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New potent and selective A_1 adenosine receptor agonists

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Abstract—Thiirane analogs of ENAdo have been synthesised and found to be extremely potent and selective A_1 adenosine receptor agonists.
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1. Introduction

Adenosine is an effective agent for the treatment of paroxysmal supraventricular tachycardias (PSVT).[1,2] It acts via extracellular receptors (A_1, A_{2A}, A_{2B}, A_3) to mediate a range of physiological effects. Cardiovascular effects include a slowing of heart rate (negative chronotropic effect), prolonged of AV nodal conduction (negative dromotropic effect), depressed atrial contractility (negative inotropic effect), anti-β-adrenergic effects and increased coronary artery blood flow. The A_1 adenosine receptors (A_1ARs) mediate adenosine's negative dromotropic effects, which are responsible for termination of supraventricular tachycardias. The use of adenosine for the therapy of such arrhythmias has some drawbacks, which result from its short half life (e.g., recurrence of arrhythmias) and its lack of receptor subtype selectivity (e.g., vasodilatory effects). As a result, it has been postulated that longer acting adenosine agonists, which are selective for the A_1AR may be advantageous.[2,3]

Adenosine is also involved in an endogenous cardioprotective phenomenon known as ischaemic preconditioning (IPC). Ischaemic preconditioning involves the attenuation of necrotic injury that is observed when the myocardium has been exposed to brief periods of ischaemia before a prolonged ischaemic period.[4] Adenosine and A_1AR agonists, administered prior to an ischaemic insult, can reduce infarct size, arrhythmia and improve post-ischaemic cardiac function. Precise mechanisms linking A_1AR stimulation to cardioprotection are not clear. A_1ARs are expressed in cardiac myocytes and their stimulation is thought to produce beneficial cellular metabolic effects, attenuating ATP depletion and reducing cellular acidosis, thereby prolonging cell viability during ischaemia.[4] These discoveries have stimulated interest in the development of agents capable of simulating ischaemic preconditioning and affording myocardial protection. A_1AR agonists may also act as cardioprotective agents due to their ability to inhibit lipolysis and reduce plasma fatty acid and triglyceride levels.[4]

In the search for potent and selective A_1AR agonists we reported the design and synthesis of ENAdo (**1**).[5,6] Other adenosine analogs with oxygenated cycloalkyl N^6-substituents [e.g., CVT-510 (**2**) and GR79236 (**3**)] have also been found to confer high selectivity for the A_1AR.[7–9] The more potent *S-endo* isomer of ENAdo (**1**) was found to degrade on silica to form polar breakdown products.[6] This process was believed to involve epoxide opening and cyclisation on N1. A similar process was observed for 1,3-dipropyl-8-[2-(5,6-epoxy)norbornyl]xanthine (ENX). In this case, the epoxide was involved in an intramolecular cyclisation with N9 under

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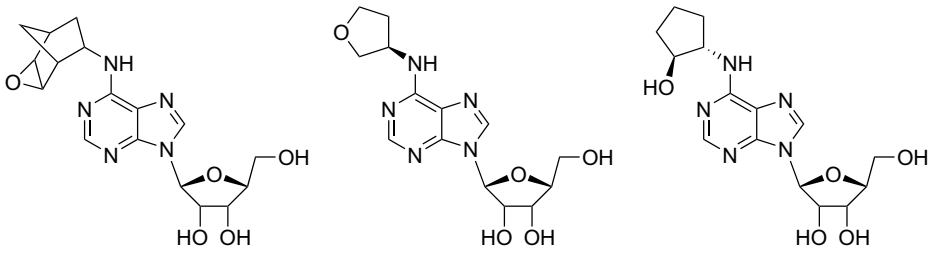
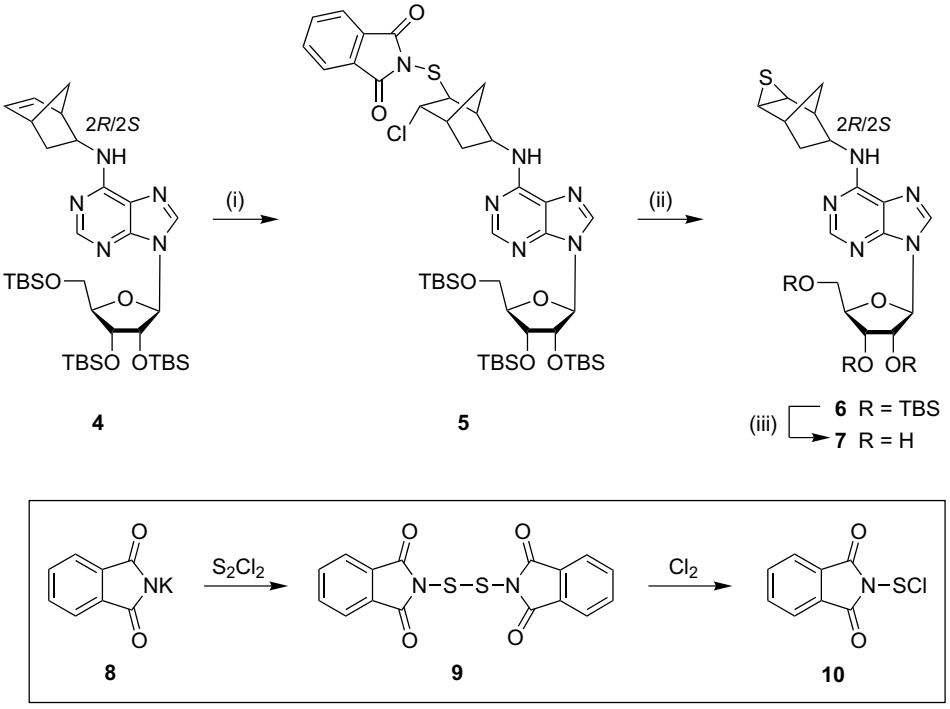


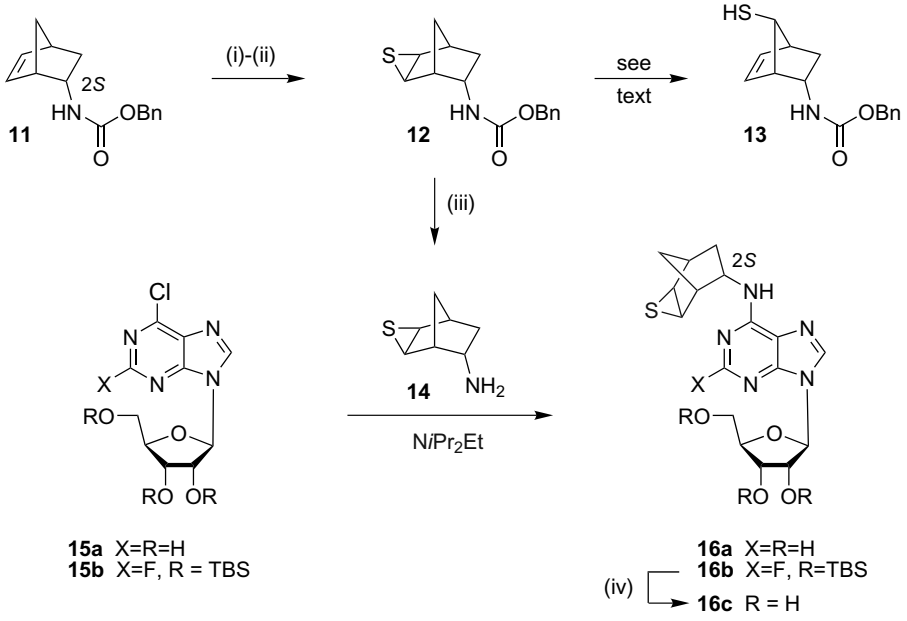
acidic conditions.[10] In contrast, compounds of this type proved to be surprisingly resistant to nucleophilic ring opening, presumably due to the norbornyl hindering the approach of the nucleophile.[11] In this study we targeted thiirane analogs of ENAdo (**1**), which were hoped to be more resistant to acid-catalysed ring opening and cyclisation. Thiiranes are generally less reactive towards electrophilic reagents than the corresponding epoxides as a result of the lower polarity of the C–S bond (relative to C–O) and lower electron density of the sulfur.[12,13] Additionally, epithionorbornanes have been reported to be stable to column chromatography and no decomposition or isomerisation was observed at 130 °C (GPLC).[14]

2. Results and discussion

The initial target compound, N^6-(*exo*-5,6-epithionorborn-2-yl)adenosine (**7**), was prepared from N^6-(*exo*-norborn-5-en-2-yl)adenosine[15] using the approach outlined in Scheme 1. The thiirane moiety was introduced using methodology for the conversion of alkenes to episulfides developed by Bombala and Ley.[16] In this approach, the alkene is treated with phthalimide-*N*-sulfenyl chloride (**10**) and the resultant addition product is reduced with lithium aluminium hydride to afford the corresponding episulfide. Phthalimide-*N*-sulfenyl chloride (**10**) was prepared by reacting potassium phthalimide (**8**) with sulfur monochloride (S_2Cl_2) at room temperature to produce *N*,*N*-dithiobis(phthalimide) (**9**). Bubbling chlorine gas through the solution of this species (**9**) in chloroform gave the desired sulfenyl chloride (**10**). In our hands, this reaction required more forcing conditions, a longer reaction time and proceeded in lower yield than was previously reported.[16] Once in hand, phthalimide-*N*-sulfenyl chloride (**10**) was reacted with the alkene moiety of N^6-(*exo*-5,6-epithionorborn-2-yl)adenosine (**4**) to afford the expected addition product (**5**) in 74% yield. Reaction of this species with $LiAlH_4$ effected the desired cyclisation and afforded



Scheme 1. Reagents: (i) Phth-SCl, NEt_3, MeOH; (ii) $LiAlH_4$, THF; (iii) NBu_4F, Et_2O.



Scheme 2. Reagents: (i) Phth-SCl, NEt_3, MeOH; (ii) $LiAlH_4$, THF; (iii) KOH, MeOH/H_2O; (iv) NH_4F, MeOH.

the thiirane (**6**) in reasonable yield (54%). Deprotection of the silyl groups with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran afforded the thiirane analogue of ENAdo, N^6-(*exo*-5,6-epithionorborn-2-yl) adenosine (**7**) in good yield (84%).

In order to facilitate the synthesis of more complex purine and ribose modified adenosine with optically pure N^6-(5,6-epithionorborn2-y-l) groups, a more convergent synthesis was developed. In the approach the 5,6-epithionorborn-2-yl and purine riboside units were assembled separately and coupled late in the synthesis. More specifically, 2*S*-*endo*-amino-5,6-*exo*-epithionorbornane (**14**) was prepared and coupled with the appropriately substituted 6-chloropurine riboside prior to deprotection of the ribose hydroxyl groups (Scheme 2). 2*S*-*endo*-Amino-5,6-*exo*-epithionorbornane (**14**), was synthesised from 2*S*-*endo*-aminonorborn-5-ene using a sequence involving amine protection, episulfide formation and deprotection. Benzyloxycarbonyl (CBZ) was employed as the protecting group for the amine. This group was chosen as it was expected to be stable to the subsequent reaction conditions (electrophilic addition of the sulfenyl chloride and $LiAlH_4$ reduction) and able to be cleaved without disrupting the thiirane (e.g., by hydrogenolysis). The CBZ protecting group was incorporated in good yield (80%) by reaction of the aminonorbornene with benzylchloroformate and 2 M NaOH in CH_2Cl_2.[17] Reaction of protected 2*S*-*endo*-aminonorborn-5-ene (**11**) with phthalimide-*N*-sulfenyl chloride (**10**) afforded the expected adduct, 2*S*-*endo*-benzyloxycarbonylamino-5-*endo*-chloro-6-*exo*-(*N*-phthali- midothio)norbornane in 57% yield. Reduction with $LiAlH_4$ produced the protected 2-amino-5,6-epithionorbornane (**12**) in good yield (79%).

Removal of the CBZ group proved more problematic. Catalytic hydrogenolysis is the most common method for cleavage of CBZ groups. This method is particularly useful as the by-products of hydrogenation are carbon dioxide and toluene, which are readily separated. There have been a variety of H-donors, which have been reported in the deprotection of CBZ groups including H_2,[18] cyclohexene,[19] formic acid,[20] *cis*-decalin,[21] 1,4-cyclohexadiene[22] and ammonia.[23] Catalytic hydrogenolysis with Pd/C and H_2 gas failed to remove the CBZ moiety. This result was not unexpected as the deprotection of sulfur containing amino acids can fail due to catalyst poisoning.[21] It had been reported that cleavage of CBZ groups from peptides that possess an amino acid containing sulfur such as methionine or cysteine, could be effected by catalytic transfer hydrogenation with palladium black (Pd^0) as catalyst and with formic acid as the H-donor.[24] Unfortunately when the thiirane (**12**) was reacted with Pd^0 no CBZ cleavage was observed, and the thiirane (**12**) was found to have undergone rearrangement. The mechanism of rearrangement presumably involved acid-catalysed ring opening of the thiirane and subsequent rearrangement to **13**. Likewise, attempted deprotection of the CBZ group with trimethylsilyl iodide in acetonitrile resulted in rearrangement to **13**.

It had been reported that cleavage of CBZ groups could be achieved by reaction with 40% aqueous potassium hydroxide (KOH) in MeOH at reflux.[18] The optimum ratio of KOH solution to methanol was found to be 1:1 for the best solubility of the starting material (**12**). A reasonable yield (~72%) of a mixture of the deprotected amine, 2*S*-*endo*-amino-5,6-*exo*-epithionorbornane (**14**) and the by-product of the reaction, benzyl alcohol was obtained. The ratio of the desired product (**14**) to benzyl alcohol in the extract was 1:1.5, the recovery of less of the amine (**14**) than the benzyl alcohol presumably due to the relatively high solubility of the amine (**14**) in the water layer. This amine was used without further purification.

Coupling of the crude amine (**14**) with 6-chloropurine riboside (**15a**) was completed using Hünig's base as acid

scavenger and *t*-BuOH as the solvent.[15] Purification by column chromatography afforded N^6-(2*S*-*endo*-5,6-epithionorborn-2-yl)adenosine (**16a**).

The introduction of a 2-fluoro group on the purine ring had been observed to increase selectivity and efficacy for the A_1AR in previous studies.[15] The 2-fluoro purine (**15b**) was synthesised as previously described, and coupled to the crude amine (**14**). Deprotection of the TBS groups was achieved with NH_4F and gave the desired compound for evaluation, 2-fluoro-N^6-(2*S*-*endo*-5,6-epithionorborn-2-yl)adenosine (**16c**).

Compounds **7**, **16a** and **16c** were tested for affinity and agonist activity at the A_1AR in DDT cells (Table 1). Compared to N^6-cyclopentyladenosine (CPA), compound **7** has a slightly lower affinity (1.5-fold) whereas **16a** and **16b** have six and ninefold higher affinities, respectively. These derivatives were more potent (3–12-fold) than CPA for inhibiting (−)isoproterenol-stimulated cAMP accumulation and all produced the same maximal response as CPA. At the A_{2A}AR in PC-12 cells, compounds **7**, **16a** and **16c** have low micromolar affinities that were 13.6–14.8-fold lower than for the standard CGS21680 (Table 2). Similarly, each of the compounds was less potent than CGS21680 for stimulating cAMP accumulation and each produced less than the maximal response. This indicates that these compounds are partial agonists at the A_{2A}AR.

Table 1. K_i, IC_{50} and intrinsic activity (IA) (nM) for adenosine derivatives at the A_1AR from DDT_1 MF-2 cells

Compound	K_i	IC_{50}	IA
CPA	18 ±3 (7)	2.4±1.2 (16)	1.00
7	27 ±3 (6)	0.89±0.02 (9)	1.00±0.03 (9)
16a	3.6±1 (5)	1.2±0.7 (4)	1.04±0.06 (4)
16c	2.7±1 (5)	0.43±0.19 (9)	1.03±0.03 (9)

The affinity (K_i values) was calculated from the concentration of the compounds that inhibited [^{3}H]CPX binding to the A_1AR by 50% in DDT cell membranes. The potency (IC_{50} values) is the concentration of compounds that inhibited (−)isoproterenol (1 μM) stimulated cAMP accumulation by 50% in DDT cells. The intrinsic activity is the maximal inhibition of (−)isoproterenol-stimulated cAMP accumulation as compared to the maximum inhibition by CPA, which was set as 1.00. Numbers in parentheses are the *N*. The maximal CPA inhibition of (−)isoproterenol-stimulated cAMP accumulation was 85±2%.

Table 2. K_i, EC_{50} and intrinsic activity (IA) (nM) for adenosine derivatives at the A_{2A}AR from PC-12 cells

Compound	K_i	EC_{50}	IA
CGS21680	284±56 (6)	25±4 (15)	1.00
7	3866±142 (4)	541±99 (6)	0.77±0.05 (6)*
16a	4207±857 (5)	636±76 (6)	0.76±0.08 (6)**
16c	4003±1331 (3)	502±502 (8)	0.76±0.06 (8)*

The K_i values were calculated from the concentration of the compounds that inhibited [^{3}H]ZM241385 binding to the A_{2A}AR by 50% in PC-12 cell membranes. The EC_{50} values are the concentration of compounds that elicited 50% of the maximal cAMP accumulated in PC-12 cells. The intrinsic activity is the maximal stimulation of cAMP accumulation as compared to the maximum produced by CGS 21680, which was set at 1.00. Numbers in parentheses are the *N*. The maximal CGS 21680-stimulated cAMP accumulation was 11.5±1.8 nmol/mg protein/10 min. $^*p < 0.02$ and $^{**}p < 0.007$ compared to CGS 21680.

Table 3. Affinity of **16c** (nM) at human adenosine receptors

Compound	A_1	A_{2A}	A_{2B}	A_3
16c	0.15±0.09	812±163	>100,000	175±10

These data show that compounds **7**, **16a** and **16c** have high affinity and potency for the A_1AR while acting as full agonists at this receptor. As expected, the *endo*-isomer (**16a**) has a higher affinity for the A_1AR than the *exo*-isomer (**7**). Although 2-fluoro substitution of the *endo*-isomer did not change the affinity for the A_1AR, its potency for inhibiting cAMP accumulation increased slightly. In contrast, these compounds show relatively low affinity and potency at the A_{2A}AR and are partial agonists at this receptor. Compound **16c** has the highest selectivity for the A_1AR as compared to the A_{2A}AR based upon affinity (1482-fold) or potency for the cellular response (1167-fold). Overall, **16c** has the favourable characteristics of high affinity, potency and selectivity for the A_1AR suggesting that additional derivatives may optimise selective agonist properties for this receptor subtype.

Based upon the data for compound **16c** using DDT and PC-12 cells, this compound was further tested for affinity at the human recombinant A_1, A_{2A}, A_{2B} and A_3AR's (Table 3). This compound had the highest affinity (subnanomolar) at the A_1AR and high selectivity for this receptor subtype. Its affinity for the human A_1AR was 18-fold higher than for the A_1AR in DDT cells (hamster) highlighting the species difference in this receptor.

3. Conclusions

In conclusion, an efficient convergent approach of the synthesis of N^6-(5,6-epithionorborn-2-yl)adenosines has been developed. The N^6-(5,6-epithionorborn-2-yl) substituent has been found to confer high A_1AR potency and selectivity. N^6-(2*S*-*endo*-5,6-Epithionorborn-2-yl)-2-fluoroadenosine (**16c**) was found to be one of the most potent and selective A_1AR agonists reported to date.

4. Experimental

^{1}H and ^{13}C NMR spectra were obtained with a Varian 300 MHz Unity Plus spectrometer at 288.976 and 75.437 MHz, respectively; or with a JEOL 400 MHz Eclipse Plus FT NMR spectrometer at 399.784 and 100.534 MHz, respectively. High resolution ES MS data was collected using a Bruker BioApex 47e FTMS with an Analytica Electrospray Source. Melting points were determined on a Reichert Hot-Stage microscope and are uncorrected. Electrospray mass spectra were obtained on a Micromass Platform instrument with Masslynx software. Thin layer chromatography was carried out using 0.2 mm plates using Merck silica gel 60 F254.

Column chromatography was achieved using Merck silica gel 60, particle size 0.063–0.200 mm, 70–230 mesh.

4.1. N^6-*exo*-(*endo*-5-Chloro-*exo*-6-(*N*-phthalimidothio)norborn-2-yl)-2′,3′,5′-tris-*O*-TBS-adenosine (5)

Phthalimide-*N*-sulfenyl chloride (**10**, 77 mg, 0.36 mmol) was dissolved in $CHCl_3$ (1 mL) and added dropwise to **4** (92 mg, 0.13 mmol) in $CDCl_3$ (7 mL). The reaction was stirred for 100 h then evaporated in vacuo and chromatographed (petroleum ether/EtOAc, 4:1) to afford **5** (88 mg, 74%) as an oil. ^{1}H NMR ($CDCl_3$): δ −0.30, −0.27, −0.05, −0.03, 0.14, 0.15 (6×s, 6×3H, $SiCH_3$), 0.76, 0.78, 0.96 (3×s, 3×3H, Bu^t), 0.95–0.99 (m, 1H, H-7a″), 1.11–1.13 (m, 1H, H-3x″/H-3n″), 1.55–1.67 (m, 1H, H-3x″/H-3n″), 1.81–1.92 (m, 1H, H-7s″), 2.43 (br s, 1H, H-4″/H-1″), 3.43–3.47 (m, 1H, H-6″), 3.83 (dd, 1H, H-5a′/H-5b′), 4.04 (dd, 1H, H-5a′/H-5b′), 4.15 (t, 1H, H-4′), 4.23–4.27 (m, 2H, H-5″), 4.39 (t, 1H, H-3′), 4.83 (t, 1H, H-2′), 4.86 (br s, 1H, H-2″), 5.99 (d, 1H, H-1′), 7.77–8.02 (m, 6H, ArH, H-2, H-8). ^{13}C NMR ($CDCl_3$): δ −5.5, −5.2, −5.1, −4.7, −4.2, −4.1, 17.8, 18.0, 18.5, 26.2, 26.4, 26.6, 30.9, 32.2, 33.7, 45.2, 60.0, 64.0, 64.0, 73.9, 77.3, 87.5, 89.3, 121.2, 124.8, 128.6, 133.3, 134.3, 136.1, 140.8, 155.2, 170.8.

4.2. N^6-*exo*-(*exo*-5,6-Epithionorborn-2-yl)-2′,3′,5′-tris-*O*-TBS-adenosine (6)

Compound **5** (230 mg, 0.25 mmol) dissolved in THF (4 mL) was cooled to −78 °C and $LiAlH_4$ in Et_2O (1 M, 0.3 mL) was added dropwise. The reaction was allowed to stir at this temperature for 15 min then gradually warmed to rt. The reaction was quenched with water (15 mL) then extracted with Et_2O (3×15 mL). The organic layer was dried ($MgSO_4$) and evaporated in vacuo. Purification by column chromatography (petroleum ether/EtOAc, 4:1) to afford **6** (99 mg, 54%) as an oil. ^{1}H NMR ($CDCl_3$): δ −0.24, −0.21, −0.05, −0.04, 0.09, 0.13 (6×s, 6×3H, $SiCH_3$), 0.80, 0.92, 0.95 (3×s, 3×3H, Bu^t), 0.91–0.96 (m, 1H, H-7a″), 1.25–1.36 (m, 1H, H-3x″/H-3n″), 1.69–1.72 (m, 1H, H-3x″/H-3n″), 2.02–2.07 (m, 1H, H-7s″), 2.56 (br s, 1H, H-4″/H-1″), 2.62–2.70 (m, 2H, H-5″/H-6″), 2.93 (br s, 1H, H-4″/H-1″), 3.77 (dd, 1H, H-5a′/H-5b′), 4.04–4.14 (m, 2H, H-2″, H-5a′/H-5b′), 4.28–4.33 (m, 1H, H-4′), 4.64–4.70 (m, 1H, H-3′), 4.64–4.70 (t, 1H, H-2′), 5.75 (br s, 1H, NH), 6.00–6.03 (m, 1H, H-1′), 8.12 (s, 1H, H-2/H-8), 8.37 (s, 1H, H-2/H-8). ^{13}C NMR ($CDCl_3$): δ −5.4, −5.1, −5.0, −4.8, −4.7, −4.4, 17.8, 18.0, 18.5, 22.7, 25.6, 25.8, 26.0, 29.7, 32.2, 32.6, 44.0, 47.9, 52.6, 62.5, 72.0, 75.9, 85.5, 88.2, 120.0, 132.9, 139.0, 152.8, 153.6.

4.3. N^6-(*exo*-5,6-Epithionorborn-2-yl)adenosine (7)

The thiirane **6** (95 mg, 0.13 mmol) was stirred in for 24 h in a 1 M solution of TBAF in THF (0.6 mL). The reaction mixture was evaporated in vacuo and purification by reverse phase column chromatography (MeOH/H_2O/NH_3, 60:40:0.1) then on silica gel ($CHCl_3$/MeOH, 9:1) afforded the title compound **7** (43 mg, 84%) as a transparent oil. ^{1}H NMR ($CDCl_3$): δ 0.88 (d, 1H, H-7a″), 1.56–1.62 (m, 2H, H-3n″, H-3x″), 2.01 (d, 1H, H-7s″), 2.52 (s, 1H, H-1″/H-4″), 2.53 (s, 1H, H-1″/H-4″), 2.82 (dd, 1H, H-5″, H-6″), 3.70 (d, 1H, H-5a′/H-5b′), 3.88 (d, 1H, H-5a′/H-5b′), 4.04–4.19 (m, 1H, H-2″), 4.26 (s, 1H, H-4′), 4.43 (d, 1H, H-3′), 4.92 (br s, 1H, H-2′), 5.84 (d, 1H, H-1′), 6.40 (br s, 1H, NH), 7.91 (br s, 1H, H-2/H-8), 8.20 (br s, 1H, H-2/H-8). ^{13}C NMR ($CDCl_3$): δ 25.6, 33.7, 35.1, 37.7, 38.1, 38.5, 54.9, 63.4, 72.6, 75.5, 88.2, 91.2, 121.3, 141.6, 149.1, 153.2, 155.1. HR-ES MS calcd for $C_{17}H_{22}N_5O_4S^+$ (M + 1) 392.1393, found 392.1388.

4.4. *NN*′-Dithiobis(phthalimide) (9)[16]

To a stirred suspension of potassium phthalimide (**8**, 5.11 g, 28 mmol) in CH_2Cl_2 (25 mL) at 0 °C was added a solution of S_2Cl_2 (1.1 mL, 14 mmol) in CH_2Cl_2 (5 mL). The reaction was allowed to warm to rt then heated briefly to reflux. After 3 h the reaction mixture was filtered, the filtrate was evaporated in vacuo and washed with petroleum ether (2×10 mL) and water (2×10 mL) then dried in vacuo. Recrystallisation from $CHCl_3$ afforded the title compound **9** product (4.78 g, 49%) as translucent needle like crystals (mp 232–234 °C) (lit.[16] 229–230 °C). ^{1}H NMR ($CDCl_3$): δ 8.01 (s, ArH). ^{13}C NMR (CD_3COCD_3): δ 124.9, 136.2, 206.2.

4.5. Phthalimide-*N*-sulfenyl chloride (10)[16]

Compound **9** (3.391 g, 9.5 mmol) was suspended in $CHCl_3$ (40 mL). The reaction was heated to 55 °C and Cl_2 gas was bubbled through for a total of 13.5 h over two days. Nitrogen was bubbled through the solution and the reaction was evaporated in vacuo to afford the product **10** (2.75 g, 100%) as a yellow solid (mp 115–118 °C) (lit.[16] mp 115–117 °C). ^{1}H NMR ($CDCl_3$): δ 7.75–7.93 (m, 2H, ArH), 8.02–8.06 (m, 2H, ArH). ^{13}C NMR ($CDCl_3$): δ 124.9, 135.7, 165.5.

4.6. 2*S*-*endo*-Benzyloxycarbonylaminonorborn-5-ene (11)

2*S*-*endo*-Aminonorborn-5-ene hydrochloride (0.998 g, 6.85 mmol) in CH_2Cl_2 (30 mL) and 2 M NaOH (10.3 mL) were cooled to 0 °C. Benzyl chloroformate (1.0 mL, 7.0 mmol) in CH_2Cl_2 (10 mL) was added over 10 min. The reaction was stirred for 3 h at room temperature then the organic layer was separated, washed successively with dilute HCl (20 mL) and water (30 mL), dried ($MgSO_4$) and evaporated in vacuo to afford a viscous oil. Column chromatography (petroleum ether/EtOAc, 10:1) afforded the title compound **11** (1.483 g, 89%) as a white solid (mp 41–42 °C). ^{1}H NMR ($CDCl_3$): δ 0.66–0.72 (m, 1H, H-3x), 1.32 (dd, 1H, H-7s), 1.46 (dd, 1H, H-7a), 2.16–2.24 (m, 1H, H-3n), 2.82 (br s, 1H, H-4), 3.04 (br s, 1H, H-1), 4.29–4.37 (m, 1H, H-2), 4.50 (br s, 1H, NH), 5.07 (s, 2H, CH_2), 5.98–6.01 (m, 1H, H-5), 6.32–6.35 (m, 1H, H-6), 7.26–7.34 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$): δ 34.8, 42.0, 45.7, 48.0, 50.4, 65.8, 127.4, 127.5, 127.9, 131.0, 136.2, 139.1, 155.5.

4.7. 2*S*-*endo*-Benzyloxycarbonylamino-5-*endo*-chloro-6-*exo*-(*N*-phthalimidothio)norbornane

Phthalimide-*N*-sulfenyl chloride (**10**, 1.296 g, 5.33 mmol) was dissolved in CH_2Cl_2 (8 mL) and added dropwise to a

solution of **11** (1.195 g, 4.91 mmol) in CH_2Cl_2 (7 mL). The reaction was stirred for 21 h then filtered and chromatographed, (petroleum ether/EtOAc, 10:1) to afford the desired product (1.38 g, 57%) as a white foam (mp 59–62 °C). ^{1}H NMR ($CDCl_3$): δ 1.46–1.53 (m, 1H, H-3n), 1.64 (d, 1H, H-7s), 1.94–2.03 (m, 2H, H-3x/H-7a), 2.48 (br s, 1H, H-4), 2.58 (br s, 1H, H-1), 3.56–3.59 (m, 1H, H-6), 4.07–4.12 (m, 1H, H-2), 4.29–4.33 (m, 1H, H-5), 4.92–5.07 (m, 3H, CH_2/NH), 7.17–7.28 (m, 5H, ArH CBZ), 7.80–7.86 (m, 4H, ArH phthalimide). ^{13}C NMR ($CDCl_3$): δ 28.7, 35.6, 44.6, 44.9, 51.8, 54.0, 66.1, 66.8, 123.4, 123.9, 128.1, 128.5, 131.7, 134.1, 134.6, 136.1, 155.9, 168.2.

4.8. 2*S*-*endo*-Benzyloxycarbonylamino-5,6-*exo*-epithionorbornane (12)

2*S*-*endo*-Benzyloxycarbonylamino-5-*endo*-chloro-6-*exo*-(*N*-phth-alimidothio)norbornane (0.671 g, 1.47 mmol) was dissolved in THF (15 mL) and added dropwise to a stirred suspension of $LiAlH_4$ (56 mg, 1.48 mmol) in THF (6 mL) at −78 °C. The reaction was maintained at that temperature for 10 min then allowed to warm to −25 °C when it was quenched with water (8 mL). The mixture was extracted with Et_2O dried (Na_2SO_4) and evaporated in vacuo to afford a pink semi-solid. Purification with column chromatography (petroleum ether/EtOAc, 4:1) packed on SiO_2 gave **12** (318 mg, 79%) as a white solid (mp 66–69 °C). ^{1}H NMR ($CDCl_3$): δ 0.79–0.90 (m, 2H, H-3n/H-7a), 1.58 (d, 1H, H-7s), 2.17–2.27 (m, 1H, H-3x), 2.43–2.45 (m, 1H, H-4), 2.72–2.75 (m, 1H, H-1), 2.84 (d, 1H, H-5/H-6), 2.97 (d, 1H, H-5/H-6), 4.12–4.20 (m, 1H, H-2), 4.92–5.02 (br s, 1H, NH), 5.10 (s, 2H, CH_2), 7.29–7.35 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$): δ 27.3, 32.8, 35.9, 37.1, 37.9, 41.1, 53.1, 66.7, 128.07, 128.12, 128.5, 136.2, 155.9.

4.9. 2*S*-*endo*-Benzyloxycarbonylamino-7-thionorbor-5-ene (13)

The thiirane **12** (35 mg, 0.13 mmol) was dissolved in 4.4% formic acid in MeOH (10 mL) and passed through a 1.5 cm plug of Pd^0 at ~1 mL/min twice. The resultant material was evaporated in vacuo and purification by column chromatography (petroleum ether/EtOAc, 4:1) afforded **13** as a transparent oil (28 mg, 80%). ^{1}H NMR ($CDCl_3$): δ 0.66–0.72 (m, 1H, H-3n), 1.45–1.48 (m, 1H, H-7a), 2.11–2.25 (m, 1H, H-3x), 2.83 (br s, 1H, H-1/H-4), 3.04 (br s, 1H, H-1/H-4), 4.28–4.43 (m, 1H, H-2), 5.07 (br s, 2H, CH_2), 5.99–6.02 (m, 1H, H-5/H-6), 6.33–6.36 (m, 1H, H-5/H-6), 7.30–7.34 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$): δ 35.7,42.5, 46.2, 48.6, 50.9, 66.5, 128.1, 128.1, 128.5, 131.5, 136.3, 140.0, 155.8.

4.10. 2*S*-*endo*-Amino-5,6-*exo*-epithionorbornane (14)

A mixture of 2*S*-*endo*-benzyloxycarbonylamino-5,6-*exo*-epithionorbornane **12** (89 mg, 0.32 mmol) in 40% KOH (4 mL) and MeOH (4 mL) was heated at reflux for 6 h. The MeOH was removed in vacuo and the mixture was extracted with Et_2O (3×25 mL), dried (Na_2SO_4) and evaporated in vacuo to afford a mixture of benzyl alcohol and the desired product **14** (49 mg, ~72%) in a ratio of 1.5:1, as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.74–0.80 (m, 2H, H-3n/H-7a), 1.54–1.58 (m, 1H, H-7s), 2.06–2.14 (m, 1H, H-3x), 2.39–2.42 (m, 2H, H-1, H-4), 2.89 (d, 1H, H-5/H-6), 3.10 (d, 1H, H-5/H-6), 3.44–3.49 (m, 1H, H-2). Benzyl alcohol 1.99 (br s, 1H, OH), 4.67 (s, 2H, CH_2), 7.27–7.37 (m, 5H, ArH). ^{13}C NMR ($CDCl_3$): δ 28.0, 33.3, 37.6, 38.1, 38.7, 43.2, 53.9. Benzyl alcohol 65.1, 126.9, 127.5, 128.5, 141.0.

4.11. N^6-(2*S*-*endo*-5,6-Epithionorborn-2-yl)adenosine (16a)

A mixture of 6-chloropurine riboside (**15a**, 47 mg, 0.16 mmol), 2*S*-*endo*-amino-5,6-*exo*-epithionorbornane (49 mg, ~0.18 mmol) and N(*i*-Pr)$_2$Et (0.3 mL, 1.7 mmol) was heated to reflux for 19 h. The reaction was evaporated in vacuo and purified by column chromatography ($CHCl_3$/MeOH, 95:5) afforded the title compound **16a** (17 mg, 27%) as a transparent solid (mp 204 °C dec). ^{1}H NMR ($CDCl_3$): δ 0.92 (d, 1H, H-7a″), 1.11–1.28 (m, 1H, H-3n″), 1.64 (d, 1H, H-7s″), 2.29–2.38 (m, 1H, H-3x″), 2.51 (d, 1H, H-4″), 2.91 (d, 1H, H-1″), 2.95 (d, 1H, H-5″), 3.03 (d, 1H, H-6″), 3.65 (d, 1H, H-5a′/H-5b′), 3.92 (d, 1H, H-5a′/H-5b′), 4.27 (s, 1H, H-4′), 4.35 (d, 1H, H-3′), 4.58–4.66 (m, 1H, H-2″), 4.87 (t, 1H, H-2′), 5.77 (d, 1H, H-1′), 7.84 (br s, 1H, H-2/H-8), 8.22 (br s, 1H, H-2/H-8). HR-ES MS calcd for $C_{17}H_{22}N_5O_4S^+$ (M + 1) 392.1393, found 392.1382.

4.12. 2′,3′,5′-Tris-*O*-TBS-2-fluoro-N^6-(2*S*-*endo*-5,6-epithionorborn-2-yl)adenosine (16b)

A mixture of **15b** (135 mg, 0.21 mmol), the amine (**14**, 23 mg, ~0.16 mmol) and N(*i*-Pr)$_2$Et (0.2 mL, 1.2 mmol) in *t*-BuOH (7 mL) was heated to reflux for 17 h. The reaction mixture was evaporated in vacuo and purified by column chromatography (hexane/EtOAc, 30:1) afforded the title compound **16b** (43 mg, 36%) as a transparent oil. ^{1}H NMR ($CDCl_3$): δ −0.10, 0.00, 0.10, 0.11, 0.12, 0.13, (6×s, 6×3H, $SiMe_3$), 0.85, 0.93, 0.95 (3×s, 3×3H, Bu^t), 0.86–0.91 (m, 1H, H-3n″), 1.12 (d, 1H, H-7s″/H-7a″), 1.66 (d, 1H, H-7s″/H-7a″), 2.27–2.38 (m, 1H, H-3x″), 2.51 (br s, 1H, H-1″/H-4″), 2.89 (d, 1H, H-5″/H-6″), 2.96 (br s, 1H, H-1″/H-4″), 3.05 (d, 1H, H-5″/H-6″), 3.79 (dd, 1H, H-5a′/H-5b′), 4.05 (dd, 1H, H-5a′/H-5b′), 4.12–4.14 (m, 1H, H-4′), 4.31 (t, 1H, H-3′), 4.64 (t, 1H, H-2′), 4.69–4.77 (br m, 1H, H-2″), 5.91 (d, 1H, H-1′), 8.17 (br s, 1H, H-8). ^{13}C NMR ($CDCl_3$): δ −5.5, −5.4, −5.0, −4.8, −4.7, −4.4, 17.9, 18.0, 18.5, 25.7, 25.8, 26.0, 33.1, 35.8, 37.3, 38.0, 41.1, 53.1, 62.1, 71.3, 75.6, 85.1, 88.8, 118.1, 138.7, 149.7 (J = 18.9 Hz), 155.8 (J = 20.2 Hz), 159.3 (J = 211 Hz).

4.13. N^6-(2*S*-*endo*-5,6-Epithionorborn-2-yl)-2-fluoroadenosine (16c)

Compound **16b** (39 mg, 0.05 mmol) and NH_4F (115 mg, 3.1 mmol) were heated to ~60 °C in MeOH (4 mL) for 23 h. The reaction mixture was evaporated in vacuo and purified by column chromatography ($CHCl_3$/MeOH, 95:5) to yield **16c** (16 mg, 75%) as a white solid (mp 249–251 °C dec). ^{1}H NMR (CD_3OD): δ 0.97 (d, 1H,

H-7a″), 0.86–0.91 (m, 1H, H-3n″), 1.66 (d, 1H, H-7s″), 2.27–2.38 (m, 1H, H-3x″), 2.51 (br s, 1H, H-1″/H-4″), 2.89 (d, 1H, H-5″/H-6″), 2.96 (br s, 1H, H-1″/H-4″), 3.05 (d, 1H, H-5″/H-6″), 3.79 (dd, 1H, H-5a′/H-5b′), 4.05 (dd, 1H, H-5a′/H-5b′), 4.12–4.14 (m, 1H, H-4′), 4.31 (t, 1H, H-3′), 4.64 (t, 1H, H-2′), 4.69–4.77 (br m, 1H, H-2″), 5.91 (d, 1H, H-1′), 8.17 (br s, 1H, H-8). ^{13}C NMR ($CDCl_3$): δ 28.1, 33.7, 36.0, 37.9, 39.4, 42.4, 54.5, 63.2, 72.2, 75.5, 87.7, 90.8, 119.4, 141.4, 145.8 (J = 23.3 Hz), 157.7 (J = 20.4 Hz), 160.5 (J = 212 Hz). ES MS calcd 410.1298 (M+H), found 410.1294.

5. Drug solutions

Stock solutions of the compounds were prepared in DSMO at a concentration of 10 mM and stored frozen. On the day of use, the stock solution was diluted with Hank's balanced salt solution for cAMP assays or 50 mM Tris–HCl buffer at pH 7.4 for receptor assays. Control incubations contained the same final concentration of DMSO.

6. Cell culture and biochemical assays

Hamster smooth muscle DDT_1 MF-2 (DDT) and rat pheochromcytoma PC-12 cells were grown as monolayers and used one day preconfluent. Cell membranes for receptor assays and intact cell suspensions for cAMP assays were prepared as reported.[15] The K_i values for the compounds at the A_1AR in DDT cell membranes and the A_{2A}AR in PC-12 membranes were determined from displacement of specific [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine and [^{3}H]ZM241385, respectively. The concentration of compounds that inhibited (−)isoproterenol-stimulated cAMP by 50% (IC_{50}) in DDT cells and stimulated half-maximal cAMP accumulation (EC_{50}) in PC-12 cells was determined as previously described.[15]

7. Data analysis

The IC_{50} and EC_{50} values for cAMP accumulation were determined by nonlinear regression analysis of the concentration–response using GraphPad software (San Diego, CA, USA). The concentration of compounds that inhibited specific radioligand binding by 50% (IC_{50}) was determined by nonlinear regression analysis using the GraphPad software. The K_i values were calculated from the IC_{50} using the Cheng and Prusoff conversion.[25] The intrinsic activity (IA) of the compounds is expressed as a fraction of the maximal response for each compound as compared to the maximum for the standards comprising CPA and CGS 21680 for the A_1 and A_{2A}AR responses, respectively. Statistical analysis of the data was performed using the Student's t-test and differences were considered significant if $p < 0.05$.

8. Radioligand binding to recombinant human receptors

Competitive binding assay were performed using recombinant human receptors with the following radioligands; [^{125}I]-ABA (A_1AR), [^{3}H]-ZM241385 (A_{2A}AR), [^{125}I]-ABOPX (A_{2B}AR), [^{125}I]-ABA (A_3AR), as described previously.[26] The K_i values determined for human A_1 and A_3 receptors represent binding to the G protein coupled high affinity conformational state that is detected by the use of an agonist radioligand.[27] K_i values were calculated from the IC_{50} as described previously.[28]

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