Fluorosulfonyl-Substituted Xanthines as Selective Irreversible Antagonists for the A_1-Adenosine Receptor

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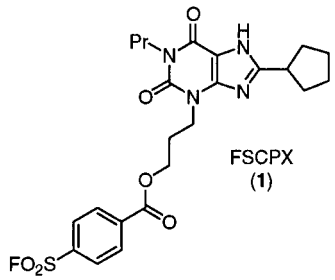
FSCPX (**1**) has been reported to be a potent, selective, and irreversible antagonist for the A_1-adenosine receptor (AR). To obtain an irreversible A_1AR antagonist with potentially better stability and to further elucidate the effects of linker structure on the pharmacological characteristics, several new analogues were targeted in which the labile ester linkage of **1** was replaced by more stable functionalities. In particular, alkyl and amide linkers between the xanthine pharmacophore and the reactive 4-fluorosulfonylphenyl group were explored. The data showed that the chemical composition of the linker affects the affinity and apparent irreversible binding to the A_1AR. Overall, compound **23b** appeared to have the most advantageous characteristics as a potential irreversible ligand for the A_1AR. These include relatively high affinity for the A_1AR as compared to the A_{2A}AR, concentration-dependent and selective apparent irreversible binding to the A_1AR, and ease of removal of unbound ligand from biological membranes. These properties indicate that **23b** has the potential to be a useful tool for further study of the structure and function of the A_1AR.

Introduction

Adenosine receptors (AR) are distributed throughout the body where they mediate the many physiological functions of the autocoid adenosine.[1−3] These effects include inhibition of neuronal firing, release of neurotransmitters, inhibition of platelet aggregation, cardiac depression, and vasodilation, vasoconstriction, and immunosuppressant effects. For the study of receptor structure and function, chemoreactive and photoreactive irreversible antagonists are useful tools. Desirable characteristics of a chemoreactive antagonist include a pharmacophore that provides affinity and selectivity for the receptor and a reactive moiety that provides covalent incorporation into the receptor allowing for the control of receptor concentrations over a wide range. These characteristics have made chemoreactive antagonists useful in a variety of studies including the identification and mapping of ligand-binding sites,[4] the physiological function of a receptor and its subtypes,[5] the relationship between receptor occupancy and a tissue or cellular response,[6] as well as the kinetics of the turnover and cellular processing of receptors.[7]

In 1994, a series of compounds were synthesized in which several reactive moieties were attached to the N-3 position of the xanthine ring in order to develop irreversible antagonists for the A_1AR subtype.[8] The apparent irreversible binding of these ligands is dependent upon the reactive moiety and its location. For example, the 3-bromoacetoxypropyl ester and 3-fluorosulfonylphenyl derivatives were found to have relatively high affinity for the A_1AR but showed little or no apparent irreversible binding to the receptor. On the other hand, 8-cyclopentyl-3-(3-((4-fluorosulfonylbenzoyl)-oxy)propyl)-1-propylxanthine (FSCPX, **1**),[8,9] incorporating the chemoreactive 4-fluorosulfonylphenyl moiety, was found to have high affinity and selectivity for the A_1AR as compared to the A_{2A}AR. This compound also produced a concentration-dependent reduction in the maximal binding of [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX) to the A_1AR of DDT_1 MF-2 cells indicating that it is an irreversible ligand. The utility of **1** to study the A_1AR is indicated by recent reports where the apparent irreversible nature of this compound was used to determine the relationship between receptor number and a tissue response.[10,11] Although compound **1** binds to the A_1AR in an apparent irreversible manner using in vitro biological preparations, the linkage between the 4-fluorosulfonylphenyl moiety and the xanthine ring contains an ester group which has the potential to be cleaved by esterases either in vivo or in preparations containing significant enzyme activity. Cleavage at the ester linkage would render the ligand inactive in terms of potential irreversible receptor binding. To obtain an irreversible A_1AR antagonist with potentially better stability and to further elucidate the effects of linker structure on the pharmacological characteristics, several analogues of **1** were targeted in which this labile ester linkage was replaced by more



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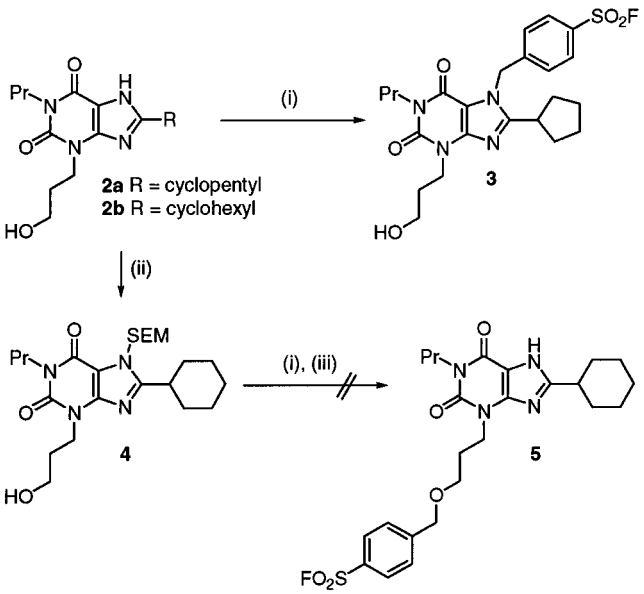
Scheme 1[a]



[a] Reagents: (i) NaH or KH, $BrCH_2C_6H_4SO_2F$, Bu_4NI, DMF; (ii) NaH, SEM-Cl, DMF; (iii) aq HCl.

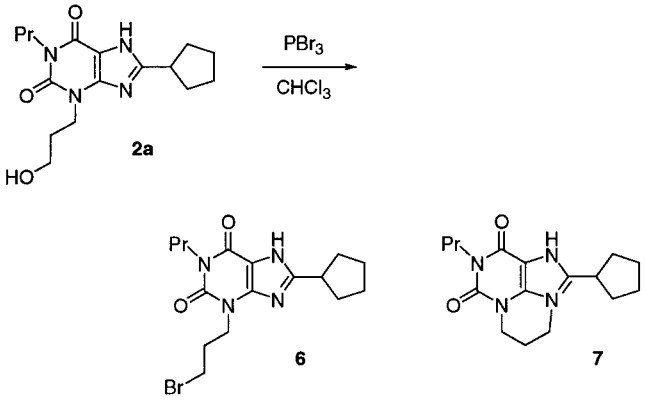
stable functionalities. Synthetic routes to ether, alkyl, and amide linkers between the xanthine pharmacophore and the reactive 4-fluorosulfonylphenyl group were explored. All target xanthines possessed 1-propyl substitution and either 8-cyclopentyl or 8-cyclohexyl substitution to enhance pharmacophore affinity for the A_1AR. All new compounds were characterized for affinity and apparent irreversible binding at the A_1AR and A_{2A}AR subtypes.

Chemistry

Ether Linker. Scheme 1 depicts the synthetic route to analogues of **1** with an ether linkage between a 4-fluorosulfonylbenzyl moiety and the xanthine. Attempts to selectively *O*-alkylate 8-cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (**2**) with 4-fluorosulfonylbenzyl bromide[12] proved unsuccessful as *N*-alkylation in the 7-position occurred in preference to *O*-alkylation under a range of conditions. To overcome this problem it was decided to protect N-7 prior to formation of the ether linkage. Although the protecting groups trialled initially (acetyl and tritylthio) blocked the hydroxyl group, selective protection of N-7 was eventually achieved using the [2-(trimethylsilyl)ethoxy]methyl protecting group.[13−15] Reaction of 3-(3-hydroxypropyl)xanthine (**2a**) with [2-(trimethylsilyl)ethoxy]methyl chloride in the presence of sodium hydride in DMF gave a 43% yield of the desired product. This process was later optimized using **2b** (potassium carbonate proved to be a superior base) to afford a 98% yield of the N-7-protected xanthine.

Formation of an ether linkage between 4-fluorosulfonylbenzyl bromide to the N-7-protected 3-(3-hydroxypropyl)xanthine **4** under typical Williamson conditions (sodium or potassium hydride in DMF or THF) proved unsuccessful, returning starting material. Addition of tetrabutylammonium iodide to generate 4-fluorosulfonylbenzyl iodide in situ had no effect. The reduced reactivity of the alkyl halide may be explained by the electron-withdrawing effect of the fluorosulfonyl group,

Scheme 2



while intramolecular hydrogen bonding may have lowered the reactivity of the hydroxyl group of **4**.

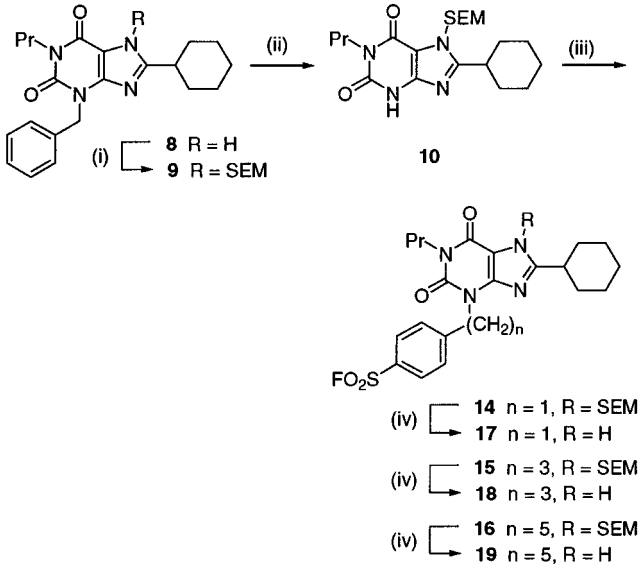
Because of the low reactivity of the 4-fluorosulfonylbenzyl bromide toward nucleophilic attack by the alkoxide formed from **4**, an alternative approach was adopted where the alkoxide was formed on the 4-fluorosulfonylbenzyl moiety. Bromination of 8-cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (**2a**) was attempted using phosphorus tribromide in chloroform (Scheme 2). While bromination occurred the product, 3-(3-bromopropyl)-8-cyclohexyl-1-propylxanthine (**6**), underwent a facile intramolecular substitution to afford the cyclized product **7**.

Rather than pursue this target further, it was decided to adopt an alternative synthetic strategy in which the 4-fluorosulfonylphenyl linker unit was prepared separately and attached directly to N-3. This approach offers the flexibility to accommodate a variety of linkers and offers the advantage of a more convergent approach. The latter is particularly valuable as it avoids the need to optimize the attachment of the 4-fluorosulfonylphenyl group at the end of a long linear synthesis (over 10 steps from allylurea).

Alkane Linker. Scheme 3 depicts a revised synthetic strategy to target compounds **17**−**19** containing one-, three-, and five-methylene spacers where the reactive 4-fluorosulfonylphenyl moiety is assembled separate from the xanthine heterocycle prior to attachment and subsequent deprotection. Debenzylation of compound **9** initially proved difficult. Attempts to N-3 debenzylate with either activated Pd−C catalyst or Pearlmans catalyst [$Pd(OH)_2$] with hydrogen at 50 psi were unsuccessful. The desired target was eventually synthesized using activated palladium on carbon in methanol with ammonium formate[16] as the hydrogen source. This reaction worked best when the reagents were thoroughly mixed (by dissolving in methanol and then evaporating the methanol) and then heated in the melt at 140 °C. It should be noted that this reaction gave little or no product on some attempts, possibly due to some contaminant (undetectable by NMR) poisoning the catalyst.

The alkyl linker with a one-methyl spacer (compound **11**) was synthesized by literature methods.[12] Construction of the alkyl linkers with the three- and five-methylene spacers (compounds **12** and **13**, respectively) was achieved by chlorosulfonation of 1-phenylpropyl bromide and 1-phenylpentyl bromide with chlorosulfonic acid, followed by halogen exchange with potassium

Scheme 3[a]



[a] Reagents: (i) K_2CO_3, SEM-Cl, DMF; (ii) Pd−C, NH_4CO_2, MeOH; (iii) K_2CO_3, $Br(CH_2)_nC_6H_4SO_2F$ (**11** $n = 1$, **12** $n = 3$, **13** $n = 5$), DMF; (iv) aq HCl.

fluoride in refluxing dioxane. The five-methylene spacer required initial bromination of 5-phenyl-1-pentanol with phosphorus tribromide as this bromide was not commercially available. Attachment of the reactive moieties to the SEM-protected species **10** produced the penultimate compounds **14**−**16**. This conversion was effected using potassium carbonate in DMF with the corresponding alkyl bromide with yields between 39% and 51%. Subsequent deprotection with aqueous HCl in ethanol produced the target compounds **17**−**19** in respectable yields (66−82%).

Amide Linker. A similar synthetic approach to the one used for the synthesis of **1**[8] was initially pursued to prepare analogues with an amide linker. It was thought that only slight modifications to this approach would allow the preparation of 3-(3-aminopropyl)xanthine that could be reacted with 4-fluorosulfonylbenzoyl chloride to give the desired target. However, hydroboration−amination of 3-allyl-8-cyclopentyl-1-propylxanthine returned a complex mixture of products, and attempts to convert the hydroxyl group of **2a** to the corresponding amine were also unsuccessful.

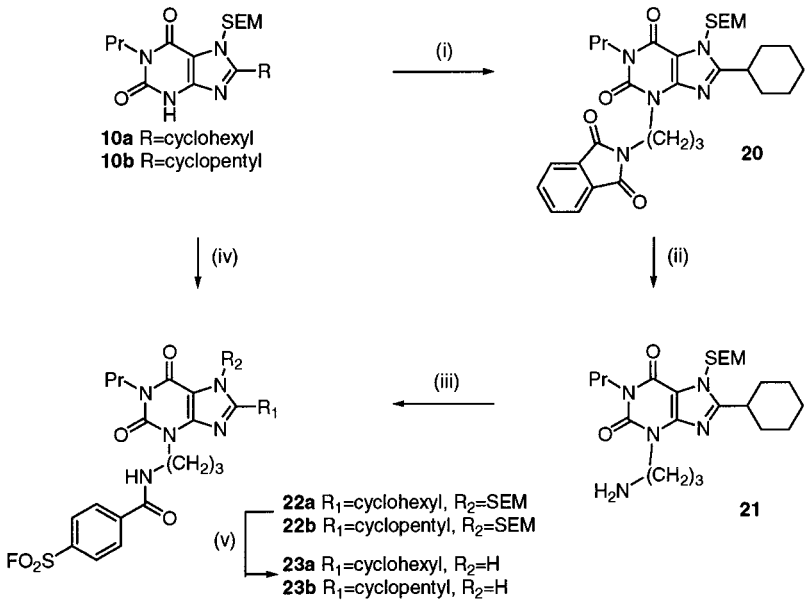
An alternative synthesis was devised, whereby 1-phthalimidopropyl bromide was attached directly to the SEM-protected xanthine **10**, to afford the 3-substituted xanthine **20** (Scheme 4). Compound **20** was heated in a pressure bomb at 50 °C with methylamine for 14 h to afford the desired amine in 56% yield. This conversion was also achieved in identical yield by refluxing **20** in hydrazine monohydrate for 15 h. Coupling of 4-fluorosulfonylbenzoic acid to the amine **21** using *N*,*N*-diisopropylethylamine (DIPEA) and the water-soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) for activation afforded the penultimate compound **22a**, which was deprotected to give the desired target **23a**.

A more convergent synthesis with construction of the reactive 3-(4-fluorosulfonylbenzamido)propyl group separate from the xanthine heterocycle was also investigated (Scheme 4). Amide coupling of 4-fluorosulfonylbenzoic acid (**24**) and 3-bromopropylamine hydrobromide with 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) activation and DIPEA afforded **25** in 63% yield. Initial attempts focused on coupling 4-fluorosulfonylbenzoic acid with 3-amino-1-propanol, followed by bromine exchange with the alcohol. Amide coupling using EEDQ activation with DIPEA yielded the alcohol in 83% yield, which was then brominated in 59% yield with *N*-bromosuccinimide, triphenylphosphine, and pyridine. It was later found that this unit could be prepared more efficiently by coupling 4-fluorosulfonylbenzoic acid with 3-bromopropylamine hydrobromide directly. Alkylation of *N*-(3-bromopropyl)-4-fluorosulfonylbenzamide (**25**) by the xanthine **10** produced the desired 3-substituted xanthine **22** in 87% yield. Subsequent removal of the SEM protecting group with aqueous HCl in ethanol produced the target compound **23** in 96% yield.

Pharmacology

The reactive xanthine derivatives were tested for affinity and apparent irreversible binding at the A_1AR in DDT cells and A_{2A}AR in PC-12 cells. Affinity was determined as the concentration of derivative that inhibited radioligand binding to the receptor by 50% (IC_{50}). Apparent irreversible binding was determined by incubating the derivatives with the cells followed by cell washing to remove unbound ligand and then determining the K_D and B_{max} for radioligand binding in cell membranes. Similar to that previously reported, compound **1** inhibited [^{3}H]DPCPX binding to the A_1AR in the low-nanomolar range and reduced the receptor content after pretreatment with 5 nM (Table 1). This is consistent with the compound binding irreversibly to the A_1AR. However, preincubation with a higher concentration of **1** (100 nM) reduced the receptor content further but decreased the K_D for the radioligand to the receptors remaining. This suggests that under the standard preincubation and cell washing conditions that residual ligand was retained by the membranes. The C-8 cyclohexylxanthine with a single-methylene linker between the N-3 of the xanthine ring and the 4-fluorosulfonylphenyl moiety (**17**) has an IC_{50} for inhibition of [^{3}H]DPCPX binding of 165 nM. Increasing the number of methylenes in the linker to three (**18**) or five (**19**) did not significantly change the IC_{50} as compared to **17**. Derivative **17** did not show apparent irreversible binding to the A_1AR because the B_{max} for [^{3}H]DPCPX binding did not change after preincubation with this compound at a relatively high concentration followed by cell washing. However, pretreatment of cells with 200 nM **18** or **19** reduced the receptor content by <15% and <50%, respectively indicating that apparent irreversible binding of these two derivatives is relatively weak. Since the five-methylene spacer of compound **19** should allow the fluorosulfonyl group to access the same part of the receptor as the corresponding group of compound **1**, we believe that the reduced apparent irreversible binding may have resulted from the electron-releasing linker deactivating the fluorosulfonyl group to nucleophilic attack. It is also possible that the ester carbonyl of **1** may be involved in some intermolecular interaction with the receptor that holds the fluorosulfonyl group in a favorable position for a reaction to take place. To increase the reactivity of the fluorosulfonylphenyl group,

Scheme 4[a]



[a] Reagents: (i) K_2CO_3, $Br(CH_2)_3Phth$, DMF; (ii) method A − $MeNH_2$, MeOH, method B − $NH_2NH_2{\cdot}H_2O$; (iii) $HO_2CC_6H_4SO_2F$, EDCI, DIPEA; (iv) $Br(CH_2)_3NHC(O)C_6H_4SO_2F$, K_2CO_3, DMF; (v) aq HCl.

Scheme 5

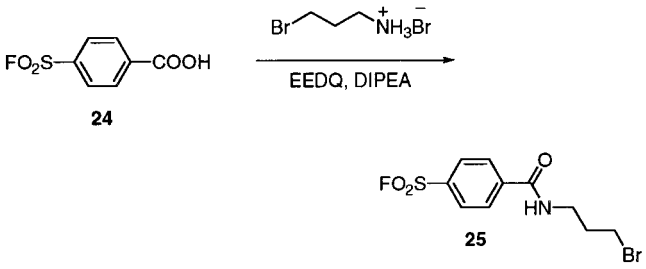


Table 1. Ligand Binding to the A_1AR

no.	IC_{50} vs [^{3}H]DPCPX, nM	% loss of specific [^{3}H]DPCPX binding (concn)[a]	K_D [^{3}H]DPCPX binding post-xanthine, nM[b]
1	11.8 ± 3.2	67 ± 3 (5)	0.33 ± 0.05
		77 ± 4 (100)	0.71 ± 0.04
17	165 ± 35	0 (200)	0.32 ± 0.04
18	112 ± 10	11 ± 6 (200)	0.37 ± 0.08
19	101 ± 37	43 ± 0.8 (200)	0.81 ± 0.09
23a	24.9 ± 7.6	16 ± 5 (0.05)	0.28 ± 0.02
		43 ± 5 (0.1)	0.27 ± 0.03
		64 ± 2 (5)	0.37 ± 0.03
		82 ± 2 (100)	0.34 ± 0.05
23b	21 ± 7	33 ± 4 (0.05)	0.26 ± 0.03
		64 ± 3 (0.1)	0.24 ± 0.03
		74 ± 4 (5)	0.36 ± 0.06
		83 ± 3 (100)	0.31 ± 0.09

[a] DDT_1 MF-2 cells were pretreated with the indicated xanthine derivative at the concentration (nM) in parentheses, and after 10 cell wash cycles membranes were assayed for B_{max} and K_D as described in the Experimental Section. Control B_{max} from individual experiments ranged from 326 to 571 fmol/mg protein. [b] Control K_D was 0.34 ± 0.03 nM. Data are the mean ± SE, n = 3−6.

an electron-withdrawing amide linker was targeted. It was predicted that the electron-withdrawing effects of the amide would be more similar to those of compound **1** and would increase the reactivity of the fluorosulfonyl moiety.

Xanthine derivative **23a**, which contains an amide linkage between the xanthine ring and the reactive moiety, inhibited [^{3}H]DPCPX binding to the A_1AR with an IC_{50} of 24.9 nM. Interestingly, compound **23a** only differs from **19** by the exchange of two methylenes for an amide in the linker, and this resulted in a 4-fold decrease in the IC_{50} for **23a**. This suggests that the amide may provide additional contact points with the receptor to increase the affinity. Pretreatment of DDT cells with **23a** resulted in a concentration-dependent decrease in the A_1AR content indicating that **23a** is an irreversible ligand (Table 1). Furthermore, at the highest pretreatment concentration used (100 nM), the K_D for [^{3}H]DPCPX binding did not change for the receptors remaining suggesting that, unlike **1**, compound **23a** easily washed out of the membrane preparation. Replacing the C-8 cyclohexyl group of **23a** with a cyclopentyl (**23b**) did not alter the IC_{50} of the latter for the A_1AR, although its IC_{50} for the A_{2A}AR was 6.6-fold higher as compared to that for **23a** (Table 2). Similar to **23a**, **23b** produced a concentration-dependent decrease in the A_1AR content and it appeared to wash out the membrane preparation relatively easily. This was indicated by the lack of change in K_D value for [^{3}H]DPCPX binding to the receptors remaining after cell pretreatment with a high concentration (100 nM) of **23b**.

Table 2. Ligand Binding to the A_{2A} AR

no.	IC_{50} vs [^{3}H]ZM214385, μM	% loss of specific [^{3}H]ZM241385 binding (concn)[a]	K_D [^{3}H]ZM241385 binding post-xanthine, nM[b]
1	1.2 ± 0.2	44 ± 11 (0.2)	0.23 ± 0.08
17	>10	ND	ND
18	>10	ND	ND
19	>10	ND	ND
23a	0.42 ± 0.05	63 ± 5 (0.2)	0.27 ± 0.05
23b	2.8 ± 0.4	65 ± 7 (0.2)	0.34 ± 0.03

[a] PC-12 cells were pretreated with the indicated xanthine derivative at the concentration (μM) in parentheses. The cells were then washed 8 times and the membranes were assayed for B_{max} and K_D as described in the Experimental Section. Control B_{max} was 3.4 ± 0.3 pmol/mg protein. [b] Control K_D was 0.25 ± 0.03 nM. Data are the mean ± SE, n = 3−4. ND, not determined.

The binding of **17**−**19** to the A_{2A}AR was rela-

tively weak as the IC_{50} values for the inhibition of [^{3}H]ZM241385 binding were greater than 10 μM (Table 2). Because of the high IC_{50} values of these compounds for the A_{2A}AR, they were not tested for irreversible binding. In contrast, the IC_{50} of **23a** for the A_{2A}AR was in the mid-nanomolar range, whereas the IC_{50} values for **1** and **23b** were in the low-micromolar range. Upon the basis of the ratio of the IC_{50} values for binding to the A_1AR and A_{2A}AR, compounds **23a**, **23b**, and **1** are 17-, 132-, and 105-fold more selective for the A_1AR over the A_{2A}AR, respectively. Pretreatment of PC-12 cells with 200 nM **23a**, **23b**, or **1** reduced the B_{max} of [^{3}H]ZM241385 binding without a change in the K_D value of the radioligand for the receptors remaining. This indicated that these three compounds bound to the A_{2A}AR in an irreversible manner. However, in keeping with their IC_{50} selectivity for the A_1AR, a 40-fold higher concentration of **23a**, **23b**, and **1** was required to produce similar apparent irreversible binding to the A_{2A}AR as 5 nM did for the A_1AR. Therefore, **23a**, **23b**, and **1** selectively bind to the A_1AR in an apparent irreversible manner.

In summary, a series of new N-3 xanthine derivatives were synthesized and tested for affinity and apparent irreversible binding at the A_1AR and A_{2A}AR. The data indicated that the chemical composition of the linker between the N-3 position of the xanthine ring and the 4-fluorosulfonylphenyl moiety contributed to the affinity and apparent irreversible binding to the A_1AR. Furthermore, the selectivity of apparent irreversible binding to the A_1AR as compared to the A_{2A}AR was enhanced by substitution of a cyclohexyl ring for a cyclopentyl moiety at the C-8 position of the xanthine ring. Overall, compound **23b** appeared to have the most advantageous characteristics as an apparent irreversible ligand for the A_1AR. These include relatively high affinity and selectivity for the A_1AR as compared to the A_{2A}AR, concentration-dependent and selective apparent irreversible binding to the A_1AR, and ease of removal (washout) of unbound ligand from biological membranes. As a result, compound **23b** has the potential to be a useful tool to further study the structure and function of the A_1AR.

Experimental Section

Merck Kieselgel 60 and 60 F_{254} were used for column and thin-layer chromatography. Melting points were determined on 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 TMS as an internal standard. Electrospray mass spectral data was obtained on a Fisons VG Micromass Platform II spectromter. 8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (**2a**), 8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (**2b**), and 4-fluorosulfonylbenzyl bromide (**11**) were prepared using literature methodology.[8,12]

8-Cyclopentyl-7-((4-fluorosulfonylbenzoyl)oxy)-3-(3-hydroxypropyl)-1-propylxanthine (3). 8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (100 mg, 0.31 mmol) was dissolved in dry DMF (3 mL). Sodium hydride (15 mg, 0.62 mmol) in dry DMF (2 mL) was added dropwise and the solution was stirred at ambient temperature for 15 min. 4-Fluorosulfonylbenzyl bromide (87 mg, 0.34 mmol) was added and stirring was continued for a further 4 h. The reaction was quenched with water (20 mL) and extracted with chloroform (3 × 20 mL). Column chromatography using ethyl acetate/hexane (1:1) as an eluent afforded pure **3** in 67% yield: ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H, $CH_2CH_2CH_3$), 1.47−1.86 (m, 12H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2OH$, 4 × cyclopentyl CH_2), 3.27 (m, 1H, cyclopentyl C*H*), 3.45 (dt, 2H, $CH_2CH_2CH_2OH$), 3.77 (t, 2H, $CH_2CH_2CH_3$), 4.04 (t, 2H, $CH_2CH_2CH_2OH$), 4.53 (t, 1H, O*H*), 5.76 (s, 2H, CH_2Ar), 7.50, 8.12 (2 × d, 4H, phenyl); ^{13}C NMR (DMSO-d_6) δ 11.6, 20.9, 25.7, 31.4, 32.1, 36.1, 42.3, 47.2, 58.9, 106.4, 128.6, 129.4, 131.0 (d), 146.8, 148.0, 150.8, 154.5, 157.7.

8-Cyclohexyl-3-(3-hydroxypropyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (4). Anhydrous potassium carbonate (206 mg, 1.5 mmol) was added to 8-cyclohexyl-3-(3-hydroxypropyl)-1-propylxanthine (391 mg, 1.2 mmol) in DMF (12 mL) at room temperature and the mixture stirred for 1 h. 2-(Trimethylsilyl)ethoxymethyl chloride (230 μL, 1.3 mmol) was added dropwise via a syringe and the mixture stirred for 30 min at room temperature. The mixture was filtered and evaporated to dryness, affording a viscous yellow oil: yield 535 mg, 98%; ^{1}H NMR ($CDCl_3$) δ −0.04 (s, 9H, Si(CH_3)$_3$), 0.88−0.96 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.24−1.96 (m, 14H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2OH$, 5 × cyclohexyl CH_2), 2.85 (m, 1H, C*H*), 3.46 (t, 2H, $CH_2CH_2CH_2OH$), 3.66 (t, 2H, CH_2CH_2Si), 3.95 (t, 2H, $CH_2CH_2CH_3$), 4.25 (t, 2H, $CH_2CH_2CH_2OH$), 5.73 (s, 2H, NCH_2O); ^{13}C NMR ($CDCl_3$) δ −1.5, 11.3, 17.8, 21.2, 25.4, 25.9, 31.0, 31.5, 36.0, 39.5, 42.9, 57.5, 66.7, 72.6, 106.0, 148.0, 151.2, 154.9, 159.8. In the DMSO spectra: 4.53 (br s, 1H, $CH_2CH_2CH_2O$*H*).

Bromination of 8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (6). To a solution of 98% PPh_3 (376 mg, 1.4 mmol) and 99% NBS (280 mg, 1.6 mmol) in anhydrous dichloromethane (8 mL) at 0 °C was added a solution of 8-cyclopentyl-3-(3-hydroxypropyl)xanthine (104 mg, 0.32 mmol) in anhydrous dichloromethane (8 mL). The mixture was allowed to warm to room temperature with stirring for 20 h. Ethyl acetate (150 mL) was added and the mixture washed with a solution of saturated $NaHCO_3$ (2 × 100 mL) and brine (100 mL). The organic fraction was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to afford the crude as a brown solid. The crude mixture was purified by column chromatography using ethyl acetate/petroleum ether (3:2) to afford the pure product: yield 107 mg, 86%; ^{1}H NMR (DMSO-d_6) 0.84 (t, 3H, $CH_2CH_2CH_3$), 1.47−2.03 (m, 10H, $CH_2CH_2CH_3$, 4 × cyclopentyl CH_2), 2.21 (m, 2H, $CH_2CH_2CH_2$Br), 3.12 (m, 1H, C*H*), 3.53 (m, 2H, 2 × $CH_2CH_2CH_2$Br), 3.81 (t, 2H, $CH_2CH_2CH_3$), 4.08 (t, 2H, $CH_2CH_2CH_2$Br); ^{13}C NMR (DMSO-d_6) δ 11.0, 20.1, 20.7, 25.1, 30.6, 35.3, 38.7, 41.0, 42.2, 107.0, 138.2, 149.1, 151.6, 154.3; ES/MS (+) 383.1. The cyclized compound **7** was also isolated: ^{1}H NMR (DMSO-d_6) 0.84 (t, 3H, $CH_2CH_2CH_3$), 1.45−2.02 (m, 10H, $CH_2CH_2CH_3$, 4 × cyclopentyl CH_2), 2.19 (m, 2H, (O)CNCH$_2$C*H*$_2$CH$_2$N), 3.20 (m, 1H, C*H*), 3.81 (m, 4H, $CH_2CH_2CH_3$, (O)CNCH$_2$CH$_2$C*H*$_2$N), 4.02 (t, 2H, (O)CNC*H*$_2$CH$_2$CH$_2$N); ^{13}C NMR (DMSO-d_6) δ 11.1, 20.6, 20.9, 25.1, 30.5, 35.9, 38.7, 39.9, 41.7, 111.6, 138.7, 150.0, 150.3, 156.5; ES/MS (+) 303.3.

Representative Procedure for SEM Protection. Anhydrous potassium carbonate (5.7 mmol) was added to the N-7 free xanthine (4.6 mmol) in DMF (30 mL), and the mixture stirred for 2 h at room temperature. 2-(Trimethylsilyl)-ethoxymethyl chloride (5.5 mmol) was added dropwise via a syringe and the mixture stirred for 0.5−20 h. The mixture was filtered to remove excess potassium carbonate and evaporated down. The residue was dissolved in methanol, adhered to silica and purified by column chromatography using petroleum ether/ethyl acetate (6:1).

3-Benzyl-8-cyclohexyl-1-propyl-7-(2-(trimethylsilyl)-ethoxymethyl)xanthine (9a). The title compound (white solid) was prepared in 97% yield using the representative procedure for SEM protection: mp 96−97 °C; ^{1}H NMR (DMSO-d_6) δ −0.10 (s, 9H, Si(CH_3)$_3$), 0.78−0.84 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.22−1.86 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 2.92 (m, 1H, C*H*), 3.58 (t, 2H, CH_2CH_2Si), 3.82 (t, 2H, $CH_2CH_2CH_3$), 5.15 (s, 2H, CH_2Ph), 5.70 (s, 2H, NCH_2O), 7.23−7.34 (m, 5H, phenyl); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.3, 20.8, 25.2, 25.3, 31.2, 34.9, 42.0, 45.7, 65.5, 72.1, 105.6, 127.4, 127.7, 128.4, 136.9, 147.3, 150.3, 154.1, 159.5.

3-Benzyl-8-cyclopentyl-1-propyl-7-(2-(trimethylsilyl)-ethoxymethyl)xanthine (9b). The title compound (colorless oil) was prepared in 93% yield using the representative procedure for SEM protection: ^{1}H NMR ($CDCl_3$) δ −0.03 (s, 9H, Si(CH_3)$_3$), 0.89−0.96 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.62−1.74, 1.91−2.09 (2 × m, 10H, $CH_2CH_2CH_3$, 4 × cyclopentyl CH_2), 3.29 (m, 1H, CH), 3.66 (t, 2H, CH_2CH_2Si), 3.95 (t, 2H, $CH_2CH_2CH_3$), 5.26 (s, 2H, CH_2Ph), 5.72 (s, 2H, NCH_2O), 7.25−7.33, 7.55−7.58 (2 × m, 5H, phenyl); ^{13}C NMR ($CDCl_3$) δ −1.5, 11.2, 17.8, 21.2, 25.7, 32.5, 36.7, 42.8, 46.3, 66.4, 72.5, 106.7, 127.6, 128.3, 129.2, 136.8, 147.7, 151.0, 154.8, 159.7.

Representative Procedure for Debenzylation. The 3-benzylxanthine (1.8 mmol) was refluxed in dry methanol (30 mL) with ammonium formate (18.6 mmol) and activated 10% Pd−C under an atmosphere of argon for 2 h. The reflux condenser was removed and the solvent vapor allowed to escape while under a stream of nitrogen. The residue was then heated at 140 °C for 1.5 h. The residue was dissolved in methanol and filtered over a Celite pad. The filtrate was then concentrated down to afford a pure white solid.

8-Cyclohexyl-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (10a). The title compound (white solid) was prepared in 89% yield using the representative procedure for N-3 debenzylation: mp 138−139 °C; ^{1}H NMR (DMSO-d_6) δ −0.10 (s, 9H, Si(CH_3)$_3$), 0.78−0.85 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.18−1.84 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 2.87 (m, 1H, CH), 3.55 (t, 2H, CH_2CH_2Si), 3.76 (t, 2H, $CH_2CH_2CH_3$), 5.66 (s, 2H, NCH_2O), 11.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.2, 20.8, 25.3, 25.4, 31.3, 34.9, 41.1, 65.4, 71.8, 105.4, 146.9, 150.7, 154.9, 159.5.

8-Cyclopentyl-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (10b). The title compound (colorless oil) was prepared in 67% yield using the representative procedure for N-3 debenzylation: ^{1}H NMR (DMSO-d_6) δ −0.08 (s, 9H, Si(CH_3)$_3$), 0.84−0.92 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.61−2.08 (2 × m, 10H, $CH_2CH_2CH_3$, 4 × cyclopentyl CH_2), 3.23 (m, 1H, CH), 3.61 (t, 2H, CH_2CH_2Si), 3.93 (t, 2H, $CH_2CH_2CH_3$), 5.70 (s, 2H, NCH_2O), 11.71 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ −1.6, 11.1, 17.7, 21.1, 25.5, 32.4, 36.7, 42.1, 66.4, 72.4, 107.9, 146.2, 151.2, 155.3, 159.9.

3-(4-Fluorosulfonylphenyl)propyl Bromide (12). 3-Phenylpropyl bromide (497 mg, 2.5 mmol) was dissolved in dry chloroform (10 mL) and chlorosulfonic acid (1.7 mL) added dropwise. The mixture was stirred at room temperature for 20 h and poured over ice (100 mL). Water (100 mL) was added and the aqueous layer extracted with ethyl acetate (3 × 40 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated down to afford the 4-chlorosulfonyl intermediate (666 mg, 2.2 mmol) as a pale yellow oil. The residue was dissolved in 1,4-dioxane (10 mL) and refluxed for 3 h with potassium fluoride (487 mg, 8.4 mmol). The mixture was allowed to cool to room temperature and poured over ice (100 mL). Water (50 mL) was added and the aqueous phase extracted with chloroform (3 × 40 mL). The organic extracts were dried ($MgSO_4$), filtered and the solvent removed under vacuum to afford the crude: yield 537 mg, 77%; ^{1}H NMR (DMSO-d_6) δ 2.09−2.18 (m, 2H, $PhCH_2CH_2CH_2$), 2.87 (m, 2H, $PhCH_2CH_2CH_2$), 3.51 (m, 2H, $PhCH_2CH_2CH_2$), 7.64, 8.04 (2 × d, 4H, phenyl); ^{13}C NMR ($CDCl_3$) δ 33.1, 33.5, 34.0, 128.6, 129.2 (d), 130.4, 150.6.

5-(4-Fluorosulfonylphenyl)pentyl Bromide (13). 5-Phenyl-1-pentanol (99%, 965 mg, 5.8 mmol) was cooled on an ice bath. Phosphorus tribromide (170 μL, 1.8 mmol) was added and the mixture was stirred on ice for 15 min, then at room temperature for 2 h, and at 60 °C for 26.5 h. Ice water (50 mL) and brine (50 mL) were added and the aqueous layer extracted with ether (100 mL). The ether extract was washed with brine (50 mL), dried ($MgSO_4$), filtered and purified by column chromatography using petroleum ether with 2% ethyl acetate, affording the alkyl bromide as a clear liquid: yield 1.2 g, 89%; ^{1}H NMR ($CDCl_3$) δ 1.46−1.54, 1.62−1.69, 1.90 (3 × m, 6H, CH_2), 2.64 (t, 2H, $PhCH_2CH_2CH_2CH_2CH_2$), 3.42 (t, 2H, $PhCH_2CH_2CH_2CH_2CH_2$), 7.18−7.32 (m, 5H, phenyl); ^{13}C NMR ($CDCl_3$) δ 27.8, 30.6, 32.7, 33.8, 35.7, 125.7, 128.3, 128.4, 142.3.

5-Phenylpentyl bromide (1.3 g, 5.8 mmol) was dissolved in dry chloroform (25 mL) and chlorosulfonic acid (4.0 mL) added dropwise. The mixture was stirred at room temperature for 16 h and poured over ice (100 mL). Water (100 mL) was added and the aqueous layer extracted with ethyl acetate (3 × 100 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated down to afford the 4-chlorosulfonyl intermediate (1.9 g, 5.8 mmol) as a brown oil. The residue was dissolved in 1,4-dioxane (20 mL) and refluxed for 3 h with potassium fluoride (1.3 g, 23 mmol). The mixture was allowed to cool to room temperature and poured over ice (250 mL). Water was added and the aqueous phase extracted with chloroform (3 × 100 mL). The organic extracts were dried ($MgSO_4$), filtered and purified by column chromatography using petroleum ether/ethyl acetate (6:1): yield 1.5 g, 82%; ^{1}H NMR ($CDCl_3$) δ 1.48−1.56, 1.64−1.75, 1.86−1.95 (3 × m, 6H, CH_2), 2.76 (t, 2H, $PhCH_2CH_2CH_2CH_2CH_2$), 3.41 (t, 2H, $PhCH_2CH_2CH_2CH_2CH_2$), 7.43, 7.93 (2 × d, 4H, phenyl); ^{13}C NMR ($CDCl_3$) δ 27.6, 30.0, 32.4, 33.5, 35.8, 128.6, 129.6, 151.2.

Representative Procedure for N-3 Alkylation. The N-3 free xanthine (0.75 mmol) was stirred in dry DMF (10 mL) with potassium carbonate (1.0 mmol) for 1 h. A solution of the alkyl bromide (0.86 mmol) in dry DMF (5 mL) was added and the mixture stirred for 1 h − 3 days, while being monitored by TLC. The reaction mixture was filtered, adhered to silica and purified by column chromatography using petroleum ether/ethyl acetate (4:1).

8-Cyclohexyl-3-(4-fluorosulfonylbenzyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (14). The title compound was prepared in 51% yield using the representative procedure for N-3 alkylation: ^{1}H NMR (DMSO-d_6) δ −0.09 (s, 9H, Si(CH_3)$_3$), 0.82 (t, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.18−1.84 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 2.92 (m, 1H, CH), 3.59 (t, 2H, CH_2CH_2Si), 3.82 (t, 2H, $CH_2CH_2CH_3$), 5.30 (s, 2H, CH_2Ph), 5.71 (s, 2H, NCH_2O), 7.69, 8.10 (2 × d, 4H, phenyl); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.2, 20.8, 25.2, 25.3, 31.2, 34.9, 42.1, 45.4, 65.6, 72.2, 105.7, 128.7, 129.2, 130.4 (d), 146.1, 147.1, 150.4, 154.1, 159.5.

8-Cyclohexyl-3-(3-(4-fluorosulfonylphenyl)propyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (15). The title compound was prepared in 28% yield using the representative procedure for N-3 alkylation: ^{1}H NMR (DMSO-d_6) δ −0.10 (s, 9H, Si(CH_3)$_3$), 0.81 (t, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.19−1.85 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 2.09 (m, 2H, $CH_2CH_2CH_2$Ph), 2.79 (t, 2H, $CH_2CH_2CH_2$Ph), 2.90 (m, 1H, CH), 3.56 (t, 2H, CH_2CH_2Si), 3.76 (t, 2H, $CH_2CH_2CH_3$), 4.02 (t, 2H, $CH_2CH_2CH_2$Ph), 5.66 (s, 2H, NCH_2O), 7.54, 7.94 (2 × d, 4H, phenyl); ^{13}C NMR (DMSO-d_6) δ −1.6, 11.1, 17.2, 20.7, 25.2, 25.3, 27.6, 31.2, 32.0, 34.8, 41.8, 42.0, 65.4, 72.0, 105.5, 128.1, 128.8 (d), 129.0, 129.9, 147.2, 150.2, 151.1, 154.0, 159.2.

8-Cyclohexyl-3-(5-(4-fluorosulfonylphenyl)pentyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (16). The title compound was prepared in 45% yield using the representative procedure for N-3 alkylation: ^{1}H NMR (DMSO-d_6) δ −0.10 (s, 9H, Si(CH_3)$_3$), 0.78−0.84 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.13−1.78 (m, 18H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_2CH_2$Ph, $CH_2CH_2CH_2CH_2CH_2$Ph, $CH_2CH_2CH_2CH_2CH_2$Ph, 5 × cyclohexyl CH_2), 2.71 (t, 2H, $CH_2CH_2CH_2CH_2CH_2$Ph), 2.90 (m, 1H, CH), 3.56 (t, 2H, CH_2CH_2Si), 3.81 (t, 2H, $CH_2CH_2CH_3$), 3.98 (t, 2H, $CH_2CH_2CH_2CH_2CH_2$Ph), 5.69 (s, 2H, NCH_2O), 7.57, 8.00 (2 × d, 4H, phenyl); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.2, 20.8, 25.2, 25.3, 26.9, 29.5, 31.2, 34.7, 34.8, 41.8, 42.2, 65.5, 72.0, 105.5, 128.4, 128.6 (d), 130.2, 147.4, 150.3, 152.2, 154.1, 159.4.

Representative Procedure for SEM Deprotection. The SEM-protected compound (0.28 mmol) was refluxed in a 120 °C oil bath with 1 M HCl (3.0 mL) and ethanol (6 mL) for 5.5 h. The mixture was evaporated down under reduced pressure, partitioned between water (pH adjusted to 7 with NaOH) and chloroform and further extracting with chloroform. The organic

extracts were dried ($MgSO_4$), filtered and evaporated down, affording a creamy solid which was recrystallized from ethanol and water.

8-Cyclohexyl-3-(4-fluorosulfonylbenzyl)-1-propylxanthine (17). The title compound was prepared in 73% yield using the representative procedure for SEM deprotection: mp 254−255 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H, $CH_2CH_2CH_3$,), 1.15−1.90 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 2.70 (m, 1H, C*H*), 3.81 (t, 2H, $CH_2CH_2CH_3$), 5.28 (s, 2H, CH_2Ph), 7.67, 8.08 (2 × d, 4H, phenyl), 13.21 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 11.2, 20.8, 25.3, 25.3, 30.9, 37.6, 42.2, 45.6, 106.1, 128.7, 129.0, 130.3 (d), 146.4, 147.2, 150.8, 153.9, 158.4.

8-Cyclohexyl-3-(3-(4-fluorosulfonylphenyl)propyl)-1-propylxanthine (18). The title compound was prepared in 82% yield using the representative procedure for SEM deprotection: mp 182−183 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 3H, $CH_2CH_2CH_3$), 1.18−1.89 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 2.08 (m, 2H, $CH_2CH_2CH_2$Ph), 2.70 (tt, 1H, C*H*), 2.80 (t, 2H, $CH_2CH_2CH_2$Ph), 3.76 (t, 2H, $CH_2CH_2CH_3$), 4.00 (t, 2H, $CH_2CH_2CH_2$Ph), 7.56, 7.95 (2 × d, 4H, phenyl), 13.03 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 11.2, 20.8, 25.3, 27.8, 31.0, 32.2, 37.5, 42.0, 42.3, 106.0, 128.2, 128.8 (d), 130.1, 147.4, 150.6, 151.2, 153.8, 158.1.

8-Cyclohexyl-3-(5-(4-fluorosulfonylphenyl)pentyl)-1-propylxanthine (19). The title compound was prepared in 66% yield using the representative procedure for SEM deprotection: mp 147−148 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (t, 3H, $CH_2CH_2CH_3$), 1.14−1.84 (m, 18H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_2CH_2CH_2$Ph, $CH_2CH_2CH_2CH_2CH_2CH_2CH_2$Ph, 5 × cyclohexyl CH_2), 2.64−2.74 (m, 3H, $CH_2CH_2CH_2CH_2CH_2$Ph, C*H*), 3.80 (t, 2H, $CH_2CH_2CH_3$), 3.95 (t, 2H, $CH_2CH_2CH_2CH_2CH_2CH_2$Ph), 7.57, 8.00 (2 × d, 4H, phenyl), 13.06 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 11.1, 20.8, 25.3, 25.3, 27.0, 29.6, 31.0, 34.8, 37.5, 41.9, 42.4, 106.0, 128.4, 128.7 (d), 130.2, 147.6, 150.6, 152.2, 153.9, 158.2.

8-Cyclohexyl-3-(3-phthalimidopropyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (20). The title compound was prepared in quantitative yield using the representative procedure for N-3 alkylation: mp 96−97 °C; ^{1}H NMR (DMSO-d_6) δ −0.11 (s, 9H, Si(CH_3)$_3$), 0.81 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.02−1.87 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 2.03 (m, 2H, $CH_2CH_2CH_2$Phth), 2.81 (m, 1H, C*H*), 3.54 (t, 2H, CH_2CH_2Si), 3.61 (t, 2H, $CH_2CH_2CH_2$Phth), 3.77 (t, 2H, $CH_2CH_2CH_3$), 4.00 (t, 2H, $CH_2CH_2CH_2$Phth), 5.62 (s, 2H, NCH_2O), 7.83 (s, 4H, phenyl); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.2, 20.7, 25.1, 25.2, 26.3, 31.0, 34.8, 35.1, 41.8, 65.4, 72.0, 105.6, 122.9, 131.5, 134.3, 147.1, 150.3, 154.1, 159.3, 167.8.

3-(3-Aminopropyl)-8-cyclohexyl-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (21). Method A. A solution of 8-cyclohexyl-3-(3-phthalimidopropyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethylxanthine (109 mg, 0.18 mmol) in dry methanol (1.5 mL) and methylamine (40% in methanol, 1.0 mL, 13 mmol) was heated in a pressure apparatus in an oil bath at 50 °C for 14 h. The solvent was removed under vacuum to afford the crude as a yellow oil which was purified by column chromatography using petroleum ether/isopropyl alcohol/ammonia (80:20:1.5): yield 48 mg, 56%; ^{1}H NMR (DMSO-d_6) δ −0.10 (s, 9H, Si(CH_3)$_3$), 0.78−0.85 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.19−1.85 (m, 14H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2NH_2$, 5 × cyclohexyl CH_2), 2.48−2.52 (m, 2H, $CH_2CH_2CH_2NH_2$), 2.91 (m, 1H, C*H*), 3.57 (t, 2H, CH_2CH_2Si), 3.82 (t, 2H, $CH_2CH_2CH_3$), 4.03 (t, 2H, $CH_2CH_2CH_2NH_2$), 5.70 (s, 2H, NCH_2O); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.2, 20.8, 25.2, 25.3, 30.9, 31.2, 34.9, 38.2, 40.2, 65.5, 72.0, 105.5, 147.4, 150.4, 154.2, 159.4; ES/MS (+) 464.4.

Method B. A solution of 8-cyclohexyl-3-(3-phthalimidopropyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethylxanthine (109 mg, 0.18 mmol) in dry methanol (1.5 mL) and hydrazine monohydrate (1.0 mL, 21 mmol) was refluxed for 15 h. NaOH (3 M, 20 mL) was added and the mixture extracted with ethyl acetate (3 × 20 mL). The organic fractions were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography using petroleum ether/isopropyl alcohol (80:20), plus 1.5% NH_4OH: yield 48 mg, 56%.

8-Cyclohexyl-3-(3-(4-fluorosulfonylbenzamido)propyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (22a). Method A. A solution of 3-(3-aminopropyl)-8-cyclohexyl-1-propyl-7-(2-(trimethylsilyl)ethoxymethylxanthine (55 mg, 0.12 mmol) and 4-fluorosulfonylbenzoic acid (95%, 27 mg, 0.13 mmol) in dry DMF (7.5 mL) was cooled over ice. EDCI (25 mg, 0.13 mmol) was added and the mixture stirred on ice for 1.5 h, then at room temperature for 2.5 h. The mixture was cooled on ice again and *N*,*N*-diisopropylethylamine (22 μL, 0.13 mmol) was added. Stirring was continued on ice for 1 h then at room temperature for 16.5 h. Ethyl acetate (30 mL) was added and the solution washed with H_2O (3 × 25 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent removed under vacuum to afford a clear yellow oil which was purified by column chromatography using petroleum ether/ethyl acetate (3:2): yield 22 mg, 29%; mp 98−103 °C; ^{1}H NMR (DMSO-d_6) δ −0.09 (s, 9H, Si(CH_3)$_3$), 0.78−0.85 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 0.92−1.08, 1.18−1.76 (2 × m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 1.95 (m, 2H, $CH_2CH_2CH_2NHCO$), 2.84 (m, 1H, C*H*), 3.27−3.34 (m, 2H, $CH_2CH_2CH_2NHCO$), 3.56 (t, 2H, CH_2CH_2Si), 3.81 (t, 2H, $CH_2CH_2CH_3$), 4.08 (t, 2H, $CH_2CH_2CH_2NHCO$), 5.67 (s, 2H, NCH_2O), 8.16, 8.26 (2 × d, 4H, phenyl), 8.88 (t, 1H, N*H*CO); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.2, 20.8, 25.1, 25.3, 27.4, 31.1, 34.8, 36.8, 40.6, 41.9, 65.5, 72.0, 105.6, 128.6, 129.0, 133.4 (d), 141.6, 147.3, 150.3, 154.2, 159.3, 164.2.

Method B. The title compound was prepared in 87% using the representative procedure for N-3 alkylation: ^{1}H NMR (DMSO-d_6) δ −0.09 (s, 9H, Si(CH_3)$_3$), 0.78−0.85 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 0.92−1.08, 1.18−1.76 (2 × m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 1.95 (m, 2H, $CH_2CH_2CH_2NHCO$), 2.84 (m, 1H, C*H*), 3.27−3.34(m, 2H, $CH_2CH_2CH_2NHCO$), 3.56 (t, 2H, CH_2CH_2Si), 3.81 (t, 2H, $CH_2CH_2CH_3$), 4.08 (t, 2H, $CH_2CH_2CH_2NHCO$), 5.67 (s, 2H, NCH_2O), 8.16, 8.26 (2 × d, 4H, phenyl), 8.88 (t, 1H, N*H*CO); ^{13}C NMR (DMSO-d_6) δ −1.5, 11.1, 17.2, 20.8, 25.1, 25.3, 27.4, 31.1, 34.8, 36.8, 40.6, 41.9, 65.5, 72.0, 105.6, 128.6, 129.0, 133.4 (d), 141.6, 147.3, 150.3, 154.2, 159.3, 164.2.

8-Cyclopentyl-3-(3-(4-fluorosulfonylbenzamido)propyl)-1-propyl-7-(2-(trimethylsilyl)ethoxymethyl)xanthine (22b). The title compound was prepared in 56% yield using representative procedure for N-3 alkylation: ^{1}H NMR ($CDCl_3$) δ −0.03 (s, 9H, Si(CH_3)$_3$), 0.89−0.97 (m, 5H, $CH_2CH_2CH_3$, CH_2CH_2Si), 1.60−2.14 (m, 12H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2NHCO$, 4 × cyclopentyl CH_2), 3.24 (m, 1H, C*H*), 3.42 (m, 2H, $CH_2CH_2CH_2NHCO$), 3.66 (t, 2H, CH_2CH_2Si), 3.99 (t, 2H, $CH_2CH_2CH_3$), 4.26 (t, 2H, $CH_2CH_2CH_2NHCO$), 5.73 (s, 2H, NCH_2O), 8.10−8.16 (m, 4H, phenyl); ^{13}C NMR ($CDCl_3$) δ −1.5, 11.3, 17.8, 21.3, 25.5, 27.8, 32.7, 35.8, 36.8, 40.2, 42.9, 66.6, 72.6, 106.8, 128.4, 128.7, 135.1 (d), 141.6, 147.5, 151.8, 154.6, 159.9, 165.0.

8-Cyclohexyl-3-(3-(4-fluorosulfonylbenzamido)propyl)-1-propylxanthine (23a). The title compound was prepared in 96% yield using the general procedure for SEM deprotection: mp 258−259 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, $CH_2CH_2CH_3$), 1.04−1.80 (m, 12H, $CH_2CH_2CH_3$, 5 × cyclohexyl CH_2), 1.95 (m, 2H, $CH_2CH_2CH_2NHCO$), 2.60 (tt, 1H, C*H*), 3.26−3.32 (m, 2H, $CH_2CH_2CH_2NHCO$), 3.81 (t, 2H, $CH_2CH_2CH_3$), 4.06 (t, 2H, $CH_2CH_2CH_2NHCO$), 8.15, 8.25 (2 × d, 4H, phenyl), 8.87 (t, 1H, N*H*CO), 13.03 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 11.2, 20.8, 25.2, 25.3, 27.4, 30.9, 36.8, 37.5, 40.7, 42.0, 106.0, 128.6, 129.0, 133.4 (d), 141.6, 147.5, 150.6, 153.9, 158.1, 164.3.

8-Cyclopentyl-3-(3-(4-fluorosulfonylbenzamido)propyl)-1-propylxanthine (23b). The title compound was prepared in 71% yield using the general procedure for SEM deprotection: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.87 (m, 3H, $CH_2CH_2CH_3$), 1.55−1.71 (m, 10H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2NHCO$, 4 × cyclopentyl CH_2), 3.09 (m, 1H, C*H*), 3.38 (m, 2H, $CH_2CH_2CH_2NHCO$), 3.89 (t, 2H, $CH_2CH_2CH_3$), 4.16 (t, 2H, $CH_2CH_2CH_2NHCO$), 8.06 (m, 4H, phenyl); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 11.0, 21.1, 25.1, 27.2, 32.2, 36.3, 39.4, 41.0, 43.1, 106.6, 128.4, 128.5, 134.9 (d), 141.1, 147.6, 151.4, 154.7, 158.3, 165.5.

***N*-(3-Bromopropyl)-4-fluorosulfonylbenzamide (25).** A solution of 4-(fluorosulfonyl)benzoic acid (95%, 565 mg, 2.6 mmol), 3-bromopropylamine hydrobromide (98%, 758 mg, 3.4 mmol) and EEDQ (735 mg, 3.0 mmol) in anhydrous DMF (20 mL) was cooled to 0 °C on an ice bath. *N*,*N*-Diisopropylethylamine (920 μL, 5.3 mmol) was added slowly and the mixture stirred a further 8 h on ice and 8 h at room temperature. Water (50 mL) was added and the mixture extracted with ethyl acetate (3 × 50 mL). The organic extracts were washed with HCl (2%, 3 × 25 mL), dried ($MgSO_4$), filtered and purified by column chromatography using petroleum ether/ethyl acetate (2:1): yield 534 mg, 63%; ^{1}H NMR ($CDCl_3$) δ 2.24 (m, 2H, $NHCH_2CH_2CH_2$), 3.51 (t, 2H, $NHCH_2CH_2CH_2$), 3.67 (m, 2H, $NHCH_2CH_2CH_2$), 6.58 (br s, 1H, N*H*), 8.06 (m, 4H, phenyl); ^{13}C NMR ($CDCl_3$) δ 30.8, 31.7, 39.1, 128.2, 128.8, 135.5 (d), 140.9, 165.4.

Biochemical Methods. Solutions. Stock solutions of the xanthine derivatives were prepared by dissolving in DMSO to give a concentration of 10 mM. On the day of use, the compounds were diluted to the desired concentration with Hank's balanced salt solution (HBSS). Control incubations contained the same final concentration of DMSO.

Cell Culture, Pretreatments, and Membrane Isolation. DDT_1 MF-2 and PC-12 cells were grown as monolayers as described previously.[8] Cells were subcultured weekly and used in experiments at 1 day preconfluence. Cell pretreatments were performed by washing the monolayers twice with HBSS (2 × 10 mL) and incubation of the cells with 10 mL of HBSS containing the indicated concentration of xanthine derivative at 37 °C for 20 min. At the end of the incubation, the HBSS was aspirated and replaced with 10 mL of ligand-free HBSS. After 5 min at room temperature, the HBSS was replaced with another 10 mL of HBSS. This washing procedure was performed 10 times with DDT cells and 8 times with PC-12 cells. At the end of the washing procedure, cell membranes were prepared as previously described[8] and the protein content was determined by the Bradford[17] method using bovine serum albumin as standard.

Radioligand Binding Assays. The A_1AR content of DDT cell membranes was determined by specific [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine as previously reported.[18] The A_{2A}AR of PC-12 membranes was determined by a modification of a previously reported procedure.[19] Briefly, cell membranes (15−25 μg 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 units/mL adenosine deaminase, 0.1−3 nM [^{3}H]ZM241385 with and without 50 μM 5′-*N*-ethylcarboxamidoadenosine (NECA) for 120 min at 25 °C. At the end of the incubation, each suspension was diluted with 3 mL of ice-cold 10 mM Tris-HCl buffer at pH 7.4, and the membranes with bound radioligand were collected by filtration under reduced pressure. The filters were washed with an additional 6 mL of ice-cold buffer and placed in a vial with 3 mL of Scinti-Verse BD, and the radioactivity was determined using a liquid scintillation counter. Specific [^{3}H]ZM241385 binding to the A_{2A}AR was calculated as the difference between total binding in the absence of NECA and the nonspecific binding determined in the presence of NECA. In displacement of [^{3}H]ZM241385 experiments using the xanthine derivatives, the concentration of the radioligand was 1.5 nM. All binding assays were performed in triplicate.

The concentration of compounds which inhibited specific radioligand binding by 50% (IC_{50}) was determined by nonlinear regression analysis of the data using the GraphPad Inplot program (GraphPad Software, San Diego, CA). The dissociation constant (K_D) and maximal radioligand binding (B_{max}) were determined by nonlinear regression analysis of saturation data plotted by the method of Scatchard.[20]

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Supporting Information Available: Microanalyses for compounds **17**−**19** and **23a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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