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Synthesis and pharmacological evaluation of novel 1,3,8- and 1,3,7,8-substituted xanthines as adenosine receptor antagonists

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ABSTRACT

A number of novel xanthines bearing a variety of substituents at positions 1, 3, 7 and 8 were prepared and evaluated for their binding affinity to the human adenosine receptor A_1, A_{2A}, A_{2B} and A_3 subtypes. Several of the 1,3,8- and 1,3,7,8-substituted xanthines showed moderate-to-high affinity at human A_{2B} and A_1 receptors, with the most active compound (**14q**) having a pK_i of 7.57 nM for hA_{2B} receptors and a selectivity over hA_{2A} receptors of 8.1-fold and hA_1 receptors of 3.7-fold.

1. Introduction

Adenosine is an endogenous non-selective agonist that activates all four subtypes of adenosine receptors (AdoR): A_1, A_{2A}, A_{2B} and A_3.[1] Adenosine receptors have been recognized as playing an important role in chronic inflammatory airway conditions such as asthma, chronic obstructive pulmonary disease and fibrosis.[2,3] Experimental evidence, such as the increase in the adenosine concentration in hypoxia and cellular inflammation in the bronchoalveolar fluids of asthmatics and in plasma (upon contact with allergens), has highlighted the key role that adenosine and its A_{2B} receptors play in asthma.[4–6] Theophylline (**1**), which has a well-established role in the therapy of asthma, selectively blocks the AMP-induced bronchoconstriction in asthmatics. The bronchodilating effect of theophylline and its structural analogue enprofylline (**2**) has been attributed to a selective antagonism of the A_{2B}-AdoR.[6] In addition, the recent discovery that A_{2B}-AdoR are functionally active on both human airway smooth muscle cells and lung fibroblast cells provides further support for the role of A_{2B}-AdoR in inflammation and asthma.[4,7] Therefore, antagonists at the A_{2B}-AdoR would provide a novel approach to the management and treatment of asthma and chronic obstructive pulmonary disease (Fig. 1).

Despite the fact that a number of high-affinity A_{2B}-AdoR antagonists have been reported, very few have shown high affinity and selectivity for A_{2B}-AdoR relative to A_1-, A_{2A}- and A_3-AdoR.[8–11] The xanthine-based A_{2B} antagonists currently show the most promising combination of high affinity and selectivity[12] and, as a result, in recent years new and more selective high-affinity A_{2B} antagonists with a xanthine scaffold have been developed. These compounds include **3** (PSB-601),[13] **4** (PSB-603; first A_{2B} antagonist with subnanomolar affinity)[14] and **5** (CVT-6883).[15] Compound **5** is currently being evaluated for cardiopulmonary disease in a phase I clinical study and is the first A_{2B} antagonist in clinical trials.[15]

Recently, we reported a series of a new 1,3,8-trisubstituted xanthines (compounds **6** and **7**; Fig. 2) some of which showed interesting affinity values and selectivity for A_{2B}-AdoR.[16,17]

The work reported here was carried out as part of a line of investigation directed at finding a potentially selective, high affinity A_{2B}-AdoR antagonist through the preparation of a series of xanthines bearing appropriate and/or more substituents in several positions of the xanthine nucleus. The new adenosine analogues of xanthine have structural variations at position 1 (alkyl or functionalized alkyl substituents), position 3 (alkyl or functionalized alkyl or aryl or heteroaryl substituents), position 7 (unsubstituted or methyl substituent) and position 8 (aryl or heteroaryl substituents) of the xanthine nucleus (Schemes 1 and 2).

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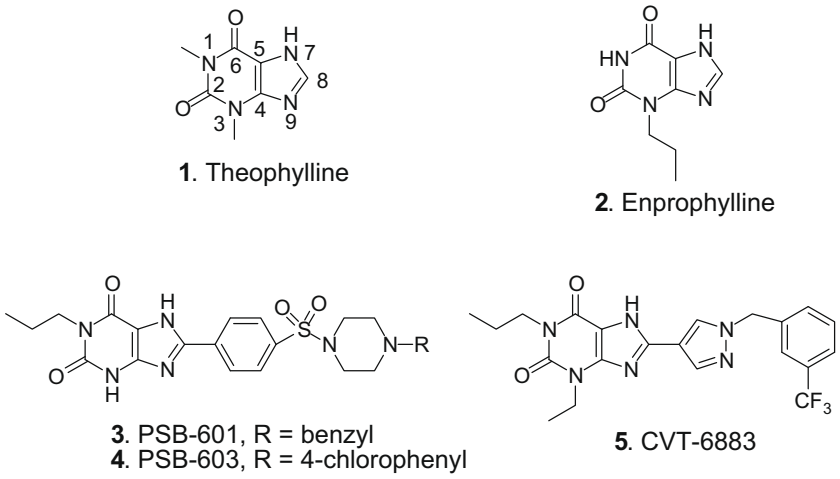
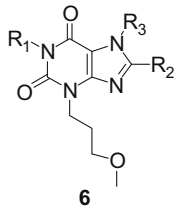
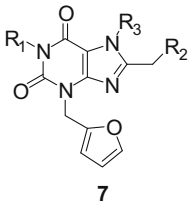


Figure 1. Classic and prototypical xanthine-derived A2B receptor antagonists.



6

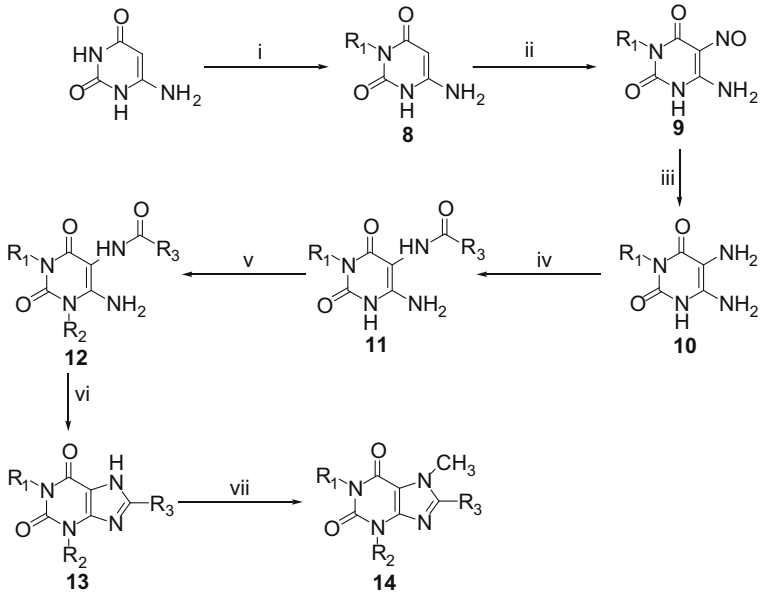
R_1 = ethyl, propyl, isobutyl, pentyl, 2-methoxyethyl, 2-ethoxyethyl, 2-(ethylthio)ethyl,2-(ethylsulfinyl)ethyl, 2-(ethylsulfonyl)ethyl.
R_2 = phenyl, furan-2-yl, thiophen-2-yl, biphenyl-4-yl, furfuryl, pyrrol-2-yl, cyclopentyl, 3-phenylpropyl.
R_3 = H, methyl.



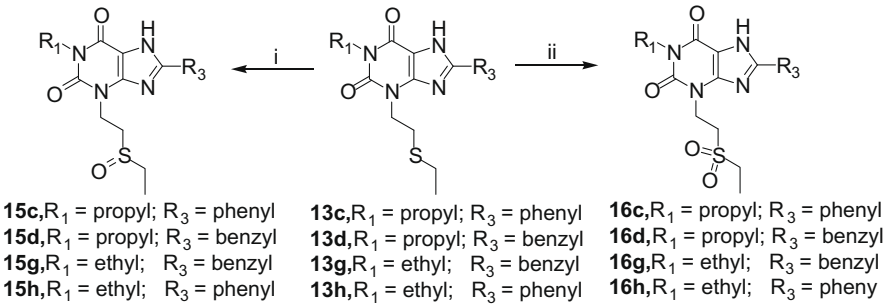
7

R_1 = methyl, ethyl, propyl, isobutyl, pentyl, cyclopropylmethyl, prop-2-ynyl, allyl, 2-methoxyethyl, 2-ethoxyethyl, 2-(methylthio)ethyl,2-(ethylthio)ethyl, 2-(ethylsulfinyl)ethyl, 2-(ethylsulfonyl)ethyl.
R_2 = phenyl, furan-2-yl, thiophen-2-yl, 2,6-difluorophenyl.
R_3 = H, methyl.

Figure 2.



Scheme 1. Reagents and conditions: (i) (a) HMDS, $(NH_4)_2SO_4$, reflux, 2 h, (b) R_1X, toluene, reflux, 90 min, (c) $Na_2S_2O_3$, H_2O, rt; (ii) $NaNO_2$, 50% aq AcOH, 80 °C, 45 min; (iii) $Na_2S_2O_4$, 35% aq NH_4OH, 60 °C, 1 h; (iv) R_3CO_2H, DIC, MeOH, rt, 1 h; (v) R_2X, K_2CO_3, DMF, 50 °C, 48 h; (vi) NaOH, MeOH, reflux, 2 h; (vii) CH_3I, K_2CO_3, DMF, 100 °C, 5 h.



Scheme 2. Reagents and conditions: (i) Oxone®, aliquat, CH_3CN, H_2O, CH_2Cl_2, rt. (ii) AcOH, 30% H_2O_2, rt.

2. Chemistry

The synthesis of the target 1,3,8-trisubstituted-1*H*-purine-2,6-(3*H*,9*H*)-diones, (**13** and **14**) is outlined in Scheme 1. Firstly, 3-substituted 6-aminouracils, **8**, were prepared by alkylation of silylated 6-aminouracil with alkyl iodides in high yields via a silylated intermediate.[18] Standard nitrosation of compounds **8** with sodium nitrite in acetic acid leading to compounds **9**, was followed by reduction with sodium dithionite to give 3-substituted 5,6-diaminouracils **10**.[19] These compounds were subsequently condensed with the corresponding carboxylic acid to introduce the 8-substituent of the xanthines to afford compounds **11**.[20] Alkylation or arylalkylation of uracil derivatives **11** was easily performed under mild conditions in dimethylformamide using alkyl or arylalkyl bromides or iodides and potassium carbonate.[20] Finally, the imidazole ring was cyclized by heating under reflux a mixture of compounds **12** and 2.5 N NaOH in MeOH to afford xanthines **13a–t**.[16,17,20]

The 7-methylated derivatives **13c**, **13e** and **13m–r** were obtained by methylation of the corresponding xanthines with excess methyl iodide in dimethylformamide in the presence of potassium carbonate.[16,17,20]

Oxidation of the corresponding xanthines to the sulfoxides **15c**, **15d**, **15g** and **15h** and sulfones **16c**, **16d**, **16g** and **16h** (Scheme 2) was achieved by previously reported literature procedures.[21]

3. Results and discussion

The affinity (pK_i or displacement percentage) values of the xanthine derivatives **13**, **14**, **15** and **16**, at cloned human adenosine receptors expressed in CHO (hA_1), HeLa cells (hA_{2A} and hA_3) and HEK-293 cells (hA_{2B}), are given in Table 1.[22] The radioligand [^{3}H]DPCPX was used for competition binding assays on A_1 and A_{2B} receptors, [^{3}H]ZM241385 was used for A_{2A} receptors whereas [^{3}H]NECA was used for A_3 receptors. The affinity values of compounds that did not fully displace specific radioligand binding at 1 μM are given only in terms of displacement percentage. These compounds showed moderate-to-low affinity for expressed human A_1, A_{2A}, A_{2B} and A_3 receptors. However, the results shown in Table 1 enable certain trends to be highlighted concerning the SAR in this group of xanthines.

The 1,3,8-substituted xanthine derivatives (**13a–t**) showed moderate-to-low affinity for A_1 and A_{2B} receptors and even lower affinity for A_3 and A_{2A} receptors. The best results were obtained on introducing an ethyl group in position 1 of the xanthine ring, a thiophen-2-ylmethyl or 2-(ethylthio)ethyl group in position 3 and an aromatic substituent bonded directly or a heteroaromatic unit attached through a methylene bridge to position 8 of the xanthine ring. Of the 20 compounds prepared, only **13p** (R_1 = ethyl, R_2 = R_3 = thiophen-2-ylmethyl) had an interesting A_{2B}/A_{2A} selectivity level (28.9), although the affinity values for both receptors are reasonably low and in any case very similar for the different AdoRs assayed.

A series of 8-methyl derivatives was prepared with the aim of assessing the influence of methylation of the nitrogen at position 7 of the xanthine ring. The results indicate that methylation of position 7 has a different effect depending on the receptor. For example, in the case of the A_3 receptors a significant decrease in the affinity levels was observed whereas the affinity values for the A_1 receptors showed irregular behavior. This is exemplified by compounds **14c** and **14e** (R_1 = propyl), which showed extremely low affinity in comparison to the demethylated analogues (**13c** and **13e**), and compounds **14m**, **14p**, **14q** and **14r** (R_1 = ethyl), for which the affinity values showed a marked increase. For the A_{2B} and A_{2A} receptors a significant increase in affinity levels was obtained (**14m**, **14n**, **14p**, **14q** and **14r**). However, this increase in affinity was accompanied by a significant decrease in A_{2B}/A_{2A} selectivity. The best results were obtained for compound **14q** (R_1 = ethyl, R_2 = thiophen-2-ylmethyl, R_3 = furfuryl, R_4 = methyl), which showed pK_i values of 7.57 and 6.66 against A_{2B} and A_{2A}, respectively, and had a $K_i(A_{2B})/K_i(A_{2A})$ ratio of 8.1-fold, and compound **14n** (R_1 = ethyl, R_2 = benzyl, R_3 = furfuryl, R_4 = methyl), which showed pK_i values of 6.55 and 5.76 against A_{2B} and A_{2A}, respectively, and had a $K_i(A_{2B})/K_i(A_{2A})$ ratio of 6.2-fold.

Finally, oxidation of the sulfur atom of the 2-(ethylthio)ethyl chain (**13c**, **13d**, **13g** and **13h**) resulted in a significant decrease in the affinity at all the AdoRs assayed (**15c**, **15d**, **15g** and **15h**; **16c**; **16d**, **16g** and **16h**).

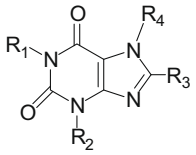
4. Conclusions

In summary, a set of novel xanthine derivatives (compounds **13a–t**, **14c**, **14e**, **14m–r**, **15c–d**, **15g–h**, **16c–d** and **16g–h**) has been synthesized and their affinities for A_1, A_{2A}, A_{2B} and A_3 receptors have been evaluated. These compounds showed moderate-to-low affinity for all receptors with low levels of selectivity, with the exception of compound **13p** [1-ethyl-3,8-bis(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione; A_{2A}/A_{2B} ratio = 28.9, A_1/A_{2B} ratio = 4.9; A_3/A_{2B} ratio = 5.9]. The biological results also show that the presence of an additional methyl group at position 7 (**14m–n** and **14p–r**) is beneficial for A_1, A_{2B} and A_{2A} affinities. This result confirms the trend found in previous studies carried out by our group.[17] Despite the moderate-to-low affinity results obtained for these compounds, an important pool of new xanthine derivatives has been synthesized, characterized an evaluated biologically.

5. Experimental

All chemicals were of reagent grade and were obtained from Aldrich Chemical Co. and used without further purification. Where 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

Table 1
Chemical structures and binding affinities[a] at hA_1, hA_{2A}, hA_{2B} and hA_3 AdoRs of xanthine derivatives **13**, **14**, **15** and **16**



General structure of compounds 13, 14, 15 and 16

Compound	R_1	R_2	R_3	R_4	hA_1	hA_{2A}	hA_{2B}	hA_3	A_{2B} selectivity		
									hA_{2A}/hA_{2B}	hA_1/hA_{2B}	hA_3/hA_{2B}
13a	Propyl	Ethoxyethyl	Phenyl	H	39%	6%	24%	47%	–	–	–
13b	Propyl	Ethoxyethyl	Benzyl	H	22%	13%	5%	2%	–	–	–
13c	Propyl	2-(Ethylthio)ethyl	Phenyl	H	7.03	5.84	6.72	6.93	7.6	0.5	0.6
13d	Propyl	2-(Ethylthio)ethyl	Benzyl	H	33%	8%	12%	1%	–	–	–
13e	Propyl	Tetrahydrofuran-2-ylmethyl	Phenyl	H	6.65	37%	64%	6.87	–	–	–
13f	Propyl	Tetrahydrofuran-2-ylmethyl	Benzyl	H	2%	24%	32%	10%	–	–	–
13g	Ethyl	2-(Ethylthio)ethyl	Benzyl	H	34%	0%	5%	10%	–	–	–
13h	Ethyl	2-(Ethylthio)ethyl	Phenyl	H	6.83	12%	6.32	6.39	–	0.3	0.9
13i	Methyl	Cyclohexylmethyl	Thiophen-2-ylmethyl	H	42%	11%	2%	2%	–	–	–
13j	Methyl	Cyclohexylmethyl	Thiophen-2-yl	H	6.66	29%	34%	41%	–	–	–
13k	Cyclohexylmethyl	Cyclohexylmethyl	Thiophen-2-ylmethyl	H	4%	50%	1%	16%	–	–	–
13l	Cyclohexylmethyl	Cyclohexylmethyl	Thiophen-2-yl	H	2%	14%	10%	2%	–	–	–
13m	Ethyl	Benzyl	Thiophen-2-ylmethyl	H	22%	6%	52%	39%	–	–	–
13n	Ethyl	Benzyl	Furfuryl	H	23%	9%	34%	30%	–	–	–
13o	Ethyl	Benzyl	2,6-Difluorobenzyl	H	23%	1%	8%	1%	–	–	–
13p	Ethyl	Thiophen-2-ylmethyl	Thiophen-2-ylmethyl	H	6.17	5.40	6.86	6.09	28.9	4.9	5.9
13q	Ethyl	Thiophen-2-ylmethyl	Furfuryl	H	6.39	19%	30%	42%	–	–	–
13r	Ethyl	Thiophen-2-ylmethyl	2,6-Difluorobenzyl	H	28%	21%	52%	38%	–	–	–
13s	Ethyl	2-(Ethylthio)ethyl	Biphenyl-4-yl	H	7%	4%	22%	17%	–	–	–
13t	Ethyl	2-Ethoxyethyl	Biphenyl-4-yl	H	19%	28%	46%	17%	–	–	–
14c	Propyl	2-(Ethylthio)ethyl	Phenyl	Methyl	17%	2%	26%	1%	–	–	–
14e	Propyl	Tetrahydrofuran-2-ylmethyl	Phenyl	Methyl	38%	25%	41%	6.20	–	–	–
14m	Ethyl	Benzyl	Thiophen-2-ylmethyl	Methyl	6.41	6.12	6.43	6%	2.1	1.1	–
14n	Ethyl	Benzyl	Furfuryl	Methyl	29%	5.76	6.55	14%	6.2	–	–
14o	Ethyl	Benzyl	2,6-Difluorobenzyl	Methyl	21%	28%	48%	4%	–	–	–
14p	Ethyl	Thiophen-2-ylmethyl	Thiophen-2-ylmethyl	Methyl	7.02	6.91	7.45	1%	3.5	2.7	–
14q	Ethyl	Thiophen-2-ylmethyl	Furfuryl	Methyl	7.00	6.66	7.57	22%	8.1	3.7	–
14r	Ethyl	Thiophen-2-ylmethyl	2,6-Difluorobenzyl	Methyl	6.92	6.75	6.87	20%	1.3	0.9	–
15c	Propyl	2-(Ethylsulfinyl)ethyl	Phenyl	H	6.71	9%	6.23	6.61	–	–	0.4
15d	Propyl	2-(Ethylsulfinyl)ethyl	Benzyl	H	10%	7%	1%	18%	–	–	–
15g	Ethyl	2-(Ethylsulfinyl)ethyl	Benzyl	H	5%	0%	0%	3%	–	–	–
15h	Ethyl	2-(Ethylsulfinyl)ethyl	Phenyl	H	29%	20%	32%	0%	–	–	–
16c	Propyl	2-(Ethylsulfonyl)ethyl	Phenyl	H	7.03	3%	39%	7.03	–	–	–
16d	Propyl	2-(Ethylsulfonyl)ethyl	Benzyl	H	15%	13%	2%	15%	–	–	–
16g	Ethyl	2-(Ethylsulfonyl)ethyl	Benzyl	H	6%	1%	55%	6%	–	–	–
16h	Ethyl	2-(Ethylsulfonyl)ethyl	Phenyl	H	35%	0%	18%	35%	–	–	–

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

silica gel plates (Merck 60 F_{254}, 0.25 mm) type E. Chromatographic spots were visualized by UV light or with Hanessian reagent.[23] Melting points (uncorrected) were measured in glass capillary tubes on a Stuart Scientific SMP3 electro thermal apparatus. 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 center of the solvent peak. Coupling constants (J values) are given in hertz (Hz). Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet), br s (broad singlet), v s (virtual singlet), dt (doublet of triplets), q (quartet), qt (quintet), sex (sextet). 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, N, H).

5.1. General procedure for the preparation of xanthines 13

The carboxamide **12** (3 mmol) was dissolved in a mixture of MeOH (100 mL) and 20% aqueous NaOH (20 mL) and the mixture was heated under reflux for 2 h. After cooling, the solution was acidified to pH 4–5 with concentrated HCl. The resulting precipitate was filtered off, washed with H_2O, and air-dried under vacuum to afford xanthines **13**.

5.1.1. 3-(2-Ethoxyethyl)-8-phenyl-1-propyl-1*H*-purine-2,6-(3*H*,9*H*)-dione, 13a

Yield 88%. White solid. Mp = 227–229 °C (H_2O). IR (KBr) ν (cm^{-1}) = 3166, 2967, 2880, 1695, 1654, 1555, 1523, 1470. ^{1}H NMR (DMSO): 13.62 (br s, 1H, D_2O exchang. NH), 8.20–8.10 (m, 2H, ArH), 7.57–7.47 (m, 3H, ArH), 4.21–4.13 (t, J = 6.0 Hz, 2H, 1-H_2 Pr), 3.96–3.88 (t, J = 7.4 Hz, 2H, 1′-H_2), 3.78–3.70 (t, J = 6.0 Hz, 2H, 2′-H_2), 3.52–3.40 (q, J = 7.0 Hz, 2H, 4′-H_2), 1.66–1.56 (q, J = 7.3 Hz, 2H, 2-H_2 Pr), 1.12–1.08 (t, J = 7.0 Hz, 3H, 5′-H_2), 0.92–0.88 (t, J = 7.4 Hz, 3H, CH_3 Pr). Anal. Calcd for $C_{18}H_{22}N_4O_3$

(342.39): C, 63.14; H, 6.48; N, 16.36. Found: C, 63.35; H, 6.21; N, 16.11.

5.1.2. 8-Benzyl-3-(2-ethoxyethyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 13b

Yield 85%. White solid. Mp 165–166 °C (H_2O). IR (KBr) ν (cm^{-1}) = 3156, 2967, 2867, 1702, 1645, 1495. ^1H NMR (DMSO): 13.66 (br s, 1H, D_2O exchang. NH), 7.30–7.22 (m, 5H, ArH), 4.14–4.09 (t, J = 6.0 Hz, 2H, 1-H_2 Pr), 4.04 (s, 2H, CH_2–Ph), 3.85–3.79 (t, J = 7.4 Hz, 2H, 1′-H_2), 3.65–3.60 (t, J = 6.0 Hz, 2H, 2′-H_2), 3.46–3.38 (q, J = 7.0 Hz, 2H, 4′-H_2), 1.58–1.52 (q, J = 7.3 Hz, 2H, 2-H_2 Pr), 1.00–0.94 (t, J = 7.0 Hz, 3H, 5′-H_2), 0.88–0.82 (t, J = 7.4 Hz, 3H, CH_3 Pr). Anal. Calcd for $C_{19}H_{24}N_4O_3$ (356.42): C, 63.97; H, 6.73; N, 15.71. Found: C, 64.13; H, 6.51; N, 15.94.

5.1.3. 3-(2-(Ethylthio)ethyl)-8-phenyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 13c

Yield 56%. White solid. Mp 223–225 °C (H_2O). IR (KBr) ν (cm^{-1}) = 3184, 2962, 1726, 1697, 1651, 1553, 1467. ^1H NMR (DMSO): 13.86 (br s, 1H, D_2O exchang. NH), 8.12–8.09 (m, 2H, 2-H and 6-H ArH), 7.50–7.48 (m, 3H, 3-H, 4-H and 5-H ArH), 4.23–4.19 (t, J = 7.3 Hz, 2H, 1-H_2 Pr), 3.88–3.83 (t, J = 7.3 Hz, 2H, 1′-H_2), 2.89–2.85 (t, J = 7.3 Hz, 2H, 2′-H_2), 2.66–2.59 (q, J = 7.3 Hz, 2H, 4′-H_2), 1.61–1.54 (q, J = 7.3 Hz, 2H, 2-H_2 Pr), 1.23–1.18 (t, J = 7.3 Hz, 3H, 5′-H_2), 0.89–0.84 (t, J = 7.3 Hz, 2H, CH_3 Pr). ^{13}C NMR and DEPT (DMSO): 154.37 (C8), 150.93 (C6), 150.26 (C2), 148.41 (C4), 130.62 (C4 Ph), 129.31 (C3 and C5 Ph), 128.87 (C1 Ph), 126.72 (C2 and C6 Ph), 108.65 (C5), 54.15 (NCH_2), 42.52 (C1 Pr), 28.20 (CH_2CH_2S), 24.88 (S–CH_2), 21.18 (C2 Pr), 14.81 (SCH_2CH_3), 11.50 (CH_3 Pr). Anal. Calcd for $C_{18}H_{22}N_4O_2S$ (358.46): C, 60.31; H, 6.19; N, 15.63. Found: C, 60.15; H, 6.31; N, 15.82.

5.1.4. 8-Benzyl-3-(2-(ethylthio)ethyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 13d

Yield 51%. White solid. Mp 256–258 °C (H_2O). IR (KBr) ν (cm^{-1}) = 3152, 2963, 1700, 1645, 1556, 1497. ^1H NMR (DMSO): 13.52 (br s, 1H, D_2O exchang. NH), 7.28–7.16 (m, 5H, ArH), 4.12–4.07 (t, J = 7.4 Hz, 2H, 1-H_2 Pr), 4.04 (s, 2H, CH_2–Ph), 3.82–3.77 (t, J = 7.3 Hz, 2H, 1′-H_2), 2.80–2.75 (t, J = 7.3 Hz, 2H, 2′-H_2), 2.55–2.47 (q, J = 7.4 Hz, 2H, 4′-H_2), 1.58–1.46 (sext, J = 7.4 Hz, 2H, 2-H_2 Pr), 1.14–1.09 (t, J = 7.4 Hz, 3H, 5′-H_2), 0.85–0.80 (t, J = 7.4 Hz, 2H, CH_3 Pr). ^{13}C NMR and DEPT (DMSO): 154.21 (C8), 152.98 (C6), 150.91 (C2), 137.37 (C4), 128.95 (C4 Ph), 128.82 (C3 and C5 Ph), 126.99 (C2 and C6 Ph), 106.63 (C5), 42.43 (NCH_2), 42.36 (C1 Pr), 34.61 (CH_2Ph), 28.22 (CH_2CH_2S), 24.87 (S–CH_2), 21.17 (C2 Pr), 14.76 (SCH_2CH_3), 11.48 (CH_3 Pr). Anal. Calcd for $C_{19}H_{24}N_4O_2S$ (372.48): C, 61.27; H, 6.49; N, 15.04. Found: C, 61.41; H, 6.28; N, 15.19.

5.1.5. 8-Phenyl-1-propyl-3-((tetrahydrofuran-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13e

Yield 90%. White solid. Mp 261–262 °C (H_2O). IR (KBr) ν (cm^{-1}) = 3188, 2962, 1699, 1655, 1520, 1470. ^1H NMR (DMSO): 13.45 (br s, 1H, D_2O exchang. NH), 8.14–8.11 (m, 2H, ArH), 7.52–7.50 (m, 3H, ArH), 4.35–3.62 (m, 6H, 2′-H_2 + 1″-H_2 Pr + +5′-H_2), 1.95–1.54 (m, 6H, 3′-H_2 + 4′-H_2 + 2″-H_2 Pr), 0.91–0.85 (t, J = 7.3 Hz, 3H, CH_3 Pr). Anal. Calcd for $C_{19}H_{22}N_4O_3$ (354.41): C, 64.39; H, 6.26; N, 15.81. Found: C, 64.07; H, 6.59; N, 15.71.

5.1.6. 8-Benzyl-1-propyl-3-((tetrahydrofuran-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13f

Yield 74%. White solid. Mp 186–187 °C (H_2O). IR (KBr) ν (cm^{-1}) = 3033, 2965, 1701, 1653, 1557, 1500. ^1H NMR (DMSO): 13.43 (br s, 1H, D_2O exchang. NH), 7.31–7.22 (m, 5H, ArH), 4.27–3.56 (m, 6H, 2′-H_2 + 1″-H_2 Pr + +5′-H_2), 4.04 (s, 2H, CH_2Ph), 1.91–1.49 (m, 6H, 3′-H_2 + 4′-H_2 + 2″-H_2 Pr), 0.88–0.82 (t, J = 7.4 Hz, 3H, CH_3 Pr). Anal. Calcd for $C_{20}H_{24}N_4O_3$ (368.43): C, 65.20; H, 6.57; N, 15.21. Found: C, 64.96; H, 6.77; N, 15.49.

5.1.7. 8-Benzyl-1-ethyl-3-(2-(ethylthio)ethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13g

Yield 79%. White solid. Mp 191–193 °C (DMF/H_2O). IR (KBr) ν (cm^{-1}) = 3031, 2974, 1696, 1654, 1557, 1500. ^1H NMR (DMSO): 13.54 (br s, 1H, D_2O exchang. NH), 7.28–7.16 (m, 5H, ArH), 4.11–4.06 (t, J = 7.3 Hz, 2H, 1-H_2 Et), 4.04 (s, 2H, CH_2–Ph), 3.91–3.84 (q, J = 7.0 Hz, 2H, 1′-H_2), 2.80–2.75 (t, J = 7.3 Hz, 2H, 2′-H_2), 2.55–2.47 (m, 2H, 4′-H_2), 1.14–1.05 (m, 6H, 5′-H_2 and CH_3 Et). ^{13}C NMR and DEPT (DMSO): 153.98 (C8), 152.97 (C6), 150.70 (C2), 148.10 (C4), 137.38 (C4 Ph), 128.94 (C3 and C5 Ph), 126.97 (C2 and C6 Ph), 106.67 (C5), 42.45 (NCH_2), 35.96 (CH_2Ph), 34.60 (C1 Et), 28.22 (CH_2CH_2S), 24.88 (S–CH_2), 14.77 (SCH_2CH_3), 13.48 (CH_3 Et). Anal. Calcd for $C_{18}H_{22}N_4O_2S$ (358.46): C, 60.31; H, 6.19; N, 15.63. Found: C, 60.17; H, 6.33; N, 15.41.

5.1.8. 1-Ethyl-3-(2-(ethylthio)ethyl)-8-phenyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 13h

Yield 72%. White solid. Mp 239–241 °C (DMF/H_2O). IR (KBr) ν (cm^{-1}) = 3154, 2975, 1706, 1649, 1466. ^1H NMR (DMSO): 13.86 (br s, 1H, D_2O exchang. NH), 8.12–8.09 (m, 2H, ArH), 7.52–7.43 (m, 3H, ArH), 4.23–4.18 (t, J = 7.4 Hz, 2H, 1-H_2 Et), 3.97–3.90 (q, J = 7.0 Hz, 2H, 1′-H_2), 2.89–2.85 (t, J = 7.3 Hz, 2H, 2′-H_2), 2.67–2.59 (q, J = 7.4 Hz, 2H, 4′-H_2), 1.24–1.19 (t, J = 7.4 Hz, 2H, 2-H_2 Et), 1.16–1.11 (t, J = 7.0 Hz, 2H, 5′-H_2). Anal. Calcd for $C_{17}H_{20}N_4O_2S$ (344.43): C, 59.28; H, 5.85; N, 16.27. Found: C, 59.57; H, 6.01; N, 15.98.

5.1.9. 3-(Cyclohexylmethyl)-1-methyl-8-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13i

Yield 30%. White solid. Mp 232–233 °C (H_2O). IR (KBr) ν (cm^{-1}) = 2921, 2850, 1706, 1652, 1501, 1420. ^1H NMR (DMSO): 13.12 (br s, 1H, D_2O exchang. NH), 7.39–7.37 (m, 1H, C_4H_3S), 6.96–6.94 (m, 2H, C_4H_3S), 4.25 (s, 2H, CH_2–C_4H_3S), 3.84–3.81 (d, J = 7.3 Hz, 2H, N–CH_2), 3.22 (s, 3H, CH_3), 2.01–1.81 (m, 1H, Cyclohex), 1.80–1.51 (m, 5H, Cyclohex), 1.21–0.90 (m, 5H, Cyclohex). Anal. Calcd for $C_{18}H_{22}N_4O_2S$ (358.46): C, 60.31; H, 6.19; N, 15.63. Found: C, 60.58; H, 6.47; N, 15.38.

5.1.10. 3-(Cyclohexylmethyl)-1-methyl-8-(thiophen-2-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13j

Yield 52%. White solid. Mp 317–319 °C (MeOH/H_2O). IR (KBr) ν (cm^{-1}) = 3134, 2922, 2851, 1700, 1661, 1534, 1484. ^1H NMR (DMSO): 13.17 (br s, 1H, D_2O exchang. NH), 7.87–7.86 (d, J = 3.5 Hz, 1H, C_4H_3S), 7.71–7.69 (d, J = 4.7 Hz, 1H, C_4H_3S), 7.20–7.16 (m, 1H, C_4H_3S), 3.86–3.83 (d, J = 7.3 Hz, 2H, N–CH_2), 3.24 (s, 3H, CH_3), 1.90–1.85 (m, 1H, Cyclohex), 1.62–1.51 (m, 5H, Cyclohex), 1.19–0.93 (m, 5H, Cyclohex). Anal. Calcd for $C_{17}H_{20}N_4O_2S$ (344.43): C, 59.28; H, 5.85; N, 16.27. Found: C, 59.59; H, 5.53; N, 15.98.

5.1.11. 1,3-Bis(cyclohexylmethyl)-8-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13k

Yield 49%. White solid. Mp 237–239 °C (EtOH/H_2O). IR (KBr) ν (cm^{-1}) = 2924, 2851, 1703, 1647, 1558, 1529. ^1H NMR (DMSO): 13.47 (br s, 1H, D_2O exchang. NH), 7.42–7.39 (m, 1H, C_4H_3S), 6.98–6.96 (m, 2H, C_4H_3S), 4.28 (s, 2H, CH_2–C_4H_3S), 3.86–3.83 (d, J = 7.0 Hz, 2H, N–CH_2), 3.77–3.74 (d, J = 7.0 Hz, 2H, N–CH_2), 1.94–1.80 (m, 1H, Cyclohex), 1.73–1.47 (m, 11H, Cyclohex), 1.13–0.96 (m, 10H, Cyclohex). Anal. Calcd for $C_{24}H_{32}N_4O_2S$ (440.60): C, 65.42; H, 7.32; N, 13.72. Found: C, 65.17; H, 7.12; N, 14.01.

5.1.12. 1,3-Bis(cyclohexylmethyl)-8-(thiophen-2-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13l

Yield 53%. Violet solid. Mp 313–315 °C (^{i}PrOH/Et_2O). IR (KBr) ν (cm^{-1}) = 3119, 2923, 2851, 1695, 1649, 1531, 1484. ^{1}H NMR (DMSO): 13.85 (br s, 1H, D_2O exchang. NH), 7.89–7.84 (m, 1H, C_4H_3S), 7.72–7.70 (m, 1H, C_4H_3S), 7.20–7.17 (m, 2H, C_4H_3S), 3.86–3.83 (d, J = 7.0 Hz, 2H, N–CH_2), 3.76–3.74 (d, J = 7.0 Hz, 2H, N–CH_2), 1.91–1.75 (m, 1H, Cyclohex), 1.73–1.44 (m, 11H, Cyclohex), 1.12–0.89 (m, 10H, Cyclohex). Anal. Calcd for $C_{23}H_{30}N_4O_2S$ (426.58): C, 64.76; H, 7.09; N, 13.13. Found: C, 64.96; H, 7.31; N, 13.35.

5.1.13. 3-Benzyl-1-ethyl-8-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13m

Yield 33%. White solid. Mp 236–237 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3130, 2945, 1700, 1648, 1559, 1498. ^{1}H NMR (DMSO): 13.53 (br s, 1H, D_2O exchang. NH), 7.37–7.22 (m, 6H, ArH + 1H C_4H_3S), 6.94–6.92 (m, 2H, C_4H_3S), 5.14 (s, 2H, CH_2–C_4H_3S), 4.25 (s, 2H, CH_2–Ph), 3.93–3.86 (q, J = 7.0 Hz, 2H, 1-H_2 Et), 1.11–1.07 (t, J = 7.0 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 154.21 (C8), 152.15 (C6), 150.86 (C2), 148.57 (C4), 138.94 (C1 C_4H_3S), 137.29 (C1 Ph), 128.74 (C3 and C5 Ph), 128.04 (C4 C_4H_3S), 127.71 (C2 and C6 Ph), 127.28 (C3 C_4H_3S), 126.54 (C4 Ph), 125.53 (C5 C_4H_3S), 107.93 (C5), 46.34 (CH_2 Ph), 36.14 (CH_2 Et), 29.12 (CH_2 C_4H_3S), 13.51 (CH_3 Et). Anal. Calcd for $C_{19}H_{18}N_4O_2S$ (366.44): C, 62.28; H, 4.95; N, 15.29. Found: C, 62.55; H, 4.77; N, 15.41.

5.1.14. 3-Benzyl-1-ethyl-8-(furan-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13n

Yield 25%. White solid. Mp 231–233 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3034, 1700, 1652, 1559, 1506, 1457. ^{1}H NMR (DMSO): 13.51 (br s, 1H, D_2O exchang. NH), 7.54–7.53 (d, J = 1.0 Hz, 1H, 5-H C_4H_3O), 7.32–7.20 (m, 5H, ArH), 6.38–6.36 (q, J = 2.9, 1.0 Hz, 1H, 4-H C_4H_3O), 6.21–6.20 (d, J = 2.9 Hz, 1H, 3-H C_4H_3O), 5.13 (s, 2H, CH_2–C_4H_3O), 4.10 (s, 2H, CH_2–Ph), 3.94–3.87 (q, J = 7.0 Hz, 2H, 1-H_2 Et), 1.12–1.07 (t, J = 7.0 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 154.17 (C8), 150.88 (C6), 150.11 (C2), 149.37 (C2 C_4H_3O), 148.71 (C4), 142.60 (C5 C_4H_3O), 137.30 (C1 Ph), 128.74 (C3 and C5 Ph), 127.88 (C2 and C6 Ph), 127.66 (C4 Ph), 111.02 (C4 C_4H_3O), 107.60 (C3 C_4H_3O), 106.56 (C5), 46.32 (CH_2Ph), 36.13 (CH_2 Et), 27.93 ($CH_2C_4H_3O$), 13.51 (CH_3 Et). Anal. Calcd for $C_{19}H_{18}N_4O_3$ (350.37): C, 65.13; H, 5.18; N, 15.99. Found: C, 62.86; H, 5.45; N, 16.16.

5.1.15. 3-Benzyl-8-(2,6-difluorobenzyl)-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 13o

Yield 65%. White solid. Mp 296–299 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3031, 1700, 1646, 1500, 1470. ^{1}H NMR (DMSO): 1300 (br s, 1H, D_2O exchang. NH), 7.44–7.09 (m, 8H, ArH), 5.06 (s, 2H, CH_2–Ph), 4.13 (s, 2H, CH_2–PhF_2), 3.94–3.88 (m, 2H, 1-H_2 Et), 1.13–1.07 (m, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 163.36 (C2 and C6 $C_6H_3F_2$), 154.03 (C8), 150.98 (C6), 150.86 (C2), 148.16 (C4), 137.26 (C1 Ph), 129.88, 129.74, 128.64, 128.40, 127.76, 111.98 and 111.66 (ArH), 106.90 (C5), 46.32 (CH_2 Ph), 36.18 (CH_2 Et), 22.25 ($CH_2C_6H_3F_2$), 13.56 (CH_3 Et). Anal. Calcd for $C_{21}H_{18}F_2N_4O_2$ (396.39): C, 63.63; H, 4.58; N, 14.13. Found: C, 63.92; H, 4.77; N, 14.41.

5.1.16. 1-Ethyl-3,8-bis(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13p

Yield 49%. White solid. Mp 234–235 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3148, 1702, 1654, 1558, 1498. ^{1}H NMR (DMSO): 13.54 (br s, 1H, D_2O exchang. NH), 7.38 (m, 2H, C_4H_3S), 7.12 (m, 1H, C_4H_3S), 6.96–6.94 (m, 3H, C_4H_3S), 5.27 (s, 2H, CH_2–C_4H_3S), 4.27 (s, 2H, CH_2–C_4H_3S), 3.91–3.88 (d, J = 6.3 Hz, 2H, 1-H_2 Et), 1.11–1.07 (t, J = 6.3 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 156.07 (C8), 153.27 (C6), 150.85 (C2), 148.99 (C4), 138.10 and 137.95 (C2 C_4H_3S), 128.91, 127.49, 127.01, 126.81, 126.50 and 125.46 (C_4H_3S), 107.48 (C5), 41.63 and 37.45 ($CH_2C_4H_3S$), 30.04 (CH_2 Et), 13.71 (CH_3 Et). Anal. Calcd for $C_{17}H_{16}N_4S_2O_2$ (372.47): C, 54.82; H, 4.33; N, 15.04. Found: C, 55.13; H, 4.46; N, 14.93.

5.1.17. 1-Ethyl-8-(furan-2-ylmethyl)-3-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13q

Yield 38%. Beige solid. Mp 283–284 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3094, 1703, 1653, 1558, 1498, 1458. ^{1}H NMR (DMSO): 13.55 (s, 1H, D_2O exchang. NH), 7.55 (s, 1H, 5-H C_4H_3O), 7.39–7.38 (m, 1H, 3-H C_4H_3S), 7.09–7.08 (m, 1H, 5-H C_4H_3S), 6.94–6.91 (m, 1H, 4-H C_4H_3S), 6.39–6.38 (m, 1H, 4-H C_4H_3O), 6.24–6.23 (m, 1H, 3-H C_4H_3O), 5.26 (s, 2H, CH_2–C_4H_3S), 4.12 (s, 2H, CH_2–C_4H_3O), 3.94–3.87 (q, J = 7.0 Hz, 2H, 1-H_2 Et), 1.12–1.07 (t, J = 7.0 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 155.85 (C8), 151.25 (C6), 150.68 (C2), 149.39 (C4), 148.12 (C2 C_4H_3O), 142.63 (C5 C_4H_3O), 138.13 (C2 C_4H_3S), 128.83 (C4 C_4H_3S), 126.99 (C3 C_4H_3S), 126.47 (C5 C_4H_3S), 111.06 (C4 C_4H_3O), 108.01 (C3 C_4H_3O), 107.45 (C5), 41.61 ($CH_2C_4H_3S$), 37.38 (CH_2 Et), 28.83 ($CH_2C_4H_3O$), 13.62 (CH_3 Et). Anal. Calcd for $C_{17}H_{16}N_4O_3S$ (356.40): C, 57.29; H, 4.52; N, 15.72. Found: C, 57.01; H, 4.89; N, 15.85.

5.1.18. 8-(2,6-Difluorobenzyl)-1-ethyl-3-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13r

Yield 36%. Beige solid. Mp 283–284 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3031, 1702, 1648, 1627, 1558, 1499, 1471. ^{1}H NMR ($CDCl_3$): 13.53 (br s, 1H, D_2O exchang. NH), 7.41–7.34 (m, 2H, 4-H ArH and 3-H C_4H_3S), 7.12–7.07 (m, 2H, 3-H and 5-H ArH), 6.98 (s, 1H, 5-H C_4H_3S), 6.89–6.88 (m, 1H, 4-H C_4H_3S), 5.17 (s, 2H, CH_2–C_4H_3S), 4.11 (s, 2H, CH_2–ArH), 3.91–3.85 (q, J = 6.7 Hz, 2H, 1-H_2 Et), 1.10–1.05 (t, J = 6.7 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT ($CDCl_3$): 160.21 (C2 and C6 $C_6H_3F_2$), 153.75 (C8), 151.12 (C6), 150.46 (C2), 147.84 (C4), 138.55 (C2 C_4H_3S), 136.75 (C1 Ph), 129.42, 128.07, 126.90, 126.59, 112.01 and 111.68 (ArH and C2–C3–C4–C5 C_4H_3S), 107.82 (C5), 40.92 ($CH_2C_6H_3F_2$), 36.08 (CH_2 Et), 22.19 ($CH_2C_6H_3F_2$), 13.49 (CH_3 Et). Anal. Calcd for $C_{19}H_{16}F_2N_4O_2S$ (402.42): C, 56.71; H, 4.01; N, 13.92. Found: C, 58.03; H, 3.85; N, 14.14.

5.1.19. 8-(Biphenyl-4-yl)-1-ethyl-3-(2-(ethylthio)ethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 13s

Yield 58%. White solid. Mp 277–281 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3157, 1690, 1645, 1569, 1552, 1531, 1472. ^{1}H NMR (DMSO): 14.27 (br s, 1H, D_2O exchang. NH), 8.19–8.15 (d, J = 8.4 Hz, 2H, ArH), 7.81–7.77 (d, J = 8.4 Hz, 2H, ArH), 7.72–7.69 (d, J = 7.2 Hz, 2H, ArH), 7.48–7.36 (m, 3H, ArH), 4.22–4.16 (t, J = 7.1 Hz, 2H, 1-H_2 Et), 3.95–3.87 (q, J = 7.0 Hz, 2H, 1′-H_2), 2.8–2.82 (t, J = 7.0 Hz, 2H, 2′-H_2), 2.66–2.57 (q, J = 7.5 Hz, 2H, 4′-H_2), 1.22–1.16 (t, J = 6.5 Hz, 3H, 5′-H_2), 1.13–1.08 (t, J = 6.8 Hz, 3H, CH_3 Et). ^{13}C NMR and DEPT (DMSO): 154.20 (C8), 151.30 (C6), 150.82 (C2), 150.10 (C4), 142.33, 140.15 and 133.54 (C Biphenylyl), 129.10 (C5), 129.40, 129.38, 127.56, 127.51, 127.36, 127.09 and 126.56 (CH Biphenylyl), 66.81 (NCH_2), 65.24 (CH_2CH_2S), 28.36 (CH_2 Et), 25.07 (SCH_2CH_3), 14.93 (SCH_2CH_3), 13.58 (CH_3 Et). Anal. Calcd for $C_{23}H_{24}N_4O_2S$ (420.53): C, 65.69; H, 5.75; N, 13.32. Found: C, 65.33; H, 6.07; N, 13.41.

5.1.20. 8-(Biphenyl-4-yl)-3-(2-ethoxyethyl)-1-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 13t

Yield 77%. White solid. Mp 271–273 °C (^{i}PrOH). IR (KBr) ν (cm^{-1}) = 3164, 1691, 1645, 1597, 1551, 1531, 1472. ^{1}H NMR (DMSO): 14.23 (br s, 1H, D_2O exchang. NH), 8.23–8.20 (d, J = 8.1 Hz, 2H, ArH), 7.83–7.80 (d, J = 8.1 Hz, 2H, ArH), 7.76–7.73 (d, J = 7.4 Hz, 2H, ArH), 7.52–7.37 (m, 3H, ArH), 4.25–4.21 (t, J = 6.8 Hz, 2H, 1-H_2 Et), 3.98–3.92 (q, J = 6.5 Hz, 2H, 1′-H_2), 3.74–

3.70 (t, J = 6.5 Hz, 2H, 2′-H_2), 3.55–3.47 (q, J = 6.8 Hz, 2H, 4′-H_2), 1.17–1.12 (t, J = 6.5 Hz, 3H, CH_3 Et), 1.07–1.02 (t, J = 6.8 Hz, 3H, 5′-H_2). ^{13}C NMR and DEPT (DMSO): 154.25 (C8), 151.3410 (C6), 150.92 (C2), 150.04 (C4), 142.09, 139.58 and 133.79 (C Biphenylyl), 129.39 (C5), 128.34, 128.11, 127.56, 127.48, 127.10, 127.06 and 126.89 (CH Biphenylyl), 66.62 (CH_2CH_2O), 65.64 (OCH_2CH_3), 36.23 (NCH_2), 35.94 (CH_2 Et), 15.46 (OCH_2CH_3), 13.60 (CH_3 Et). Anal. Calcd for $C_{23}H_{24}N_4O_3$ (404.46): C, 68.30; H, 5.98; N, 13.85. Found: C, 68.02; H, 6.16; N, 13.99.

5.2. General procedure for the methylation of xanthines 13

To a suspension of K_2CO_3 (1.2 mmol) in dry DMF (10 mL) was added the corresponding xanthine **13** (1 mmol) and the mixture was shaken at room temperature for 15 min and at 60 °C for a further 15 min. Once the mixture had reached room temperature CH_3I (1 mmol) was added. The reaction mixture was heated at 100 °C for 2 h and then allowed to cool down to room temperature. Water was added and the resulting precipitate was filtered off, washed with water and dried under vacuum.

5.2.1. 3-(2-(Ethylthio)ethyl)-7-metyl-8-phenyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 14c

Yield 75%. Yellow solid. Mp 97–99 °C (cyclohexane/Et_2O). IR (KBr) ν (cm^{-1}) = 2958, 2924, 2853, 1699, 1655, 1537, 1452, 1429. ^{1}H NMR ($CDCl_3$): 7.69–7.66 (m, 2H, ArH), 7.53–7.51 (m, 3H, ArH), 4.39–4.32 (q, J = 7.4 Hz, 2H, 1″-H_2 Pr), 4.06 (s, 3H, N–CH_3), 4.02–3.97 (t, J = 7.4 Hz, 2H, 1′-H_2), 2.97–2.92 (m, 2H, 2′-H_2), 2.72–2.65 (q, J = 7.4 Hz, 2H, 4′-H_2), 1.74–1.66 (q, J = 7.4 Hz, 2H, 2″-H_2 Pr), 1.32–1.27 (t, J = 7.4 Hz, 2H, 3″-H_2 Pr), 1.00–0.95 (t, J = 7.4 Hz, 2H, 5′-H_2). Anal. Calcd for $C_{19}H_{24}N_4O_2S$ (372.48): C, 61.27; H, 6.49; N, 15.04. Found: C, 61.02; H, 6.68; N, 15.25.

5.2.2. 7-Methyl-8-phenyl-1-propyl-3-((tetrahydrofuran-2-yl)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 14e

Yield 78%. White solid. Mp 220–224 °C (cyclohexane). IR (KBr) ν (cm^{-1}) = 2958, 1692, 1536, 1451, 1428. ^{1}H NMR ($CDCl_3$): 7.65–7.61 (m, 2H, ArH), 7.49–7.46 (m, 3H, ArH), 4.54–4.49 (m, 2H, 2′-H_2), 4.32–4.23 (m. 2H, 1″-H_2 Pr), 4.00 (s, 3H, CH_3), 4.03–3.72 (m, 4H, N–CH_2 + 5′-H_2), 2.05–1.62 (m, 6H, 3′-H_2 + 4′-H_2 + 2″-H_2 Pr), 0.96–0.94 (t, J = 7.4 Hz, 3H, CH_3 Pr). Anal. Calcd for $C_{20}H_{24}N_4O_3$ (368.43): C, 65.20; H, 6.57; N, 15.21. Found: C, 65.49; H, 6.33; N, 15.57.

5.2.3. 3-Benzyl-1-ethyl-7-methyl-8-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 14m

Yield 89%. Beige solid. Mp 108–110 °C (hexane). IR (KBr) ν (cm^{-1}) = 3027, 1700, 1654, 1540, 1490, 1448, 1426. ^{1}H NMR ($CDCl_3$): 7.48–7.45 (m, 1H, C_4H_3S), 7.26–7.13 (m, 5H, ArH), 6.89–6.86 (m, 1H, C_4H_3S), 6.78–6.77 (m, 1H, C_4H_3S), 5.19 (s, 2H, CH_2–C_4H_3S), 4.25 (s, 2H, CH_2–Ph), 3.99–3.93 (q, J = 7.0 Hz, 2H, 1-H_2 Et), 1.17–1.12 (t, J = 7.0 Hz, 3H, 2-H_2 Et). Anal. Calcd for $C_{20}H_{20}N_4O_2S$ (380.46): C, 63.04; H, 5.30; N, 14.73. Found: C, 62.87; H, 5.55; N, 14.96.

5.2.4. 3-Benzyl-1-ethyl-8-(furan-2-ylmethyl)-7-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 14n

Yield 88%. Yellow solid. Mp 118–120 °C (hexane). IR (KBr) ν (cm^{-1}) = 3029, 1700, 1653, 1559, 1541, 1497, 1448. ^{1}H NMR ($CDCl_3$): 7.45–7.42 (m, 1H, 5-H C_4H_3O), 7.28–7.20 (m, 5H, ArH), 6.27–6.24 (m, 1H, 4-H C_4H_3O), 6.09–6.06 (m, 1H, 3-H C_4H_3O), 5.18 (s, 2H, CH_2–C_4H_3O), 4.10 (s, 2H, CH_2–Ph), 4.02–3.94 (q, J = 7.0 Hz, 2H, 1-H_2 Et), 3.84 (s, 3H, N–CH_3), 1.17–1.12 (t, J = 7.0 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT ($CDCl_3$): 155.21 (C8), 151.03 (C6), 150.45 (C2), 149.17 (C2 C_4H_3O), 147.35 (C4), 142.37 (C5 C_4H_3O), 138.64 (C1 Ph), 129.59 (C3 and C5 Ph), 128.93 (C2 and C6 Ph), 126.85 (C4 Ph), 111.97 (C4 C_4H_3O), 111.64 (C3 C_4H_3O), 108.72 (C5), 40.82 (CH_2Ph), 36.79 (CH_2 Et), 32.51 (NCH_3), 21.05 ($CH_2C_4H_3O$), 13.70 (CH_3 Et). Anal. Calcd for $C_{20}H_{20}N_4O_3$ (364.40): C, 65.92; H, 5.53; N, 15.38. Found: C, 66.21; H, 5.21; N, 15.51.

5.2.5. 3-Benzyl-8-(2,6-difluorobenzyl)-1-ethyl-7-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 14o

Yield 85%. White solid. Mp 159–161 °C (hexane). IR (KBr) ν (cm^{-1}) = 3019, 1703, 1654, 1595, 1540, 1474, 1444. ^{1}H NMR ($CDCl_3$): 7.48–7.44 (m, 2H, ArH), 7.34–7.21 (m, 4H, ArH), 6.99–6.89 (m, 2H, ArH), 5.15 (s, 2H, CH_2–Ph), 4.13 (s, 2H, CH_2–PhF_2), 4.07–4.00 (q, J = 7.0 Hz, 2H, 1-H_2 Et), 3.95 (s, 3H, N–CH_3), 1.22–1.18 (t, J = 7.0 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 163.12 (C2 and C6 $C_6H_3F_2$), 155.09 (C8), 151.31 (C6), 150.23 (C2), 147.35 (C4), 137.05 (C1 Ph), 129.81, 129.65, 128.68, 128.09, 111.95 and 111.61 (ArH), 108.83 (C5), 46.72 (CH_2 Ph), 36.80 (CH_2 Et),32.14 (NCH_3), 21.04 ($CH_2C_6H_3F_2$), 13.69 (CH_3 Et). Anal. Calcd for $C_{22}H_{20}F_2N_4O_2$ (410.42): C, 64.38; H, 4.91; N, 13.65. Found: C, 64.61; H, 5.15; N, 13.33.

5.2.6. 1-Ethyl-7-methyl-3,8-bis(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 14p

Yield 81%. Beige solid. Mp 85–88 °C (hexane). IR (KBr) ν (cm^{-1}) = 2983, 1700, 1653, 1558, 1540, 1508, 1490. ^{1}H NMR ($CDCl_3$): 7.06–7.00 (m, 3H, C_4H_3S), 6.77–6.68 (m, 3H, C_4H_3S), 5.23 (s, 2H, CH_2–C_4H_3S), 4.14 (s, 2H, CH_2–C_4H_3S), 3.90–3.81 (d, J = 7.0 Hz, 2H, 1-H_2 Et), 3.67 (s, 3H. N–CH_3), 1.05–1.00 (t, J = 7.0 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 156.11 (C8), 151.54 (C6), 150.87 (C2), 147.23 (C4), 138.39 and 137.40 (C2 C_4H_3S), 128.78, 127.52, 126.97, 126.47, 126.39 and 125.51 (C_4H_3S), 108.32 (C5), 41.11 and 36.85 ($CH_2C_4H_3S$), 32.42 (NCH_3), 28.43 (CH_2 Et), 13.70 (CH_3 Et). Anal. Calcd for $C_{18}H_{18}N_4S_2O_2$ (386.09): C, 55.94; H, 4.69; N, 14.50. Found: C, 55.66; H, 4.92; N, 14.77.

5.2.7. 1-Ethyl-8-(furan-2-ylmethyl)-7-methyl-3-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 14q

Yield 86%. Yellow solid. Mp 121–123 °C (hexane). IR (KBr) ν (cm^{-1}) = 3000, 2898, 1702, 1653, 1540, 1490, 1443. ^{1}H NMR ($CDCl_3$): 7.16 (s, 1H, 5-H C_4H_3O), 7.07–7.00 (m, 1H, 3-H and 5-H C_4H_3S), 6.73 (s, 1H, 4-H C_4H_3S), 6.15 (s, 1H, 4-H C_4H_3O), 5.98 (s, 1H, 3-H C_4H_3O), 5.22 (s, 2H, CH_2–C_4H_3S), 4.00 (s, 2H, CH_2–C_4H_3O), 3.90–3.84 (q, J = 6.7 Hz, 2H, 1-H_2 Et), 1.06–1.01 (t, J = 6.7 Hz, 3H, 2-H_2 Et). ^{13}C NMR and DEPT (DMSO): 155.79 (C8), 151.15 (C6), 150.41 (C2), 149.51 (C4), 147.98 (C2 C_4H_3O), 142.65 (C5 C_4H_3O), 138.21 (C2 C_4H_3S), 128.71 (C4 C_4H_3S), 126.95 (C3 C_4H_3S), 126.37 (C5 C_4H_3S), 111.13 (C4 C_4H_3O), 108.15 (C3 C_4H_3O), 107.98 (C5), 41.10 ($CH_2C_4H_3S$), 36.85 (CH_2 Et), 32.45 (NCH_3), 27.23 ($CH_2C_4H_3O$), 13.70 (CH_3 Et). Anal. Calcd for $C_{18}H_{18}N_4O_3S$ (370.43): C, 58.36; H, 4.90; N, 15.12. Found: C, 58.07; H, 4.70; N, 15.43.

5.2.8. 8-(2,6-Difluorobenzyl)-1-ethyl-7-methyl-3-(thiophen-2-ylmethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 14r

Yield 91%. Brown solid. Mp 177–179 °C (hexane). IR (KBr) ν (cm^{-1}) = 3031, 1705, 1654, 1628, 1595, 1540, 1474, 1444, 1324, 1265, 1230, 1207, 1060, 1019, 761. ^{1}H NMR ($CDCl_3$): 7.22–6.81 (m, 6H, ArH and C_4H_3S), 5.25 (s, 2H, CH_2–C_4H_3S), 4.07 (s, 2H, CH_2–ArH), 3.99–3.96 (m, 2H, 1-H_2 Et), 3.88 (s, 3H, N–CH_3), 1.16–1.10 (m, 3H, 2-H_2 Et). Anal. Calcd for $C_{20}H_{18}F_2N_4O_2S$ (416.45): C, 57.68; H, 4.36; N, 13.45. Found: C, 57.91; H, 4.11; N, 13.63.

5.3. General procedure for the oxidation of xanthines 13 to sulfoxides 15

Oxone® (0.67 mmol) and aliquat (three drops) in a mixture of H_2O (6 mL) and CH_2Cl_2 (4 mL) were added to a solution of the corresponding xanthine **13** (1 mmol) in CH_3CN (0.4 mmol) at 0 °C. The

reaction mixture was stirred at room temperature for 7 h. The reaction mixture was then poured into EtOAc (100 mL) and the organic layer was washed twice with H_2O, dried (Na_2SO_4) and the solvents evaporated under reduced pressure to leave a residue, which was purified by recrystallization.

5.3.1. 3-(2-(Ethylsulfinyl)ethyl)-8-phenyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 15c

Yield 89%. White solid. Mp 226–227 °C (CH_3CN). IR (KBr) ν (cm^{-1}) = 3173, 2963, 1694, 1654, 1467. ^1H NMR (DMSO): 13.87 (br s, 1H, D_2O exchang. NH), 8.13–8.10 (m, 2H, 2-H and 6-H ArH), 7.52–7.44 (m, 3H, 3-H, 4-H and 5-H ArH), 4.45–4.40 (t, J = 7.3 Hz, 2H, 1-H_2 Pr), 3.87–3.82 (t, J = 7.3 Hz, 2H, 1′-H_2), 3.31–3.02 (m, 2H, 2′-H_2), 2.94–2.66 (m, 2H, 4′-H_2), 1.63–1.51 (q, J = 7.4 Hz, 2H, 2-H_2 Pr), 1.19–1.14 (t, J = 7.4 Hz, 3H, 5′-H_2), 0.89–0.84 (t, J = 7.4 Hz, 2H, CH_3 Pr ^{13}C NMR and DEPT (DMSO): 153.97 (C8), 150.92 (C6), 150.15 (C2), 148.07 (C4), 130.61 (C4 Ph), 129.28 (C3 and C5 Ph), 128.99 (C1 Ph), 126.80 (C2 and C6 Ph), 108.04 (C5), 49.01 (NCH_2), 44.91 (C1 Pr), 42.61 (CH_2CH_2SO), 38.00 ($SOCH_2$), 21.19 (C2 Pr), 11.50 (CH_3 Pr), 6.74 ($OSCH_2CH_3$). Anal. Calcd for $C_{18}H_{22}N_4O_3S$ (374.46): C, 57.74; H, 5.92; N, 14.92. Found: C, 57.95; H, 5.81; N, 15.07.

5.3.2. 8-Benzyl-3-(2-(ethylsulfinyl)ethyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 15d

Yield 83%. White solid. Mp 186–189 °C (CH_3CN). IR (KBr) ν (cm^{-1}) = 3032, 2963, 1699, 1645, 1497. ^1H NMR (DMSO): 13.48 (br s, 1H, D_2O exchang. NH), 7.30–7.19 (m, 5H, ArH), 4.38–4.30 (m, 2H, 1-H_2 Pr), 4.04 (s, 2H, CH_2–Ph), 3.83–3.78 (t, J = 7.4 Hz, 2H, 1′-H_2), 3.18–2.98 (m, 2H, 2′-H_2), 2.85–2.64 (m, 2H, 4′-H_2), 1.57–1.50 (q, J = 7.4 Hz, 2H, 2-H_2 Pr), 1.15–1.13 (t, J = 7.4 Hz, 3H, 5′-H_2), 0.86–0.84 (t, J = 7.5 Hz, 2H, CH_3 Pr). ^{13}C NMR and DEPT (DMSO): 154.85 (C8), 153.71 (C6), 151.22 (C2), 136.89 (C4), 128.93 (C4 Ph), 128.8 (C3 and C5 Ph), 127.04 (C2 and C6 Ph), 106.95 (C5), 48.97 (NCH_2), 44.85 (C1 Pr), 42.47 (CH_2Ph), 34.65 (CH_2CH_2SO), 25.12 ($SOCH_2$), 21.16 (C2 Pr), 11.52 (CH_3 Pr), 6.65 ($SOCH_2CH_3$). Anal. Calcd for $C_{19}H_{24}N_4O_3S$ (388.48): C, 58.74; H, 6.23; N, 14.42. Found: C, 58.55; H, 6.41; N, 14.27.

5.3.3. 8-Benzyl-1-ethyl-3-(2-(ethylsulfinyl)ethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 15g

Yield 87%. White solid. Mp 192–193 °C (CH_3CN). IR (KBr) ν (cm^{-1}) = 3144, 3090, 3034, 2979, 1703, 1656, 1586, 1556, 1498. ^1H NMR (DMSO): 13.81 (br s, 1H, D_2O exchang. NH), 7.16–7.07 (m, 5H, ArH), 4.24–4.15 (q, J = 7.1 Hz, 2H, 1-H_2 Et), 3.90 (s, 2H, CH_2Ph), 3.76–3.70 (q, J = 7.0 Hz, 2H, 2′-H_2), 3.07–2.86 (m, 2H, 1-H_2 Et), 2.67–2.34 (m, 2H, 4′-H_2), 1.06–0.99 (m, 3H, 5′-H_2), 0.97–0.92 (q, J = 7.0 Hz, 2H, CH_3 Et). Anal. Calcd for $C_{18}H_{22}N_4O_3S$ (374.46): C, 57.74; H, 5.92; N, 14.96. Found: C, 57.45; H, 5.77; N, 15.21.

5.3.4. 1-Ethyl-3-(2-(ethylsulfinyl)ethyl)-8-phenyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 15h

Yield 90%. White solid. Mp 246–247 °C (CH_3CN). IR (KBr) ν (cm^{-1}) = 3154, 2976, 1698, 1659, 1519, 1470. ^1H NMR (DMSO): 13.91 (br s, 1H, D_2O exchang. NH), 7.95–7.92 (m, 2H, 2-H and 6-H ArH), 7.36–7.30 (m, 3H, 3-H, 4-H and 5-H ArH), 4.27–4.21 (t, J = 7.0 Hz, 2H, 1-H_2 Et), 3.79–3.70 (q, J = 7.0 Hz, 2H, 2′-H_2), 3.13–2.87 (m, 2H, 1-H_2 Et), 2.73–2.49 (m, 2H, 4′-H_2), 1.08–1.02 (t, J = 7.4 Hz, 3H, 5′-H_2), 1.01–0.92 (q, J = 7.4 Hz, 2H, CH_3 Et). Anal. Calcd for $C_{17}H_{20}N_4O_3S$ (360.43): C, 56.65; H, 5.59; N, 15.54. Found: C, 56.91; H, 5.38; N, 15.26.

5.4. General procedure for the oxidation of xanthines 13 to sulfones 16

A mixture of the corresponding xanthine **10** (0.35 mmol), AcOH (0.18 mmol) and 30% H_2O_2 (0.24 mL) was stirred at room temperature for 24 h. H_2O (30 mL) was then added and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with H_2O, dried (Na_2SO_4) and the solvents evaporated under reduced pressure to leave a residue, which was purified by recrystallization.

5.4.1. 3-(2-(Ethylsulfonyl)ethyl)-8-phenyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 16c

Yield 80%. White solid. Mp 270–272 °C (EtOH/Et_2O). IR (KBr) ν (cm^{-1}) = 3167, 2968, 1702, 1655, 1519, 1467. ^1H NMR (DMSO): 13.91 (br s, 1H, D_2O exchang. NH), 8.14–8.11 (m, 2H, 2-H and 6-H ArH), 7.54–7.50 (m, 3H, 3-H, 4-H and 5-H ArH), 4.49–4.44 (t, J = 7.3 Hz, 2H, 1-H_2 Pr), 3.88–3.83 (t, J = 7.3 Hz, 2H, 1′-H_2), 3.58–3.53 (t, J = 7.3 Hz, 2H, 2′-H_2), 3.29–3.22 (q, J = 7.4 Hz, 2H, 4′-H_2), 1.64–1.52 (sex, J = 7.4 Hz, 2H, 2-H_2 Pr), 1.27–1.22 (t, J = 7.4 Hz, 3H, 5′-H_2), 0.90–0.85 (t, J = 7.4 Hz, 2H, CH_3 Pr). ^{13}C NMR and DEPT (DMSO): 154.25 (C8), 150.89 (C6), 149.45 (C2), 147.68 (C4), 131.43 (C4 Ph), 130.66 (C3 and C5 Ph), 129.32 (C1 Ph), 127.93 (C2 and C6 Ph), 107.91 (C5), 48.55 (NCH_2), 46.72 (C1 Pr), 42.62 ($CH_2CH_2SO_2$), 36.92 (SO_2CH_2), 21.16 (C2 Pr), 11.52 (CH_3 Pr), 6.48 ($SO_2CH_2CH_3$). Anal. Calcd for $C_{18}H_{22}N_4O_4S$ (390.46): C, 55.37; H, 5.68; N, 14.35. Found: C, 55.55; H, 5.43; N, 14.19.

5.4.2. 8-Benzyl-3-(2-(ethylsulfonyl)ethyl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 16d

Yield 74%. White solid. Mp 216–217 °C (EtOH/Et_2O). IR (KBr) ν (cm^{-1}) = 3144, 3090, 3030, 2964, 1699, 1639, 1560, 1497, 1451. ^1H NMR (DMSO): 13.83 (br s, 1H, D_2O exchang. NH), 7.32–7.11 (m, 5H, ArH), 4.40–4.35 (t, J = 7.3 Hz, 2H, 1-H_2 Pr), 4.07 (s, 2H, CH_2–Ph), 3.85–3.80 (t, J = 7.3 Hz, 2H, 1′-H_2), 3.50–3.45 (t, J = 7.3 Hz, 2H, 2′-H_2), 3.22–3.15 (q, J = 7.4 Hz, 2H, 4′-H_2), 1.58–1.50 (sex, J = 7.4 Hz, 2H, 2-H_2 Pr), 1.22–1.16 (t, J = 7.4 Hz, 3H, 5'-H_2), 0.89–0.83 (t, J = 7.4 Hz, 2H, CH_3 Pr). Anal. Calcd for $C_{19}H_{24}N_4O_4S$ (404.48): C, 56.42; H, 5.98; N, 13.85. Found: C, 56.15; H, 6.17; N, 14.14.

5.4.3. 8-Benzyl-1-ethyl-3-(2-(ethylsulfonyl)ethyl)-1*H*-purine-2,6(3*H*,7*H*)-dione, 16g

Yield 72%. Pink solid. Mp 181–184 °C (EtOH/Et_2O). IR (KBr) ν (cm^{-1}) = 3087, 3029, 2982, 1709, 1659, 1584, 1553. ^1H NMR (DMSO): 13.50 (br s, 1H, D_2O exchang. NH), 7.36–7.19 (m, 5H, ArH), 4.38–4.33 (t, J = 7.1 Hz, 2H, 1-H_2 Et), 4.05 (s, 2H, CH_2Ph), 3.92–3.85 (q, J = 7.1 Hz, 2H, 1′-H_2), 3.5843.41 (m, 2H, 2′-H_2), 3.21–3.13 (q, J = 7.1 Hz, 2H, 4′-H_2), 1.29–1.05 (m, 6H, 5′-H_2 + CH_3 Et). Anal. Calcd for $C_{18}H_{22}N_4O_4S$ (376.43): C, 55.37; H, 5.68; N, 14.35. Found: C, 55.51; H, 5.40; N, 14.29.

5.4.4. 1-Ethyl-3-(2-(ethylsulfonyl)ethyl)-8-phenyl-1*H*-purine-2,6(3*H*,7*H*)-dione, 16h

Yield 75%. White solid. Mp 272–273 °C (hexane/Et_2O). IR (KBr) ν (cm^{-1}) = 3172, 2982, 1701, 1660, 1519. ^1H NMR (DMSO): 13.92 (br s, 1H, D_2O exchang. NH), 8.14–8.10 (m, 2H, 2-H and 6-H ArH), 7.55–7.46 (m, 3H, 3-H, 4-H and 5-H ArH), 4.48–4.44 (t, J = 7.0 Hz, 2H, 1-H_2 Et), 3.97–3.90 (q, J = 7.0 Hz, 2H, 1′-H_2), 3.58–3.53 (t, J = 7.4 Hz, 2H, 2′-H_2), 3.29–3.22 (q, J = 7.4 Hz, 2H, 4′-H_2), 1.27–1.22 (t, J = 7.4 Hz, 3H, 5′-H_2), 1.16–1.11 (t, J = 7.0 Hz, 2H, CH_3 Et). Anal. Calcd for $C_{17}H_{20}N_4O_4S$ (376.43): C, 54.24; H, 5.36; N, 14.88. Found: C, 53.89; H, 5.61; N, 14.69.

5.5. Biochemistry and pharmacology

5.5.1. Radioligand binding assays

Radioligand binding competition assays were performed in vitro using A_1, A_{2A}, A_{2B} and A_3 human receptors expressed in transfected CHO (hA_1), HeLa (hA_{2A} and hA_3) and HEK-293 (hA_{2B}) cells as previously described.[24] A brief description is given below.

5.5.1.1. Human A_1 receptors. Adenosine A_1 receptor competition binding experiments were carried out in membranes from CHO-A_1 cells (Euroscreen, Gosselies, Belgium) labeled with 2 nM [^{3}H]DPCPX. Non-specific binding was determined in the presence of 10 μM (R)-PIA. The reaction mixture was incubated at 25 °C for 60 min.

5.5.1.2. Human A_{2A} receptors. Adenosine A_{2A} receptor competition binding experiments were carried out in membranes from HeLa-A_{2A} cells labeled with 3 nM [^{3}H]ZM241385. Non-specific binding was determined in the presence of 50 μM NECA. The reaction mixture was incubated at 25 °C for 30 min.

5.5.1.3. Human A_{2B} receptors. Adenosine A_{2B} receptor competition binding experiments were carried out in membranes from HEK-293-A_{2B} cells (Euroscreen, Gosselies, Belgium) labeled with 35 nM [^{3}H]DPCPX. Non-specific binding was determined in the presence of 400 μM NECA. The reaction mixture was incubated at 25 °C for 30 min.

5.5.1.4. Human A_3 receptors. Adenosine A_3 receptor competition binding experiments were carried out in membranes from HeLa-A_3 cells. Labeled with 30 nM [^{3}H]NECA. Non-specific binding was determined in the presence of 100 μM (R)-PIA. The reaction mixture was incubated at 25 °C for 180 min. After each incubation time samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin–Elmer, Madrid, Spain).

5.5.1.5. Data analysis. The −log of the inhibition constant (pK_i) of each compound was calculated by the Cheng–Prusoff equation

$$K_i = IC_{50}/(1 + [L]/K_D)$$

where IC_{50} is the concentration of compound that displaces the binding of radioligand by 50%, [L] is the free concentration of radioligand and K_D is the dissociation constant of each radioligand. IC_{50} values were obtained by fitting the data with non-linear regression using Prism 2.1 software (GraphPad, San Diego, CA). For those compounds that showed either little affinity or poor solubility a percentage of inhibition of specific biding at 1 μM is reported.

Results are the mean of 3 experiments (n = 3) each performed in duplicate.

Selectivity is defined by the ratio K_i A_{2A}/K_i A_{2B}.

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References and notes

1. Fredholm, B. N.; Ijzerman, A. P.; Jacobson, K. A.; Klotz, K.-N.; Linden, J. *Pharmacol. Rev.* **2001**, *53*, 527.
2. Berge, M.; Hylkeman, M.; Verslius, M.; Pastma, D. S. *Drugs R&D* **2007**, *8*, 13.
3. Akkari, R.; Burbiel, J. C.; Hockemeyer, J.; Mueller, C. E. *Curr. Top. Med. Chem.* **2006**, *6*, 1375.
4. Zhong, H.; Bellardinelli, L.; Zeng, D. *Am. J. Respir. Cell Mol. Biol.* **2004**, *30*, 118.
5. Zhong, H.; Bellardinelli, L.; Zeng, D. *Am. J. Respir. Cell Mol. Biol.* **2006**, *5*, 587.
6. Holgate, S. T. *Br. J. Pharmacol.* **2005**, *145*, 1009.
7. Zhong, H.; Bellardinelli, L.; Maa, T.; Zeng, D. *Am. J. Resp. Cell Mol. Biol.* **2004**, *32*, 2.
8. Beukers, M.; Meurs, I.; Ijzerman, A. *Med. Res. Rev.* **2006**, *26*, 667.
9. Zablocki, J.; Elzein, E.; Kalla, R. *Expert Opin. Ther. Pat.* **2006**, *16*, 1347.
10. Cacciari, B.; Pastorin, G.; Bolcato, C.; Spalluto, G.; Bacilieri, M.; Moro, S. *Mini-Rev. Med. Chem.* **2005**, *5*, 1053.
11. Baraldi, P. G.; Tabrizi, M. A.; Preti, D.; Bovero, A.; Romagnoli, R.; Fruttarolo, F.; Zaid, N. A.; Moorman, A. R.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. *J. Med. Chem.* **2004**, *47*, 1434.
12. Baraldi, P. G.; Tabrizi, M. A.; Gessi, S.; Borea, P. A. *Chem. Rev.* **2008**, *108*, 238. and references cited herein.
13. Yan, L.; Bertarelli, D. C.; Hayallah, A. M.; Meyer, H.; Klotz, K. N.; Müller, C. E. *J. Med. Chem.* **2006**, *49*, 4384.
14. Borrmann, T.; Hinz, S.; Bertarelli, D. C.; Li, W.; Florin, N. C.; Scheiff, A. B.; Müller, C. E. *J. Med. Chem.* **2009**, *52*, 3994.
15. Elzein, E.; Kalla, R. V.; Perry, T.; Gimbel, A.; Zeng, D.; Lustig, D.; Leung, K.; Zablocki, J. *J. Med. Chem.* **2008**, *51*, 2267.
16. Nieto, M. I.; Balo, M. C.; Brea, J.; Caamaño, O.; Cadavid, M. I.; Fernández, F.; García-Mera, F.; López, C.; Rodríguez-Borges, J. E. *Bioorg. Med. Chem.* **2009**, *17*, 3426.
17. Balo, M. C.; Brea, J.; Caamaño, O.; Fernández, F.; García-Mera, F.; López, C.; Loza, M. I.; Nieto, M. I.; Rodríguez-Borges, J. E. *Bioorg. Med. Chem.* **2009**, *17*, 6755.
18. Müller, C. E. *Tetrahedron Lett.* **1991**, *32*, 6539.
19. Müller, C. E. *Synthesis* **1993**, 125.
20. Müller, C. E.; Sandoval-Ramírez, J. *Synthesis* **1995**, 1295.
21. Fortes, C. C.; Garrote, C. F. D. *Synth. Commun.* **1997**, *27*, 2993.
22. Cunha, R. A.; Constantino, M. D.; Ribeiro, J. A. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1999**, *359*, 295.
23. Touchstone, J. *In Advances in Thin-Layer Chromatography*; Wiley: New York, 1982.
24. Stefanachi, A.; Nicolotti, O.; Leonetti, F.; Cellamare, S.; Campagna, F.; Loza, M. I.; Brea, J. M.; Mazza, F.; Gavuzzo, E.; Carotti, A. *Bioorg. Med. Chem.* **2008**, *16*, 9780.