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New Irreversible Adenosine A_1 Antagonists Based on FSCPX

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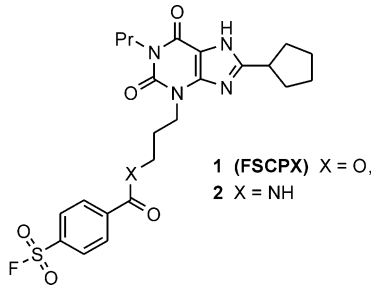
Abstract—FSCPX (**1**) and its amide analogue (**2**) have been reported to exhibit potent and selective irreversible antagonism of the A_1 adenosine receptor (A_1AR) when used in in vitro biological preparations. In order to obtain an irreversible A_1AR antagonist with improved stability, analogues of FSCPX incorporating the chemoreactive 4-(fluorosulfonyl)phenyl moiety separated from the xanthine pharmacophore by a ketone linkage were explored. Compounds **4a**–**c** exhibited improved affinity for the A_1AR and concentration-dependent irreversible binding to the A_1AR.

Adenosine receptors (AR's) are distributed widely throughout the mammalian body where they mediate the many functions of the purine nucleoside adenosine.[1–3] These responses are mediated via the four distinct AR subtypes, namely A_1, A_{2A}, A_{2B}, and A_3.[4,5] These effects include release of neurotransmitters,[6] inhibition of neuronal firing, inhibition of lipolysis, cardiac depression and vasodilation,[7] vasoconstriction and immunosuppresant effects.[3]

Irreversible alkylating ligands are useful tools for the study of receptor structure and function, including the measurement of receptor reserve[8–10] and the identification and mapping of ligand-binding sites.[11,12] Desirable characteristics of irreversible ligands include high efficiency of incorporation into the receptor allowing for the control of receptor concentrations over a wide range, a pharmacophore that provides affinity and selectivity for the receptor and a reactive moiety that is reactive enough to provide covalent incorporation into the receptor but is stable enough that it can be used under a wide variety of biological conditions.

Fluorosulfonyl substituted xanthines have been synthesised where the chemoreactive 4-(fluorosulfonyl)phenyl moiety is attached to N3 of the xanthine ring via ester (FSCPX, **1**)[13] and amide (compound **2**)[14] linkages. All compounds exhibited high affinity for the A_1AR, high selectivity for the A_1AR over the A_{2A}AR, and irreversibly blocked the binding of [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]CPX) to the A_1AR of DDT_1 MF-2 cells (Table 1).[13–16]



Although compounds **1** and **2** bind to the A_1AR irreversibly, the ester linkage of FSCPX (**1**) and the amide linkage of **2** have the potential to be cleaved by esterase or amidase enzymes, when used in vivo or in preparations containing significant enzyme activity. Cleavage of the fragment containing the reactive moiety would obviously render the ligand inactive in terms of irreversible binding. Consistent with this possibility is the recent report indicating that FSCPX is rapidly degraded in rat blood.[17]

In order to obtain an irreversible A_1AR antagonist with increased stability toward enzyme cleavage, we prepared an analogue with an alkyl chain [–$(CH_2)_5$–] between the 4-(fluorosulfonyl)phenyl and the xanthine ring.[14] While

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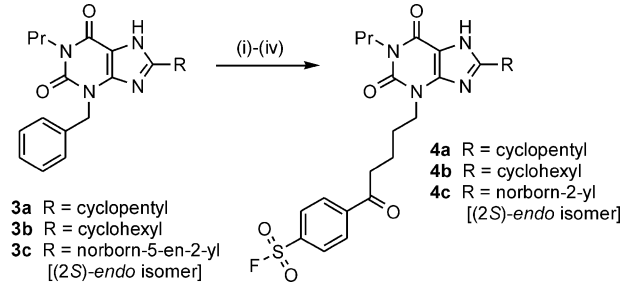
Table 1. Ligand binding to the A_1AR from DDT_1 MF2 cells

Compd	IC_{50} versus [^{3}H]DPCPX (nM)	% Loss of specific [^{3}H]DPCPX binding (concn)[b]	K_D [^{3}H]DPCPX binding post-xanthine (nM[c])
1[a]	11.8±3.2	67±3 (5) 77±4 (100)	0.33±0.05 0.71±0.04
2[a]	21±7	74±4 (5) 83±3 (100)	0.36±0.06 0.31±0.09
4a	8.7±3.9	51±3 (5) 73±0.7 (100)	0.53±0.02 0.46±0.06
4b	16.8±3.5	56±8 (5) 78±3 (100)	0.40±0.04 0.31±0.06
4c	3.9±0.9	50±5 (5) 81±2 (100)	0.53±0.02 0.41±0.04

[a]Data taken from Beauglehole et al.[14]
[b]DDT_1 MF-2 cells were incubated without (control) and with the indicated xanthine derivative at the concentration (nM) in parentheses, and after 10 cell wash cycles, membranes were prepared and assayed for [^{3}H]DPCPX B_{max} and K_D. Control B_{max} from individual experiments ranged from 267 to 593 fmol/mg protein.
[c]Control K_D was 0.49±0.06 nM. Data are the mean±SE, n=3–6.

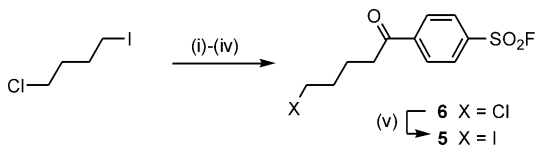
this linker was expected to be relatively inert, the compound exhibited poor irreversible binding characteristics. This was attributed to the electronic effect of the linker on the reactivity of the fluorosulfonyl group to substitution reactions. It was predicted that a ketone linkage would be more resistant to enzymatic cleavage and would possess similar electronic properties to FSCPX (**1**) and its amide analogue **2**. In order to maximise pharmacophore affinity and selectivity for the A_1AR, target xanthines possessing 8-cyclopentyl, 8-cyclohexyl, and 8-(norborn-2-yl) substitution were targeted.[18]

3-Benzyl xanthines with the appropriate C8 substituent (**3a**–**c**) were synthesised via literature methods.[19,20] Optically pure (2*S*)-*endo*-norborn-5-ene-2-carboxylic acid, prepared using methodology developed by Helmchen et al.[21] was used in the synthesis of **3c**. Scheme 1 depicts the synthetic route used to prepare targets with a ketone linkage separating the 4-(fluorosulfonyl)phenyl moiety from the xanthine ring. Alkylation with [2-(trimethylsilyl)ethoxy]methyl chloride (SEM-Cl) and K_2CO_3 then protected N-7 of the 3-benzyl-8-cycloalkyl-1-propylxanthines (**3a**–**c**, 45–97%). Subsequent debenzylation at N3 with 10% palladium on carbon and ammonium formate in refluxing methanol[22] (40–89%) provided a convenient attachment point for N3 substituents. In the case of compound **3c**, reduction of the alkene moiety of the 8-norborn-5-en-2-yl group proceeded concomitantly with the debenzylation. Attachment of the chemoreactive N3 substituent with the alkyl iodide **5** and potassium carbonate in DMF provided the penultimate compounds in moderate yields (34–60%). Initial attempts at N3 alkylation using an identical procedure to that used in the preparation of compound **2**[14] (xanthine, alkyl halide, potassium carbonate and DMF) resulted in successful coupling with concurrent hydrolysis of the fluorosulfonyl moiety to the corresponding sulfonic acid. As the fluorosulfonyl moiety is relatively stable in water (commonly recrystallised from aqueous ethanol), formation of the sulfonic acid presumably involved initial formation of the iodosulfonyl prior to hydrolysis. This result was somewhat surprising as the fluorosulfonyl proved to be stable to refluxing sodium iodide in acetone. Therefore, it was predicted that formation of the iodosulfonyl was occurring during concentration on workup. Successful alkylation was achieved by pouring the reaction mixture into a solution of silver nitrate to remove free iodide ions immediately after the reaction was complete. Subsequent SEM deprotection with aqueous hydrochloric acid afforded the target compounds **4a**–**c**[23–25] in good yield (52–89%).



Scheme 1. Reagents and conditions: (i) K_2CO_3, SEM-Cl, DMF; (ii) Pd/C, NH_4CO_2H, MeOH; (iii) K_2CO_3, $I(CH_2)_4C(O)C_6H_4SO_2F$ (**5**), DMF; (iv) aq HCl.

Scheme 2 depicts construction of the N3 substituent and formation of the ketone functionality via a highly functionalised organocopper reagent. Following activation of the zinc with 4 mol% of 1,2-dibromoethane and 3 mol% of trimethylsilyl chloride, a regioselective zinc insertion occurs into the carbon iodide bond of 1-chloro-4-iodobutane.[26] GC showed no evidence of zinc insertion into the carbon-chlorine bond. The organozinc iodide is then transformed into the organocopper species via a transmetallation reaction with the THF soluble copper salt CuCN·2LiCl, to form the zinc–copper reagent, tentatively represented as RCu(CN)ZnX.[26] The zinc–copper reagent displays an enhanced reactivity toward organic electrophiles, and reacts rapidly with acyl chlorides in THF at 0 °C over 4–12 h to form ketones.[26] Compound **6** was produced in an overall yield of 54% with respect to 4-(fluorosulfonyl)benzoyl chloride. Alkyl chlorides of this type failed to alkylate N3 under a variety of conditions. In order to overcome this problem a halogen exchange was performed on **6** to increase the reactivity of the alkyl halide for subsequent substitution.



Scheme 2. Reagents and conditions: (i) Zn, THF, 45 °C, 2.5 h; (ii) CuCN·2LiCl, −60 °C to 0 °C, 5 min; (iii) −78 °C to 0 °C, 4 h; (iv) $FO_2SC_6H_4COCl$, −78 to 0 °C, 4 h; (v) NaI, acetone.

Initially, efforts to synthesise the bromo analogue of **5** were focused on benzylic oxidation of 3-(4-(fluorosulfonyl)phenyl)pentyl bromide due to its availability in our laboratory. Attempts at benzylic oxidation with selenium dioxide in dioxane[27,28] and sodium bismuthate in acetone[29] returned starting material, while ceric ammonium nitrate in acetic acid[30] resulted in oxidation of the benzylic position to the corresponding alcohol.

All target xanthines were tested for biological activity via their ability to inhibit the binding of [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX) to the A_1AR of DDT_1 MF-2 cells as described in detail previously.[14,31] Affinity was determined as the concentration of ligand required to inhibit radioligand binding to the receptor by 50% (IC_{50}). Irreversible binding was determined by incubating the cells with antagonist for 30 min at 30 °C followed by 10 cell washing cycles to remove unbound ligand and then determining the binding maximum (B_{max}) and equilibrium dissociation constant (K_D) for the radioligand using cell membranes.[32–35]

As previously reported, FSCPX (**1**) and its amide analogue **2** have low nanomolar potency for binding to the A_1AR, and reduce the receptor content in a concentration dependent manner.[13,14] The reduction in receptor content is consistent with **1** and **2** binding irreversibly to the A_1AR. Compounds **4a**–**c** containing a ketone linkage separating the 4-(fluorosulfonyl)phenyl moiety and xanthine ring, exhibited high affinity for the A_1AR as its IC_{50} for the inhibition of [^{3}H]DPCPX binding were 8.7, 16.8, and 3.9 nM for the 8-cyclopentyl, 8-cyclohexyl, and 8-(2*S*-*endo*)-norborn-2-yl, respectively (Table 1). These potencies are in the same range as that reported for **1** and **2**.[14] Previously, it has been shown that the linker between the 4-(fluorosulfonyl)phenyl and the xanthine ring contribute to the ligand affinity for the A_1AR. Thus, an ester (**1**) and amide (**2**) in the linker were about 10- and 5-fold more potent for the receptor than an alkyl linker.[14] The present data show that replacement of the amide or ester with a more stable ketone in the linker does not decrease the affinity of the ligand for the receptor.

Pretreatment of DDT_1 cells with 5 or 100 nM of **4a**, **4b**, or **4c** reduced the receptor content of the cell membranes by 50–56 and 73–81%, respectively, with no change in the K_D for [^{3}H]DPCPX binding to the receptors remaining. These data suggest that compounds **4a**–**c** produce a concentration-dependent decrease in the A_1AR of DDT_1 cells by binding to the receptor in an irreversible manner. The lack of change in the K_D value for [^{3}H]DPCPX binding after pretreatment with the higher concentration of **4a**–**c** indicates that these compounds wash out of the cells relatively easily. This is in contrast to FSCPX (**1**) that has previously been reported to be more resistant to washout.[14] The ease of cell washout for **4a**–**c** provides a distinct advantage for these irreversible antagonists as compared to FSCPX.

An efficient synthetic approach has been developed for 3-substituted xanthines of this type in which the xanthine and reactive moiety plus linker are constructed separately and joined prior to the final deprotection step. All synthetic steps proceeded in moderate to excellent yield, making this approach readily amenable to gram scale preparation of the desired targets. The xanthines **4a**–**c** were found to be highly potent A_1AR antagonists that appear to bind to the receptor in an irreversible manner. These characteristics are advantageous in their use as irreversible ligands for the A_1AR. Furthermore, because **4a**–**c** contain a biologically stable ketone linker between the reactive moiety and the xanthine ring, it is likely that they will be useful for in vivo studies to further elucidate the function and regulation of the A_1AR.

Acknowledgements

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23. Characterisation of **4a**: ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 2H, $CHCH_3$), 1.64–1.99, 2.11–2.17 (m, 14H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_2CO$, $CH_2CH_2CH_2CH_2CO$, 4×cyclopentyl CH_2), 3.18 (t, 2H, $CH_2CH_2CH_2CH_2CO$), 3.28 (tt, 1H, CH), 4.02 (t, 2H, $CH_2CH_2CH_3$), 4.24 (t, 2H, $CH_2CH_2CH_2CH_2CO$), 8.12, 8.20 (2×d, 4H, phenyl), 12.52 (br s, 1H, N^7H), ^{13}C NMR ($CDCl_3$) δ 11.4, 20.6, 21.4, 25.5, 27.4, 32.5, 38.6, 40.0, 43.6, 43.8, 106.5, 128.9, 129.1, 136.5 (d), 142.2, 147.3, 150.7, 155.4, 158.7, 198.2, Anal. ($C_{24}H_{24}FN_4O_5S$) C, H, N: calcd, 57.13, 5.79, 11.1, found, 57.29, 5.94, 10.72.
24. Characterisation of **4b**: ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 2H, $CH_2CH_2CH_3$), 1.23–2.05 (m, 16H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_2CO$, $CH_2CH_2CH_2CH_2CO$, 5′cyclohexyl CH_2), 2.86 (tt, 1H, CH), 3.17 (t, 2H, $CH_2CH_2CH_2CH_2CO$), 4.04 (t, 2H, $CH_2CH_2CH_3$), 4.22 (t, 2H, $CH_2CH_2CH_2CH_2CO$), 8.12, 8.19 (2×d, 4H, phenyl), 12.52 (br s, 1H, N^7H), ^{13}C NMR ($CDCl_3$) δ 11.4, 20.5, 21.3, 25.6, 25.9, 27.2, 31.4, 38.4, 38.7, 42.9, 43.3, 106.5, 128.8, 129.0, 136.4 (d), 142.2, 148.7, 151.0, 155.5, 159.7, 198.1, Anal. ($C_{25}H_{31}FN4O5S\cdot\frac{1}{2}H_2O\cdot\frac{1}{2}EtOAc$) C, H, N: calcd, 56.73, 6.35, 9.80, found, 56.73, 6.22, 9.99.
25. Characterisation of **4c**: ^{1}H NMR (CDCl3) δ 0.98 (t, 2H, $CH_2CH_2CH_3$), 1.10–1.97 (m, 14H, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_2CO$, $CH_2CH_2CH_2CH_2CO$, H3′, H5′, H6′, H7′), 2.36 (br s, 1H, H4′), 2.76 (br s, 1H, H1′), 3.17 (t, 2H, $CH_2CH_2CH_2CH_2CO$), 3.35 (m, 1H, H2′), 4.01 (t, 2H, $CH_2CH_2CH_3$), 4.25 (t, 2H, $CH_2CH_2CH_2CH_2CO$), 8.11, 8.19 (2×d, 4H, phenyl), 12.47 (br s, 1H, N^7H), ^{13}C NMR ($CDCl_3$) δ 11.4, 20.5, 21.3, 24.1, 27.3, 29.2, 32.6, 37.1, 38.5, 40.2, 40.7, 42.5, 43.6 (×2), 106.6, 128.8, 129.0, 136.4 (d), 142.1, 147.5, 150.7, 155.2, 157.4, 198.1, Anal. ($C_{26}H_{31}FN_4O_5S$) C, H, N: calcd, 58.85, 5.89, 10.56, found 58.79, 5.97, 10.20.
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