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Bioorganic & Medicinal Chemistry Letters 14 (2004) 3793–3797
Bioorganic & Medicinal Chemistry Letters

Structure–affinity relationships of 5′-aromatic ethers and 5′-aromatic sulfides as partial A_1 adenosine agonists, potential supraventricular anti-arrhythmic agents ☆

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Received 23 February 2004; revised 27 April 2004; accepted 28 April 2004
Available online 25 May 2004

Abstract—Atrial fibrillation (AF) is the most commonly encountered sustained clinical arrhythmia with an estimated 2.3 million cases in the US (2001). A_1 adenosine receptor agonists can slow the electrical impulse propagation through the atrioventricular (AV) node (i.e., negative dromotropic effect) resulting in prolongation of the stimulus-to-His bundle (S-H) interval to potentially reduce ventricular rate. Compounds that are full agonists of the A_1 adenosine receptor can cause high grade AV block. Therefore, it is envisioned that a compound that is a partial agonist of the A_1 adenosine receptor could avoid this deleterious effect. 5′ Phenyl sulfides (e.g., **17**, $EC_{50} = 1.26\,\mu M$) and phenyl ethers (e.g., **28**, $EC_{50} = 0.2\,\mu M$) are partial agonists with respect to their AV nodal effects in guinea pig isolated hearts. Additional affinity, GTPγS binding data suggesting partial activity of the A_1 adenosine receptor, and PK results for 5′ modified adenosine derivatives are shown.

Adenosine is an endogenous ligand that has affinity for all four adenosine receptor (AdoR) subtypes: A_1, A_{2A}, A_{2B}, and A_3. Each subtype is coupled through a G-protein to the cAMP producing enzyme adenylate cyclase either in an inhibitory (A_1 and A_3 subtypes) or stimulatory manner (A_{2A} and A_{2B} subtypes).[1–8] Numerous physiological responses are mediated via local adenosine production that is dependent on both the concentration of adenosine produced and the proximity to the various AdoR subtypes, given that adenosine has an exceedingly short half-life in plasma.[9] Physiological effects mediated by AdoR subtypes have been widely studied: A_{2A} AdoR—coronary vasodilatation (used as a pharmacological stress agent)[10–12] as well as anti-inflammatory properties,[13] A_{2B} AdoR—angiogenesis[14] and A_3 AdoR—cardioprotection.[15] A_1 AdoR's mediate the negative chronotropic, dromotropic, inotropic, and anti-lipolytic effects of adenosine and adenosine derivatives.[16–18] Adenosine and other full AdoR agonists, such as tecadenoson (**1**, Fig. 1), slow atrioventricular (AV) nodal conduction (i.e., negative dromotropic effect) and therefore provide an approach toward the

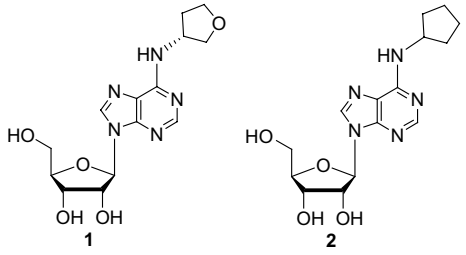

Figure 1. Tecadenoson (**1**) and CPA (**2**).

Keywords: Partial A1 agonist; Arrhythmias; PSVT.
☆ Supplementary data associated with this article can be found, in the online version, at 10.1016/j.bmcl.2004.04.096
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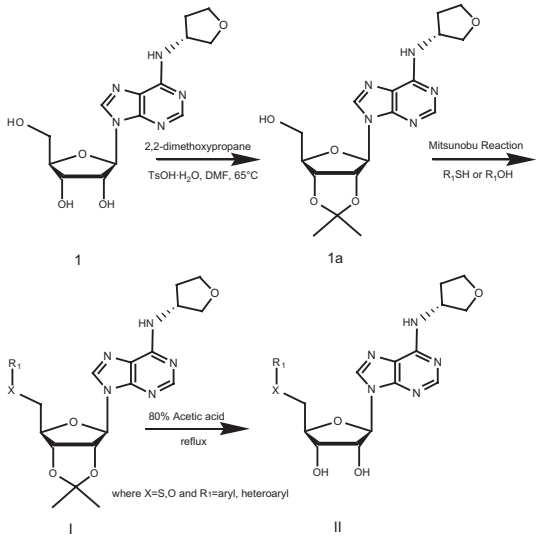
doi:10.1016/j.bmcl.2004.04.096

treatment of paroxysmal supraventricular tachycardias (i.e., PSVT).[19]

Although adequate for abrupt conversion of PSVT to normal sinus rhythm, the potential of these full agonists for chronic rate control is limited by the potential of these compounds to cause severe high degree AV block and bradycardia.[20,21] It was envisioned that a partial A_1 AdoR agonist could provide therapeutic effect on the AV node to achieve control of ventricular rate without the fear of causing high degree AV block. Partial agonists, by definition, are compounds that cause a submaximal response. In this example, a partial agonist of the A_1 AdoR will prolong AV nodal conduction to achieve rate control, but would not cause AV block at higher concentrations.

Over the last few years a considerable effort has been directed toward the discovery of potent and selective partial A_1 AdoR agonists.[22,23] IJzerman and others have reported that substitution at the 5′ or C-8 position, or the deletion of 2′ or 3′ hydroxyl groups on N^6-cyclopentyladenosine (CPA) **2** (Fig. 1) can lead to partial agonism.[23] Studies done by Belardinelli et al.[24,25] have identified CVT-2759 (**3**), a 5′-methyl carbamate of **1**, as a partial agonist at the A_1 AdoR. Encouraged by the above, we have undertaken efforts to synthesize more analogs that included 5′-aromatic ethers and 5′-aromatic sulfides of **1**. We describe our initial efforts[26] in preparing adenosine analogs with high affinity toward the A_1 AdoR as well as possessing suitable pharmacokinetics.

A typical preparation of 5′-aromatic ethers and 5′-aromatic sulfides of **1** was as follows (Scheme 1): compound **1** was treated with 2,2-dimethoxypropane to protect the 2′ and 3′ hydroxyl groups to furnish compound **1a**. Compound **I** was obtained by reacting **1a** with aryl/heteroaryl thiols and alcohols employing the Mitsunobu reaction.[27] Deprotection of the isopropylidene group with 80% acetic acid gave the final product **II**.[28] All compounds were purified by preparative TLC plate (2–10% methanol/dichloromethane) until only one spot by TLC and pure by ^{1}H NMR.



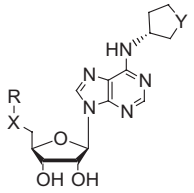
Scheme 1. Reagents: (a) 2,2-dimethoxypropane, $TsOH{\cdot}H_2O$, DMF, 65 °C; (b) PPh_3, DIAD, THF, aryl/heteroaryl thiols/alcohols; (c) 80% acetic acid/H_2O, reflux.

All compounds were screened for their binding affinities (K_i low, G-protein uncoupled receptor) for the A_1 AdoR and the results are shown in Table 1. As a general trend, heteroaryl sulfides had lower affinities and elevated GTPγS values indicative of compounds that would be full agonists—leading to AV block at higher concentrations—including substituted imidazoles **4** and **5**, pyrimidine **6**, pyridine **7**, benzothiazoles **8** and **9**, substituted benzothiazoles **10** and **11**, and benzoxazole **12**. However, the smaller thiazole **13** (with a N^6-cyclopentyl substituent) had an excellent affinity (K_i low = 166 nM), although its GTPγS value was 105% of CPA, indicating the likelihood of full agonism. Its N^6-tetrahydrofuranyl counterpart **14** had a comparable affinity (K_i low = 158 nM) and its GTPγS value was also elevated, 106% of CPA. Compound **15** (the 4′-methylthiazole analog of **14**) while having a lower GTPγS value of 86%, exhibited extremely poor affinity (K_i low value >5000 nM). Heteroaryl ether **16**, a methyl substituted isoxazole, displayed an excellent binding affinity (K_i low = 192 nM), a moderate GTPγS binding of 84%, but as with the analogous heteroaryl sulfides it was determined to be a full agonist leading to AV block at higher concentrations in guinea pig isolated heart preparations.

Compound **17**, a 2-fluorophenyl sulfide with a N^6-tetrahydrofuranyl substituent showed favorable K_i high (79 nM) and K_i low (506 nM) values along with a moderate GTPγS value (72%) suggesting that this compound may be a partial agonist. It is interesting to note that the 3-fluorophenyl (*meta*) analog of **17**, compound **18**, had three-fold less affinity for the A_1 AdoR than **17** while the 2,4-difluorophenyl analog of **17**, compound **19**, had 11-fold less affinity for the A_1 AdoR than **17**. The GTPγ S values for these three compounds were all between 72% and 86%. The N^6-cyclopentyl substituted direct analog of **17**, compound **20**, had excellent affinity (K_i low value = 287 nM) and a GTPγS value of 90%, nonetheless it was determined to be a full agonist in guinea pig isolated heart preparations. Compound **21** (*ortho* methyl analog of **17**) had four-fold less affinity than **17** while **22** (*ortho* chloro analog of **17**) had comparable affinity to **17**. As well, it should be noted that disubstituted phenyl sulfides such as **19**, **24**, and **25** had reduced affinities for the A_1 AdoR, indicating that the optimal phenyl was monosubstituted, preferably at the 2-position—however the size of the *ortho* substituent also played a role as compound **27** (trifluoromethyl analog of **17**) was inactive.

With respect to phenyl ethers, compound **28**, a 2-fluorophenyl ether with a N^6-tetrahydrofuranyl substituent, showed excellent K_i high (12.7 nM) and K_i low (117 nM) values along with a moderate GTPγS value (78%) suggesting that this compound may be a partial agonist. Benzyl ether **29** had excellent affinity (K_i low value = 165 nM) though its GTPγS value (98% of CPA) indicated potential full agonism. The N^6-cyclopentyl substituted direct analog of **28**, compound **30** had a

Table 1. Biological data for 5′-aromatic ethers and 5′-aromatic sulfides of N^6-substituted adenosine derivatives



Compound	R	X	Y	K_i high[b,d,e] (nM)	*n*	K_i low[b,c,d,e] (nM)	*n*	GTPγS[a] (% of CPA)
1	H	O	O	3.25 ± 0.74	6	543 ± 182	4	93
2	H	O	CH_2	0.8		19.5 ± 12.0	2	100[f]
3	CONHMe	O	O	167 ± 38	6	3612 ± 1937	5	76
4	2-(N-Me-imidazole)	S	O			4191 ± 922	5	84
5	2-(N-Me-imidazole)	S	CH_2			1338	1	112
6	2-Pyrimidine	S	O			913 ± 517	3	89
7	2-Pyridine	S	O			894 ± 659	3	110
8	2-Benzthiazole	S	O			400 ± 38	3	100
9	2-Benzthiazole	S	CH_2			1773 ± 209	3	95
10	2-(5′-OMe-benzthiazole)	S	CH_2	222	1	354 ± 113	3	94
11	2-(6′-OMe-benzthiazole)	S	CH_2			1666 ± 556	3	105
12	2-(6′-Cl-benzoxazole)	S	O			1945 ± 1316	4	99
13	2-Thiazole	S	CH_2	0.93 ± 0.92	3	166 ± 75	3	105
14	2-Thiazole	S	O	21 ± 0	2	158 ± 21	3	106
15	2-(4′-Me-thiazole)	S	O			>5000	2	86
16	CH_2-3-(5′-Me-isoxazole)	O	O	2.73 ± 0.49	4	192	1	84
17	2-FPh	S	O	79 ± 42	3	506 ± 111	4	72
18	3-FPh	S	O	180 ± 23	2	1571 ± 245	3	84
19	2,4-diFPh	S	O	971 ± 139	3	5497 ± 180	2	86
20	2-FPh	S	CH_2	33	1	287 ± 125	4	90
21	2-MePh	S	O	160 ± 41	2	1143 ± 426	4	81
22	2-ClPh	S	O	96 ± 11	2	338 ± 98	3	74
23	2,6-diClPh	S	O	102 ± 45	2	614 ± 78	3	89
24	2,6-diMePh	S	O	243 ± 107	2	5855 ± 1208	2	12
25	2-Cl,6-MePh	S	O	233 ± 98	2	7292 ± 3241	2	18
26	2,4-diFPh	S	O			494 ± 69	3	88
27	2-CF_3Ph	S	O	2400 ± 16	2	6250 ± 1767	2	n/a
28	2-FPh	O	O	12.7 ± 1.5	3	117 ± 46	4	78
29	Bn	O	O	7.5 ± 1.9	4	165	1	98
30	2-FPh	O	CH_2			4394	1	n/a
31	2-ClPh	O	O			473 ± 329	3	62
32	2-MePh	O	O			503 ± 262	3	61
33	3-FPh	O	O			604 ± 241	2	70
34	4-FPh	O	O			343 ± 137	3	71

[a] Some compounds with high K_i-low values were not tested in the GTPγS assay.
[b] The affinities of compounds for A_1 AdoR were determined in competition studies, where the binding of either ^{3}H-CCPA (0.8 nM, for K_i-high values) or the ^{3}H-CPX (0.5 nM, for K_i low values) to DDT membranes were displaced by increasing concentrations of compounds.
[c] In the K_i-low assays, GTPγS was included in the reaction to uncouple receptors from G-proteins.
[d] Data shown are average ± standard deviation of experiments (n = number of experiments).
[e] In each experiment, six concentrations of compounds were tested in duplicate. The activation of G-proteins by A_1 agonists was assessed by measuring the binding of [^{35}S]GTPγS to DDT membranes in the presence of 1 μM CPA or 10 μM compounds.
[f] CPA stimulated [^{35}S]GTPγS binding was used as 100%.[29]

remarkably lower affinity (K_i low value of 4.4 nM) for the A_1 AdoR. The *ortho* chloro analog (compound **31**) had a reasonable binding affinity and reduced GTPγS value (62%) relative to **28** (78%). As with the phenyl sulfide series, the *ortho* methyl analog (compound **32**) showed four-fold weaker affinity for A_1 AdoR as compared to **28**, as did compounds with fluoro substitution that was either *meta* substituted (compound **33**) or *para* (compound **34**).

As noted above, several compounds (e.g., **17**, a 2-fluorophenyl sulfide and **28**, a 2-fluorophenyl ether, both with a N^6-tetrahydrofuranyl substituent) showed excellent K_i high and K_i low values along with moderate GTPγS values. As shown in Table 2, the EC_{50} value for **17** is 1260 nM while the value for **28** of 200 nM is six-fold more potent. As a result of these encouraging affinity and efficacy results, various pharmacokinetic data was obtained for these two compounds and is shown in Table 2. Both compounds exhibited reasonable pharmacokinetic profiles relative to adenosine, though **28** showed much greater bioavailability than **17**. Oral dosing of **17** (4 mpk) and **28** (2 mpk) in rat yielded F = 12% for **17** but a much greater value of 81% for **28**. The oral elimination half-lives were comparable, 1.6 h for **28** compared to 1.3 h for **17**. However, with rat IV dosing

Table 2. Pharmacokinetic data for selected compounds

Compound	K_i high (nM)	K_i low (nM)	EC_{80} (nM)	Pharmacokinetic data		
					Oral	IV
1	3.25 ± 0.74	543 ± 182	41			
3	167 ± 38	3612 ± 1937	2800			
17	79 ± 42	506 ± 111	1260	Dose (mg/kg)	4	1
				Cmax (ng/mL)	107	—
				F% (%)	12.3	—
				CLp (mL/min/kg)	—	69.4
				Elim. *t* 1/2 (h)	1.3	0.4
28	12.7 ± 1.5	117 ± 46	200	Dose (mg/kg)	2	0.5
				Cmax (ng/mL)	60.3	—
				F% (%)	81	—
				CLp (mL/min/kg)	—	202
				Elim. *t* 1/2 (h)	1.6	0.3

the CLp was 202 mL/min/kg for **28** (0.5 mpk) while the CLp was 69 mL/min/kg for **17** (1 mpk).

In summary, **28** possesses the traits that are desirable in a compound that could be a partial agonist of the A_1 AdoR, that is excellent affinity, moderate GTPγS and sub-maximal efficacy in the guinea pig isolated heart. While **28** has been the most 'potent partial' A_1 AdoR agonist in our experience to date, a minor metabolic product of phenyl ether **28** is **1**, a known full agonist.[30] Thus—for the ether class of compounds—this clearly demonstrates the desire to find equally or more potent partial agonists that can unequivocally avoid this cleavage to furnish **1**. However, the pharmacological consequences of this minor metabolite have yet to be determined and work in this area is ongoing.

Acknowledgements

We thank Dr. Brent Blackburn and Dr. Luiz Belardinelli for helpful discussion.

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28. A stirred solution of **1** (5.21 g, 15.5 mmol), catalytic *p*-toluenesulfonic acid monohydrate (150 mg, 0.79 mmol) and 2,2-dimethoxypropane (15.0 mL, 122 mmol) in *N*,*N*-dimethylformamide (65 mL) was heated at 65 °C for 16 h. After heating, excess *p*-toluenesulfonic acid was neutralized to pH 7 with several drops of concentrated ammonium hydroxide and solvent was removed in vacuo. Recrystallization (ethyl acetate/methanol) furnished **1a** (3.67 g, 9.73 mmol). To a stirred solution of PS-triphenylphosphine (Argonaut Technologies© polymer bound PPh_3, 280 mg, 0.46 mmol) in anhydrous THF (2 mL) was sequentially added **1a** (107 mg, 0.284 mmol) followed by a 1 M solution of diisopropylazodicarboxylate (0.53 mL, 0.53 mmol). After stirring for 30 min, 6-chloro-2-benzoxazolethiol (53 mg, 0.29 mmol) as a solution in anhydrous THF (1 mL) was added dropwise and the reaction contents refluxed for 16 h. Reaction contents were filtered, solvent removed in vacuo and the crude material was purified by preparative TLC (5% methanol/dichloromethane) to yield isopropylidene protected **12** (136 mg, 0.250 mmol). Isopropylidene protected **12** (136 mg, 0.250 mmol) was dissolved in 80% acetic acid/water and heated at 90 °C for 16 h. Solvent was removed in vacuo, and the crude material was purified by preparative TLC (5% methanol/dichloromethane) to furnish **12** as a white powder (23 mg, 0.046 mmol).
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30. LC/MS-MS data suggested that trace levels of **1** (<1 ng/mL) were detected in plasma. These levels represent equal or less than 1% of that of **28** in plasma at the same time point. Based on LC/MS–MS data (provided in Supporting information) this plasma level of **1** is a metabolite of **28**, not a trace impurity in **28**.