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

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ABSTRACT

The synthesis of an important set of 3-furfurylxanthine derivatives is described. Binding affinities were determined for rat A_1 and human A_{2A}, A_{2B} and A_3 receptors. Several of the 3-furfuryl-7-methylxanthine derivatives showed moderate-to-high affinity at human A_{2B} receptors, the most active compound (**10d**) having a K_i of 7.4 nM for hA_{2B} receptors, with selectivities over rA_1 and hA_{2A} receptors up to 14-fold and 11-fold, respectively. Affinities for hA_3 receptors were very low for all members of the set.

1. Introduction

Adenosine modulates many important physiological functions, that affect the cardiovascular, renal, immune and central nervous systems. In recent years the search for adenosine analogues that show agonistic or antagonistic properties against adenosine receptors has intensified. These receptors belong to a superfamily of rhodopsin-like G protein-coupled receptors (GPCRs), of which four subtypes are known and designated as A_1, A_{2A}, A_{2B} and A_3. The receptors modulate the activity of adenylate cyclase either by stimulation (A_{2A} and A_{2B}) or inhibition (A_1 and A_3) of the activity.[1] All of the subtypes have been cloned, and each receptor can therefore be studied individually by use of recombinant systems.

In the past decade a large body of experimental evidence has been obtained that supports the idea that adenosine plays an important role in allergic asthma.[2–5] Patients with asthma and bronchitis show higher concentrations of adenosine in bronchoalveolar fluid than control subjects, suggesting that adenosine may be a marker of pulmonary inflammation. In in vivo assays, both inhaled adenosine and its precursor 5-adenosine monophosphate (AMP) cause bronchoconstriction in atopic and asthmatic patients, but not in normal control subjects. Furthermore, dipyridamole, which is an inhibitor of the cellular adenosine reuptake, increases bronchospasms induced by adenosine in asthmatic patients, an effect that may be inhibited by theophylline, an antagonist of adenosine receptors.[6]

The presence of A_{2B} receptors has been demonstrated in bronchoalveolar mastocytes, a finding that supports the hypothesis that adenosine participates in the physiopathology of asthma through activation of these receptors.[4,7] As a result A_{2B} receptor antagonists can be proposed as potential antiasthmatic drugs.

Several groups have designed and tested many xanthine derivatives with the aim of discovering new, potent and A_{2B}-selective ligands.[8–11] Good initial results have been obtained by Jacobson and coworkers in the series of 8-(4-substitutedphenyl)xanthines, with terms such as XAC (**1**),[12] a potent adenosine receptors (AR) antagonist, and MRS 1754 (**2**), that was shown to be selective for human A_{2B} (hA_{2B}) AR versus hA_1, hA_{2A}, and hA_3 subtypes of AR.[13] Excellent results in terms of both hA_{2B} AR affinity and selectivity have also been obtained by another group for the xanthine derivative **3**.[14]

Even though a number of A_{2B} AR antagonists have been reported, only a few have shown high affinity and selectivity for the A_{2B} AR relative to the A_1, A_{2A}, and A_3 subtypes of AR. In a first foray into this field, we studied a short series of new [1,2,4]triazolo[1,5-*c*]quinazoline[15] analogues of triazoloquinazoline **4**, a strong

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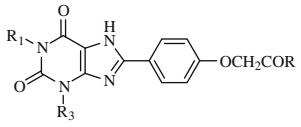
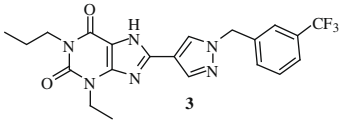
antagonist for A_{2A} and A_{2B} AR,[4] but unfortunately our compounds showed poor activities. More promising results were found in a complete study over the design, synthesis, structure–activity relationships (SAR) and structure–selectivity relationships (SSR) of a large series of 9-deaza- and 9-OH-9-deazaxanthines.[16] Recently, we have reported a series of new 1-alkyl-8-substituted-3-(3-methoxypropyl)xanthines,[17] bearing the same 8-(N-substituted-phenoxyacetamido) moiety proposed by Jacobson,[18] and a series of 1,3-dialkyl-8-(N-substituted-benzyloxycarbonylamino)-9-deazaxanthines,[19] bearing an isosteric group of the Jacobson motif. In both series, a number of compounds are endowed with nanomolar affinities for hA_{2B}, hA_1 and/or hA_{2A} AR subtypes.



1, XAC: $R_1 = R_3 = CH_2CH_2CH_3$; $R = NH(CH_2)_2NH_2$
2, MRS 1754: $R_1 = R_3 = CH_2CH_2CH_3$; $R = NHC_6H_4$-4-CN



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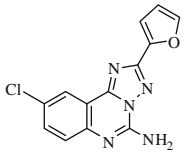
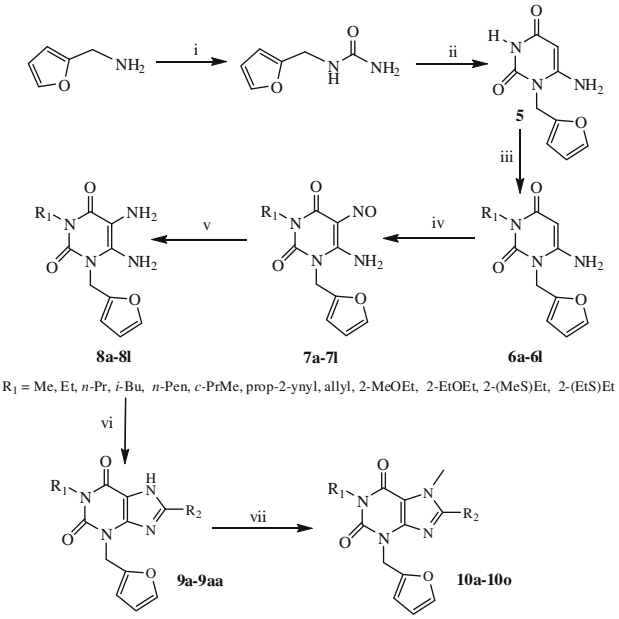


4, CGS 15943

The study reported here was carried out as part of a line of investigation directed at finding a potentially selective, high affinity A_{2B}-AR antagonist through the preparation of a series of xanthines bearing appropriate and/or more simple substituents in several positions of the xanthine nucleus, while exploring their SAR and SSR. The new adenosine analogues of xanthine type present structural variations at position 1 (alkyl or functionalized alkyl substituents), position 7 (unsubstituted or methyl substituent) and position 8 (aryl or heteroaryl substituents) of the xanthine nucleus, with a furfuryl substituent at position 3 (Schemes 1 and 2). The rationale behind this election was the strong adenosine antagonist activity reported for xanthines such as **1–3** combined with earlier reports that 3-furfurylxanthines have shown interesting bronchodilating and vasodilatadors effects,[20] as well as the presence of the 2-furyl motif at the triazoloquinazoline CGS 15943 (**4**).

2. Chemistry

The target compounds **9(a–aa)** and **10(a–o)** were synthesized as illustrated in Scheme 1. The 5,6-diamino-1-furfuryl-3-substituted uracils **8** were synthesized according to previously described methods. Thus, 1-furfurylurea was condensed with cyanoacetic acid[21,22] to give uracil **5** (79%). Direct alkylation of this uracil was performed with 15% aqueous NaOH and the appropriate alkyl halide[21,23] or by heating under reflux with $(NH_4)_2SO_4$ in HMDS and the subsequent addition of I_2 and the appropriate halide[17,24] to give the corresponding 1,3-disubstituted-6-aminouracil **6**. Standard nitrosation of **6(a–l)** with sodium nitrite in acetic acid was followed by reduction with sodium dithionite to give diaminouracils 8(a–**l**). Finally, condensation of diaminouracils **8** 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 **9**. The 7-methyl-



Scheme 1. Reagents and conditions: (i) KOCN, H_2SO_4; (ii) $NCCH_2CO_2H$; (iii) (a) *Method A*: RX, NaOH, EtOH; (b) *Method B*: $(NH_4)_2SO_4$, HMDS, I_2, RX, to, $Na_2S_2O_3$, H_2O; (iv) $NaNO_2$, AcOH; (v) $Na_2S_2O_4$, NH_4OH; (vi) a) R_2CO_2H, DIC, MeOH, rt, 0.5 h; (b) 2.5 N NaOH, MeOH, reflux, 10 min–1 h; (vii) CH_3I, NaH, DMF, 60 °C, 2 h.

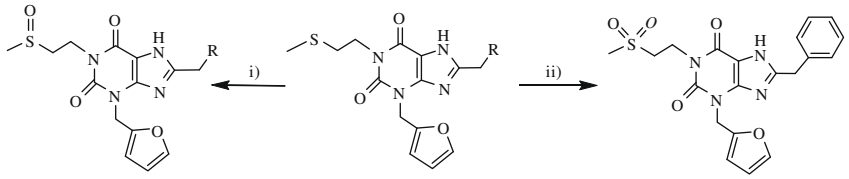
ated derivatives **10(a–o)** were obtained by methylation of **9(a–g)**, **9(l,m)**, **9(p–t)** and **9y** with excess methyl iodide in DMF in the presence of NaH.

Oxidation of the xanthines **9(r–aa)** (Scheme 2) to the corresponding sulfoxides **11(a–c)** and sulfone **12** was achieved by following previously reported literature procedures.[17,19,25]

3. Test results and discussion

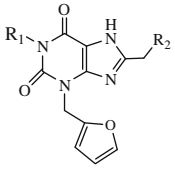
The affinity (pK_i or displacement percentage) values of the 3-furfurylxanthine derivatives **9(a–aa)**, **10(a–o)**, **11(a–c)** and **12**, at cloned human adenosine receptors expressed in HeLa cells (hA_{2A} and hA_3) and HEK-293 cells (hA_{2B}) and at rat A_1 receptors in membranes from rat cortex,[26] are given in Tables 1 and 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 receptors.[15,17] The affinity values of compounds that did not fully displace specific radioligand binding at 1 μM are given only in terms of displacement percentage. The biological methods employed are fully described into the Supplementary data including a representative binding curve for compound **10d** at hA_{2B} receptors.

The 7-unsubstituted xanthine derivatives (Table 1) compounds showed moderate-to-low affinity for hA_{2B} and rA_1 receptors, a lesser affinity for hA_{2A} receptors, and an almost complete lack of affinity for hA_3 receptors. Results shown in Table 1 enable certain trends to be deduced concerning the SAR and SSR for this group of xanthines. For example, in as much as these compounds showed some level of affinity for the receptors in question, an increase in the size of the 1-alkyl chain from two to three or more carbon atoms (compounds **9(f–m)**) yielded analogues with decreased A_{2B} and A_{2A} receptors binding affinity relative to the 1-methyl or 1-ethyl analogues **9(a–d)**, while most of them retained or even increased their affinity for A_1 receptors, giving rise to members with the selectivity A_{2B}/A_1 reversed (**9(f–h,j–l)**). However, the presence of an unsaturation in the 1-alkyl chain, see compounds **9(n–p)**, or the replacement of a methylene group by a sulfur atom in that alkyl chain, see compounds **9(w–aa)**, lead to an improvement of the



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

Table 1
Chemical structures and binding affinities[a] at hA_{2B}, hA_{2A}, rA_1 and hA_3 ARs of 1,8-disubstituted 3-furfurylxanthine derivatives



Compound	R_1	R_2	R_2	hA_{2B}	hA_{2A}	rA_1
9a	Metil	Furan-2-yl	6.50	6.37	14%	6%
9b	Ethyl	Phenyl	6.79	5.98	41%	5%
9c	Ethyl	Furan-2-yl	6.90	6.28	24%	18%
9d	Ethyl	Thiophen-2-yl	6.92	6.44	6.33	3%
9e	Propyl	Phenyl	6.93	6.38	6.52	9%
9f	Propyl	Furan-2-yl	66%	33%	6.38	3%
9g	Propyl	Thiophen-2-yl	68%	19%	6.47	2%
9h	Isobutyl	Phenyl	33%	13%	6.31	8%
9i	Isobutyl	Thiophen-2-yl	44%	11%	n.d.[b]	n.d.
9j	Pentyl	Phenyl	47%	7%	6.06	24%
9k	Pentyl	Thiophen-2-yl	17%	10%	21%	11%
9l	Cyclopropylmethyl	Thiophen-2-yl	42%	12%	6.46	21%
9m	Cyclopropylmethyl	2,6-Difluorophenyl	59%	6%	14%	7%
9n	Prop-2-ynyl	Thiophen-2-yl	6.57	6.35	6.66	11%
9o	Prop-2-ynyl	2,6-Difluorophenyl	7.05	6.34	6.36	5%
9p	Allyl	Thiophen-2-yl	6.26	5.84	23%	4%
9q	Allyl	2,6-Difluorophenyl	24%	11%	3%	1%
9r	2-Methoxyethyl	Phenyl	65%	32%	27%	5%
9s	2-Methoxyethyl	Thiophen-2-yl	70%	34%	17%	2%
9t	2-Methoxyethyl	2,6-Difluorophenyl	70%	23%	28%	4%
9u	2-Ethoxyethyl	Phenyl	31%	2%	25%	6%
9v	2-Ethoxyethyl	Thiophen-2-yl	27%	1%	n.d.	n.d.
9w	2-(Methylthio)ethyl	Thiophen-2-yl	7.03	5.68	6.82	8%
9x	2-(Methylthio)ethyl	2,6-Difluorophenyl	6.70	5.91	6.70	2%
9y	2-(Ethylthio)ethyl	Phenyl	6.08	11%	6.40	14%
9z	2-(Ethylthio)ethyl	Furan-2-yl	6.19	17%	42%	4%
9aa	2-(Ethylthio)ethyl	Thiophen-2-yl	6.56	4%	32%	13%
11a	2-(Ethylsulfinyl)ethyl	Phenyl	9%	11%	1%	26%
11b	2-(Ethylsulfinyl)ethyl	Furan-2-yl	13%	5%.	n.d.	n.d.
11c	2-(Ethylsulfinyl)ethyl	Thiophen-2-yl	33%	10%	n.d.	n.d.
12	2-(Ethylsulfonyl)ethyl	Phenyl	20%	1%	2%	1%

[a] Binding affinity is expressed as pK_i or displacement percentage at 1 μM where indicated. The SEM was always lower than 10%.
[b] Not determined.

affinity values for A_{2B} receptors, while selectivities with regard to both A_{2A} and A_1 receptors remained low. Finally, oxidation of the sulfur atom of the 1-(alkylthioalkyl) chain of xanthines **9(y–aa)**, resulted in the loss of binding affinity at all the AR assayed [compounds **11(a–c)** and **12**].

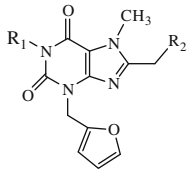
On the other hand, the 7-methylxanthine derivatives (Table 2) showed increased values of affinity for hA_{2B}, hA_{2A} and rA_1 receptors, while keeping an almost complete lack of affinity for hA_3 receptors. Generally speaking, A_{2B}/A_{2A} and A_{2B}/A_1 selectivities improved for this series, though not spectacularly.

Thus, compound **10d** showed high affinity at A_{2B} receptors (K_i = 7.4 nM), with 11-fold and 14-fold selectivities over A_{2A} and A_1 receptors, respectively. Maximum (39-fold) A_{2B}/A_{2A} selectivity was reached (compound **10i**) at the cost of a decrease in A_{2B} affinity (K_i = 41 nM) and in A_{2B}/A_1 selectivity (2.6-fold). A more balanced situation can be seen for compound **10m**, with intermediate affinity at A_{2B} receptors (K_i = 28 nM) and 25-fold and 14-fold selectivities over A_{2A} and A_1 receptors, respectively. Still, in some cases affinity for A_1 receptors was equal (**10o**) or even slightly greater (**10e**) than for A_{2B} receptors.

4. Conclusions

In summary, an important set of xanthine derivatives, compounds **9(a–aa)**, **10(a–o)**, **11(a–c)** and **12,** have been synthesized

Table 2
Chemical structures and binding affinities[a] at hA_{2B}, hA_{2A}, rA_1 and hA_3 ARs of 1,8-disubstituted 3-furfuryl-7-methylxanthine derivatives



Compound	R_1	R_2	hA_{2B}	hA_{2A}	rA_1	hA_3 (%)	A_{2B}/A_{2A} ratio[b]	A_{2B}/A_1 ratio
10a	Methyl	Furan-2-yl	7.49	6.83	6.28	45	4.57	16.2
10b	Ethyl	Phenyl	7.89	7.05	7.54	16	6.90	2.24
10c	Ethyl	Furan-2-yl	7.86	6.98	6.97	11	7.60	7.76
10d	Ethyl	Thiophen-2-yl	8.13	7.08	6.99	5	11.2	13.8
10e	Propyl	Phenyl	7.36	6.31	7.57	41	11.2	0.62
10f	Propyl	Furan-2-yl	7.72	6.51	6.88	41	16.2	6.92
10g	Propyl	Thiophen-2-yl	7.83	6.62	6.75	65	16.2	12.0
10h	Cyclopropylmethyl	Thiophen-2-yl	7.45	6.31	7.0	65	13.8	2.82
10i	Cyclopropylmethyl	2,6-Difluorophenyl	7.33	5.74	6.92	1	38.9	2.75
10j	Allyl	Thiophen-2-yl	7.67	6.51	6.88	12	14.5	6.17
10k	Allyl	2,6-Difluorophenyl	7.67	6.42	6.88	23	17.8	6.17
10l	2-Methoxyethyl	Phenyl	7.21	6.04	6.76	5	14.8	2.82
10m	2-Methoxyethyl	Thiophen-2-yl	7.56	6.16	6.40	4	25.1	14.4
10n	2-Methoxyethyl	2,6-Difluorophenyl	7.41	6.31	6.71	1	12.6	5.02
10o	2-(Ethylthio)ethyl	Thiophen-2-yl	7.05	6.05	7.05	52	10	1.0

[a] Binding affinity is expressed as pK_i or displacement percentage at 1 μM where indicated. The SEM was always lower than 10%.
[b] Affinity ratios were calculated on the basis of K_i values.

and their affinities for four subtypes of AR have been evaluated. The pharmacological activity data (Tables 1 and 2) show unequivocally that the presence of an additional methyl group at position 7 proved to be beneficial for A_{2B}, A_{2A} and A_1 affinities. This is a rather surprising finding, at the light of a short but illustrative precedent found in a related series of xanthines.[17] Also important is the fact that affinities for A_{2B} receptors at the one digit nM level can be found for a combination of appropriate substituents at several positions of the xanthine scaffold, without adhering to the 8-(N-substituted-phenoxyacetamido) motif proposed by Jacobson.[18] Despite the low A_{2B}/A_{2A} and A_{2B}/A_1 selectivity ratios found for these compounds, this work gives a deeper insight into the universe of structural variability which significantly affects the biological properties here studied.

5. Experimental

All chemicals 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 given as s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet), br s (broad singlet), v s (virtual singlet), dt (double triplet), q (quadruplet), qt (quintuplet), sex (sextet). 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-furfuryluracils 6(a–l)

5.1.1. Method A

A mixture of 6-amino-1-furfuryluracil (1 mmol), 15% NaOH (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. 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 H_2O, dried (Na_2SO_4) and evaporated to dryness to give the corresponding 3-substituted 6-amino-1-furfuryluracils, which in most cases were used in subsequent steps without further purification.

5.1.1.1. 6-Amino-1-furfuryl-3-propyluracil (6c). Method A, alkylating agent: propyl bromide, reaction time 6 h, foamy solid, yield 54%. ^{1}H NMR (DMSO-d_6) δ: 7.58 (d, J = 0.9 Hz, 1H, 5-H C_4H_3O), 6.85 (s, 2H D_2O exchange, NH_2), 6.38 (dd, J = 3.1, 1.9 Hz, 1H, 4-H C_4H_3O), 6.30 (d, J = 3.1 Hz, 1H, 3-H C_4H_3O), 5.05 (s, 2H, CH_2–C_4H_3O), 4.66 (s, 1H, 5-H), 3.66 (t, J = 7.4 Hz, 2H, NCH_2), 1.47 (sex, J = 7.4 Hz, 2H, CH_2CH_3), 0.79 (t, J = 7.4 Hz, 3H, CH_3). HRMS m/z calcd for $C_{12}H_{15}N_3O_3$, 249.1113; found, 227.1130.

5.1.2. Method B

A suspension of 6-amino-1-furfuryluracil (1.0 g, 7.8 mmol) and $(NH_4)_2SO_4$ (0.05 g) in hexamethyldisilazane (HMDS, 10 mL) was heated under reflux for 2 h and during this time the mixture became homogeneous. Excess HMDS was distilled off, at first under atmospheric pressure and then under vacuum. The product was allowed to cool to 70 °C, a temperature above the melting point of the compound as it had to be liquid for the subsequent step. A cat-

alytic amount of I_2 (ca. 8 mg) was dissolved in the product and an 80% toluene solution of the corresponding organic bromide (1.0 mL, 9.0 mmol) was added. The mixture was heated in an oil bath under reflux for (1.5–3 h) and then was allowed to cool to room temperature and a solution of $Na_2S_2O_3$ (1.5 g) in H_2O (5 mL) was added. The flask was cooled in an ice bath and a saturated aqueous $NaHCO_3$ (ca. 40 mL), was added in small portions over a period of 15 min with vigorous stirring until effervescence ceased, and the gelatinous mixture had turned into a suspension. The precipitate was filtered off, washed with cold water (15 mL), toluene (10 mL), and ether (10 mL).

5.1.2.1. 6-Amino-1-furfuryl-3-(prop-2-ynyl)uracil (6g). Method B, alkylating agent: prop-2-ynyl bromide, reaction time 1 h, pink solid, yield 38%. ^{1}H NMR ($CDCl_3$) δ: 7.38 (v s, 1H, 5-H C_4H_3O), 6.41 (v s, 1H, 3-H C_4H_3O), 6.30 (v s, 1H, 4-H C_4H_3O), 5.01 (s, 2H, C*H*$_2$–C_4H_3O), 4.70 (s, 1H, 5-H), 3.46–3.45 (m, 2H, CHCC*H*$_2$), 2.21 (s, 1H, C*H*CCH$_2$), 2.16 (br s, 2H, D_2O exchange, NH_2). Anal. Calcd for $C_{12}H_{11}N_3O_3$, (245.23): C, 58.77; H, 4.52; N, 17.13. Found: C, 59.04; H, 4.76; N, 17.40.

5.2. General procedure for the preparation of 3-substituted 6-amino-1-furfuryl-5-nitrosouracils 7(a–l)

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-furfuryluracil **6** (6 mmol) in 50% aqueous 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 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-furfuryl-5-nitrosouracil (7b)

Reaction time 24 h, violet solid, mp 192–194 °C (EtOAc), yield 54%. ^{1}H NMR (DMSO-d_6) δ: 3.24 (br s, 1H, D_2O exchange, N*H*H), 9.27 (br s, 1H, D_2O exchange, NH*H*), 7.61 (d, J = 0.8 Hz, 1H, 5-H C_4H_3O), 6.45 (d, J = 3.0 Hz, 1H, 3-H C_4H_3O), 6.40 (dd, J = 3.0, 1.8 Hz, 1H, 4-H C_4H_3O), 5.13 (s, 2H, C*H*$_2$–C_4H_3O), 3.93 (q, J = 7.0 Hz, 2H, C*H*$_2$CH$_3$), 1.06 (t, J = 7.0 Hz, 3H, CH_3). Anal. Calcd for $C_{11}H_{12}N_4O_4$, (264.23): C, 50.00; H, 4.58; N, 21.20. Found: C, 50.42; H, 4.69; N, 21.09.

5.3. General procedure for the preparation of 3-substituted 5,6-diamino-1-furfuryluracils 8(a–l)

$Na_2S_2O_4$ (16 mmol) was added in small portions to a well-stirred suspension of the corresponding 3-substituted 6-amino-1-furfuryl-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 to 4 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-furfuryluracil (8b)

Reaction time 2.5 h, green solid, yield 91%. ^{1}H NMR (DMSO-d_6) δ: 7.31 (d, J = 1.7 Hz, 1H, 5-H C_4H_3O), 6.39 (d, J = 3.1 Hz, 1H, 3-H C_4H_3O), 6.31 (dd, J = 3.1, 1.9 Hz, 1H, 4-H C_4H_3O), 5.11 (br s, 2H, D_2O exchange, NH_2), 5.03 (s, 2H, C*H*$_2$–C_4H_3O), 3.90 (q, J = 7.1 Hz, 2H, C*H*$_2$CH$_3$), 2.24 (br s, 2H, D_2O exchange, N*H*$_2$), 1.17 (t, J = 7.1 Hz, 3H, CH_3). Anal. Calcd for $C_{11}H_{14}N_4O_3$ (250.25): C, 52.79; H, 5.64; N, 22.39. Found: C, 53.04; H, 5.86; N, 22.53.

5.4. General procedure for the preparation of xanthines 9(a–aa)

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 **8(a–l)** (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 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 precipitated and was filtered off, washed with H_2O and purified by crystallization or washing with the appropriate solvent.

5.4.1. 3,8-Difurfuryl-1-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (9a)

Reaction time 30 min, white solid, mp 204–206 °C (MeOH), yield 18%. IR (KBr) ν (cm^{-1}): 3144, 3092, 3033, 1708, 1656, 1555, 1504, 1411, 1398, 1288, 1222, 1151, 1004, 939, 825, 760, 507. ^{1}H NMR ($CDCl_3$) δ: 12.52 (s, 1H, D_2O exchange, NH), 7.32–7.29 (m, 2H, 2 × (5-H C_4H_3O), 6.43 (d, J = 3.0 Hz, 1H, 3-H C_4H_3O), 6.31–6.28 (m, 2H, 2 C_4H_3O), 6.21 (d, J = 2.6 Hz, 1H, C_4H_3O), 5.31 (s, 2H, NC*H*$_2$–C_4H_3O), 4.27 (s, 2H, C*H*$_2$–C_4H_3O), 3.42 (s, 3H, CH_3). ^{13}C NMR and DEPT ($CDCl_3$) δ: 156.05 (C8), 151.39 (C6), 151.32 (C2), 149.82 (C4), 149.37 (C2 C_4H_3O), 148.96 (C2 C_4H_3O), 142.83 (C5 C_4H_3O) 142.64 (C5 C_4H_3O), 111.05, 110.85, 109.98 and 108.07 (2C3 and 2C4 C_4H_3O), 107.55 (C5), 40.27 (*C*H$_2$–C_4H_3O), 28.86 (*C*H$_2$–C_4H_3O), 28.74 (CH_3). MS (EI) m/z (%): 326 (M, 6), 148 (1), 269 (3), 84 (13), 82 (6), 81 (100), 78 (1), 68 (2), 54 (1), 53 (12), 52 (2), 51 (2). Anal. Calcd for $C_{16}H_{14}N_4O_4$ (326.30): C, 58.89; H, 4.32; N, 17.17. Found: C, 59.12; H, 4.61; N, 17.42.

5.5. General procedure for the methylation of compounds 9(a–g), 9(l,m), 9(p–t) and (9y)

To a suspension of NaH (1.2 mmol) in dry DMF (10 mL) was added the corresponding 1*H*-purine-2,6(3*H*,7*H*)-dione **9** (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 was heat 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 H_2O and dried under vacuum.

5.5.1. 3,8-Difurfuryl-1,7-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (10a)

White solid, mp 121–123 °C, yield 58%. IR (KBr) ν (cm^{-1}): 3433, 3146, 2956, 1709, 1660, 1542, 1502, 1446, 1411, 1336, 1171, 1124, 1012, 971, 760, 737. ^{1}H NMR ($CDCl_3$) δ: 7.41 (v s, 1H, 5-H C_4H_3O), 7.33 (v s, 1H, 5-H C_4H_3O), 6.44–6.41 (m, 1H, C_4H_3O), 6.37–6.29 (m, 2H, 2C_4H_3O), 6.16 (d, J = 3.1 Hz, 1H, C_4H_3O), 5.27 (s, 2H, NC*H*$_2$–C_4H_3O), 4.20 (s, 2H, C*H*$_2$–C_4H_3O), 3.92 (s, 3H, CH_3), 3.39 (s, 3H, CH_3). ^{13}C NMR and DEPT ($CDCl_3$) δ: 155.85 (C8), 151.49 (C6), 150.02 (C2), 149.82 (C4), 149.37 (C2 C_4H_3O), 147.61 (C2 C_4H_3O), 142.74 (C5 C_4H_3O), 142.65 (C5 C_4H_3O), 111.11 (C_4H_3O), 110.81 (C_4H_3O), 109.79 (2C C_4H_3O), 107.96 (C5), 39.97 (*C*H$_2$–C_4H_3O), 30.10 (*C*H$_2$–C_4H_3O), 28.74 (CH_3), 27.29 (CH_3). MS (EI) m/z (%): 340 (M 9), 326 (23), 316 (39), 256 (20), 153 (20), 141 (17), 127 (21), 125 (21), 113 (24), 111 (25), 99 (29), 85 (51), 83 (24), 80 65), 70 (67), 68 (49), 56 (100), 54 (19). Anal. Calcd for $C_{17}H_{16}N_4O_4$ (340.34): C, 60.00; H, 4.74; N, 16.46. Found: C, 59.72; H, 4.44; N, 16.32.

5.6. General procedure for the oxidation of sulfides 9(y–aa) to sulfoxides 11(a–c), respectively

OXONE® (0.67 mmol) and aliquat (three drops) in a mixture of H_2O (5.8 mL) and CH_2Cl_2 (3.8 mL) were added to a solution of the corresponding 1-[2-(ethylthio)ethyl]-1*H*-purine-2,6(3*H*,7*H*)-dione **9(y–aa)** (1 mmol) in CH_3CN (0.4 mL) cooled to 0 °C, and the mixture was stirred at room temperature for the time quoted below. 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 that was purified by recrystallization or column chromatography on silica gel.

5.6.1. 8-Benzyl-1-[2-(ethylsulfinyl)ethyl]-3-furfuryl-1*H*-purine-2,6(3*H*,7*H*)-dione (11a)

White solid, mp 165–167 °C (CH_3CN), yield 65%. IR (KBr) ν (cm^{-1}): 3140, 3088, 2969, 1704, 1658, 1602, 1556, 1503, 1453,1409, 1268, 1215, 1155, 1105, 1018, 980, 770, 754, 729, 697. ^{1}H NMR ($CDCl_3$) δ: 12.36 (s, 1H, D_2O exchange, NH), 7.34–7.19 (m, 6H, 2-H, 3-H, 4-H, 5-H, 6-H C_6H_5 and 5-H C_4H_3O), 6.41 (d, J = 3.2 Hz, 1H, 3-H C_4H_3O), 6.29 (dd, J = 3.1, 1.9 Hz, 1H, 4-H C_4H_3O), 5.28 (s, 2H, CH_2–C_4H_3O), 4.45–4.22 (m, 2H, CH_2N), 4.18 (s, 2H, CH_2–C_6H_5), 3.31–3.22 (m, 1H, SOC*H*H), 3.02–2.73 (m, 3H, CH_2SOCH*H*), 1.32 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR and DEPT ($CDCl_3$) δ: 154.85 (C8), 154.35 (C6), 151.32 (C2), 149.86 (C4), 149.08 (C2 C_4H_3O), 142.80 (C5 C_4H_3O), 136.57 (C1 C_6H_5), 129.26 and 129.15 (C2, C3, C5, C6 C_6H_5), 127.46 (C4 C_6H_5), 110.86 (C4 C_4H_3O), 109.94 (C3 C_4H_3O), 107.46 (C5), 50.19 ($SOCH_2$), 45.92 (*C*H_2–C_4H_3O), 40.24 (CH_2SO) 36.33 (*C*H_2–C_6H_5), 35.70 (NCH_2), 7.17 (CH_3). Anal. Calcd for $C_{21}H_{22}N_4O_4S$ (426.49): C, 59.14; H, 5.20; N, 13.14; S, 7.52. Found: C, 59.55; H, 5.47; N, 13.45; S, 7.12.

5.7. 8-Benzyl-1-[2-(ethylsulfonyl)ethyl]-3-furfuryl-1*H*-purine-2,6(3*H*,7*H*)-dione (12)

A mixture of the 1-[2-(ethylthio)ethyl]-1*H*-purine-2,6(3*H*,7*H*)-dione **9y** (0.14 g, 0.35 mmol), HOAc (0.18 mL) 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 solvent was evaporated under reduced pressure to leave a solid residue (0.15 g) that was purified by recrystallization.

Compound **12:** White solid, mp 210–212 °C (MeOH), yield 97%. IR (KBr) ν (cm^{-1}): 3176, 3110, 3036, 1705, 1667, 1553, 1495, 1347, 1289, 1121, 1013, 782, 696. ^{1}H NMR ($CDCl_3$) δ: 11.84 (s, 1H, D_2O exchange, NH), 7.35–7.22 (m, 6H, 2-H, 3-H, 4-H, 5-H, 6-H C_6H_5 and 5-H C_4H_3O), 6.43 (d, J = 3.2 Hz, 1H, 3-H C_4H_3O), 6.31 (dd, J = 3.2, 1.9 Hz, 1H, 4-H C_4H_3O), 5.31 (s, 2H, CH_2–C_4H_3O), 4.48 (t, J = 3.2 Hz, 2H, CH_2N), 4.24 (s, 2H, CH_2–C_6H_5), 3.33 (t, J = 7.3 Hz, 2H, SO_2CH_2), 3.08 (q, J = 7.5 Hz, 2H, CH_2SOCH_2), 1.38 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR and DEPT ($CDCl_3$) δ: 154.70 (C8), 154.49 (C6), 150.54 (C2), 149.16 (C4), 149.12 (C2 C_4H_3O), 142.49 (C5 C_4H_3O), 135.64 (C1 C_6H_5), 128.91 and 128.76 (C2, C3, C5, C6 C_6H_5), 127.33 (C4 C_6H_5), 110.49 (C4 C_4H_3O), 109.70 (C3 C_4H_3O), 106.70 (C5), 48.99 (*C*H_2–C_4H_3O), 47.25 (SO_2CH_2), 39.90 (CH_2SO_2), 35.31 (*C*H_2–C_6H_5), 35.18 (NCH_2), 6.52 (CH_3). MS (EI) *m/z* (%): 442 (M, 13), 279 (2), 278 (4), 250 (3), 226 (2), 91 (9), 82 (6), 81 (100), 53 (15). Anal. Calcd for $C_{22}H_{24}N_4O_5S$ (456.51): C, 57.88; H, 5.30; N, 12.27; S, 7.02. Found: C, 57.35; H, 5.62; N, 12.44; S, 7.48.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.034.

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