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XH-14 Analogues as Adenosine Antagonists

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Abstract—Analogues of the potent adenosine antagonist 5-(3′-hydroxypropyl)-7-methoxy-2-(3′-methoxy-4′-hydroxyphenyl)benzo[*b*]furan-3-carbaldehyde (XH-14, **1**) with alternate substituents in the 2-, 5- and 7-positions have been synthesised. The affinity of these compounds for the A_1 adenosine receptor has been evaluated using a [^{3}H]CPX competitive binding assay. This structure-activity study highlighted the importance of the 3-formyl and 5-(3-hydroxypropyl) moieties for high receptor affinity.

Introduction

Adenosine is an important regulatory compound which mediates a wide range of physiological effects in the cardiac, nervous and immune systems.[1,2] Adenosine interacts with extracellular receptors which are coupled to a variety of secondary messenger systems, including enzymes and ion channels. Four adenosine receptor subtypes (termed A_1, A_{2a}, A_{2b}, and A_3 AR) have been defined based on their pharmacological properties and molecular cloning. Although an enormous effort has been directed toward the development of therapeutic agents based on adenosine, relatively few compounds have entered trials or been approved for clinical use.[2] Adenosine agonists typically produce side effects resulting from the widespread distribution of adenosine receptors in the body and are also known to cause tachyphylaxis (receptor desensitisation).[3] Adenosine antagonists may have improved therapeutic potential since their effects are dependant on the level of endogenous purinergic tone, rather than the absolute distribution of receptors.[4] It has been proposed that A_1 selective antagonists with good bioavailability could be useful agents for the treatment of ischaemic bradyarrhythmias, cardiac arrest and renal disease.[4]

The relatively potent adenosine antagonist XH-14 (**1**) was isolated from the plant, *Salvia miltiorrhiza*, commonly known as Danshen.[5,6] Aqueous extracts of this plant have long been used as a traditional Chinese medicine for angina pectoris and acute myocardial infarction. This compound is the first known nitrogen free adenosine receptor ligand and has the advantage of relatively high water solubility. Since the initial reports which focused on the isolation, total synthesis and A_1 adenosine receptor affinity, benzofuran adenosine antagonists of this type have remained largely unexplored. Therefore, the aim of our research was to investigate the structure–activity relationships of this interesting class of compounds with respect to their interaction with the A_1 adenosine receptor. The approach chosen towards this end involved the modification of XH-14 based on known requirements for receptor blockade, developed largely from the structure–activity relationships of xanthines. In the case of xanthines, 1,3-dipropyl and 8-cycloalkyl/aryl substitution produced dramatic increases in receptor affinity. The effect of these 1,3 and 8-substituents on A_1 receptor affinity is exemplified by a comparison of 1,3-dipropyl-8-phenylxanthine (**2**) and theophylline (1,3-dimethylxanthine). 1,3-Dipropyl-8-phenylxanthine exhibited over 800 times greater affinity than theophylline in a [^{3}H]-N^6-(phenylisopropyl)adenosine competitive binding assay (K_i values of 10 and 8470 nM, respectively).[7] Since XH-14 acts at the same receptor site as these xanthines it was thought that incorporation of propyl and phenyl substituents in the appropriate positions may produce benzofuran antagonists with improved binding affinity (Fig. 1).

Key words: Adenosine antagonist; benzofuran; A_1 adenosine receptor.
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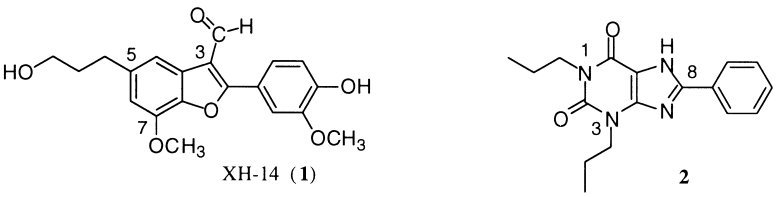
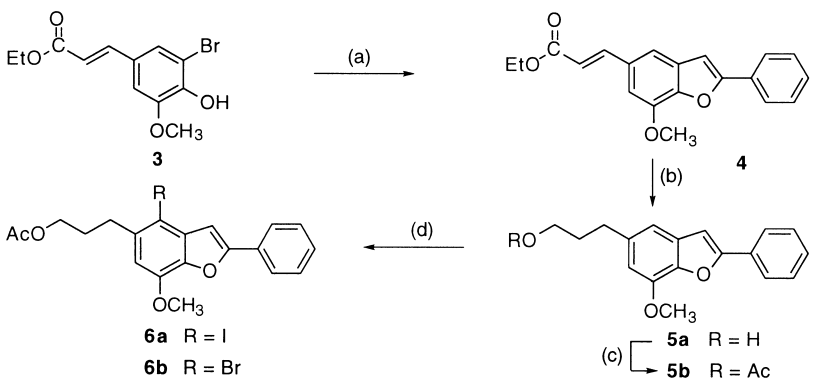


Figure 1.

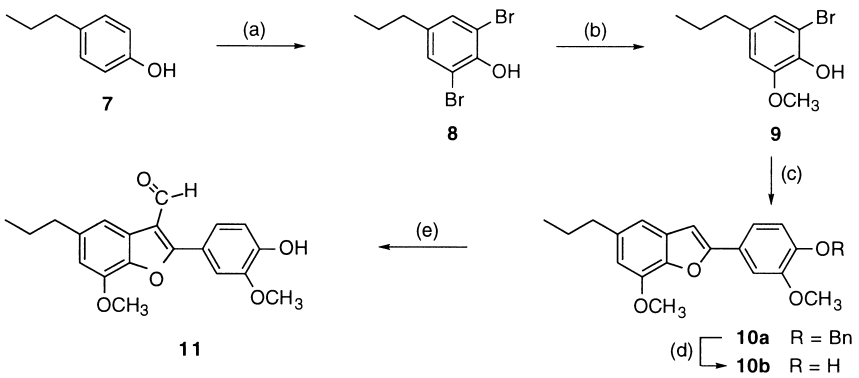
Results and Discussion

The synthetic approach used to prepare a 2-phenyl analogue of the natural product, XH-14 (**1**) is outlined in Scheme 1. Briefly, this involved coupling ethyl 3-methoxy-4-hydroxy-5-bromocinnamate (**3**) with cuprous phenylacetylide to afford 2-phenyl-7-methoxy-5-(2-ethoxycarbonyl-*E*-ethenyl)benzo[*b*]furan (**4**). Reduction of the α,β-unsaturated ester moiety using lithium aluminium hydride followed by protection of the resultant alcohol yielded the benzo[*b*]furan **5b**. Introduction of a 3-formyl substituent was attempted using the modified Gatterman–Adams reaction developed by Yang et al.[6] A range of conditions were trialed, but in each case only deprotected starting material (**5a**) was isolated. In order to overcome this problem an indirect method involving halogenation and formylation[8,9] of the resultant aryl halide was considered. Iodination of **5b** using iodide and silver sulphate afforded a crystalline product. NMR and MS spectra confirmed that iodination had occurred, but the position of the iodide was unable to be unambiguously assigned. A crystal structure was obtained[10] that indicated the iodine substituted for H4 rather than reacting at the 3-position as desired. Modelling studies on the benzo[*b*]furan **1** and related compounds indicated the 3-position was electronically favoured for electrophilic substitution, but the 4-position was sterically favoured.[6] This postulation was supported by experimental data in which bulky electrophiles reacted at C4 and small electrophiles at C3. Based on this information we attempted to brominate **5b** in the hope that the smaller halogen would give the desired regioselectivity. The careful addition of bromine (1.1 molar equivalents) to compound **5b** at 0 °C produced one major product. Comparison of the ^{1}H NMR with that of **6a** indicated the bromine had also substituted at the 4-position. Other approaches involving halogenation-formylation-dehalogenation of the benzofuran **5b** or coupling of the *ortho*-bromophenol **3** with phenylpropargyl aldehyde under the conditions developed by Larock[11] were contemplated. Fortunately closely related benzofurans were able to be formylated (see Schemes 2 and 3) which enabled the importance of the 3-formyl group for adenosine receptor affinity to be assessed and obviated the need to persist with these alternatives.

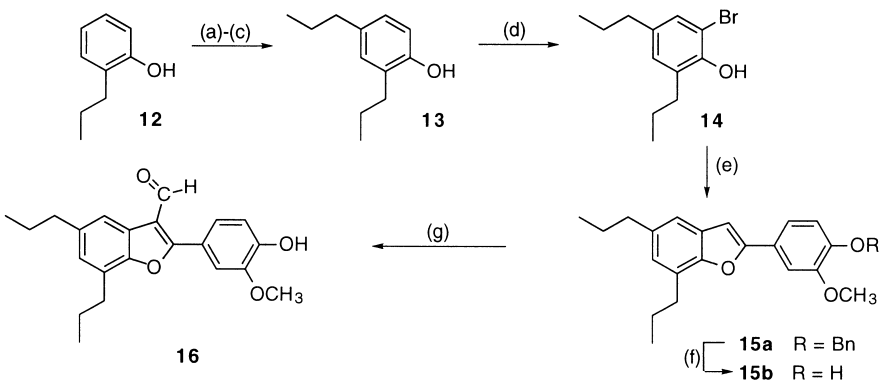
In order to prepare an analogue of XH-14 (**1**) with a propyl (rather than 3-hydroxypropyl) side chain via the cuprous acetylide coupling approach it was necessary to prepare the *ortho*-bromophenol **9** (Scheme 2). This was initially attempted via the bromination of 2-methoxy-4-propylphenol, however regioselectivity problems induced us to turn to the alternative sequence which involved bromination of 4-propylphenol followed by nucleophilic displacement of bromine. Once obtained, **9** was coupled with cuprous (4-benzyloxy-3-methoxyphenyl) acetylene and the resultant benzo[*b*]furan deprotected to afford **10b**. In contrast to the 2-phenylbenzo[*b*]furan **5b**, this compound underwent a Gattermann–Adams reaction to give the desired product **11** in 40% yield. The



Scheme 1. (a) PhC≡CCu, pyridine; (b) $LiAlH_4$, THF; (c) Ac_2O, pyr; (d) see text.



Scheme 2. (a) Br_2, CH_2Cl_2; (b) MeOH, BaO, $CuCl_2$, DMF; (c) (4-BnO-3-OMe)PhC≡CCu, pyridine; (d) H_2, Pd/C, AcOH/THF; (e) $Zn(CN)_2$, KCl, HCl, Et_2O then H_2O/EtOH.



Scheme 3. (a) CH_3CH_2COCl; (b) $AlCl_3$, CS_2, Δ; (c) Zn(Hg), EtOH/aq HCl; (d) Br_2, AcOH; (e) (4-BnO-3-OMe)PhC≡CCu, pyridine; (f) H_2, Pd/C, AcOH, THF; (g) $Zn(CN)_2$, KCl, HCl, Et_2O then H_2O/EtOH.

higher reactivity of **10b** relative to that observed for **5b** may be a result of mesomeric factors associated with the 2-(4-hydroxyphenyl) substituent. Similarly, Yang et al. argued that XH-14 analogues bearing this substituent at C2 were more reactive as a result the negative charge of the phenolate oxygen being delocalised over the 3- and 4-positions, thus making the benzofuran ring system more electron rich.[6]

A 5,7-dipropylbenzo[*b*]furan analogue of XH-14 (**1**) was prepared using a similar synthetic approach (Scheme 3). The key intermediate in this synthesis was 6-bromo-2,4-dipropylphenol (**14**), which was prepared from 2-propylphenol in four steps. The additional propyl side chain was constructed by *O*-acylation, Fries rearrangement, Clemmensen reduction all of which proceeded in good yield. Bromination of the resultant dipropylphenol afforded **14**, which was coupled with cuprous (4-benzyloxy-3-methoxyphenyl)acetylene to form the benzo[*b*]-furan ring system. After deprotection, a formyl group was introduced into the 3-position via a Gattermann–Adams reaction (49% yield).

In order to allow a comparison of the receptor affinity of these analogues with the natural product, a supply of XH-14 (**1**) was prepared using a new synthetic approach[12] developed in our laboratory. The final precursor in this synthesis, 5-(3-hydroxypropyl)-7-methoxy-2-(3′-methoxy-4′-hydroxyphenyl)benzo[*b*]furan (**17**), was also evaluated at the A_1 adenosine receptor.

The affinity of the compounds outlined above for the A_1 adenosine receptor was measured using a competitive binding assay which employed [^{3}H]8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]CPX) as a reference. The results are listed in Table 1.

Conclusions

The natural product XH-14 (**1**) was found to bind the A_1 adenosine receptor with relatively high affinity ($K_i = 50$ nM). However, this value for the binding affinity of **1** was somewhat lower than the value originally reported by Yang et al. ($IC_{50} = 17$ nM[6]). This disparity maybe attributed to species differences between the A_1

Table 1. Dissociation constants (K_i) for the A_1 adenosine receptor in DDT_1 MF-2 cell membranes

Compd	K_i (μM)
1 (XH-14)	0.05 ± 0.02
5a	9.5 ± 2.1
6a	4.9 ± 1.5
10b	> 50.0
11	2.2 ± 90.3
15b	7.4 ± 0.9
16	> 50.0
17 (XH-14-CHO)	8.4 ± 0.4

DDT_1 MF-2 cells were incubated with 2.5 nM [^{3}H]CPX and various concentrations of the compounds for 90 min at 25 °C. The K_i values were calculated from the concentration of the compounds that inhibited specific [^{3}H]CPX binding by 50%. Each value is the mean ± SE, $n = 3$.

adenosine receptors used in the binding assays; we used cells derived from a steroid-induced leiomyoscarcoma of the vas deferens of an adult Syrian Hamster, while Yang and co-workers used bovine cerebral cortex cells as a source of A_1 receptors.

From the structure–activity data it is immediately apparent that all modifications made to XH-14 significantly diminished receptor affinity. Since analogous modifications (incorporation of propyl and phenyl substituents) produced increased affinity in the case of xanthine antagonists it is fair to conclude that benzofuran and xanthine ring systems interact with the adenosine receptor in a different orientation (i.e. not via a 'superimposed' model in which the 6:5 fused heterocycles bind in the same orientation). The importance of the 3-formyl substituent was highlighted by a comparison of compounds with and without this substituent. XH-14 bound the receptor with two orders of magnitude higher affinity than the corresponding analogue which lacked a formyl group (compare **1** with **17**). The 5-propylbenzofuran **10b** possessed negligible affinity for the receptor ($K_i > 50$ μM), but after a 3-formyl was incorporated receptor affinity increased to 2.2 μM. The hydroxyl attached to the 5-propyl side chain of XH-14 is also required for high affinity binding. The XH-14 analogue which was devoid of this hydroxyl (compound **11**) showed a ~40 fold lower receptor affinity. Analogues of 5-(3-hydroxypropyl)-7-methoxy-2-phenylbenzo[*b*]furan (**5a**) in which this hydroxyl was lacking (**10b**) or acetylated (**5b**) also exhibited significantly diminished activity.

Experimental

Solvents were either AR grade or distilled prior to use. Diethyl ether and pyridine were dried by distillation over sodium wire and either used fresh or stored over 4 Å sieves. All reactions were performed under an inert (N_2) atmosphere. Merck Kieselgel 60 and 60 F_{254} were used for column and thin layer chromatography, respectively. Melting points (mp) were determined on an Electrothermal melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz Unity Plus spectrometer using $CDCl_3$ as the solvent and TMS as an internal standard. FAB mass spectra were measured on a Jeol JMS-DX300 mass spectrometer and processed on an MSS data system.

5-(2′-(Ethoxycarbonyl)-*E*-ethenyl)-7-methoxy-2-phenylbenzo[*b*]furan (4). Cuprous phenylacetylide (7.91 g, 48 mmol) was dissolved in dry pyridine (120 mL). Ethyl 3-methoxy-4-hydroxy-5-bromocinnamate[6] (**3**, 14.48 g, 48 mmol) in dry pyridine (120 mL) was added and the mixture was refluxed for 20 h. After cooling, the pyridine was evaporated under reduced pressure and the residue extracted with chloroform (500 mL). The chloroform was washed with water (150 mL), dried over magnesium sulphate, filtered and evaporated to give the crude product. Column chromatography on silica gel using dichloromethane:petroleum ether 40-60 (1:3) as an eluent afforded pure **4** (9.48 g, 61%); mp 159–161 °C; ^{1}H NMR δ 1.35 (t, 3H, $J = 7.0$ Hz, CH_3), 4.06 (s, 3H, OCH_3), 4.28 (q, 2H, $J = 7.0$ Hz, CH_2), 6.41 (d, 2H, $J = 15.9$ Hz, CH = CH-Ar), 6.98 (s, 1H, ArH), 7.00 (s, 1H, ArH), 7.34 (s, 1H, CH), 7.37 (d, 1H, $J = 7.3$ Hz, H-4′), 7.44 (t, 2H, $J = 7.3$ Hz, H-3′,5′), 7.75 (d, 1H, $J = 15.9$ Hz, CH = CH-Ar), 7.87 (d, 2H, $J = 7.3$ Hz, H-2′,6′); ^{13}C NMR δ 56.2, 60.4, 101.6, 105.6, 114.7, 117.1, 125.1, 128.3, 128.6, 128.9, 129.9, 130.7, 131.2, 145.3, 145.4, 145.5, 157.1.

5-(3′-Hydroxypropyl)-7-methoxy-2-phenylbenzo[*b*]furan (5a). A solution of lithium aluminium hydride (42.2 mL, 1.0 M, 42.2 mmol) in THF (70 mL) was cooled to −15 °C on an ice bath. 5-(2-(Ethoxycarbonyl)-*E*-ethenyl)-7-methoxy-2-phenylbenzo[*b*]furan (**4**, 8.0 g, 24.8 mmol) in THF (124 mL) was added dropwise over 30 min. The reaction mixture was then stirred at room temperature for 24 h before being quenched with cold 5% H_2SO_4. Extraction with ether (3×120 mL), washing with brine (2×80 mL), drying over Na_2SO_4 and evaporation afforded a crude solid. Purification was effected by column chromatography using petroleum ether:$CHCl_3$:EtOH (40:10:1) followed by recrystallisation from ethanol/water (5.93 g, 84%); mp 97–98 °C; ^{1}H NMR δ 1.71 (s, 1H, OH), 1.95 (m, 2H, $CH_2CH_2CH_2OH$), 2.79 (t, 2H, $J = 7.7$ Hz, $CH_2CH_2CH_2OH$), 3.72 (t, 2H, $J = 6.4$, $CH_2CH_2CH_2OH$), 4.05 (s, 3H, OCH_3), 6.66 (s, 1H, H3), 6.95 (s, 1H, H4/6), 7.01 (s, 1H, H4/6), 7.35–7.89 (m, 5H, ArH); ^{13}C NMR δ 32.4, 34.7, 56.2, 62.3, 101.5, 107.7, 112.5, 125.0, 128.5, 128.7, 130.4, 130.9, 137.6, 142.7, 144.9, 156.21.

5-(3′-Acetoxypropyl)-7-methoxy-2-phenylbenzo[*b*]furan (5b). Compound **5a** (1.52 g, 5.38 mmol) was dissolved in dry pyridine (10 mL). Acetic anhydride (2.5 mL) was added and the reaction was stirred at room temperature for 24 h. The solvent was removed under vacuum and the crude product chromatographed using dichloromethane:hexane (2:1) to afford the desired product (1.6 g, 86%); mp 63–64 °C; ^{1}H NMR δ 2.02 (m, 2H, J = 6.6, 7.7 Hz, $CH_2CH_2CH_2OAc$), 2.07 (s, 3H, Ac), 2.76 (t, 2H, J = 7.7 Hz, $CH_2CH_2CH_2OAc$), 4.05 (s, 3H, OCH_3), 4.13 (t, 2H, J = 6.6 Hz, $CH_2CH_2CH_2OAc$), 6.64 (s, 1H, H3), 6.95 (s, 1H, H4/6), 6.99 (br s, 1H, H4/6), 7.31–7.88 (m, 5H, ArH); ^{13}C NMR δ 21.0, 30.7, 32.5, 56.2, 63.9, 101.4, 107.6, 112.5, 125.0, 128.5, 128.7, 130.4, 130.9, 136.9, 142.8, 144.9, 156.3, 171.2.

5-(3′-Acetoxypropyl)-4-iodo-7-methoxy-2-phenylbenzo[*b*]-furan (6a). Compound **5b** (0.6 g, 1.85 mmol), silver sulphate (0.58 g, 1.86 mmol) and iodine (0.47 g, 1.85 mmol) were stirred in dry chloroform (10 mL) for 16 h at room temperature. During this period the colour of the reaction mixture was changed from reddish brown to clear. Water (20 mL) was added and the reaction mixture was extracted with chloroform (3×20 mL). Drying, filtration and evaporation of the organic phase afforded a yellowish solid. Purification by column chromatography using dichloromethane: hexane (2:1) yielded pure **6a** (0.81 g, 92%); mp 134–135 °C; ^{1}H NMR δ 1.99 (m, 2H, $CH_2CH_2CH_2OAc$), 2.10 (s, 3H, Ac), 2.89 (t, 2H, J = 7.8 Hz, $CH_2CH_2CH_2OAc$), 4.04 (s, 3H, OCH_3), 4.17 (t, 2H, J = 6.5 Hz, $CH_2CH_2CH_2OAc$), 6.72 (s, 1H, H3), 6.95 (s, 1H, H6), 7.27–7.90 (m, 5H, ArH).

5-(3′-Hydroxypropyl)-4-bromo-7-methoxy-2-phenylbenzo-[*b*]furan (6b). Compound **6a** (126 mg, 0.388 mmol) was dissolved in dichloromethane (8 mL) and cooled to −5 °C on an ice–salt bath. Bromine (68 mg, 0.426 mmol) was added and the reaction was stirred for 1 h. Evaporation of the solvent afforded a brown solid. Purification via column chromatography using dichloromethane:hexane (1:1.25) as an eluent yielded **6b** (137 mg, 87%); mp 155–157 °C; ^{1}H NMR δ 2.01 (m, 2H, $CH_2CH_2CH_2OAc$), 2.09 (s, 3H, Ac), 2.89 (t, 2H, J = 7.7 Hz, $CH_2CH_2CH_2OAc$), 4.04 (s, 3H, OCH_3), 4.16 (t, 2H, J = 6.6 Hz, $CH_2CH_2CH_2OAc$), 6.68 (s, 1H, H3), 7.03 (s, 1H, H6), 7.37–7.89 (m, 5H, ArH); ^{13}C NMR δ 21.0, 29.3, 32.3, 56.4, 63.8, 102.2, 105.7, 109.0, 125.1, 128.8, 129.8, 132.5, 135.4, 142.2, 144.3, 156.6, 171.2.

2,6-Dibromo-4-propylphenol (8). 4-Propylphenol (27.9 mmol) was dissolved in dichloromethane (30 mL) and cooled to 0 °C. Bromine (8.92 g, 55.8 mmol) was added dropwise and the reaction was stirred for 2 h at 0 °C. Water (20 mL) was added and the organic layer separated. After further extraction of the aqueous portion (CH_2Cl_2, 3×30 mL), the organic portions were combined, dried and evaporated to afford a crude oil. Purification by chromatography using DCM:hexane (1:1) yielded pure **8** (7.25 g, 88%); ^{1}H NMR δ 0.92 (t, 3H, J = 7.4 Hz, $CH_2CH_2CH_3$), 1.59 (m, 2H, $CH_2CH_2CH_3$), 2.47 (t, 2H, J = 7.6 Hz, $CH_2CH_2CH_3$), 5.73 (s, 1H, OH), 7.25 (s, 2H, H3,5); ^{13}C NMR δ 13.6, 24.4, 36.5, 109.5, 131.8, 137.3, 147.2.

2-Bromo-6-methoxy-4-propylphenol (9). 2,6-Dibromo-4-propylphenol (5.49 g, 18.7 mmol) and BaO (14.32 g, 93.4 mmol) were refluxed in dry MeOH (100 mL) for 3 h. The methanol was evaporated under reduced pressure and the residue was taken up in dry DMF (50 mL). Copper(II)chloride (2.51 g, 18.7 mmol) was added and the reaction was brought to reflux to ensure all solids were immersed and temperature was maintained at 100 °C for 4 h. After this period the solvent was removed under reduced pressure and the residue partitioned between EtOAc (100 mL) and water (100 mL). The aqueous portion was further extracted with EtOAc (2×100 mL) before the organic portions were combined, dried ($MgSO_4$), filtered and evaporated to give a crude brown oil. After column chromatography using hexane:DCM (2:1) as an eluent, the desired product was obtained in 42% yield as an orange oil (1.74 g of starting material was also recovered from the column); mp 78–80 °C; ^{1}H NMR δ 0.94 (t, 3H, J = 7.6 Hz, $CH_2CH_2CH_3$), 1.61 (m, 2H, J = 7.6, 7.6 Hz, $CH_2CH_2CH_3$), 2.50 (t, 2H, J = 7.6 Hz, $CH_2CH_2CH_3$), 3.89 (s, 3H, OCH_3), 5.76 (s, 1H, OH), 6.62 (s, 1H, H3), 6.91 (br s, 1H, H5); ^{13}C NMR δ 13.7, 24.6, 37.4, 56.2, 107.8, 110.3, 124.1, 135.4, 140.9, 146.9.

2-(4′-Benzyloxy-3′-methoxyphenyl)-7-methoxy-5-propyl-benzo[*b*]furan (10a). A solution of cuprous acetylide (2.33 g, 7.75 mmol) in dry pyidine (40 mL) was added to 2-bromo-6-methoxy-4-propylphenol (1.9 g, 7.75 mmol) in dry pyridine (20 mL) and the mixture was refluxed for 15 h. The pyridine was removed under reduced pressure and the residue was taken up in chloroform (50 mL) and washed with water (50 mL). The aqueous layer was extracted with chloroform (2×50 mL) and the combined chloroform portions were dried, filtered and evaporated under reduced pressure to give a brown oil. This was purified by column chromatography (DCM:hexane, 1:1) to afford the product as a white solid (2.27 g, 73%); mp 115–118 °C; ^{1}H NMR δ 0.98 (t, 3H, J = 6.4 Hz, $CH_2CH_2CH_3$), 1.70 (m, 2H, $CH_2CH_2CH_3$), 2.66 (t, 2H, J = 7.6 Hz, $CH_2CH_2CH_3$), 4.00 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 5.21 (s, 2H, CH_2Ph), 6.62 (s, 1H, H3), 6.83–7.48 (m, 10H, H4,6,2′,5′,6′, CH_2Ph); ^{13}C NMR δ 13.9, 25.1, 38.4, 56.1, 56.2, 71.0, 100.4, 107.3, 108.6, 112.3, 113.9, 117.9, 124.0, 127.3, 127.9, 128.6, 130.6, 136.9, 138.4, 142.4, 144.6, 148.6, 149.7, 156.1.

2-(4′-Hydroxy-3′-methoxyphenyl)-7-methoxy-5-propylbenzo[*b*]furan (10b). Compound **10a** (150 mg, 0.373 mmol) was dissolved in THF (10 mL). Acetic acid (glacial, ~0.1 mL) and palladium on carbon (10%, ~20 mg) were added and the reaction was placed under an atmosphere of hydrogen. After stirring for 16 h at ambient temperature, the reaction mixture was filtered and evaporated to afford a colourless oil. Column chromatography using dichloromethane:hexane (1:1) as an eluent gave pure **10b** as a colourless oil, which crystallised upon standing (105 mg, 90%); mp 106–107 °C; ^{1}H NMR δ 0.99 (t, J = 7.3 Hz, 3H, $CH_2CH_2CH_3$), 1.71 (m, 2H, $CH_2CH_2CH_3$), 2.67 (t, 2H, J = 7.6 Hz, $CH_2CH_2CH_3$), 3.99 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 5.79 (s, 1H, OH), 6.63 (s, 1H, H3), 6.82 (s, 1H, H4/6), 6.97 (s, 1H, H4/6), 6.99 (d, 1H, J = 8.3 Hz, H5′), 7.38 (s, 1H, H2′), 7.41 (d, 1H, J = 8.3 Hz, H6′); ^{13}C NMR δ 13.9, 25.1, 38.4, 56.0, 56.1, 100.1, 107.3, 107.6, 112.3, 114.7, 118.8, 123.1, 131.0, 138.4, 142.3, 144.6, 146.2, 146.7, 156.3.

2-(4′-Hydroxy-3′-methoxyphenyl)-7-methoxy-5-propylbenzo[*b*]furan-3-carbaldehyde (11). The benzo[*b*]furan **10b** (91 mg, 0.291 mmol) was dissolved in dry ether (10 mL) and cooled on an ice–salt bath in a 50 mL, three necked round bottomed flask. A HCl generator (concd H_2SO_4 onto solid NH_4Cl) in series with a H_2SO_4 wash bottle and a safety bottle was connected to the flask. Attached to another joint was a condenser in series with a H_2SO_4 wash bottle and a safety bottle connected to the surface of aqueous NaOH. Zinc cyanide (51 mg, 0.437 mmol) and potassium chloride (15 mg) were added and HCl gas was bubbled through the solution for 1 h. The ethereal solution was combined with ethanol (10 mL) and water (20 mL) and heated at 50 °C for 1 h. Extraction with ethyl acetate (3×40 mL) followed by drying ($MgSO_4$), filtration and evaporation afforded a crude yellow oily solid. Purification was achieved via column chromatography with dichloromethane as an eluent. The pure product was a pale-yellow solid weighing 40 mg (40%); ^{1}H NMR δ 0.97 (t, 3H, J = 7.3 Hz, $CH_2CH_2CH_3$), 1.71 (m, 2H, $CH_2CH_2CH_3$), 2.70 (t, 2H, J = 7.6 Hz, $CH_2CH_2CH_3$), 4.00 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.01 (s, 1H, OH), 6.73 (s, 1H, H4/6), 7.07 (d, 1H, J = 8.3 Hz, H5′), 7.38 (s, 1H, H2′), 7,40 (d, 1H, J = 8.3 Hz, H6′), 7.65 (s, 1H, H4/6), 10.3 (s, 1H, CHO); ^{13}C NMR δ 13.8, 25.1, 38.5, 56.0, 56.3, 108.8, 111.0, 113.6, 115.0, 116.8, 120.7, 123.6, 127.1, 140.8, 141.5, 144.4, 146.9, 148.5, 165.9, 186.8; HR MS (FAB) calcd 341.13889, found 341.14012.

2,4-Dipropylphenol (13)

2′-Propylphenylpropanoate. Propanoyl chloride (7.40 mL, 75.1 mmol) was added to stirred 2-propylphenol over a period of 30 min, and the mixture stirred at room temperature for 6 h. Starting material still present. Extra propanoyl chloride (3.30 mL, 33.5 mmol) was added and the mixture stirred for a further hour at 50 °C. The resulting oil was distilled under vacuum to afford the product as a translucent liquid (9.5 g, 73%); bp 84–86 °C (1.3–1.4 mmHg); ^{1}H NMR δ 0.93 (t, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.29 (t, J = 7.6 Hz, $COCH_2CH_3$), 1.59 (m, J = 7.5 Hz, $CH_2CH_2CH_3$), 2.48 (t, J = 7.5 Hz, $CH_2CH_2CH_3$), 2.60 (q, J = 7.6 Hz, $COCH_2CH_3$), 7.01–7.24 (m, 4H, ArH); ^{13}C NMR δ 9.2, 13.9, 23.1, 27.7, 32.2, 122.2, 125.8, 126.8, 130.2, 134.2, 148.9, 172.9.

4′-Hydroxy-3′-propylphenylpropanone. 2′-Propylphenylpropanoate (2.3 g, 12.2 mmol) was added to a stirred mixture of aluminium chloride (1.8 g, 13.5 mmol) in dry carbon disulfide (12 mL) and the mixture gently refluxed for 2 h. The solvent was then removed by distillation, the mixture stirred for as long as possible with heating at 140–150 °C (for ~3 h), and allowed to cool affording a hard brown mass. This was dissolved in methanol (20 mL), hydrochloric acid (1:1, 16 mL) and water (24 mL) were added slowly, and the mixture allowed to stand overnight. The product was extracted with diethyl ether (3×100 mL) and the solvent removed under reduced pressure. The resulting brown solid was dissolved in 3 M sodium hydroxide (100 mL) and the solution washed with ether (3×100 mL). The aqeous layer was neutralised with HCl, the product extracted with ether (3×100 mL), and the solvent removed under reduced pressure. The solid was purified by column chromatography (petroleum ether:ethyl acetate, 7:2) to afford the product as a white solid (1.2 g, 51%); mp 77–78.0 °C; ^{1}H NMR δ 0.98 (t, 3H, J = 7.3 Hz, $CH_2CH_2CH_3$), 1.22 (t, 3H, J = 7.3 Hz, $COCH_2CH_3$), 1.67 (qt, 2H, J = 7.3, 7.7 Hz, $CH_2CH_2CH_3$), 2.63 (t, 2H, J = 7.7 Hz, $CH_2CH_2CH_3$), 2.97 (q, 2H, J = 7.3 Hz, $COCH_2CH_3$), 6.11 (br s, 1H, OH), 6.84 (d, 1H, J = 8.3 Hz, ArH), 7.75 (d, 1H, J = 8.3 Hz, ArH), 7.80 (s, 1H, ArH); ^{13}C NMR δ 8.7, 13.9, 22.7, 31.4, 31.9, 115.1, 128.2, 130.9, 129.0, 129.1, 159.1, 201.5.

2,4-Dipropylphenol. A solution of mercuric chloride (2.1 g) in water (150 mL) was added to mossy zinc (107.4 g) and the mixture agitated occasionally, then allowed to stand overnight. The supernatant liquid was decanted off and the zinc rinsed once with water. A mixture of water (100 mL) and concentrated hydrochloric acid (100 mL) and a solution of 4′-hydroxy-3′-propylphenylpropanone (7.7 g, 39.7 mmol) in ethanol were added the zinc, and the mixture stirred vigorously with refluxing for 69 h. Toluene (50 mL) was added to the resulting mixture and stirring was continued for a few minutes. The solution was filtered and washed with water (3×50 mL). The solvent was removed by distillation and the resulting brown liquid purified by column chromatography (petroleum ether/ethyl acetate, 5:1) to afford the desired product as an orange oil (5.8 g, 81%);

^{1}H NMR δ 0.94 (t, 3H, J=7.3 Hz, $CH_2CH_2CH_3$), 0.99 (t, 3H, J=7.3 Hz, $CH_2CH_2CH_3$), 1.64 (m, 4H, J=7.3,7.6,7.7 Hz, 2×$CH_2CH_2CH_3$), 2.51 (t, 2H, J=7.6 Hz, $CH_2CH_2CH_3$), 2.58 (t, 2H, J=7.7 Hz, $CH_2CH_2CH_3$), 4.66 (br s, 1H, OH), 6.70 (d, 2H, J=8.0 Hz, ArH), 6.90 (d, 2H, J=8.0 Hz, ArH), 6.94 (s, 1H, ArH); **13**C NMR δ 13.8, 14.0, 23.0, 24.8, 32.1, 37.3, 115.0, 126.8, 128.0, 130.3, 134.9, 151.4.

6-Bromo-2,4-dipropylphenol (14). 2,4-Dipropylphenol (5.2 g, 29.0 mmol) was added slowly to bromine (7.0 g, 43.7 mmol) in 90% acetic acid (50 mL), and the mixture stirred at room temperature for 10 h. Water (100 mL) was added to the mixture, the product extracted with ethyl acetate (3×100 mL) and washed with water (3×100 mL). The solvent was removed by reduced pressure and the resulting crude brown liquid was purified by column chromatography (petroleum ether:chloroform, 2:1) to afford the product as an orange oil (4.5 g, 61%); ^{1}H NMR δ 0.93 (t, 3H, J=7.4 Hz, $CH_2CH_2CH_3$), 0.96 (t, 3H, J=7.4 Hz, $CH_2CH_2CH_3$), 1.62 (m, 4H, J=7.4,7.6,7.7 Hz, 2×$CH_2CH_2CH_3$), 2.48 (t, 2H, J=7.6 Hz, $CH_2CH_2CH_3$), 2.62 (t, 2H, J=7.7 Hz, $CH_2CH_2CH_3$), 5.37 (s, 1H, OH), 6.87 (s, 1H, ArH), 7.12 (s, 1H, ArH); ^{13}C NMR δ 13.7, 14.0, 22.8, 24.6, 32.9, 36.9, 110.1, 128.9, 129.9, 135.7, 148.0.

5,7-Dipropyl-2-(4′-benzyloxy-3′-methoxyphenyl)benzo[*b*]-furan (15a). A solution of cuprous (4-benzyloxy-3-methoxy)phenylacetylide (0.64 g, 2.14 mmol) in dry pyridine (5 mL) was added to 6-bromo-2,4-dipropylphenol (0.55 g, 2.14 mmol) in dry pyridine (5 mL). The mixture was refluxed for 24 h. The pyridine was removed under reduced pressure and the residue was taken up in chloroform (50 mL) and washed with water (50 mL). The aqueous layer was extracted with chloroform (2×50 mL) and the combined chloroform portions were dried, filtered and evaporated under reduced pressure to give a brown oil. The crude product was purified by column chromatography using hexane:DCM (3:1); mp 97–98 °C; ^{1}H NMR δ 0.98 (t, 3H, J=7.5 Hz, $CH_2CH_2CH_3$), 1.04 (t, 3H, J=7.4 Hz, $CH_2CH_2CH_3$), 1.70 (m, 2H, $CH_2CH_2CH_3$), 1.85 (m, 2H, $CH_2CH_2CH_3$), 2.66 (t, 2H, J=7.6 Hz, $CH_2CH_2CH_3$), 2.92 (t, 2H, J=7.6 Hz, $CH_2CH_2CH_3$), 4.01 (s, 3H, OCH_3), 5.22 (s, 2H, CH_2Ph), 6.84 (s, 1H, H3), 6.91 (s, 1H, H4/6), 6.96 (d, 1H, J=9.0 Hz, H5′), 7.19 (s, 1H, H4/6), 7.30–7.52 (m, 7H, H2′,6′,CH_2Ph); ^{13}C NMR δ 13.9, 14.2, 23.1, 25.2, 32.0, 38.1, 56.1, 71.0, 100.3, 108.6, 114.1, 117.5, 117.8, 124.5, 124.9, 125.4, 127.2, 127.9, 128.6, 129.0, 136.9, 137.3, 148.5, 149.8, 152.1, 155.5.

5,7-Dipropyl-2-(4′-hydroxy-3′-methoxyphenyl)benzo[*b*]furan (15b). Compound **15a** (200 mg, 0.483 mmol) was dissolved in THF (20 mL). Acetic acid (glacial, ~0.1 mL) and palladium on carbon (10%, ~20 mg) were added and the reaction was placed under an atmosphere of hydrogen. After stirring for 16 h at ambient temperature, the reaction mixture was filtered and evaporated to afford a colourless oil. Column chromatography using dichloromethane:hexane (1:1) as an eluent gave pure **15b** as a white solid (136 mg, 87%); ^{1}H NMR δ 0.98 (t, 2H, $CH_2CH_2CH_3$), 1.04 (t, 2H, $CH_2CH_2CH_3$), 1.70 (m, 2H, $CH_2CH_2CH_3$), 1.85 (m, 2H, $CH_2CH_2CH_3$), 2.66 (t, 2H,$CH_2CH_2CH_3$), 2.92 (t, 2H, $CH_2CH_2CH_3$), 4.01 (s, 3H, OCH_3), 5.78 (s, 1H, OH), 6.83 (s, 1H, H3), 6.91 (d, 1H, J=1.1 Hz, H4/6), 7.01 (d, 1H, J=8.2 Hz, H5′), 7.19 (d, 1H, J=1.1 Hz, H4/6), 7.34 (d, 1H, J=1.7 Hz, H2′), 7.42 (dd, 1H, J=1.7, 8.2 Hz, H6′); ^{13}C NMR δ 13.8, 14.0, 23.1, 25.2, 31.7, 38.1, 56.2, 111.0, 115.1, 116.8, 119.1, 121.1, 123.4, 125.2, 125.4, 126.7, 139.6, 146.9, 148.5, 151.1, 165.5, 186.9; HR MS (FAB) calcd 325.18036, found 325.18083.

5,7-Dipropyl-2-(4′-hydroxy-3′-methoxyphenyl)benzo[*b*]-furan-3-carbaldehyde (16). The same apparatus described for the preparation of **11** was used for this reaction. The benzo[*b*]furan **15b** (214 mg, 0.660 mmol) was dissolved in dry ether (15 mL) and cooled on an ice-salt bath. Zinc cyanide (116 mg, 0.988 mmol) and potassium chloride (20 mg) were added and HCl gas was bubbled through the solution for 1 h. The ethereal solution was combined with ethanol (10 mL) and water (20 mL) and heated at 50 °C for 1 h. Extraction with ethyl acetate (3×40 mL) followed by drying, filtration and evaporation afforded a crude yellow oily solid. After purification by column chromatography with dichloromethane as an eluent the product was isolated (113 mg, 49%); mp 103–104 °C; ^{1}H NMR δ 0.96 (t, 2H, $CH_2CH_2CH_3$), 1.01 (t, 2H, $CH_2CH_2CH_3$), 1.73 (m, 2H, $CH_2CH_2CH_3$), 1.81 (m, 2H, $CH_2CH_2CH_3$), 2.69 (t, 2H,$CH_2CH_2CH_3$), 2.90 (t, 2H, $CH_2CH_2CH_3$), 4.02 (s, 3H, OCH_3), 6.06 (s, 1H, OH), 7.02 (s, 1H, H4/6), 7.10 (d, 1H, J=8.6 Hz, H5′), 7.36 (d, 1H, J=2.0 Hz, H2′), 7.43 (dd, 1H, J=2.0, 8.6 Hz, H6′), 7.89 (s, 1H, H4/6), 10.3 (s, 1H, CHO); ^{13}C NMR δ 13.8, 14.0, 23.1, 25.2, 31.7, 38.1, 56.2, 111.0, 115.1, 116.8, 119.1, 121.1, 123.4, 125.2, 125.4, 126.7, 139.6, 146.9, 148.5, 151.1, 165.5, 186.9; HR MS (FAB) calcd 353.17529, found 353.17617.

Receptor binding determinations. DDT_1 MF-2 cells were grown and cell membranes prepared as described previously.[13] The dissociation constants of the compounds were determined from displacement of radioligand binding assays. Briefly, cell membranes (0.05 mg protein) were incubated in a total volume of 0.25 mL containing 50 mM Tris–HCl buffer at pH 7.4, 5 mM $MgCl_2$, 2.5 nM [^{3}H]8-cyclopentyl-1,3-dipropylxanthine (CPX, 120 Ci/mmol, New England Nuclear) and without or with varying concentrations of the compounds for 90 min at 25 °C. Nonspecific binding was determined in

parallel assays without the compounds but containing 10 μM 8-cyclopentyltheophylline (CPT). At the end of the incubation, each suspension was diluted with 3 mL of ice-cold incubation buffer and poured onto a glass fiber filter under reduced pressure. The filters were washed with a further 6 mL of ice-cold incubation buffer, placed in a vial with 3 mL of scintillation fluid and the radioactivity counted in a liquid scintillation counter. Specific binding to the A^1-adenosine receptor was calculated as the difference between total binding in the absence of CPT and nonspecific binding determined in the presence of CPT. The concentration of compounds which inhibited specific [^{3}H]CPX binding by 50% (IC_{50}) was determined from a nonlinear regression analysis of the concentration-inhibition data (Prism, GraphPad) and the K_i for each compound was calculated from the IC_{50} using the method of Cheng and Prusoff.[14] Each binding experiment was performed in triplicate and repeated at least three times.

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References

1. Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197.
2. Jacobson, K. A.; van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407.
3. Williams, M. *Drug News & Perspectives*, **1991**, *4*, 5.
4. Collis, M. J.; Hourani, S. M. O. *TIPS Reviews*, **1993**, *14*, 360.
5. Yang, Z.; Hon, P. M.; Chui, K. Y.; Xu, Z. L.; Chang, H. M.; Lee, C. M.; Cui, Y. X.; Wong, H. N. C.; Poon, C. D.; Fung, B. M. *Tetrahedron* **1991**, *32*, 2061.
6. Yang, Z.; Liu, H. B.; Lee, C. M.; Chang, H. M.; Wong, H. N. C. *J. Org. Chem.* **1992**, *57*, 7428.
7. Jacobson, K. A.; Kiriasis, L.; Barone, S.; Bradbury, B. J.; Kammula, U.; Campagne, J. M.; Secunda, S.; Daly, J. W.; Neumeyer, J. L.; Pfleiderer, W. *J. Med. Chem.* **1989**, *32*, 1873.
8. Baillargeon, V. P.; Stille, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 452.
9. Okano, T.; Harada, N.; Kiji, J. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 2329.
10. Scammells, P. J.; Tiekink, E. Z. *Krist.* **1998**, *213*, 59.
11. Larock, R. C.; Yum, E.; Doty, M. J.; Sham, K. K. C. *J. Org. Chem.* **1995**, *60*, 3270.
12. Hutchinson, S. A.; Luetjens, H.; Scammells, P. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3081.
13. Scammells, P. J.; Baker, S. P.; Berlardinelli, L.; Olsson, R. A. *J. Med. Chem.* **1994**, *37*, 2704.
14. Cheng, Y.; Prusoff, S. *Biochem. Pharmacol.* **1973**, *22*, 3099.