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Synthesis of novel 1-alkyl-8-substituted-3-(3-methoxypropyl) xanthines as putative A_{2B} receptor antagonists

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ABSTRACT

In order to identify a high-affinity, selective antagonist for the A_{2B} subtype adenosine receptor, more than 40 1,8-disubstituted-3-(3-methoxypropyl) xanthines were prepared and evaluated for their binding affinity at recombinant human adenosine receptors, mainly of the A_{2A} and A_{2B} subtypes. Some of the 1-ethyl-3-(3-methoxypropyl)-8-aryl substituted derivatives **15(a–m)** showed moderate-to-high affinity at human A_{2B} receptors, with compound **15d** showing A_{2B} selectivity over the other A receptors assayed (A_1, A_{2A}, A_3) of 34-fold or over.

1. Introduction

Adenosine is an endogenous purine nucleoside that is present in every cell of the human body and has a wide variety of well-documented regulatory functions and physiological effects. Four distinct adenosine receptor subtypes have been identified to date (A_1, A_{2A}, A_{2B} and A_3), all of which belong to the rhodopsin family of G-protein-coupled receptors (GPCRS) that are characterized by 7-transmembrane-spanning helical domains (TM I–VII) as well as extracellular (EL) and intracellular (IL) loops.[1–4] Interaction of adenosine with its receptors initiates signal transduction pathways, including the classical adenylate cyclase effector system that utilizes cyclic adenosine monophosphate (*c*AMP) as a second messenger. Activation of the A_1 and A_3 adenosine receptors (A_1-AdoR and A_3-AdoR) inhibits adenylate cyclase activity through activation of pertussis-sensitive G_i proteins and results in a decrease in intracellular levels of *c*AMP. On the other hand, activation of the A_{2A} and A_{2B} adenosine receptors (A_{2A}-AdoR and A_{2B}-AdoR) stimulates adenylate cyclase by activation of G_s proteins and this leads to intracellular accumulation of *c*AMP. Coupling of adenosine receptors to other second messenger systems has also been described: activation of potassium and inhibition of calcium channels in cardiac muscles and neurons (A_1-AdoR); stimulation of phospholipase C (A_1-, A_{2B}-, and A_3-AdoRs); mobilization of intracellular calcium (A_3-AdoR).[3,5,6]

Adenosine receptors have been recognized as playing an important role in chronic inflammatory airway conditions such as asthma, chronic obstructive pulmonary disease (COPD) and fibrosis.[7,8] Experimental evidence, such as the increase in the adenosine concentration in hypoxia and cellular inflammation in bronchoalveolar fluids of asthmatics and in plasma (upon a contact with allergens), has highlighted the key role that adenosine and its A_{2B} receptors play in asthma.[9–11] Moreover, adenosine, in the form of AMP, induces bronchoconstriction in asthmatics but not in healthy individuals.[12] The bronchodilating effect of theophylline (**1**) and its analogue enprophylline (**2**) has been attributed to a selective antagonism of A_{2B}-AdoR.[11] The discovery that A_{2B}-AdoRs are functionally active on human airway smooth muscle cells and lung fibroblast cells provides further support for the role of A_{2B}-AdoR.[11,13] Antagonists of A_{2B}-AdoR would therefore represent a novel approach for the management and treatment of asthma and COPD.

Even though a number of high-affinity A_{2B}-AdoR antagonists have been reported, only a few have shown high affinity and selectivity for A_{2B}-AdoR relative to A_1-, A_2- and A_3-AdoRs.[14–18] Several

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research groups have synthesized 8-phenyl-substituted xanthines that have high A_{2B} affinity and selectivity against the other AdoR subtypes.[19–21] Jacobson and co-workers demonstrated that the introduction of a *para*-substituted phenyl derivative at the 8-position of the xanthine core increases the A_{2B}-AdoR affinity and selectivity in comparison to the other AdoRs, as exemplified by compound **3** (see Chart 1).[20,21]

For this reason, some years ago we began a systematic study to identify an easy, efficient and rapid synthetic pathway to fully functionalized xanthines and followed this with an investigation into the biological and pharmacological properties of the resulting compounds. Our goal was the discovery of a selective, high-affinity A_{2B}-AdoR antagonist through the preparation of xanthines bearing appropriate substituents in several positions of the xanthine nucleus, with the aim of exploring the structure–affinity (SAR) and structure–selectivity relationships (SSR). Exploratory tests made to evaluate the effect of replacement of each alkyl group (methyl, ethyl, propyl) of very simple 1,3,8-trialkylxanthines by slightly more polar, oxygen bearing alkyl groups showed a noticeable increase in the affinity towards A_{2B}-AdoR receptors when a 3-methoxypropyl group was placed at the position 3 of the xanthine. Thus, the structural variations at the xanthine nucleus initially envisaged at this work were made at position 1 (alkyl or functionalized alkyl substituents) and position 8 (aryl or heteroaryl substituents), with the 3-methoxypropyl substituent kept at position 3 (Schemes 1 and 2). Also, on the basis of the interesting results obtained by Jacobson et al.,[12] an oxy acetamido group linked through its nitrogen atom to a variety of aromatic, heteroaromatic and cycloaliphatic substituents was placed in the *para*-position of the 8-phenyl ring in a second series of xanthines, while keeping the 3-methoxypropyl group at position 3 (Scheme 3).

2. Chemistry

3-Alkyl-1-(3-methoxypropyl)-5,6-diaminouracils (**7**) were synthesized according to previously described methods. Thus, 1-(3-methoxypropyl)urea was condensed with cyanoacetic acid,[22,23] to give uracil **4** (75%). Direct alkylation of this uracil was performed with 15% aqueous NaOH and the appropriate alkyl halide,[22,24] to give the corresponding 1,3-disubstituted-6-aminouracil, **5**. Standard nitrosation of **5(a–g)**, with sodium nitrite in acetic acid, was followed by reduction with sodium dithionite to achieve diaminouracils **7(a–g)**. Finally, condensation of diaminouracils **7** with an appropriate carboxylic acid in the presence of diisopropylcarbodiimide (DIC) in MeOH, and subsequent cyclization by heating under reflux with 2.5 N NaOH in MeOH afforded the xanthines **8**.

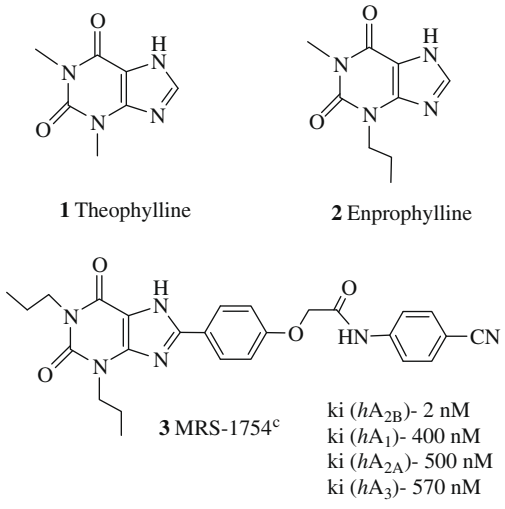


Chart 1. Xanthines with antiasthmatic (**1** and **2**) and selective A_{2B} AdoR antagonistic activities (**3**).

The 7-methylated derivatives **9a** and **9b** were obtained by methylation of **8b** and **8d** with excess methyl iodide in DMF in the presence of K_2CO_3. Oxidation of the xanthines **8q** and **8r** (Scheme 2) to the corresponding sulfoxides (**10a** and **10b**) and sulfones (**11a** and **11b**) was achieved by following previously reported literature procedures.[25]

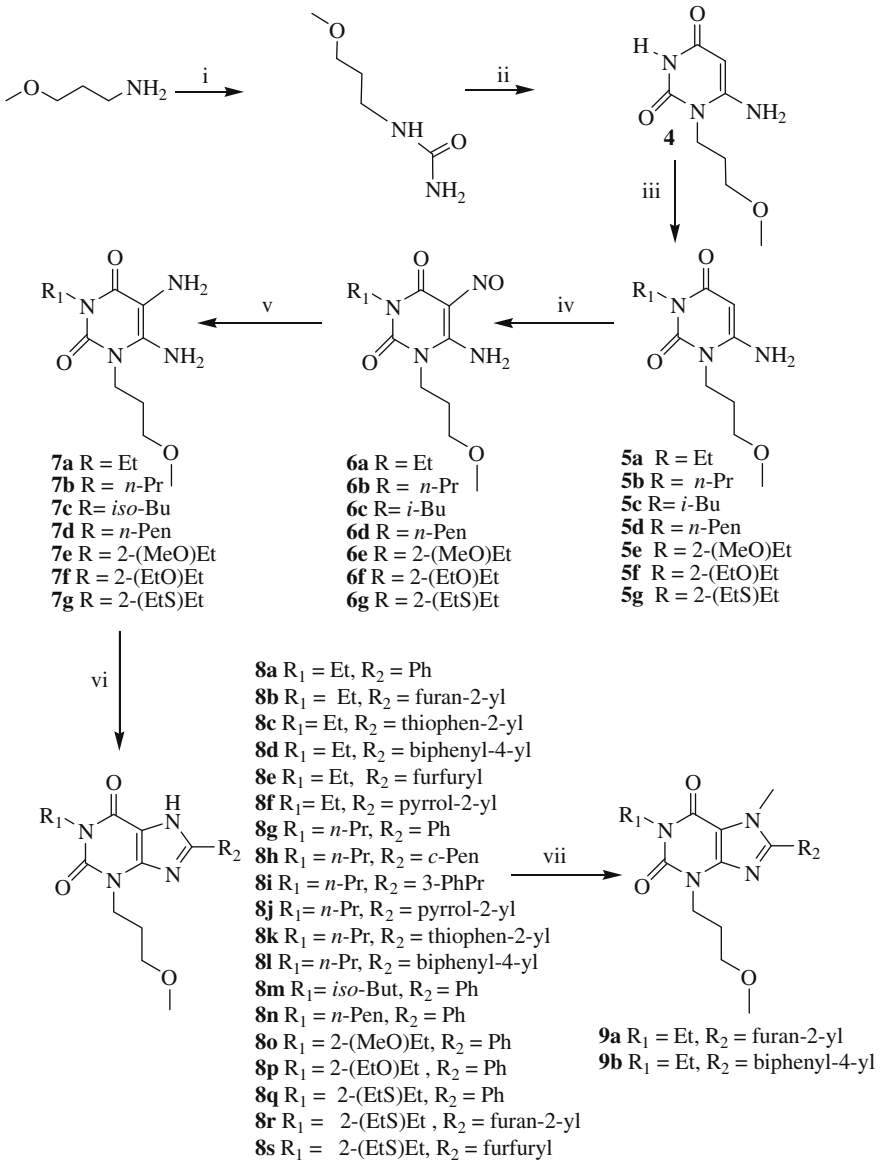
Compound **7a** was also converted into the series of xanthines **15a–15m**, which bear an amide group linked to the position 8 of the xanthine through a *para*-phenyloxymethyl unit. This transformation was achieved in a one-pot procedure, by reacting *p*-formylphenoxyacetic acid (**12**) with the corresponding amine in the presence of DIC, followed by treatment of the resulting, not isolated amides **13** with diamine **7a** in MeOH/AcOH at room temperature for 1 h, and then by oxidative cyclization of the benzylidene adduct **14** to form the imidazole ring present in **15a–15m** (Scheme 3). At our hands, this procedure proved to be not more time consuming than the alternative of preparing first a common intermediate, 2-{4-[1-ethyl-3-(3-methoxypropyl)-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl]phenoxy}acetic acid (**16**) to be condensed with each of a variety of amines, while these last condensations resulted in very low yields of compounds **15**.

3. Results and discussion

The affinity (pK_i or displacement percentage) values of the 3-(3-methoxypropyl)xanthine derivatives **8a–8s**, **9a**, **9b**, **10a**, **10b**, **11a**, **11b**, and cloned human A_{2A} and A_{2B} receptors, expressed in HeLa (A_{2A}) and HEK-293 (A_{2B}) cells, are given in Table 1.[26] These compounds showed moderate-to-low affinity for human A_{2A} and A_{2B} receptors expressed. The affinity values of compounds that did not fully displace specific radioligand binding are given only in terms of displacement percentage. However, the results shown in Table 1 enable certain trends to be deduced concerning the SAR in this group of xanthines. For example, in order for these compounds to show some level of affinity for the receptors in question they must contain a heteroaromatic unit in position 8 of the xanthine nucleus (compounds **8a**, **8l–8q**, in which a phenyl radical is in position 8, completely lack activity for both receptors). Similarly, the introduction of a methylene group between the heteroaromatic unit and the carbon at position 8 of the xanthine, cf. compounds **8b**/**8e** and **8r**/**8s**, leads to the loss of affinity for both receptors. Comparison of the affinity results for the pairs of compounds **8c**/**8k** and **8d**/**8l** shows that lengthening of the carbon chain in position 1 also leads to the loss of affinity.

The affinity (pK_i or displacement percentage) values of the 1-ethyl-3-(3-methoxypropyl)-9-susbtituted xanthine derivatives **15a–15m** at cloned human adenosine receptors expressed in HeLa (A_{2A} and A_3), and HEK-293 (A_{2B}) cells, and at rat A_1 receptors, are given in Table 2. The radioligand [^{3}H]DPCPX was used for competition binding assays on A_1 and A_{2B} receptors whereas [^{3}H]ZM241385 was used for A_{2A}, and [^{3}H]NECA for A_3.[26]

1-Ethyl-3-(3-methoxypropyl)-9-subtituted xanthine derivatives showed moderate-to-high affinity at human A_{2B} receptors with selectivity over A_{2A} receptors of up to 33.8-fold (compound **15d**). The affinity at rat A_1 and human A_3 receptors for most of these compounds showed lower pK_i values than those observed at A_{2B} receptors. It should be noted that several compounds were selective for A_{2B} receptors over A_{2A}, A_1 and A_3 receptors with selectivity indexes higher than 10-fold over each of the adenosine receptors evaluated (compounds **15b**, **15d**, **15g**, **15h** and **15k**). Compounds **15b** and **15d** proved to be excellent A_{2B} selective compounds with pK_i values higher than 8.5 at human A_{2B} receptors and a good selectivity profile over the other adenosine receptors. These



Scheme 1. Reagents and conditions: (i) KOCN, H_2SO_4, 85 °C, 1 h; (ii) (a) $NCCH_2CO_2H$, Ac_2O, 85 °C, 3 h, (b) 10% NaOH, EtOH, 80 °C, 1 h; (iii) RX, NaOH, EtOH; (iv) $NaNO_2$, AcOH rt, 2-24 h; (v) Na_2S2O_4, NH_4OH, 60 °C, 1 h; (vi) (a) R_2CO_2H, DIC, MeOH, rt, 0.5 h, (b) 2.5 N NaOH, MeOH, reflux, 10 min–9 h; (vii) CH_3I, K_2CO_3, DMF, 100 °C, 5 h.

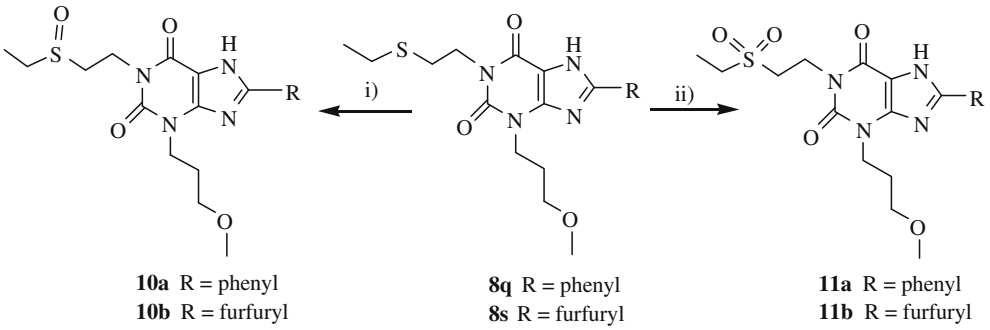
results indicate that, of the compounds synthesized, the best affinity and selectivity indexes are obtained when R is a phenylamino or bromophenylamino radical (**15b**, **15d**). The presence of other substituents on the phenylamino group, such as fluoro or cyano (**15d**, **15k**), leads to compounds that maintain similar affinity levels on the three types of receptor under investigation, albeit with a significant decrease in selectivity.

4. Conclusions

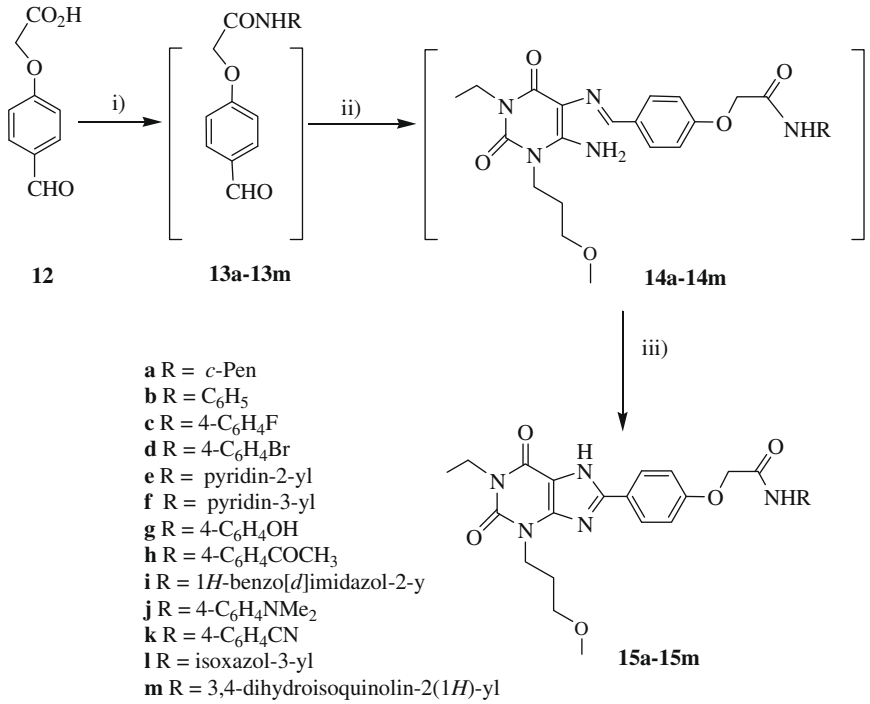
In summary, compounds **8a**–**8s**, **9a**, **9b**, **10a**, **10b**, **11a**, **11b** and **15a**–**15m** have been synthesized and their biological properties evaluated. Even though low affinity binding results were obtained for 3-(3-methoxypropyl)xanthine compounds (**8a**–**11b**), the 1-ethyl-3-(3-methoxypropyl)-9-subtituted xanthine derivatives **15a**–**15m** showed moderate-to-high affinity at human A_{2B} receptors with selectivity over A_{2A} receptors of up to 33.8-fold (compound **15d**). Even though low affinity binding results were obtained for these compounds, an important pool of compounds has been synthesized and characterized.

5. Experimental

All chemicals used were of reagent grade and were obtained from Aldrich Chemical Co. and used without further purification. When necessary, solvents were dried by standard techniques and distilled. All air-sensitive reactions were carried out under argon. Flash chromatography was performed on silica gel (Merck 60, 230–240 mesh) and analytical TLC was carried out on pre-coated silica gel plates (Merck 60 F_{254}, 0.25 mm) type E. Chromatographic spots were visualized by UV light or with Hanessian reagent.[27] Melting points (uncorrected) were measured in glass capillary tubes on a Stuart Scientific electro thermal apparatus SMP3. Infrared spectra were recorded on a Perkin–Elmer 1640 FTIR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, J in Hertz). All of the observed signals are consistent with the proposed structures. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the centre of the solvent peak. Coupling constants (J values) are given in Hertz (Hz). Spin multiplicities are



Scheme 2. Reagents and conditions: (i) Oxone^{R}, aliquat, CH_3CN, H_2O, CH_2Cl_2, rt; (ii) AcOH, 30% H_2O_2, rt.



Scheme 3. Reagents and conditions: (i) RNH_2, DIC, THF, 0 °C, 15 min; (ii) **7a**, MeOH, AcOH, rt, 1 h; (iii) *m*-CPBA, CH_3CN, rt, 24 h.

given as s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet), bs (broad singlet), dt (double triplet), q (quadruplet). Mass spectra were recorded on Hewlett–Packard HP5988A or Micromass Autospec spectrometers. Elemental analyses were performed in a FISONS EA 1108 Elemental Analyser at the University of Santiago Microanalysis Service; all results shown are within ±0.4% of the theoretical values (C, S, N, H).

5.1. General procedure for the preparation of 3-substituted 6-amino-1-(3-methoxypropyl)uracils 5a–5g

A mixture of 6-amino-1-(3-methoxypropyl)uracil (1 mmol), NaOH 15% (0.3 mL) and 95% EtOH (0.62 mL) was heated under reflux for 15 min and the corresponding alkylating agent RX (2 mmol) was added dropwise. The resulting solution was heated under reflux for 0.25–48 h, except in the preparation of **5g**, which was carried out at room temperature. The solvents were removed under reduced pressure and the residue was partitioned between $CHCl_3/H_2O$ (2/1, 6.3 mL). The organic layer was washed with water H_2O, dried (Na_2SO_4) and evaporated to dryness to give the corresponding 3-substituted 6-amino-1-(3-methoxypropyl)uracils **5a–5g**, which in most cases were used in subsequent steps without further purification. Unreacted starting material was recovered from the aqueous phase by evaporation to dryness.

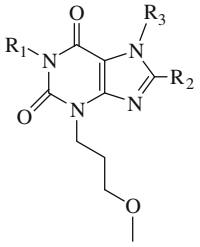
5.1.1. 6-Amino-3-ethyl-1-(3-methoxypropyl)uracil (5a)

Alkylating agent: ethyl iodide; reaction time 5 h; thick oil, yield 65%. ^{1}H NMR ($CDCl_3$): 5.43 (br s, 2H, D_2O exchan, NH_2), 4.93 (s, 1H, 5-H), 3.99–3.89 (m, 4H, 3′-H_2 and 1-H_2 C_2H_5), 3.42 (t, 2H, J = 5.6 Hz, 1′-H_2), 3.35 (s, 3H, OCH_3), 1.99 (qt, 2H, J = 5.7 Hz, 2′-H_2), 1.17 (t, 3H, J = 7.0 Hz, CH_3). ^{13}C NMR and DEPT ($CDCl_3$): 163.18 (C4), 154.97 (C6), 150.02 (C2), 78.76 (C5), 68.81 (C3′), 58.80 (OCH_3), 40.05 (C1 C_2H_5), 36.45 (C1′), 28.43 (C2′), 13.61 (CH_3). HRMS *m/z* calcd for $C_9H_{15}N_3O_3$, 213.1113; found, 213.1130.

5.2. General procedure for the preparation of 3-substituted 6-amino-1-(3-methoxypropyl)-5-nitrosouracils 6a–6g

A solution of $NaNO_2$ (18 mmol) in H_2O (7 mL) was added slowly over 15 min to a solution of the corresponding 3-substituted 6-amino-1-(3-methoxypropyl)uracil **5** (6 mmol) in 50% AcOH (30 mL) at 80 °C. The reaction mixture was stirred at room temperature. In cases where a precipitate was formed the solid was filtered off, washed with water and dried under vacuum. In cases

Table 1
Chemical structures and binding affinities[a] at human hA_{2A} and hA_{2B} AdoRs of 3-(3-methoxypropyl) xanthine derivatives



General Structure of compounds 8a–8s, 9a, 9b, 10a, 10b, 11a, 11b.

	R_1	R_2	R_3	hA_{2B}	hA_{2A}
8a	Ethyl	Phenyl	H	67%	49%
8b	Ethyl	Furan-2-yl	H	6.86	6.31
8c	Ethyl	Thiophen-2-yl	H	6.90	6.66
8d	Ethyl	Biphenyl-4-yl	H	7.15	6.28
8e	Ethyl	Furfuryl	H	17%	11%
8f	Ethyl	Pyrrol-2-yl	H	7.07	6.68
8g	Propyl	Phenyl	H	6.49	6.47
8h	Propyl	Cyclopentyl	H	62%	84%
8i	Propyl	3-Phenylpropyl	H	70%	26%
8j	Propyl	Pyrrol-2-yl	H	6.83	6.40
8k	Propyl	Thiophen-2-yl	H	77%	25%
8l	Propyl	Biphenyl-4-yl	H	66%	14%
8m	Isobutyl	Phenyl	H	30%	25%
8n	Pentyl	Phenyl	H	49%	0%
8o	2-Methoxyethyl	Phenyl	H	70%	54%
8p	2-Ethoxyethyl	Phenyl	H	21%	51%
8q	2-(Ethylthio)ethyl	Phenyl	H	41%	8%
8r	2-(Ethylthio)ethyl	Furan-2-yl	H	6.31	5.30
8s	2-(Ethylthio)ethyl	Furfuryl	H	5.7	1%
9a	Ethyl	Furan-2-yl	Methyl	1%	14%
9b	Ethyl	Biphenyl-4-yl	Methyl	6%	5%
10a	2-(Ethylsulfinyl)ethyl	Phenyl	H	11%	18%
10b	2-(Ethylsulfinyl)ethyl	Furfuryl	H	0%	12%
11a	2-(Ethylsulfonyl)ethyl	Phenyl	H	0%	20%
11b	2-(Ethylsulfonyl)ethyl	Furfuryl	H	15%	6%

[a] Binding affinity is expressed as pK_i or displacement percentage at 1 μM where indicated. pK_i and displacement percentage values had an SEM <10%.

where precipitation was only slight the aqueous solution was extracted with EtOAc. The organic phase was dried (Na_2SO_4), the solvent was removed under reduced pressure and the residue was crystallized from the appropriate solvent.

5.2.1. 6-Amino-3-ethyl-1-(3-methoxypropyl)-5-nitrosouracil (6a)

Reaction time 24 h; yield 76%, violet solid, mp = 150–152 °C (recrystallized from EtOAc). ^{1}H NMR ($CDCl_3$), 13.42 (br s, 2H D_2O exchan, NH_2), 4.14 (q, 2H, J = 7.0 Hz, 3′-H_2), 4.0 (t, 2H, J = 6.3 Hz, 1-H_2 C_2H_5), 3.45 (t, 2H, J = 5.6 Hz, 1′-H_2), 3.41 (s, 3H, OCH_3), 2.04 (qt, 2H, J = 5.9 Hz, 2′-H_2), 1.31 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR and DEPT ($CDCl_3$): 160.58 (C4), 149.63 (C6), 146.62 (C2), 138.79 (C5), 68.81 (C3′), 59.14 (OCH_3), 39.10 (C1′), 37.58 (C1 C_2H_5), 27.98 (C2′), 13.58 (CH_3). Anal. Calcd for $C_{10}H_{16}N_4O_4$ (256.26): C, 46.87; H, 6.29; N, 21.86. Found: C, 46.99; H, 6.43; N, 22.10.

5.3. General procedure for the preparation of 3-substituted 5,6-diamino-1-(3-methoxypropyl)uracils 7a–7g

$Na_2S_2O_4$ (16 mmol) was added in small portions to a well-stirred suspension of the corresponding 6-amino-1-(3-methoxypropyl)-5-nitrosouracil **6** (8 mmol) in 30% NH_4OH (25 mL) at 60 °C. On completion of the addition the reaction mixture was heated for 1 h and then cooled to 4–5 °C for 18 h. The resulting precipitate was filtered off, washed with H_2O and dried under vacuum. The volume of the filtrate was reduced to give a second crop of the corresponding diaminouracil.

5.3.1. 5,6-Diamino-3-ethyl-1-(3-methoxypropyl)uracil (7a)

Yield 76%, white solid, mp = 170–172 °C (recrystallized from EtOAc). ^{1}H NMR ($CDCl_3$): 5.56 (br s, 2H, D_2O exchan, NH_2), 4.02–3.93 (m, 4H, 3′-H_2 and 1-H_2 C_2H_5), 3.42 (t, 2H, J = 5.5 Hz, 1′-H_2), 3.38 (s, 3H, OCH_3), 2.26 (br s, 2H D_2O exchan, NH_2), 2.01 (qt, 2H, J = 5.9 Hz, 2′-H_2), 1.20 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C NMR and DEPT ($CDCl_3$): 161.83 (C4), 150.67 (C6), 150.51 (C2), 95.47 (C5), 68.74 (C3′), 58.80 (OCH_3), 40.63 (C1 C_2H_5), 36.88 (C1′), 28.33 (C2′), 13.65 (CH_3). Anal. Calcd for $C_9H_{16}N_4O_3$ (228.25): C, 47.36; H, 7.07; N, 24.55. Found: C, 47.62; H, 7.31; N, 24.89.

5.4. General procedure for the preparation of the xanthines 8a–8s

Diisopropylcarbodiimide (1 mmol) was added to a solution or suspension of the corresponding carboxylic acid (1 mmol) in anhydrous MeOH (2 mL) and this was followed by the addition of the appropriate diaminouracil **7a–7g** (1 mmol). The reaction mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure and the sticky residue was triturated with H_2O. The resulting yellow solid was filtered off and mixed with MeOH (3.5 mL) and 2.5 N NaOH (5 mL). The mixture was heated under reflux for the appropriate time in each case and allowed to cool down to room temperature. The solid was filtered off and the filtrate was adjusted to pH 6 by the addition of 2 N HCl. The corresponding 1*H*-purine-2,6(3*H*,7*H*)-dione **8** precipitated and was filtered off, washed with H_2O and purified by crystallization or washing with the appropriate solvent.

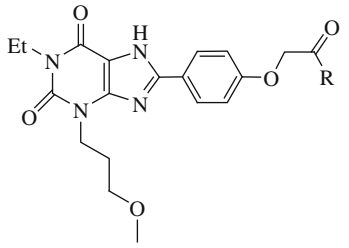
5.4.1. 1-Ethyl-3-(3-methoxypropyl)-8-phenyl-1*H*-purine-2,6(3*H*,7*H*)-dione (8a)

Reflux 10 min, yield 30%, white solid, mp = 239–241 °C (washed from MeOH). IR (KBr) ν (cm^{-1}): 3188, 2975, 2807, 1701, 1655, 1588, 1555, 1521, 1469, 1279, 1236, 1110, 1013, 818, 779, 762, 748, 520. ^{1}H NMR ($CDCl_3$): 13.08 (br s, 1H, D_2O exchan, NH), 8.35–8.32 (m, 2H, 2-H and 6-H C_6H_5), 7.51–7.48 (m, 3H, 3-H, 4-H and 5-H C_6H_5), 4.35 (t, J = 7.0 Hz, 2H, 3′-H_2), 4.24 (t, J = 7.0 Hz, 2H, 1-H_2 C_2H_5), 3.53 (t, J = 6.2 Hz, 2H, 1′-H_2), 3.34 (s, 3H, OCH_3), 2.19–2.10 (m, 2H, 2′-H_2), 1.37 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR and DEPT ($CDCl_3$): 156.11 (C8), 152.04 (C6), 151.00 (C2), 150.25 (C4), 131.01 (C3 and C5 C_6H_5), 129.21 (C1 C_6H_5), 129.19 (C4 C_6H_5), 127.40 (C2 and C6 C_6H_5), 108.56 (C5), 70.66 (C3′), 58.99 (OCH_3), 41.29 (C1′), 37.55 (C1 C_2H_5), 28.50 (C2′), 13.81 (CH_3). MS (EI) *m/z* (%): 328 (M, 41), 313 (51), 296 (10), 270 (17), 257 (74), 242 (23), 241 (15), 225 (14), 212 (34), 199 (17), 198 (100), 193 (12), 149 (12), 104 (67), 103 (13), 77 (15), 71 (14), 67 (14), 58 (12). Anal. Calcd for $C_{17}H_{20}N_4O_3$ (328.37): C, 62.18; H, 6.14; N, 17.06. Found: C, 62.55; H, 6.49; N, 17.02.

5.5. General procedure for the preparation of 8-{4-[(substituted)carbamoyl]methoxy]phenyl}-1-ethyl-3-(3-methoxypropyl)-1*H*-purine-2,6(3*H*,7*H*)-diones 15

Diisopropylcarbodiimide (4 mmol) was added to a solution of 4-formylphenoxyacetic acid (**12**, 4 mmol) in dry THF (30 mL) and the mixture was stirred for 10 min. The solution was cooled to 0 °C and the corresponding amine (4 mmol) was added in one portion and the mixture was stirred at this temperature for 15 min. The resulting *N*,*N*′-diisopropylurea was filtered off and the filtrate was evaporated to dryness. The residue was washed with hot H_2O. The solid was dissolved in MeOH (12 mL) and this solution was added to a solution of the diamine **7a** (4 mmol) in AcOH (2.5 mL) and MeOH (15 mL). The mixture was stirred for 1 h. The solid was filtered

Table 2
Chemical structures and binding affinities[a] at human A_{2B}, A_{2A}, A_3 and rat A_1 AdoRs of 1-ethyl-3-(3-methoxypropyl)-8-susbtituted xanthine derivatives



General Structure of compounds 15a–15m

	R	hA_{2B}	hA_{2A}	rA_1	hA_3	A_{2B}/A_{2A}[c] ratio	A_{2B}/A_1 ratio	A_{2B}/A_3 ratio
15a	Cyclopentylamino	7.11	7.02	n.d.[b]	n.d.	1.20	n.c.[d]	n.c.
15b	Phenylamino	8.52	7.06	7.44	5.60	28.8	13.8	832
15c	4-Fluorophenylamino	8.52	7.25	7.68	6.02	18.6	6.92	316
15d	4-Bromophenylamino	8.54	7.01	7	5.89	33.8	34.7	447
15e	Pyridin-2-ylamino	7.81	6.77	2%	69%	11.0	n.c.	n.c.
15f	Pyridin-3-ylamino	7.47	6.12	6.79	7.02	22.4	4.79	2.82
15g	4-Hydroxyphenylamino	7.81	6.38	6.76	65%	26.9	11.2	n.c.
15h	4-Acetylphenylamino	7.93	6.83	68%	6.15	12.6	n.c.	60.3
15i	1*H*-benzo[*d*]imidazol-2-ylamino	7.03	6.69	n.d.	n.d.	2.20	n.c.	n.c.
15j	4-(Dimethylamino)phenylamino	6.76	5.75	6.06	6.68	10.0	5.05	1.20
15k	4-Cyanophenylamino	8.04	6.95	6.17	6.4	12.3	74.1	44.7
15l	Isoxazol-3-ylamino	7.68	6.67	7.02	6.03	10.2	n.c.	n.c.
15m	3,4-Dihydroisoquinolin-2(1*H*)-yl	7.31	6.64	7.0	n.d.	5.00	n.c.	n.c.

[a] Binding affinity is expressed as pK_i or displacement percentage at 1 μM where indicated. pK_i and displacement percentage values had an SEM <10%.
[b] n.d.: not determined.
[c] Affinity ratios were calculated on the basis of K_i values.
[d] n.c.: not calculated due to the low affinity of the compounds at one of the receptors assayed.

off in cases where the amide precipitated from the reaction medium. In cases where a precipitate did not form, the solvents were removed under reduced pressure. In all cases, the residue (**14**) obtained (1 mmol) was suspended in CH_3CN (25 mL) and MCPBA (1 mmol) was added. The reaction mixture was stirred for 24 h and the resulting solid was filtered off.

5.5.1. 8-{4-[(Cyclopentylcarbamoyl)methoxy]phenyl}-1-ethyl-3-(3-methoxypropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (15a)

Yield 20%, beige solid, mp = 274–275 °C (recrystallized from DMF). IR (KBr) ν (cm^{-1}): 3287, 3166, 2970, 2871, 1699, 1655, 1612, 1560, 1478, 1366, 1236, 1183, 835, 763. ^{1}H NMR (DMSOd_6): 13.43 (br s, 1H, D_2O exchan, NH), 10.24 (br s, 1H, NH), 8.08 (d, J = 8.8 Hz, 2H, 2-H and 6-H C_6H_4), 7.08 (d, J = 8.8 Hz, 2H, 3-H and 5-H C_6H_4), 4.54 (s, 2H, OCH_2), 4.20–4.15 (m, 3H, 3′-H_2 and 1-H *c*-C_5H_9), 4.12–3.92 (m, 2H, 1-H_2 C_2H_5), 3.98–3.65 (m, 2H, 1′-H_2), 3.31 (s, 3H, OCH_3), 2.53–2.49 (m, 4H, *c*-C_5H_9), 1.85–1.82 (m, 2H, 2′-H_2), 1.82–1.65 (m, 4H, *c*-C_5H_9), 1.16 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.92 (CONH), 159.62 (C4 C_6H_4), 154.36 (C8), 150.64 (C6), 150.37 (C2), 147.21 (C4), 128.68 (C2 and C6 C_6H_4), 122.42 (C1 C_6H_4), 115.33 (C3 and C5 C_6H_4), 108.01 (C5), 69.32 (C3′), 66.98 (OCH_2), 58.42 (OCH_3), 41.22 (C1′), 40.65 (C1 *c*-C_5H_9), 36.05 (C1 C_2H_5), 32.43 (C2 and C5 *c*-C_5H_9), 28.90 (C3 and C4 *c*-C_5H_9), 28.05 (C2′), 12.54 (CH_3). MS (EI) *m/z* (%): 470 (12), 469 (M, 46), 454 (24), 398 (15), 339 (11), 214 (11), 120 (12), 84 (12), 71 (28), 70 (17), 69 (91), 68 (43), 67 (52), 58 (100), 57 (24), 56 (34), 55 (16), 54 (13), 53 (11). Anal. Calcd for $C_{24}H_{31}N_5O_5$ (469.54): C, 61.39, H, 6.65; N, 14.92. Found: C, 60.99; H, 6.85; N, 15.13.

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Supplementary data

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