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Affinity and intrinsic efficacy (IE) of 5′-carbamoyl adenosine analogues for the A_1 adenosine receptor—efforts towards the discovery of a chronic ventricular rate control agent for the treatment of atrial fibrillation (AF)

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Abstract—The SAR for the affinity to the A_1 adenosine receptor and relative intrinsic efficacy (IE, [^{35}S]-GTPγS binding) of a series of 5′-carbamate and 5′-thionocarbamate derivatives of tecadenoson is described. Based on this SAR, selected compounds were evaluated in guinea pig isolated hearts to determine whether they were partial or full agonists with respect to their negative dromotropism, an A_1 AdoR mediated effect. Progress towards obtaining a partial A_1 AdoR agonist to potentially control ventricular rate during atrial fibrillation has been made with the discovery of several potent partial A_1 AdoR agonists (compounds **13**, **14**, and **17**).

The most common form of atrial arrhythmia is atrial fibrillation (AF), with an estimated 2.2 million Americans having either paroxysmal (sudden onset) or persistent AF.[1] One strategy for the management of AF is ventricular rate control wherein the AF is allowed to persist while the ventricular rate is controlled. Most agents that provide ventricular rate control act by prolonging the effective refractory period (ERP) of the atrioventricular (A-V) node, thereby 'filtering' atrial electrical activity and reducing the number of impulses conducted to the ventricles.[2] The A_1 adenosine receptor (A_1 AdoR) agonists prolong the ERP in the A-V node by increasing an inwardly-rectifying potassium current and reducing the excitability of A-V nodal cells.[3] The primary rationale for partial agonism at the A_1 AdoR of the A-V node is to avoid third degree A-V block.[4]

Tecadenoson (CVT-510) **1** is a full A_1 AdoR agonist with high affinity and selectivity for the A_1 AdoR (Fig. 1 and Table 1), and it has been investigated in clinical trials for the treatment of paroxysmal supraventricular tachycardia (PSVT).[5,6] We recently reported the partial agonism at the A_1 AdoR of the A-V node with respect to the negative dromotropic effect for the 5′-*N*-methylcarbamate derivative of tecadenoson, compound **2**.[4] We have studied the SAR of affinity and relative intrinsic efficacy (IE) of a series of 5′-carbamates and 5′-thionocarbamates of tecadenoson, with a goal to find an orally active partial A_1 AdoR agonist that can be used to provide ventricular rate control during AF.

The 5′-position of tecadenoson **1** was chosen as the site to modify to potentially provide partial agonism, since others have found that the 5′-deoxy-*N*-6-cyclopentyladenosine (5′-deoxyCPA) **6** retains affinity for the A_1 AdoR (K_i = 70 nM, rat membrane) and selectivity over the A_{2A} AdoR (Fig. 1).[7] In addition, IJzerman and co-workers found that removing the hydrogen bond donor capabilities of the 5′-hydroxyl group of CPA **5** by

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conversion to a methoxyl group resulted in a partial agonist, compound **7**, with high affinity for the A_1 AdoR ($K_i = 33.7 \pm 6.9$ nM, rat membrane, Fig. 1).[8] They also found that the 5′-methylamino derivative **8** (Fig. 1) had a high IE that suggests the hydrogen bond donor capabilities of the 5′-methylamino substituent maybe contributing to IE.[8]

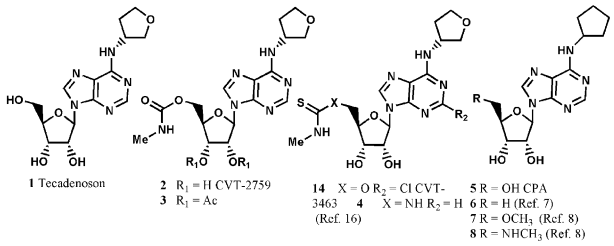
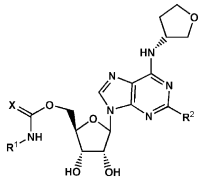


Figure 1. A_1 AdoR agonists: tecadenoson **1**, a full agonist; **2**, weak partial agonist;[4] **3**-bis pro-drug; **14**, potent partial; **4**, full agonist; **5**-CPA; **6**–**8** previously described agonists.[7,8]

By definition, a partial agonist produces a sub-maximal response even when present in a sufficiently high concentration to activate all of its receptors in a given tissue.[4,9] The concentration–response relationships for an agonist are dependent on the affinity of the agonist for the receptor, the IE of the agonist to activate the receptor, the receptor density in the tissue in question, and the efficiency of translation of receptor stimulus to response.[10] To obtain a potent partial agonist at the A_1 AdoR for a given biological response, it may require the separation of ligand–receptor interactions that provide affinity from those that are responsible for IE. We hypothesized that it is possible to increase the affinity of

Table 1. Affinity for the A_1AdoR, activity in GTPγS binding assay and negative dromotropic effect (i.e., S–H prolongation) of 5′-carbamates and 5′-thionocarbamates of tecadenoson



Compd	R^1	R^2	X	K_i (nM)[a]	GTP γS %CPA[b] (%)	S–H prolongation, relative potency[c]	Partial[d] or full
2	Me	H	O	140±33	76	+ +	Partial
12	Me	Cl	O	96±34	77	+ +	Partial
13	Me	H	S	45±11	77	+ + + +	Partial
14	Me	Cl	S	71±27	77	+ + + +	Partial
15	Et	H	O	167±78	80	—	—
16	Et	H	S	33±5	77	+ +	Full
17	Et	Cl	S	55±31	84	+ + + +	Partial
18	Pr	H	O	235±6	73	—	—
19	Pr	H	S	50±21	89	+ + +	Partial
20	Bu	H	O	1409±286	49	+	—
21	Bu	H	S	157±5	70	Inactive	—
22	*i*-Propyl	H	O	78±35	91		
23	*i*-Propyl	H	S	36±4	84	—	—
24	*c*-Propyl	H	O	209	82	—	—
25	*c*-Propyl	H	S	157±62	82	—	—
26	*c*-Butyl	H	O	306±136	67	—	—
27	*c*-Butyl	H	S	148±11	79	—	—
28	*c*-Pentyl	H	O	321	82	+ +	Partial
29	*c*-Pentyl	Cl	O	226±146	69	+ +	Partial
30	*c*-Pentyl	H	S	72	95	+ +	Full
31	*c*-Hexyl	H	O	584	80	—	—
32	*c*-Hexyl	H	S	253±1	82	—	—
33	Benzyl	H	O	1826±40	44	—	—
34	Benzyl	H	S	12±3	93	—	—
35	4-F-Benzyl	H	O	1913±423	42	—	—
36	*N*-Allyl	H	S	35±8	78	—	—
37	*N*-Allyl	H	O	524±204	48	—	—
38	*N*,*N*-DiMe	H	O	238	87	—	—
39	*N*,*N*-DiMe	H	S	205±57	85	—	—
1 tecadenoson	5′-OH	—	—	3±0.4	93	+ + + + +	Full

[a] Binding affinity for A_1AdoR was determined using DDT_1 cell membranes (hamster vas deferens smooth muscle cell line) with [^{3}H]-CCPA as the radioligand. Data shown are mean±standard deviation ($n = 2$–4).

[b] Measurement of the stimulation of binding of [^{35}S] GTPγS to G protein-coupled A_1AdoR in DDT_1 cell membranes of test compound (10 uM), as % of CPA (1 uM).

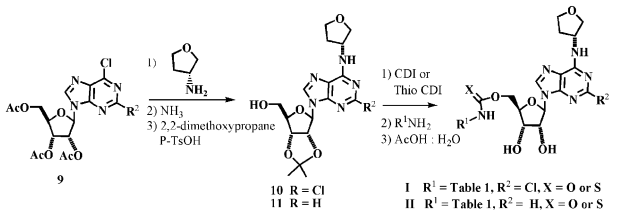
[c] The EC_{50} for agonist-induced S–H prolongation in guinea pig isolated hearts, defined as follows: + means an $EC_{50} > 5$ uM, + + means EC_{50} between 3 and 5 uM, + + + means 0.5 μM > EC_{50} between 1.0 uM, + + + + means 0.5 uM > EC_{50} between 0.51 uM, + + + + + means $EC_{50} < 0.5$ uM.

[d] An agonist whose concentration–effect relationship for prolongation of the S–H interval reaches a plateau at a concentration greater than 50-fold the concentration wherein the prolongation of the S–H interval starts. A full agonist causes second or third degree AV block in the guinea pig isolated heart.

a ligand without increasing its intrinsic efficacy. In this study, we compare the effect of structural modifications of the 5′-substituent of tecadenoson derivatives with those of known 5′deoxy-CPA analogues to understand the nature of the ligand–receptor interactions that lead to affinity versus IE. The agonist-induced increase of [^{35}S]-GTPγS binding is used as an indication of agonist efficacy in selecting or inducing an active state of the receptor that stimulates release of GDP from a G protein, thus allowing [^{35}S]-GTPγS, a stable analogue of GTP, to bind. In the [^{35}S]-GTPγS assay, the increase of [^{35}S]-GTPγS binding induced by a partial agonist is reported as a percentage of the increase of binding induced by a full agonist (e.g., CPA, **5**, Fig. 1).

The 5′-carbamate and 5′-thionocarbamate classes were prepared from the known 2′,3′,5′-triacetoxy-2,6-dichloropurine riboside[11] as shown in Scheme 1 to afford compounds **2**–**39** in Table 1. Selective addition of the commercially available (*R*)-3-aminotetrahydrofuran at the 6-position of compound **9** over the 2-position was made possible by reacting at moderate temperatures (refluxing ethanol 16–20 h) in the presence of triethylamine. Removal of the triacetate protecting groups from the intermediate addition product by treatment with ammonia in methanol yielded the 2-chloro analogue of tecadenoson. This intermediate and tecadenoson **1** were converted to the isopropylidene (IP) protected compounds **10** and **11** by treatment with *p*-toluenesulfonic acid (*p*-TsOH) and 2,2-dimethoxypropane in dimethylformamide at high temperature for an extended period of time (70 °C, 40 h) in a similar manner to published procedures.[11] Introduction of the 5′-carbamate or 5′-thionocarbamate groups was effected using an excess (4 equivalents) of carbonyldiimidazole (CDI) or thiocarbonyldiimidazole (thio CDI), respectively. After quenching the excess reagent through the addition of water, the intermediate acyl imidazolide or thiono imidazolide are reacted directly with the appropriate amine to afford the corresponding 2′,3′-IP protected 5′-carbamates or 5′-thionocarbamates of Table 1, respectively.[12] The 2′,3′-IP protecting group was removed by treatment with warm (90 °C) acetic acid/water (4:1) followed by purification of the final products which were tested for biological activity by preparative thin layer chromatography (PTLC).[13]

The SAR for the affinity to the A_1 adenosine receptor and relative IE ([^{35}S]-GTPγS binding) of a series of 5′-carbamates and 5′-thionocarbamates of tecadenoson is shown in Table 1, along with the relative potency of selected compounds to prolong the S–H interval in guinea pig isolated heart preparations. The discovery of the



Scheme 1.

previously described partial agonist, 5′-*N*-methylcarbamate **2** (Fig. 1), provided a tool to demonstrate that partial agonism of the A_1 AdoR with respect to the negative dromotropic effect at the A-V node can be separated from other A_1 AdoR-mediated effects.[4] The major challenge for us was to discover a potent partial agonist of the 5′-carbamates and 5′-thionocarbamates of tecadenoson. Our goal was to understand what structural features of the 5′-substituent contribute to affinity and/or IE, and to demonstrate that it was possible to make a potent partial agonist of the A_1 AdoR. In addition we determined the selectivity, metabolic stability, and the pharmacokinetic (PK) properties of several members of the series to assess their suitability as an orally active partial A_1 AdoR agonist to provide ventricular rate control during AF.

The structural features of 5′-methylcarbamate **2** that were varied include the length of the alkyl chain, monoalkyl versus dialkyl, oxocarbamates versus thionocarbamates, cycloalkyl ring size, introduction of a pi system, and for selected compounds, the addition of a 2-chloro substituent (Table 1). Comparing the effect of alkyl chain length within the oxo and thionocarbamate series, compounds **2**, **15**, **18**, and **20** or compounds **13**, **16**, **19**, and **21**, respectively, the trend for the effect on affinity for the A_1 AdoR was methyl = ethyl = propyl > butyl (Table 1). The NH of the 5′-carbamate and 5′-thionocarbamate maybe contributing partly to affinity, since the 5′-*N*,*N*-dimethylthionocarbamate **39** and 5′-*N*,*N*-dimethylcarbamate **38** had lower affinity than their mono-alkyl comparators, compounds **13** and **2**, respectively. When comparing the oxo- versus thionocarbamates with the same alkyl chain length, a general trend developed with the thionocarbamates having a 3–9-fold higher affinity (compounds **2** and **13**, **15** and **16**, **18** and **19**, **20** and **21**). The isopropyl analogues **22** and **23** had slightly higher affinity than the related cyclopropyl analogues **24** and **25** (Table 1). The effect of varying the ring size on affinity within the 5′-cycloalkyl carbamate and thionocarbamate series, respectively, followed the trend cyclopropyl = cyclobutyl = cyclopentyl > cyclohexyl with the exception of 5′-cyclopentyl thionocarbamate **30** that possessed higher affinity (compounds **24**–**32**, Table 1). The effect of introduction of a pi substituent was very favorable with respect to the affinity of the *N*-benzyl and *N*-allyl 5′-thionocarbamates, compounds **34** and **36**, respectively, but much less favorable with the corresponding oxo-carbamates, compounds **33** and **37** (Table 1). The introduction of the 2-chloro substituent had little effect on affinity or IE based on the following comparisons: compounds **2** and **12**, **13** and **14**, **16** and **17**, **28** and **29**. We anticipated a pronounced reduction in IE for the 2-chloro analogues based on the previously described 2′-deoxy[14] and *N*-6-alkylsulfide[15] series wherein the 2-chloro group lowered the IE, and in the case of the latter series to the point of excluding cardiovascular effects altogether.

Based on their affinity and GTPγS binding, selected members of the 5′-carbamate and 5′-thionocarbamate class were tested for their negative dromotropic effect in guinea pig isolated hearts as previously described.[4] All

of the compounds were found to recruit less GTPγS binding than the full agonist CPA (Table 1), and they would be predicted to be partial based on this assay. All of the analogues tested in the isolated heart were found to be partial with the exception of compounds **16** and **30**. This result is in contrast to the corresponding 5′-urea series (compound **4**, Fig. 1) wherein almost all derivatives were full agonists.[16] There is a trend that the removal of the hydrogen bond donor capabilities directly at the 5′-position on the agonist has a propensity to lead to an agonist with lower IE. More importantly, several compounds had an EC_{50} of <1 uM for their negative dromotropic effect. The more potent analogues from the isolated heart study were further evaluated for their selectivity of binding, and metabolic stability in an in vitro liver S-9 incubation study (Table 2). All five lead compounds demonstrated high binding selectivity for the A_1 AdoR over the A_{2A} and A_{2B} receptors, but only compounds **13** and **19** demonstrated high selectivity over the A_3 AdoR (Table 2). There is precedence for high affinity A_1 AdoR agonists to have high affinity at the A_3 AdoR as well.[7,8] The metabolic stability of each lead compound was determined by incubation with human and rat liver S-9 fractions, and the amount of parent compound remaining was recorded after 30 min of incubation. The metabolic stability of the lead compounds in human liver S-9 fraction are presented in Table 2. Similar results were obtained using rat liver S-9. Both the 2-chloro analogues **14** and **17** had $<65\%$ of the parent compound remaining indicating poor metabolic stability. The thionocarbamates **13** and **19** demonstrated high metabolic stability with at least 98% of the parent compound remaining at 30 min. However, low but significant amounts (0.5–2%) of tecadenoson were detected (Table 2). The original *N*-methylcarbamate **2** was found to be the most stable in the liver S-9 studies with only a trace of tecadenoson detected. Therefore, the parent *N*-methylcarbamate **2** and the 2′,3′-bis acetate pro-drug **3** were further evaluated for their pharmacokinetic properties following dosing in rats (Figs. 1 and 2). The 2′,3′-bis acetate pro-drug **3** provided nearly twice the C_{max} and the plasma area under the curve (AUC) as the parent compound **2** following oral dosing in rats (Fig. 2a). However, low concentrations of tecadenoson (up to 5 ng/mL) were detected in rat plasma following oral administration of

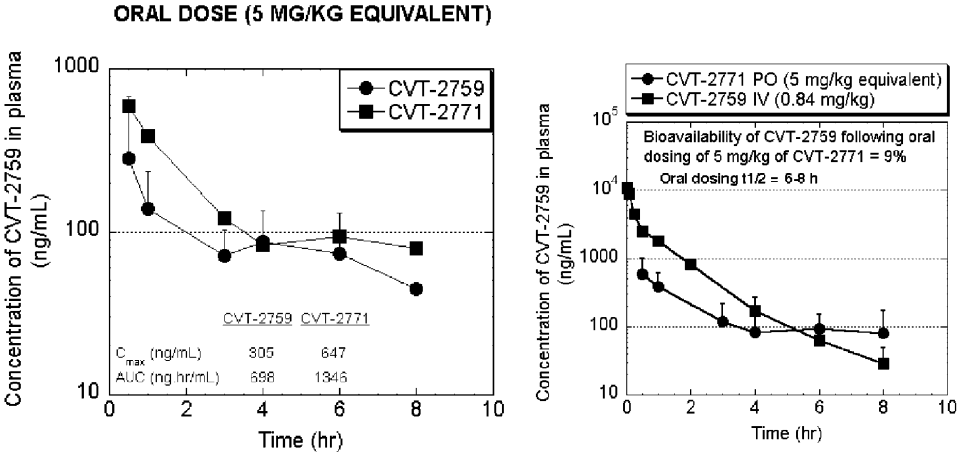


Figure 2. (panel a) Concentration of parent **2** in plasma following oral gavage in rats (5 mg per kg, MPK, or equivalent) of either the parent compound **2** (CVT-2759) or the bis-acetate pro-drug **3** (CVT-2771); (panel b) the oral bioavailability of the parent **2** (CVT-2759) following oral dosing of the bis pro-drug **3** (CVT-2771) in rats was found to be approximately 9% as determined by area under the curve measurements.

Table 2. Selectivity relative to the A_1 AdoR, metabolic stability in human liver S-9 incubation, and the percentage of tecadenoson detected at 30 min in the S-9 study

Compd	K_i ratio A_{2A}/A_1[a]	K_i ratio A_{2B}/A_1[b]	K_i ratio A_3/A_1[c]	Liver S-9 stability[d] remaining (%)	% tecadenoson detected[e]
2 (**2759**)	28	28	7.5	~100	Trace
13 (**3109**)	135	135	23	99	~0.5–1
14 (**3463**)	49	49	3.3	62	0
17 (**3462**)	56	56	5.3	41	0
19 (**3111**)	135	135	23	98	1–2

[a] Affinity for A_{2A} AdoR was determined in a competition study using ^{3}H-CGS21680 as the radioligand in the HEK-293 cells that were stably transfected with human A_{2A} AdoR (HEK-A_{2A}).
[b] Affinity for A_{2B} AdoR was determined in a competition study using ^{3}H-ZM-241385 as the radioligand in the HEK-293 cells that were stably transfected with human A_{2B} AdoR (HEK-A_{2B}).
[c] Affinity for A_3 AdoR was determined in a competition study using ^{125}I-AB-MECA as the radioligand in the CHO cells that were stably transfected with human A_3 AdoR (CHO-A_3).
[d] Compounds were incubated with 2 mg/mL of human liver microsomes (BD Bioscience/Gentest) at 37 °C for 30 min in a pH 7.4, 0.05 M phosphate buffer containing 5 mM $MgCl_2$, and 2 mM NADPH.
[e] The % of tecadenoson present after 30 min in the liver S-9 assay as determined by mass spec analysis.

either the parent compound **2** (5 mg/kg) or molar equivalents of pro-drug **3**. The absolute bioavailability of compound **2** following the oral administration of the pro-drug **3** resulted in a value of approximately 9% (Fig. 2b), and the oral half life was approximately 6–8 h.

In this study, many 5′-carbamoyl and 5′-thionocarbamoyl analogues of tecadenoson demonstrated high affinity to the A_1 adenosine receptor and had lower relative IE than the full agonist CPA based on the GTPγS binding assay. The trend for members of these classes to have lower IE maybe due to the removal of the hydrogen bond donor capabilities at the 5′-position (i.e., removal of 5′-OH). Compounds **13**, **14**, and **17** were found to be potent, partial agonists with respect to their negative dromotropic effect at the A-V node in guinea pig isolated hearts. Unfortunately, these compounds either were metabolized to the full agonist tecadenoson or had low in vitro metabolic stability (liver S-9). Encouraged by the oral availability of the weak partial **2** following dosing of the bis prodrug **3**, future efforts will attempt to incorporate more optimal pharmaceutical properties and potent, partial agonism within the same molecule.

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12. To a solution of 5′-alcohol in dry tetrahydrofuran (0.1 M) was added dry carbonyldiimidazole or thiocarbonyldiimidazole (4 equiv) at 23 °C under argon. After 1 h, 50 μL of water was added to quench excess reagent, and then the corresponding amine (1.5 equiv) was added in one portion. After 16 h, the reaction was concentrated and purified by preparative thin-layer chromatography to afford the 5′-carbamate or 5′-thionocarbamate.
13. The final compounds were purified by preparative thin layer chromatography (methanol/methylene chloride 1:19 to 1:10) to afford the 5′-carbamate or 5′-thionocarbamate in the 2′,3′-IP protected form.
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