100
9	$-CONH_2$	H	H		0.35±0.03	1.35±0.22
10	$-CONHCH_3$	H	H		0.29±0.01	1.12±0.32
11	$-CON(CH_3)_2$	H	H		0.14±0.02	0.44±0.10
12	$-CONHCH_2CH_3$	H	H		0.060±0.01	0.38±0.13
13	$-CONH\ CH_2CH_2CH_3$	H	H		0.050±0.02	0.12±0.02
14	$-CONHCH_2$Ph-4-Cl	H	H		0.21±0.01	0.71±0.08
15	$-CONHCH_2CO_2Et$	H	H		0.29±0.02	0.98±0.18
16	$-CONH$-c-C_5H_9	H	H		0.060±0.01	0.38±0.13
18				CH_2CO_2Me	>100	>100
19				H	>100	>100
20				Me	>100	>100
21				*i*-Pr	3.88±1.29	10
22				*n*-Pentyl	2.59±0.32	2.14±0.952
23				*n*-Penten-4-yl	0.47±0.08	1.71±0.14
24				*n*-Decyl	6.11±0.69	>100
25				Benzyl	1.61±0.23	1.58±0.41
26				2-Phenethyl	2.26±0.31	5.90±0.69
27				3-Phenpropyl	1.28±0.43	1.66±0.382
28				*c*-Hexylmethyl	0.22±0.04	0.88±0.07
29				2-*c*-Hexylethyl	1.10±0.24	3.43±0.42
30				3-*c*-Hexylpropyl	2.62±0.36	6.68±0.93
31				4-*t*-Bu-$PhCH_2$	5.29±1.62	2.42
32				CH_2CO_2H	>100	>100
33				$CH_2CONHMe$	>100	>100
34				$CH_2CONHEt$	>100	>100
35				$CH_2CONHPr$	>100	>100
36				$CH_2CON(Me_2)$	21±6	>100
37				CH_2CH_2OH	>100	>100
III WRC-0470					0.066±0.01	

[a]Receptor binding affinities for the Ado A_{2A} receptors were determined using [^{3}H]ZM241385 as a radioligand as described in Gao et al.[6]

tain both lipophilic and polar substituents provided good affinity for the Ado A_{2A} receptor (rat, $K_i < 350$ nM). In stark contrast, the compounds of the C-pyrazole series V did not tolerate hydrophilic substitution (see compounds **32**–**37**, Table 1) and retain affinity for the Ado A_{2A} receptor, even though they have the same relative position on the 5-membered ring pyrazole with respect to adenosine attachment as compounds **2**, **9**, and **15** of the *N*-pyrazole class. There are many possible reasons for this observed trend in binding affinities between the two classes. We hypothesize that the the N-linked pyrazole of series **IV** may have additional interactions with the Ado A_{2A} receptor, possibly through N−1, that may enhance affinity or possibly orient the side chain in a different manner than the C-pyrazole class **V**. The choice of the 2-*N*-pyrazolyl series was not entirely serendipity as structural similarities with known active Ado A_{2A} agonists were foreseen. For instance, the high affinity N-pyrazole analogues **3**, **4**, and **5** of Table 1 overlay nicely with the *N*-linked analogue **II** of Figure 1 as shown in Figure 2a. Also, as mentioned above, the *N*-pyrazole class **IV** can be construed as a constrained analogue of the Z-conformation of the 2-cyclohexylmethylidene hydrazinoadenosine analogue **III** (Fig. 1).[15] An overlay of the high affinity analogue cyclopentylamide **16** and analogue **III** is shown in Figure 2b.

The compounds of the C-pyrazole series **V** required specific lipophilic substituents to have even moderate affinity for the Ado A_{2A} receptor. This point is exemplified by the simple *N*-alkyl analogues **20**–**22** wherein the *N*-methyl analogue **20** was found to have affinity greater than 100 μM, and the isopropyl **21** and pentyl **22** analogues had modest affinity (Table 1). We would have predicted a higher affinity for the pentyl analogue **22**

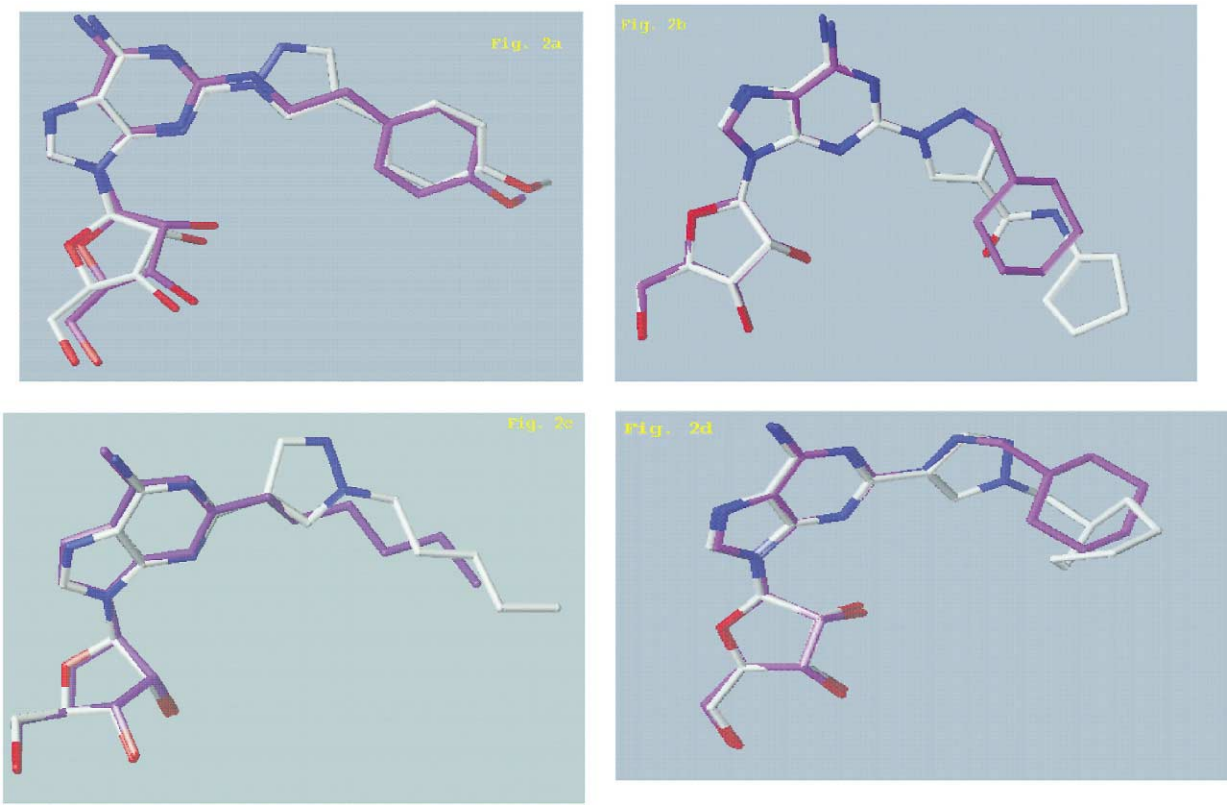


Figure 2. (a) Compound **II** (purple) and compound **4** (red, white, and blue). (b) Compound **III** (purple) and compound **16** (red, white, and blue). (c) Compound **Ib** (purple) and compound **22** (red, white, and blue). (d) Compound **III** (purple) and compound **28** (red, white, and blue).[22]

based on its favorable overlay with the high affinity Ado A_{2A} receptor ligand *trans*-hexenyl derivative **Ib** of Fig. 1 (see Fig. 2c). Similarily, *N*-benzyl **25**, *N*-2-phenethyl **26**, and *N*-3-phenpropyl **27** analogues had modest affinity for the Ado A_{2A} receptor (Table 1). It is interesting that the *N*-cyclohexylmethyl analogue **28** had the highest affinity of this series for the Ado A_{2A} receptor, even more so than the *N*-cyclohexylethyl **29** and *N*-cyclohexylpropyl **30** analogues. This fact prompted the overlay of *N*-cyclohexylmethyl analogue **28** with *c*-hexylmethylhydrazine analogue **III** in Fig. 2d.

The SAR for both N and C-pyrazole series suggest that in general the combined heteroatom linker and pi system of the *N*-pyrazole class provides more favorable affinity for the Ado A_{2A} receptor than the C-pyrazole class. As noted above, adenosine itself has a very short plasma half-life in part due to the conversion to inosine by adenosine deaminase. All of the compound binding affinities for the Ado A_{2A} receptor were tested in the presence of adenosine deaminase, and prolonged incubation of selected members in the competitive binding did not lead to a decrease in affinity. Selected members of each class were evaluated for their functional selectivity for the Ado A_{2A} receptor and coronary vasodilating effects in a separate study.[6] Compounds **10** (CVT-3146) and **22** (CVT-3033) were found to be functionally selective for the Ado A_{2A} receptor activation over Ado A_1 and A_{2B} receptors (note: both compounds were found to have low affinity for human Ado A_3 receptor $>>10\,\mu M$, and compound **10** has low affinity at the human Ado A_1 receptor with greater than 13-fold selectivity, $>16{,}460$ nM CHO cells versus 1269 nM HEK-hA_{2A} AdoR[6]), and a preliminary account of their pharmacological use as coronary vasodilators has been described.[6]

Acknowledgements

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17. To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added 2-(4-methyl)malondialdehyde (0.019 g, 0.12 mmol) and the mixture was heated at 80 °C for 3 h. The precipitate formed was collected by filtration and washed with EtOH and ether and dried.
18. The acid **38** was synthesized as follows: The ester **2** (0.50 g, 1.2 mmol) was dissolved in 30 mL DMF. To the solution was added imidazole (0.68 g, 10 mmol) and TBDMSCl (1.5 g, 10 mmol). The mixture was allowed to stir at 80 °C for 24 h. The solvent was removed and the residue was purified using column chromatography to afford the trisilyl-protected derivative of **2**.

The trisilyl derivative (0.4 g, 0.5 mmol) was then suspended in 1 mL of water and treated with 4 mL 1 N KOH/MeOH. The mixture was allowed to stir at room temperature for 72 h. The solvent was removed under reduced pressure and the residue was dissolved in 5 mL water and acidified to pH 5 using 1 N HCl. The resulting precipitate was collected by filtration and washed with water and ethyl ether to afford the trisilyl acid **38**, which was used without further purification. The 2′,3′,5′ trisilyl adenosine acid derivative of **2** (0.14 g, 0.2 mmol) was then dissolved in 5 mL dichloromethane. To the solution was added HBTU (0.19 g, 0.4 mmol), HOBt (0.076 g, 4 mmol), *N*-methylmorpholine (0.04 g, 0.4 mmol) and cat. DMAP. The mixture was allowed to stir at 23 °C for 24 h. The mixture was then washed with 10% citric acid, saturated $NaHCO_3$, brine and dried over $MgSO_4$. The solvent was removed and the residue was treated with 5 mL 0.5 N NH_4F/MeOH.[21] The solution was heated at reflux for 24 h. The solvent was evaporated and the residue was purified by preparative TLC to afford compounds **14**–**16**.
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22. Molecular modeling was performed using a Sybyl 6.6 program wherein the torsional angle about the pyrazolyl-purine bond was optimized to a local minima using the advanced computational module searching at 15 rotational degrees followed by alignment of the comparator compound using the Fit Algorithm within Sybyl.