European Journal of Medicinal Chemistry 45 (2010) 2884–2892

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and pharmacological evaluation of novel substituted 9-deazaxanthines as A_{2B} receptor antagonists

María Isabel Nieto [d], María Carmen Balo [a], José Brea [b,c], Olga Caamaño [a,c,*], Franco Fernández [a,c,**], Xerardo García-Mera [a,c], Carmen López [a,c], María Isabel Loza [b,c], José Enrique Rodríguez-Borges [e], Bernat Vidal [f]

[a] Departamento de Química Orgánica, Facultade de Farmacia, Universidade de Santiago de Compostela, Campus Sur, E-15782 Santiago de Compostela, Spain
[b] Departamento de Farmacología, Facultade de Farmacia, Universidade de Santiago de Compostela, Campus Sur, E-15782 Santiago de Compostela, Spain
[c] Instituto de Farmacia Industrial, Facultade de Farmacia, Universidade de Santiago de Compostela, Campus Sur, E-15782 Santiago de Compostela, Spain
[d] Departamento de Química Fundamental, Facultade de Química, Universidade de A Coruña, Campus da Zapateira, E-15071, A Coruña, Spain
[e] CIQ-Departamento de Química, Facultade de Ciencias, Universidade de Porto, Rua do Campo Alegre, 687, 4169-007, Porto, Portugal
[f] Medicinal Chemistry Department, Laboratorios Almirall, S.A., E-08960 St. Just Desvern, Barcelona, Spain

ARTICLE INFO

Article history:
Received 18 December 2009
Received in revised form
5 March 2010
Accepted 9 March 2010
Available online 12 March 2010

Dedicated to Professor Antonio García-Martínez on the occasion of his 70th birthday.

Keywords:
Substituted-9-deazaxanthines
Synthesis
Adenosine receptor antagonist
A_1, A_{2A}, A_{2B}, and A_3 binding affinities

ABSTRACT

A new series of 9-deazaxanthine derivatives with various substituents at the heterocyclic system were synthesized and evaluated for their binding affinities for the four human recombinant adenosine receptors, A_1–A_3 subtypes. A number of the 9-deazaxanthines derivatives **3a**–**m** showed moderate-to-high affinity for hA_{2B} receptors, with compound **3f** showing a 32-fold selectivity for A_{2B} over A_1 and a 2750-fold selectivity for A_{2B} over A_{2A}.

1. Introduction

Adenosine is an endogenous purine nucleoside that is normally increased under conditions of hypoxia or high metabolism that typically occur in pathological or stressful situations. Four diverse G-protein-coupled adenosine receptor (AdoR) subtypes (A_1, A_{2A}, A_{2B}, A_3) have been identified and characterized at biological, pharmacological and physiological levels [1].

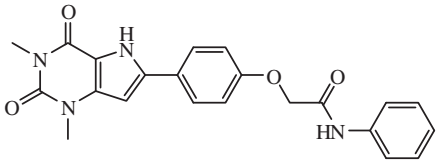
Along with purine nucleosides analogues and condensed tricyclic nitrogen heterocyclic derivatives [2–10], xanthines derivatives constitute one of the most widely exploited classes of AR (adenosine receptor) ligands [11–15]. Selective A_{2B} receptor antagonists can therefore play a role in important pathologies such as neurological (e.g., AD and dementia) and hypersensitive (e.g., asthma) disorders, and diabetes [16]. Our main interest in this challenging area has been the development of selective A_{2B} antagonists as potential anti-asthmatic agents [17–20]. After the pioneering work of Müller et al. [21] showed evidence of the existence of compounds carrying a 9-deazaxanthine (9-dAX) scaffold endowed of AdoR antagonistic activity, suitably substituted 9-dAXs that are highly potent hA_{2B} antagonist have been unveiled [19–25].

In particular, some 1,3-dialkyl-8-[4-(*N*-arylcarbamoylmethoxy)phenyl]-9-dAXs [22–24], e.g., **1**, and 1,3-dialkyl-8-[4-(*N*-arylcarbamoyloxymethyl)phenyl]-9-dAXs [20], e.g., **2**, represent

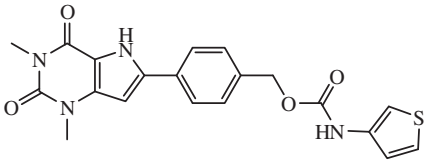
* Corresponding author at: Departamento de Química Orgánica, Facultade de Farmacia, Universidade de Santiago de Compostela, Campus Sur, E-15782 Santiago de Compostela, Spain. Tel.: +34 981563100 15047; fax: +34 981594912.
** Corresponding author at: Departamento de Química Orgánica, Facultade de Farmacia, Universidade de Santiago de Compostela, Campus Sur, E-15782 Santiago de Compostela, Spain. Tel.: +34 981563100 14942; fax: +34 981594912.
E-mail addresses: molga.caamano@usc.es (O. Caamaño), franco.fernandez@usc.es (F. Fernández).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.03.011

a novel class of AR ligands endowed with nanomolar affinities at the hA_{2B}, hA_1 and hA_{2A} AR subtypes. However, none of these ligands exhibited an acceptably high selectivity versus the hA_{2B}AR.



1

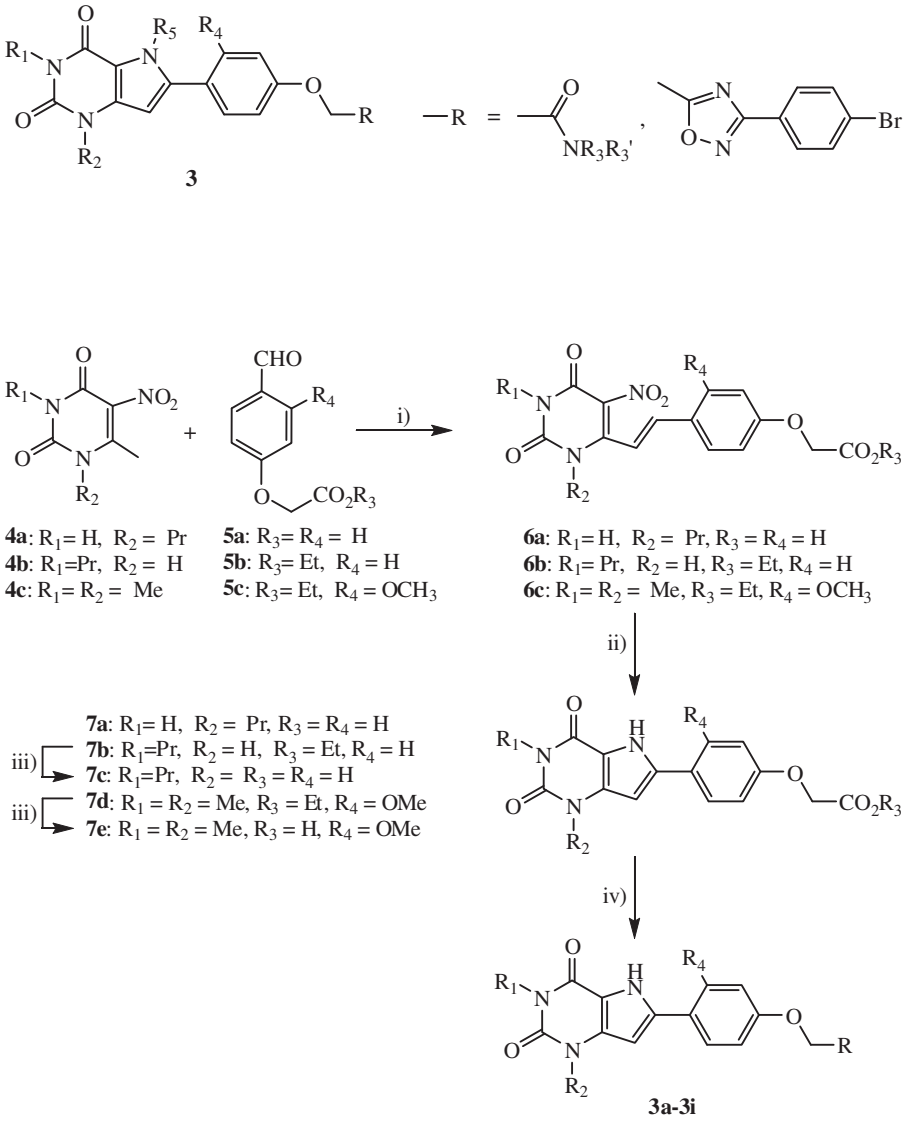


2

As a continuation of our ongoing research in the field we designed, synthesized and evaluated at AdoRs a new series of 9-dAXs (**3**), some of which differ from previously reported ligands in the combination of R_1, R_2 and/or R_4 radicals.

2. Chemistry

The synthesis of the target compounds **3a**–**m** was performed according to published procedures [20,22,26,27], as outlined in Scheme 1. The key intermediates for the synthesis of compounds **3a**–**i** were 9-dAX carboxylic acid derivatives **7a**, **7c** and **7e**, and these were prepared by the condensation of appropriate uracil derivatives **4** with the corresponding *para*-substituted benzalde-



Scheme 1. Reagents, conditions and yields: i) Dioxane, piperidine, DMAP, reflux, 5–96 h, **6a** (64%), **6b** (55%), **6c** (88%). ii) HCO_2H, $Na_2S_2O_4$, reflux, 18 h, **7a** (89%), **7b** (75%), **7d** (72%). iii) NaOH, EtOH, reflux, 1 h, **7c** (82%), **7e** (77%). iv) Appropriate amine, EDC, HOBt, TEA, DMF, rt, 5–8 days.

hydes **5** to afford the 6-styryluracils **6**, followed by cyclization to the 9-dAXs **7** and, where necessary, saponification to the free carboxylic acid. The amidation procedure for the synthesis of the 9-dAX **3a**–**i** involved condensation of 9-dAX carboxylic acids **7** and the appropriate amines in a polar aprotic solvent (DMF) in the presence of an organic base (triethylamine), 1-[3-dimethylaminopropyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt).

The synthesis of **3j** (Scheme 2) was carried out by methylation of 2-[4-(1-methyl-2,4-dioxo-3-propyl-2,3,4,5-tetrahydro-1*H*-pyrrolo[3,2-*d*]pyrimidin-6-yl)phenyloxy]acetic acid (**8**) [22] with CH_3I and Na_2CO_3 in dry DMF at 65 °C for 24 h. The ^1H NMR spectrum of the isolated product shows three singlets (at 3.89, 3.68 and 3.36 ppm, each integrating for three protons) that are assigned to the methyl groups in positions 7 and 3 (3.68 and 3.36 ppm), respectively, and to a CH_3O group of an ester (3.89 ppm), which is consistent with that, in addition to methylation at position 7, esterification of the carboxylic acid group of **8** has taken place to give **9a**. This crude product was then saponified under the conditions indicated in Scheme 2, which gave the desired acid **9b**. Reaction of this compound with 4-fluoroaniline under the previously described amidation conditions gave compound **3j**.

Finally, 7-methyl-9-dAX derivatives **3k**–**m** were prepared from the corresponding 9-dAX **10a**–**c**, [22] by treatment with CH_3I in DMF in the presence of K_2CO_3 (Scheme 3).

The physicochemical characteristics of the synthesized compounds are presented in Table 1.

3. Results and discussion

Chemical structures and human AR binding affinities of the novel 1,3,8-substituted-9-deazaxanthine derivatives **3a**–**m** are reported in Table 2. The affinity (pK_i or displacement percentage) values were determined at cloned human adenosine receptors expressed in HeLa cells (hA_{2A} and hA_3), HEK-293 cells (hA_{2B}) and CHO-A1 cells (hA_1). 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 [17,18,20]. 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 Experimental part.

All of the compounds displayed in this Table showed low affinity for human A_3 receptor. On the other hand, affinity values of our compounds for the A_1 receptor subtype point to the convenience of having a substituent at position 1 for the improvement of this property, best results being obtained when R_1 is a propyl group. Thus, compound **3g** showed maximum affinity for A_1 receptor, with a 2-fold A_1/A_{2B} selectivity.

Interestingly, affinities for the A_{2B} receptor subtype were found to be generally higher than those observed for the A_{2A} receptor. The best affinity for the A_{2B} receptor is obtained when R_1 is a propyl radical, and R_3 is 4-halophenylaminocarbonyl group [see compounds **3f**–**h**, which bear a propyl radical in position 1 and showed moderate-to-high affinity at human A_{2B} receptor (K_i in the 2.34–31.6 nM range)], with the most interesting compound **3f** showing both high affinity for human A_{2B} receptor and a 32-fold A_{2B}/A_1 selectivity and a 2750-fold A_{2B}/A_{2A} selectivity. Though not determined, selectivity over A_3 receptor is estimated to be even higher.

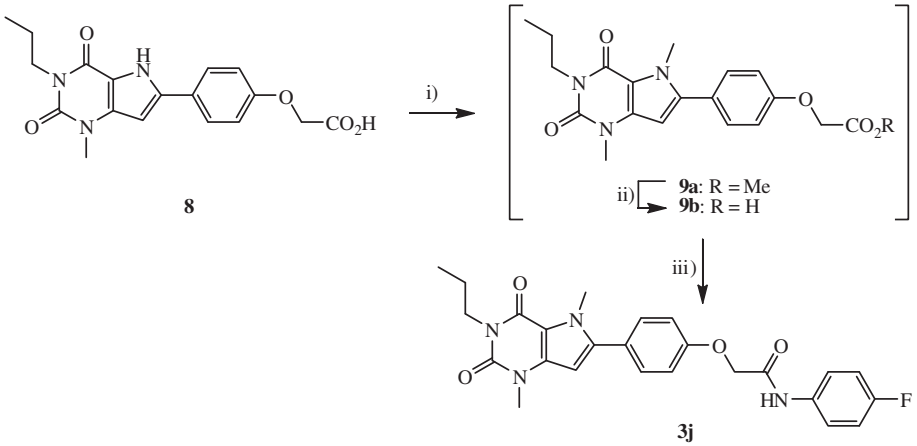
In contrast, affinity for the A_{2B} receptor decreased for the compounds **3a**, **3b** and **3d**, which contain a propyl group in position 3, (K_i in the 21.9–115 nM range), while their selectivity over A_1 and A_2 receptors increased. All these compounds had a very low affinity for human A_3 receptors, in line with previously reported findings on related structures [11,28,29].

The 7-methyl-9-dAX derivatives (compounds **3j**–**m**) were found to have a notably decreased affinity for human A_{2B} receptor with regard to their corresponding 7-demethyl counterparts (all of them with K_i in the 2.40–5.62 nM range [17,20]), as maximum affinity at this subset was found for compound **3l**, with a $K_i = 97.7$ nM.

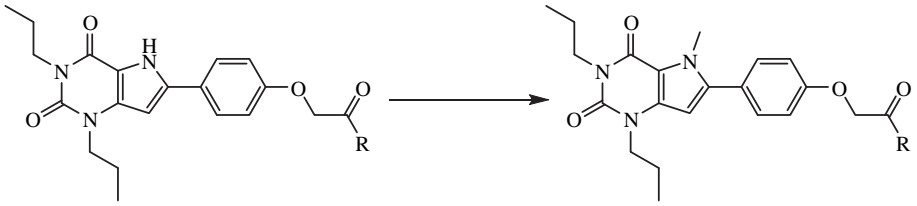
Analysis of the structure–activity relationships (SAR) shows that N-1 substituted 9-deazaxanthines derivatives present higher affinity and selectivity at A_{2B} receptor compared to their N-3 counterparts (**3a**–**e**) and to their corresponding 1,3-disubstituted analogues (**3i**–**m**). This appears to be a general trend previously put in evidence on xanthine derivatives [11,28,29].

4. Conclusion

The results reported here show that 1-alkyl-8-(4-halophenylaminocarbonylmethoxyphenyl)-9-deazaxanthines can be considered as appropriate compounds to develop potent and hopefully ligands for the ARs, especially at the A_{2B} receptor subtype. Interestingly, affinities at the A_{2B} receptor subtype were found to be generally higher than those observed at the A_{2A} receptor, especially 3-unsubstituted 9-deazaxanthine derivatives bearing a propyl group at the 1-position and a 4-halophenylaminocarbonylmethoxyphenyl residue at the 8-position, with the most selective compound **3f** having a $K_i(A_1)/K_i(A_{2B})$ ratio over 32 and a $K_i(A_{2A})/K_i(A_{2B})$ ratio over 2750.



Scheme 2. Reagents and conditions: i) CH_3I, K_2CO_3, DMF, 65 °C, 24 h. ii) EtOH, 3 N NaOH, 80 °C, 1 h. iii) 4-fluorophenylaniline, EDC, TEA, HOBt, DMF, rt, 48 h.



10a: R = 4-phenylpiperazinyl
10b: R = 3,4-dihydroisoquinolin-2(1*H*)-yl
10c: R = 4-fluorophenylamino

3k (50%)
3l (63%)
3m (61%)

Scheme 3. Reagents and conditions: a) CH_3I, K_2CO_3, DMF, 65 °C, 5–7 h.

5. Experimental

5.1. Chemistry

General information. All chemicals used 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 silica gel plates (Merck 60 F254, 0.25 mm). Melting points (uncorrected) were measured in a glass capillary tube on a Stuart Scientific electro thermal SMP3 apparatus. Infrared spectra were recorded on a Perkin-Elmer 1640 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AMX 300 spectrometer at 300 MHz using TMS as internal standard (chemical shifts in δ values, J in Hertz). Mass spectra were recorded on a Hewlett-Packard HP5988A, a Micromass Autospec spectrometers or an Electrospray interface Ion Trap Mass spectrometer. (1100 series LC/MSD Trap System Agilent, Palo Alto, Ca). Microanalyses were performed on a FISONS EA 1108 Elemental Analyser at the University of Santiago Microanalysis Service; all results shown are within 0.4% of the theoretical values.

The uracil derivatives **4** needed for the preparation of target compounds **3** were either commercially available or recently obtained by standard methods [19,30,31].

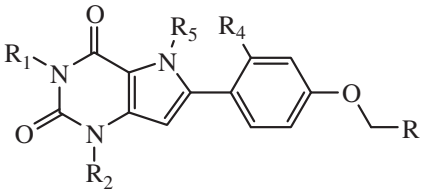
5.1.1. General procedure for the condensation between 6-methyl-5-nitrouracils ***4*** and benzaldehydes ***5***. Preparation of 1,3-dialkyl-6-styryluracil derivatives ***6a–c***

A solution of the corresponding 1,3-dialkyl-6-methyl-5-nitropyrimidine-2,4(1*H*,3*H*)-dione **4a–c** (8 mmol), the appropriate benzaldehyde **5** (8 mmol), piperidine (12 mmol) and 3 Å molecular sieves in dry dioxane (50 mL) was heated under reflux for the appropriate time under argon and then allowed to cool down to room temperature.

*5.1.1.1. 2-{4-[(E)-2-(5-Nitro-2,4-dioxo-3-propyl-1,2,3,6-tetrahydropyrimidin-4-yl)ethenyl]phenyloxy}acetic acid [**6a**].* Reaction time 48 h. 6 N HCl was added to the solution and the resulting precipitate was collected by filtration and washed with water. Yield 64%. ^{1}H NMR (DMSO-d_6): 12.25 (br s, 1H, D_2O exchange, CO_2H), 7.58 (d, J = 8.2 Hz, 2H, C_6H_4O), 7.05–6.88 (m, 4H, 2H C_6H_4O + 2H, HC=CH), 4.73 (s, 2H, CH_2O), 3.89–3.73 (m, 2H, CH_2N), 3.40 (br s, 1H, D_2O exchange, NH), 1.62–1.53 (m, 2H, $C\underline{H}_2CH_3$), 0.81 (t, J = 7.3 Hz, CH_3).

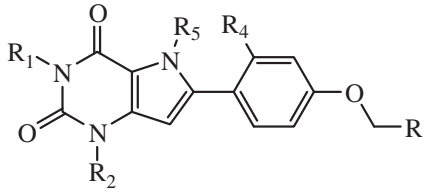
*5.1.1.2. Ethyl 2-{4-[(E)-2-(5-nitro-2,4-dioxo-1-propyl-1,2,3,6-tetrahydropyrimidin-4-yl)ethenyl]phenyloxy}acetate [**6b**].* Reaction time 96 h. 6 N HCl was added to the solution and the resulting precipitate was collected by filtration and washed with ether. Yield 55%. ^{1}H NMR (DMSO-d_6): 11.62 (s, 1H, D_2O exchange, NH), 7.74 (d, J = 16.1 Hz, 1H, CH=CH), 7.58 (d, J = 8.6 Hz, 2H, 2-H and 6-H,

Table 1
Physicochemical characteristics of synthesized 1,3,8-substituted-9-deazaxanthines **3a–m**.



Compd.	R_1	R_2	R	R_4	R_5	Molecular formula	Mol. wt	Mp (°C)	Yield (%)
3a	H	Pr	4-bromophenylaminocarbonyl	H	H	$C_{23}H_{21}BrN_4O_4$	497.34	276–278	25
3b	H	Pr	4-fluorophenylaminocarbonyl	H	H	$C_{23}H_{21}FN_4O_4$	436.44	306–308	56
3c	H	Pr	phenylaminocarbonyl	H	H	$C_{23}H_{22}N_4O_4$	418.16	277–280	37
3d	H	Pr	4-phenylpiperazinylcarbonyl	H	H	$C_{27}H_{29}N_5O_4$	487.22	280–282	20
3e	H	Pr	3,4-dihydroisoquinolin-2(1*H*)-ylcarbonyl	H	H	$C_{26}H_{26}N_4O_4$	458.20	246–248	28
3f	Pr	H	4-fluorophenylaminocarbonyl	H	H	$C_{23}H_{21}FN_4O_4$	436.44	>370	75
3g	Pr	H	4-bromophenylaminocarbonyl	H	H	$C_{23}H_{21}BrN_4O_4$	497.34	>305	20
3h	Pr	H	3-(4-bromophenyl)-1,2,4-oxadiazol-5-yl)	H	H	$C_{24}H_{20}B_rN_5O_4$	522.35	>300	32
3i	Me	Me	3-(4-bromophenyl)-1,2,4-oxadiazol-5-yl)	OMe	H	$C_{24}H_{20}BrN_5O_5$	538.35	>350	27
3j	Pr	Me	4-fluorophenylaminocarbonyl	H	Me	$C_{25}H_{25}FN_4O_4$	464.49	195–196	39
3k	Pr	Pr	4-phenylpiperazinylcarbonyl	H	Me	$C_{31}H_{37}N_5O_4$	543.66	172–174	33
3l	Pr	Pr	3,4-dihydroisoquinolin-2(1*H*)-ylcarbonyl	H	Me	$C_{30}H_{34}N_4O_4$	514.26	121–123	63
3m	Pr	Pr	4-fluorophenylaminocarbonyl	H	Me	$C_{27}H_{29}FN_4O_4$	492.54	169–171	61

Table 2
Chemical structures and binding affinities[a] at human A_{2B}, A_{2A}, A_1 and A_3 AdoRs of 1,3,8-substituted-9-deazaxanthines.



Compd.	R_1	R_2	R	R_4	R_5	hA_{2B}	hA_{2A}	hA_1	hA_3	A_{2B}/A_{2A} ratio[b]	A_{2B}/A_1 ratio[b]
3a	H	Pr	4-bromophenylaminocarbonyl	H	H	7.66	22%	41%	28%	–	–
3b	H	Pr	4-fluorophenylaminocarbonyl	H	H	7.0	36%	2%	3%	–	–
3c	H	Pr	phenylaminocarbonyl	H	H	5.98	17%	0.5%	1%	–	–
3d	H	Pr	4-phenylpiperazinylcarbonyl	H	H	6.94	33%	23%	15%	–	–
3e	H	Pr	3,4-dihydroisoquinolin-2(1*H*)-ylcarbonyl	H	H	7.10	12%	9%	1%	–	–
3f	Pr	H	4-fluorophenylaminocarbonyl	H	H	8.63	5.19	7.12	2%	2754.23	32.36
3g	Pr	H	4-bromophenylaminocarbonyl	H	H	8.45	43%	8.76	14%	–	0.49
3h	Pr	H	3-(4-bromophenyl)-1,2,4-oxadiazol-5-yl)	H	H	7.50	11%	21%	2%	–	–
3i	Me	Me	3-(4-bromophenyl)-1,2,4-oxadiazol-5-yl)	OMe	H	7.39	6.15	36%	3%	17.38	–
3j	Pr	Me	4-fluorophenylaminocarbonyl	H	Me	7.51	11%	6.74	6%	–	5.89
3k	Pr	Pr	4-phenylpiperazinecarbonyl	H	Me	36%	22%	22%	2%	–	–
3l	Pr	Pr	3,4-dihydroisoquinolin-2(1*H*)-ylcarbonyl	H	Me	7.01	29%	19%	9%	–	–
3m	Pr	Pr	4-fluorophenylaminocarbonyl	H	Me	20%	15%	9%	1%	–	–

[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] Affinity ratios were calculated on the basis of K_i values.

C_6H_4O), 6.99 (d, J = 8.5 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.82 (d, J = 16.1 Hz, 1H, CH=CH), 4.84 (s, 2H, $C\underline{H}_2O$), 4.15 (q, J = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), (3.74 (t, J = 7.4 Hz, 2H, CH_2N), 1.56–1.51 (m, 2H, $C\underline{H}_2CH_3$), 1.20 (t, J = 7.1 Hz, 2H, $OCH_2C\underline{H}_3$), 0.86 (t, J = 7.6 Hz, 3H, CH_3).

*5.1.1.3. Ethyl 2-{4-[(E)-2-(1,3-dimethyl-5-nitro-2,4-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)ethenyl]-3-methoxyphenyloxy}acetate [**6c**].* Reaction time 48 h. The resulting solution was concentrated under vacuum and the black residue was washed with EtOH/ether to give a yellow solid. Yield 88%. ^{1}H NMR (DMSO-d_6): 7.61 (d, J = 8.5 Hz, 1H, 6-H C_6H_3), 7.13 (d, 1H, J = 16.5 Hz, HC=C$\underline{H}$), 6.99 (d, 1H, J = 16.5 Hz, $\underline{H}$C=CH), 6.64 (s, 1H, 3-H C_6H_3), 6.60 (d, 1H, J = 8.5 Hz, 5-H C_6H_3,), 4.86 (s, 2H, OCH_2), 4.17 (q, 2H, J = 7.07 Hz, CH_2N), 3.82 (s, 3H, CH_3O), 3.35 (s, 3H, CH_3N), 3.22 (s, 3H, CH_3N), 1.21 (t, 3H, J = 7.07 Hz, $CH_2C\underline{H}_3$).

*5.1.2. General procedure for the reductive ring closure of 5-nitro-6-styryluracils **6a–b** to **7a**, **7b** or **7d***

To a solution of the appropriate 5-nitro-6-styryluracil derivative **6a–c** (5 mmol) in formic acid (44 mL) was slowly added sodium dithionite (25 mmol) and the mixture was heated under reflux for 18 h overnight. The resulting suspension was cooled to room temperature and poured into water. The precipitate was collected by filtration, washed with water and dried under vacuum to yield the expected compounds **7a–b** or **7d**.

*5.1.2.1. 2-[4-(2,4-Dioxo-1-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)phenyloxy]acetic acid [**7a**].* Yield 89%. ^{1}H NMR (DMSO-d_6): 13.15 (br s, 1H, CO_2H), 12.23 (br s, 1H, D_2O exchange, NH), 10.82 (br s, 1H, D_2O exchange, NH), 7.82 (d, 2H, J = 5Hz, C_6H_4O), 6.99–6.93 (m, 2H, C_6H_4O), 6.60 (s, 1H, 7-H), 4.71 (s, 2H, CH_2O), 3.81–3.77 (m, 2H, CH_2N), 1.68–1.63 (m, 2H, $NCH_2C\underline{H}_2$), 0.94 (t, 3H, J = 7.5 Hz, CH_3).

*5.1.2.2. Ethyl 2-[4-(2,4-dioxo-3-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)phenyloxy]acetate [**7b**].* Yield 75%. ^{1}H NMR (DMSO-d_6): 10.77 (s, 1H, D_2O exchange, CO_2H), 8.12 (s, 1H, D_2O exchange, NH), 7.91 (s, 1H, D_2O exchange, NH), 7.10 (d, J = 8.6 Hz, 2H, 2-H and 6-H, C_6H_4O), 6.65 (d, J = 9.0 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.17 (d, J = 2.3 Hz, 1H, 7-H), 4.52 (s, 2H, CH_2O), 3.79–3.74 (m, 2H, CH_2N), 1.59–1.50 (m, 2H, $C\underline{H}_2CH_3$), 0.88–0.82 (m, 3H, CH_3).

*5.1.2.3. Ethyl 2-[4-(1,3-dimethyl-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)-3-methoxyphenyloxy]acetate [**7d**].* Yield 72%. ^{1}H NMR (DMSO-d_6): 8.67 (br s, 1H, D_2O exchange, NH), 7.72 (d, J = 8.5 Hz, 1H, 6-H C_6H_3), 6.71 (s, 1H, 7-H), 6.60 (d, 1H, J = 8.5 Hz, 5-H C_6H_3), 6.53 (s, 1H, H-6 C_6H_3), 4.87 (s, 2H, CH_2O), 4.20 (q, 2H, J = 7.0 Hz, $C\underline{H}_2CH_3$), 3.88 (s, 3H, CH_3O), 3.41 (s, 3H, CH_3N), 3.26 (s, 3H, CH_3N), 1.24 (t, 3H, J = 7.0 Hz, $CH_2C\underline{H}_3$).

*5.1.3. General procedure for the hydrolysis of **7b** and **7d***

To a suspension of the corresponding ethyl ester **7b** or **7d** (1.60 mmol) in EtOH (12 mL) was added 2.5 N NaOH (12 mL) and the mixture was heated under reflux for 1 h. The resulting yellow solution was cooled to room temperature and was evaporated under reduced pressure to remove the EtOH. 3 N HCl was added to the resulting suspension and the precipitate was collected by filtration, washed with water and dried.

*5.1.3.1. 2-[4-(2,4-Dioxo-3-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)phenyloxy]acetic acid [**7c**].* Yield 82%. ^{1}H NMR (DMSO-d_6): 10.77 (s, 1H, D_2O exchange, CO_2H), 8.12 (s, 1H, D_2O exchange, NH), 7.91 (s, 1H, D_2O exchange, NH), 7.10 (d, J = 8.6 Hz, 2H, 2-H and 6-H, C_6H_4O), 6.65 (d, J = 9.0 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.17 (d, J = 2.3 Hz, 1H, 7-H), 4.52 (s, 2H, $C\underline{H}_2O$), 3.79–3.74 (m, 2H, CH_2N), 1.59–1.50 (m, 2H, $C\underline{H}_2CH_3$), 0.88–0.82 (m, 3H, CH_3).

*5.1.3.2. 2-[4-(1,3-Dimethyl-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)-3-methoxyphenyloxy]acetic acid [**7e**].* Yield 77%. ^{1}H NMR (DMSO-d_6): 11.93 (br s, 1H, CO_2H), 8.14 (br s, 1H, NH), 7.68 (d, J = 8.2 Hz, 1H, 6-H C_6H_3O), 6.68 (s, 1H, 3-H, C_6H_3O), 6.55 (d, 1H, J = 8.2 Hz, 5-H C_6H_3O), 6.50 (s, 1H, 7-H), 4.87 (s, 2H, CH_2O), 3.86 (s, 3H, CH_3O), 3.77 (s, 3H, CH_3N), 3.71 (s, 3H, CH_3N).

*5.1.4. General procedures for the preparation of amides **3a–j** from the appropriate carboxylic acid xanthine derivatives **7a**, **7b**, **7d** or **9b***

To a mixture of the carboxylic acid **7** (see Scheme 1) (1.24 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.28 g, 1.49 mmol), 1-hydroxybenzotriazole (0.20 g, 1.49 mmol), triethylamine (0.35 mL, 2.48 mmol) and anhydrous CH_2Cl_2 (20 mL) was added the appropriate amine (1.61 mmol) and the mixture was stirred at room temperature for 5–8 days under an argon atmosphere. The resulting solution was evaporated under reduced pressure and the residue was dissolved in DCM and washed with a saturated aqueous sodium bicarbonate. The organic phase was separated, washed with water and brine, dried (Na_2SO_4) and evaporated under reduced pressure. The resulting crude material was purified by flash column chromatography on silica gel or crystallized from H_2O/MeOH.

*5.1.4.1. 2-[4-(2,4-Dioxo-1-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d] pyrimidin-6-yl)phenyloxy]-N-(4-bromophenyl)acetamide **[3a]**.* Yield 25%, mp 276–278 °C. IR (KBr) ν (cm^{-1}): 3178, 3034, 2964, 1682, 1550, 1489, 1437, 1182, 1119, 1009, 831, 777. ^{1}H NMR (DMSO-d_6): 12.00 (s, 1H, D_2O exchange, NH), 10.53 (s, 1H, D_2O exchange, NHCO), 10.16 (s, 1H, D_2O exchange, NH), 7.67 (d, J = 8.6 Hz, 2H, 2-H and 6-H, C_6H_4Br), 7.46 (d, J = 8.6 Hz, 2H, 3-H and 5-H, C_6H_4Br), 7.34 (d, J = 8.6 Hz, 2H, 2-H and 6-H, C_6H_4O), 6.88 (d, J = 8.5 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.41 (s, 1H, 7-H), 4.58 (s, 2H, CH_2O), 3.94–3.47 (m, 2H, CH_2N), 1.50–1.05 (m, 2H, CH_2–CH_3), 0.73 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 167.30 (C), 158.49 (C), 155.56 (C), 151.55 (C), 140.04 (C), 138.46 (C), 137.97 (C), 132.24 (CH), 127.60 (CH), 124.84 (C), 122.31 (CH), 116.05 (C), 115.70 (C), 111.60 (C), 93.07 (CH), 79.86 (CH), 67.80 (CH_2), 45.94 (CH_2), 21.37 (CH_2), 11.69 (CH_3). MS (EI) m/z (%): 498 (5), 496 (M, 4), 285 (100), 284 (44), 243 (64), 213 (78), 207 (17). Anal. Calcd for $C_{23}H_{21}BrN_4O_4$ (497.34): C, 55.54; H, 4.26; Br, 16.07; N, 11.27. Found: C, 55.59; H, 4.35; Br, 15.88; N, 10.97.

*5.1.4.2. 2-[4-(2,4-Dioxo-1-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d] pyrimidin-6-yl)-phenyloxy]-N-(4-fluorophenyl)]acetamide **[3b]**.* Yield 56%, mp 306–308 °C. IR (KBr) ν (cm^{-1}): 3156, 3033, 2968, 1685, 1550, 1437, 1369, 1220,1069, 835, 777. ^{1}H NMR (DMSO-d_6): 12.20 (s, 1H, D_2O exchange, NH), 10.78 (s, 1H, D_2O exchange, NHCO), 10.16 (s, 1H, D_2O exchange, NH), 7.85 (d, J = 8.7 Hz, 2H, 2-H and 6-H, C_6H_4F), 7.65 (d, J = 9.0 Hz, 2H, 2-H and 6-H, C_6H_4O) 7.16 (t, J = 8.9 Hz, 2H, 3-H and 5-H, C_6H_4F), 7.05 (d, J = 8.8 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.62 (s, 1H, 7-H), 4.74 (s, 2H, CH_2O), 3.78 (t, J = 7.3 Hz, 2H, CH_2N), 1.65 (sext, J = 7.3 Hz, 2H, CH_2CH_3), 0.90 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.67 (C), 158.23 (C), 156.32 (C), 139.65 (C), 135.28 (C), 134.22 (C), 133.12 (C), 129.02 (C), 127.21 (CH), 124.82 (C), 121.96 (CH), 121.85 (CH) 115.78 (CH), 115.49 (CH), 115.33 (CH), 112.08 (C), 92.78 (CH), 67.32 (CH_2), 46.12 (CH_2), 20.99 (CH_2), 11.29 (CH_3). MS (EI) m/z (%): 437 (M + 1, 11), 436 (100), 364 (3), 285 (79), 284 (37), 243 (29), 213 (5), 207 (1). Anal. Calcd for $C_{23}H_{21}FN_4O_4$ (436.44): C, 63.30; H, 4.85; F, 4.35; N, 12.84. Found: C, 63.59; H, 4.55; F, 4.65; N, 13.17.

*5.1.4.3. 2-[4-(2,4-Dioxo-1-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d] pyrimidin-6-yl)phenyloxy]-N-phenyl]acetamide **[3c]**.* Yield 37%, mp 277–280 °C. IR (KBr) ν (cm^{-1}): 3181, 3034, 2966, 1684, 1548, 1490, 1439, 1381, 1184, 1118, 991, 766. ^{1}H NMR (DMSO-d_6): 12.19 (s, 1H, D_2O exchange, NH), 10.78 (s, 1H, D_2O exchange, NHCO), 10.08 (s, 1H, D_2O exchange, NH), 7.82 (d, J = 8.6 Hz, 2H, 2-H and 6-H, C_6H_5), 7.22 (t, J = 7.7 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.00–6.94 (m, 4H, 3-H and 5-H, C_6H_4O and 3-H and 5-H, C_6H_5), 6.80 (t, J = 7.1 Hz, 1H, 4-H, C_6H_5), 6.60 (d, J = 1.2 Hz, 1H, CH), 4.92 (s, 2H, CH_2O), 3.78 (t, J = 7.0 Hz, 2H, CH_2N), 1.66 (sext, J = 7.2 Hz, 2H, CH_2CH_3), 0.91 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.76 (C), 158.26 (C), 151.24 (C), 151.17 (C), 139.73 (C), 138.73 (C), 129.13 (CH), 127.28 (CH), 127.21 (CH), 124.46 (C), 124.11 (CH), 120.08 (CH), 115.38 (CH), 111.26 (C), 92.98 (CH), 67.53 (CH_2), 43.65 (CH_2), 21.01 (CH_2), 11.37 (CH_3). MS (EI) m/z (%): 419 (M + 1, 1), 418 (12), 396 (7), 147 (27), 121 (25), 107 (64), 95 (63), 91 (52), 81 (69), 69 (80), 57 (82), 55 (100). Anal. Calcd for $C_{23}H_{22}N_4O_4$ (418.16): C, 66.02; H, 5.30; N, 13.39. Found: C, 66.53; H, 5.67; N, 13.13.

*5.1.4.4. 6-[4-(4-Phenylpiperazin-1-yl)carbonylmethoxyphenyl]-1-propyl-1,5-dihydro-3H-pirrolo[3,2-d]pyrimidine-2,4-dione **[3d]**.* Yield 20%, mp 280–282 °C. IR (KBr) ν (cm^{-1}): 3431, 3183, 3034, 1684, 1553, 1490, 1466, 1230, 1183, 1025, 694. ^{1}H NMR (DMSO-d_6): 12.18 (s, 1H, D_2O exchange, NH), 10.08 (s, 1H, D_2O exchange, NH), 7.82 (d, J = 8.6 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.21 (t, J = 7.7 Hz, 2H, 3-H and 5-H, C_6H_5), 7.00–6.94 (m, 2H, 3-H and 5-H, C_6H_4O), 6.82–6.78 (m, 3H, 3-H, 4-H and 5-H, C_6H_5), 6.61 (s, 1H, 7-H), 4.92 (s, 2H, CH_2O), 3.78 (t, J = 7.0 Hz, 2H, CH_2N), 3.65–3.58 (m, 4H, $C_4H_8N_2$), 3.19–3.11 (m, 4H, $C_4H_8N_2$), 1.65 (sext, J = 7.1 Hz, 2H, CH_2CH_3), 0.90 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.05 (C), 158.43 (C), 155.16 (C), 151.17 (C), 151.06 (C), 139.77 (C), 137.62 (C), 129.30 (CH), 127.09 (CH), 124.06 (C), 119.67 (CH), 116.18 (CH), 115.25 (CH), 111.12 (C), 92.61 (CH), 66.16 (CH_2O), 48.57 ($2CH_2$), 45.56 (CH_2), 43.87 (CH_2), 40.63 (CH_2), 20.99 (CH_2), 11.28 (CH_3). MS (EI) m/z (%): 488 (M + 1, 8), 487 (33), 281 (16), 207 (20), 161 (55), 160 (21), 132 (100), 105 (20), 104 (14), 73 (10), 56 (22). Anal. Calcd for $C_{27}H_{29}N_5O_4$ (487.22): C, 66.55; H, 6.00; N, 14.36. Found: C, 66.83; H, 5.84; N, 14.11.

*5.1.4.5. 6-[4-(1,2,3,4-Tetrahydroisoquinolin-2-yl)carbonylmethoxyphenyl]-1-propyl-1,5-dihydro-3H-pyrrolo[3,2-d]pyrimidine-2,4-dione **[3e]**.* Yield 28%, mp 246–248 °C. IR (KBr) ν (cm^{-1}): 3173, 3040, 2967, 1673, 1551, 1485, 1369, 1284, 1225, 1180, 1084, 819, 768. ^{1}H NMR (DMSO-d_6): 12.32 (s, 1H, D_2O exchange, NH), 10.11 (s, 1H, D_2O exchange, NH), 7.81 (d, J = 8.4 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.18 (virtual s, 4H, C_6H_4), 7.00 (d, J = 8.1 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.60 (s, 1H, 7-H), 4.96 (s, 2H, CH_2O), 4.72–4.68 (m, 1H, 2-HH $C_9H_{10}N$), 4.66–4.61 (m, 1H, 2-HH $C_9H_{10}N$), 3.78–3.76 2H, 6-H_2 $C_4H_6N_2$), 3.70–3.68 (m, 2H, CH_2N), 2.94–2.91 (m, 2H, 5-H_2 $C_4H_6N_2$), 1.65 (sext, J = 7.0 Hz, 2H, CH_2CH_3), 0.90 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.58 (C), 158.54 (C), 155.21 (C), 151.23 (C), 139.85 (C), 137.69 (C), 133.03 (C), 128.75 (C), 127.15 (CH), 126.83 (CH), 126.57 (CH), 124.07 (C), 115.28 (CH), 111.16 (C), 92.66 (CH), 66.36 (CH_2), 45.60 (CH_2), 44.01 (CH_2), 42.13 (CH_2), 28.96 (CH_2), 21.04 (CH_2), 11.33 (CH_3). MS (EI) m/z (%): 459 (M + 1, 27), 458 (88), 284 (22), 174 (86), 146 (39), 132 (100), 130 (26), 117 (32), 115 (20), 105 (16), 104 (16), 58 (34). Anal. Calcd for $C_{26}H_{26}N_4O_4$ (458.20): C, 68.11; H, 5.72; N, 12.22. Found: C, 68.39; H, 5.94; N, 12.48.

*5.1.4.6. 2-[4-(2,4-Dioxo-3-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d] pyrimidin-6-yl)phenyloxy]-N-(4-fluorophenyl)]acetamide **[3f]**.* Yield 75%, mp > 370 °C. IR (KBr) ν (cm^{-1}): 2963, 2359, 1715, 1627, 1540, 1508, 1465, 1409, 1232, 1185, 1061, 829, 777. ^{1}H NMR (DMSO-d_6): 10.18 (s, 1H, D_2O exchange, NHCO), 7.68–7.63 (m, 2H, 2-H and 6-H, C_6H_4F), 7.51 (d, J = 8.7 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.18–7.11 (m, 4H, 3-H and 5-H, C_6H_4F and 3-H and 5-H, C_6H_4O), 6.20 (s, 1H, 7-H), 4.73 (s, 2H, CH_2O), 3.86 (s, 3H, CH_3N), 3.81 (t, J = 7.4 Hz, 2H, CH_2N), 1.56 (sext, J = 7.3 Hz, 2H, CH_2CH_3), 0.86 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.81 (CO), 158.15 (C4 C_6H_4O), 155.28 (C4 C_6H_4F), 151.42 (C4 and C2), 139.80 (C6), 135.05 (C4a), 134.16 (C1 C_6H_4F), 127.31 (C2 and C6 C_6H_4O), 124.46 (C1 C_6H_4O), 121.95 (C7a), 121.85 (C2 and C6 C_6H_4F), 115.50 (C3 and C5 C_6H_4F), 110.85 (C3 and C5 C_6H_4O), 94.75 (C7), 67.39 (OCH_2), 42.08 (NCH_2), 33.56 (NCH_3), 31.79 (NCH_3), 21.22 (CH_2CH_3), 11.51 (CH_3). MS (EI) m/z (%): 436 (M, 100), 398 (85), 394 (49), 370 (86), 328 (28), 285 (69), 283 (51), 243 (92), 111 (42) 86 (64), 72 (86), 58 (51), 43 (52). Anal. Calcd for $C_{23}H_{21}FN_4O_4$ (436.44): C, 63.30; H, 4.85; F, 4.35; N, 12.84. Found: C, 63.63; H, 4.49; F, 4.64; N, 13.07.

5.1.4.7. 2-[4-(2,4-Dioxo-3-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d] pyrimidin-6-yl)phenyloxy]-N-(4-bromophenyl)]acetamide ***[3g]***. Yield 20%, mp > 305 °C. IR (KBr) ν (cm^{-1}): 3114 2962, 1720, 1627, 1531, 1489, 1465, 1397, 1234, 1186, 830, 750, 712. ^1H NMR (DMSO-d_6): 12.04 (s, 1H, D_2O exchange, NH), 11.09 (s, 1H, D_2O exchange, NHCO), 10.23 (s, 1H, D_2O exchange, NH), 7.81 (d, J = 8.9 Hz, 2H, 3-H and 5-H, C_6H_4Br), 7.61 (d, J = 8.6 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.50 (d, J = 8.5 Hz, 2H, 2-H and 6-H, C_6H_4Br), 7.02 (d, J = 8.5 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.19 (s, 1H, 7-H), 4.74 (s, 2H, CH_2O), 3.80 (t, J = 7.3 Hz, 2H, CH_2N), 1.55 (sext, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 0.86 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.67 (C), 157.84 (C), 154.98 (C), 151.12 (C), 138.49 (C), 137.77 (C), 133.84 (C), 131.86 (CH), 127.06 (CH), 124.18 (C), 121.66 (CH), 115.40 (C), 115.04 (CH), 110.56 (C), 91.65 (CH), 67.13 (CH_2), 41.07 (CH_2), 21.05 (CH_2), 11.22 (CH_3). MS (EI) m/z (%): 498 (92), 497 (28), 496 (M, 92), 454 (42), 285 (72), 284 (47), 243 (100), 227 (47), 105 (23), 91 (20), 65 (13), 43 (16), 41 (15). Anal. Calcd for $C_{23}H_{21}BrN_4O_4$ (497.34): C, 55.54; H, 4.26; Br, 16.07; N, 11.27. Found: C, 55.63; H, 4.59; Br, 16.46; N, 11.48.

5.1.4.8. 6-{4-[3-(4-Bromophenyl)-[1,2,4]oxadiazol-5-yl]carbonylmethoxyphenyl}-3-propyl-1,5-dihydro-3H-pyrrolo[3,2-d]pyrimidine-2,4-dione ***[3h]***. Yield 32%, mp > 300 °C IR (KBr) ν (cm^{-1}): 2963, 1698, 1628, 1495, 1468, 1405, 1345, 1275, 1248, 1181, 1065, 1009, 834, 783, 742. ^1H NMR (DMSO-d_6): 12.12 (s, 1H, D_2O exchange, NH), 11.16 (s, 1H, D_2O exchange, NH), 7.95 (d, J = 8.3 Hz, 2H, 3-H and 5-H, C_6H_4Br), 7.85 (d, J = 8.5 Hz, 2H, 2-H and 6-H C_6H_4O), 7.79 (d, J = 8.3 Hz, 2H, 2-H and 6-H, C_6H_4Br), 7.13 (d, J = 8.5 Hz, 2H, 3-H and 5-H, C_6H_4O), 6.22 (s, 1H, H-7), 5.65 (s, 2H, CH_2O), 3.84–3.79 (m, 2H, CH_2N), 1.61–152 (m, 2H, $C\underline{H}_2CH_3$), 0.88 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 175.95 (C), 167.03, (C), 157.11 (C), 154.96 (C), 151.06 (C), 139.21 (C), 133.83 (C), 132.41 (CH), 128.99 (CH), 127.02 (CH), 125.36 (C), 124.89 (C), 124.82 (C), 115.05 (CH), 110.68 (C), 91.70 (CH), 60.81 (CH_2O), 40.96 (CH_2N), 20.98 ($C\underline{H}_2CH_3$), 11.17 (CH_3). MS (EI) m/z (%): 534 (4), 523 (15), 522 (4), 521 (15), 325 (34), 300 (24), 285 (28), 284 (62), 243 (78), 242 (99), 225 (29), 183 (95), 181 (87), 102 (100), 76 (22), 75 (40). Anal. Calcd for $C_{24}H_{20}BrN_5O_4$ (522.35): C, 55.18; H, 3.86; Br, 15.30; N, 13.41. Found: C, 55.36; H, 3.59; Br, 14.86; N, 13.78.

5.1.4.9. 6-[4-[3-(4-Bromophenyl)-[1,2,4]oxadiazol-5-yl]-(2-methoxy) carbonylmethoxyphenyl]-1,3-dimethyl-1,5-dihydro-3H-pyrrolo[3,2-d] pyrimidine-2,4-dione ***[3i]***. Yield 27%, mp > 350 °C. IR (KBr) ν (cm^{-1}): 3670, 3644, 1828, 1668, 1622, 1605, 1590, 1573, 1474, 1416, 1284, 1267, 1154, 1084, 985, 920, 752, 702. ^1H NMR (DMSO-d_6): 11.82 (s, 1H, D_2O exchange, NH), 8.29–7.95 (m, 2H, 3-H and 5-H, C_6H_4Br), 7.94–7.78 (m, 1H, 6-H, C_6H_4O), 7.77–7.74 (m, 2H, 2-H and 6-H, C_6H_4Br), 6.85–6.77 (m, 1H, 5-H, C_6H_4O), 6.74 (d, J = 2.1 Hz, 1H, 7-H), 6.51 (d, J = 4.4 Hz, 1H, 3-H, C_6H_4O), 5.67 (s, 2H, CH_2O), 3.87 (s, 3H, CH_3O), 3.39 (s, 3H, CH_3), 3.24 (s, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 176.64 (C), 167.78 (C), 159.20 (C), 158.24 (C), 155.14 (C), 151.32 (C), 136.23 (C), 133.54 (C), 133.18 (CH), 130.00 (CH), 129.91 (CH), 126.12 (C), 125.52 (C), 106.80 (CH), 100.54 (CH), 95.65 (CH), 79.91 (CH_2), 58.32 (CH_3), 32.41 (2 CH_3). MS (EI) m/z (%): 539 (M + 1, 23), 538 (4), 537 (22), 358 (14), 301 (24), 300 (100), 257 (24), 209 (15), 207 (11), 183 (14), 181 (12), 102 (7), 58 (6). Anal. Calcd for $C_{24}H_{20}BrN_5O_5$ (538.35): C, 53.54; H, 3.74; Br, 14.84; N, 13.01. Found: C, 53.66; H, 3.33; Br, 14.56; N, 13.38.

5.1.5. General procedure for the methylation of compounds ***8*** *and* ***10a–c***

CH_3I (0.40 mmol) was added to a stirred suspension of the corresponding 1*H*-purine-2,6(3*H*,7*H*)-dione **8** and **10a–c** (0.20 mmol) and K_2CO_3 (25 mml) in dry DMF (3 mL) and the mixture was heated at 65 °C for the time indicated. The reaction mixture was allowed to cool down to room temperature and H_2O (15 mL) was added. The resulting precipitate was filtered off, washed with H_2O and dried under vacuum.

5.1.5.1. 2-[4-(1,5-Dimethyl-2,4-dioxo3-propyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)phenyloxy]-N-(4-fluorophenyl)acetamide ***[3j]***. Yield 30%, mp 195–196 °C. IR (KBr) ν (cm^{-1}): 1687, 1640, 1539, 1505, 1444, 1233, 1183, 1062, 829, 767. ^1H NMR (DMSO-d_6): 10.18 (s, 1H, D_2O exchange, NHCO), 7.68–7.63 (m, 2H, 2-H and 6-H, C_6H_4F), 7.50 (d, J = 8.7 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.19–7.11 (m, 4H, 3-H and 5-H, C_6H_4F and 3-H and 5-H, C_6H_4O), 6.25 (s, 1H, 7-H), 4.76 (s, 2H, CH_2O), 3.86 (s, 3H, CH_3N), 3.85–3.80 (m, 2H, CH_2N), 3.34 (s, 3H, CH_3N), 1.58–1.51 (m, 2H, $C\underline{H}_2CH_3$), 0.85 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR and DEPT (DMSO-d_6): 166.59 (C), 158.25 (C), 155.28 (C), 150.89 (C), 142.64 (C), 135.72 (C), 135.04 (C), 130.72 (CH), 123.58 (C), 121.91 (CH), 121.81 (CH), 115.78 (CH), 115.50 (CH), 115.29 (CH), 110.40 (C), 94.75 (CH), 67.39 (CH_2), 42.08 (CH_2), 33.56 (CH_3), 31.79 (CH_3), 21.22 (CH_2), 11.51 (CH_3). MS (EI) m/z (%): 465 (M + 1, 28), 464 (100), 422 (63), 271 (36), 270 (31), 138 (13), 124 (30), 110 (31), 83 (13). Anal. Calcd for $C_{25}H_{25}FN_4O_4$ (464.49): C, 64.64; H, 5.42; F, 4.09; N, 12.06. Found: C, 64.23; H, 4.35; F, 3.87; N, 12.32.

5.1.5.2. 6-[4-(4-Phenylpiperazin-1-yl)carbonylmethoxyphenyl]-5-methyl-1,3-dipropyl-1,5-dihydro-3H-pyrrolo[3,2-d]pyrimidine-2,4-dione ***[3k]***. Yield 33%, mp 172–174 °C. IR (KBr) ν (cm^{-1}): 3432, 2963, 2932, 1653, 1581, 1466, 1382, 1181, 1044, 842, 769. ^1H NMR (DMSO-d_6): 7.97 (d, J = 8.4 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.72–7.60 (m, 3H, 3-H, 4-H and 5-H, C_6H_5), 7.47 (d, J = 8.3 Hz, 2H, 3-H and 5-H, C_6H_4O), 7.05 (d, J = 8.4 Hz, 2H, 3-H, 4-H and 5-H, C_6H_5), 6.27 (s, 1H, 7-H), 5.74 (virtual d, J = 1.0 Hz, 2H, $C\underline{H}_2O$), 5.08–4.88 (m, 2H, $C_4H_8N_2$), 4.46–4.41 (m, 2H, $C_4H_8N_2$), 4.09–4.01 (m, 4H, $2CH_2N$), 3.86 (virtual s, 4H, $C_4H_8N_2$), 3.54 (s, 3H, CH_3N), 1.64 (sext, J = 7.3 Hz, 2H, $C\underline{H}_2CH_3$), 1.56 (sext, J = 7.3 Hz, 2H, $C\underline{H}_2CH_3$), 0.86 (virtual q, J = 7.3 Hz, 6H, $2CH_3$). ^{13}C NMR and DEPT (DMSO-d_6): 165.57 (C), 158.73 (C), 155.34 (C), 150.71 (C), 143.84 (C), 142.85 (C), 135.18 (C), 131.04 (CH), 130.76 (CH), 130.67 (CH), 123.37 (C), 115.30 (CH), 110.74 (C), 94.76 (CH), 65.80 (CH_2), 56.47 (CH), 47.03 (CH_2), 33.61 ($2CH_3$), 21.28 (CH_2), 20.94 (CH_2), 11.43 (CH_3), 11.32 (CH_3). MS (EI) m/z (%): 543 (M + 1, 35), 543 (100), 340 (16), 226 (8), 210 (7), 189 (12), 161 (30), 132 (58), 105 (20), 104 (15). Anal. Calcd for $C_{31}H_{37}N_5O_4$ (543.66): C, 68.49; H, 6.86; N, 12.88. Found: C, 66.79; H, 6.44; N, 12.51.

5.1.5.3. 6-[4-(1,2,3,4-Tetrayidroisoquinolin-2-yl)]carbonylmethoxyphenyl]-5-methyl-1,3-dipropyl-1,5-dihydro-3H-pyrrolo[3,2-d]pyrimidine-2,4-dione ***[3l]***. Yield 63%, mp 121–123 °C. IR (KBr) ν (cm^{-1}): 3461, 2963, 2934, 1691, 1650, 1536, 1473, 1455, 1243, 1051, 1000, 767, 746. ^1H NMR (DMSO-d_6): 7.48 (d, J = 7.9 Hz, 2H, 2-H and 6-H, C_6H_4O), 7.19 (virtual s, 4H, 3-H, 5-H, C_6H_4O and 5-H, 8-H, $C_9H_{10}N$), 7.13–7.05 (m, 2H, 6-H and 7-H, $C_9H_{10}N$), 6.30 (s, 1H, 7-H), 5.01 (s, 2H, CH_2O), 4.62 (s, 2H, 1-H_2, $C_9H_{10}N$), 3.97–3.94 (m, 5H, 3-H_2, $C_9H_{10}N$ and CH_3N), 3.76–3.67 (m, 4H, $2CH_2N$), 2.98–2.88 (m, 2H, CH_2N), 2.85–2.80 (m, 2H, 4-H_2, $C_9H_{10}N$), 1.69–1.49 (m, 4H, $2C\underline{H}_2CH_3$), 1.00–0.77 (m, 6H, $2CH_3$). ^{13}C NMR and DEPT (DMSO-d_6): 166.64 (C), 158.85 (C), 155.27 (C), 150.65 (C), 142.85 (C), 135.12 (C), 134.77 (C), 133.452 (C), 133.30 (C), 130.64 (CH), 128.70 (CH), 128.76 (CH), 126.78 (CH), 126.51 (CH), 123.27 (C), 115.18 (CH), 110.48 (C), 94.69 (CH), 66.34 (CH_2), 46.33 (CH_2), 45.92 (CH_2), 43.94 (CH_2), 42.12 (CH_2), 41.98 (CH_2), 33.54 (CH_3), 28.92 (CH_2), 21.21 (CH_2), 20.87 (CH_2), 11.54 (CH_3), 11.31 (CH_3). MS (EI) m/z (%): 515 (M + 1, 34), 514 (100), 430 (11), 340 (14), 174 (21). Anal. Calcd for $C_{30}H_{34}N_4O_4$ (514.26): C, 70.02; H, 6.66; N, 10.89. Found: C, 70.45; H, 6.44; N, 10.58.

*5.1.5.4. 2-[4-(5-Methyl-2,4-dioxo-1,3-dipropyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)phenyloxy]-N-(4-fluorophenyl)acetamide [**3m**].* Yield 61%, mp 169–171 °C. IR (KBr) ν (cm^{-1}): 3347, 2962, 2935, 1696, 1650, 1531, 1507, 1477, 1455, 1409, 1239, 1227, 1065, 834, 770. ^{1}H NMR (DMSO-d_6): 10.19 (s, 1H, D_2O exchange, NHCO), 7.68–7.63 (m, 2H, 2-H and 6-H, C_6H_4F), 7.52 (d, $J = 8.3$ Hz, 2H, 2-H and 6-H, C_6H_4O), 7.19–7.11 (m, 4H, 3-H, 5-H, C_6H_4F and 3-H, 5-H, C_6H_4O), 6.30 (s, 1H, 7-H), 4.77 (s, 2H, CH_2O), 3.87 (s, 3H, CH_3N), 3.87–3.45 (m, 4H, $2CH_2N$), 1.65–1.63 (m, 2H, CH_2CH_3), 1.56–1.54 (m, 2H, CH_2CH_3), 0.90–0.83 (m, 6H, $2CH_3$). ^{13}C NMR and DEPT (DMSO-d_6): 166.58 (C), 158.50 (C), 155.27 (C), 150.64 (C), 142.71 (C), 135.11 (C), 130.78 (CH), 123.56 (C), 121.91 (CH), 121.80 (CH), 115.77 (CH), 115.48 (CH), 115.24 (CH), 110.52 (C), 94.75 (CH), 67.38 (CH_2), 46.33 (CH_2), 41.98 (CH_2), 33.55 (CH_3), 21.21 (CH_2), 20.86 (CH_2), 11.48 (CH_3),11.24 (CH_3). MS (EI) m/z (%): 493 (M + 1, 31), 492 (100), 450 (14), 409 (11), 408 (43), 378 (21), 257 (25), 256 (12), 227 (26), 124 (21), 110 (20), 58 (26). Anal. Calcd for $C_{27}H_{29}FN_4O_4$ (492.54): C, 65.84; H, 5.93; N, 11.38. Found: C, 65.59; H, 5.72; N, 11.17.

5.2. Biochemistry and pharmacology

5.2.1. 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 [25].

5.2.1.1. Human A_1 receptors. Adenosine A_1 receptor competition binding experiments were carried out in membranes from CHO-A1 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.

5.2.1.2. 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.

5.2.1.3. Human A_{2B} receptors. Adenosine A_{2B} receptor competition binding experiments were carried out in membranes fromHEK-293-A2B 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.

5.2.1.4. 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 using a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

5.2.2. Data analysis

The −log of the inhibition constant (pK_i) of each compound was calculated by the Cheng–Prusoff equation:

$$K_i = IC50/(1 + [L]/KD)$$

where IC_{50} is the concentration of compound that displaces the binding of the 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 2 experiments ($n = 2$), each performed with duplicate points.

Selectivity is defined by the ratios $K_i(A_{2A})/K_i(A_{2B})$ or $K_i(A_1)/K_i(A_{2B})$.

Acknowledgments

The authors thank Almirall Prodesfarma S.A. (Barcelona-Spain) for promoting and financially supporting this work. I. N. thanks the Xunta de Galicia for financial support under “Programa Isidro Parga Pondal”. J. B. thanks the Xunta de Galicia for financial support under “Isabel Barreto Contract”.

References

[1] B.B. Fredhom, A.P. Ijzerman, K.A. Jacobson, K.-N. Klotz, J. Linden, Pharmcol. Rev. 533 (2001) 527–552.
[2] P.G. Baraldi, P.A. Borea, Trends Pharmacol. Sci. 21 (2000) 456–459.
[3] P.G. Baraldi, B. Caccieri, S. Moro, G. Spalluto, G. Pastorin, T. Da Ros, K.-N. Klotz, K. Varani, S. Gessi, P.A. Borea, J. Med. Chem. 45 (2002) 770–780.
[4] P.G. Baraldi, M.A. Trabizi, A. Bovero, B. Avitabili, D. Preti, F. Fruttarolo, R. Romagnoli, K. Varani, P.A. Borea, Eur. J. Med. Chem. 38 (2003) 367–382.
[5] R.V. Kalla, E. Elzein, T. Perry, X. Li, V. Palle, V. Varkhedkas, A. Gimbel, T. Moa, D. Zeng, J. Zablocki, J. Med. Chem. 49 (2006) 3682–3692.
[6] E. Elzein, R.V. Kalla, X. Li, T. Perry, A. Gimbel, D. Zeng, D. Lustig, K. Leung, J. Zablocki, J. Med. Chem. 51 (2008) 2267–2278.
[7] H. Adachi, K.K. Palaniappan, A.A. Ivanov, N. Bergman, Z.-G. Gao, K.A. Jacobson, J. Med. Chem. 50 (2007) 1810–1827.
[8] K.A. Jacobson, Z.-G. Gao, Nat. Rev. Drug Discov. 5 (2006) 247–264.
[9] S. Moro, Z.-G. Gao, K.A. Jacobson, G. Spalluto, Med. Res. Rev. 26 (2006) 131–159.
[10] R.V. Kalla, J. Zablocki, M.A. Tabrici, P.G. Baraldi, Adenosine receptors in health and disease. in: C.N. Wilson, S.J. Mustafa (Eds.), Handbook of Experimental Pharmacology 193. Springer-Verlag, Berlin, 2009, pp. 99–122.
[11] A.M. Hayallah, J. Sandoval-Ramirez, U. Reith, U. Shobert, B. Preiss, B. Schumacher, J.W. Daly, C.E. Müller, J. Med. Chem. 45 (2002) 1500–1510.
[12] S.-A. Kim, M.A. Marshall, N. Melman, H.S. Kim, C.E. Müller, J. Linden, K.A. Jacobson, J. Med. Chem. 45 (2002) 2131–2138.
[13] P.G. Baraldi, M.A. Trabizi, D. Preti, A. Bovero, R. Romagnoli, F. Fruttarolo, N.A. Zaid, A.R. Moorman, K. Varani, S. Gessi, S. Merighi, P.A. Borea, J. Med. Chem. 47 (2004) 1434–1447.
[14] C.E. Müller, Farmaco 56 (2001) 77–80.
[15] Y.-C. Kim, X.-D. Ji, N. Melman, J. Linden, K.A. Jacobson, J. Med. Chem. 43 (2000) 1165–1172.
[16] H. Harada, O. Asano, Y. Hoshino, S. Yoshikawe, M. Matsukura, Y. Kabasawa, J. Nijima, Y. Kotake, N. Watanabe, T. Kawata, T. Inoue, T. Horizoe, N. Yasuda, H. Minami, K. Nagata, M. Murakami, J. Nagoaka, S. Kobayashi, I. Tanaka, S. Aba, J. Med. Chem. 44 (2001) 170–179.
[17] M.C. Balo, C. López, J.M. Brea, F. Fernández, O. Caamaño, Chem. Pharm. Bull. 55 (2007) 372–375.
[18] (a) M.I. Nieto, M.C. Balo, J. Brea, O. Caamaño, M.I. Cadavid, F. Fernández, X. García-Mera, C. López, J.E. Rodríguez-Borges, Bioorg. Med. Chem. 17 (2009) 3426–3432;
(b) M.C. Balo, J. Brea, O. Caamaño, F. Fernández, X. García-Mera, C. López, M.I. Loza, M.I. Nieto, J.E. Rodríguez-Borges, Bioorg. Med. Chem. 17 (2009) 6755–6760;
(c) J.E. Rodríguez-Borges, X. García-Mera, M.C. Balo, J. Brea, O. Caamaño, F. Fernández, C. López, M.I. Loza, M.I. Nieto, Bioorg. Med. Chem. 18 (2010) 2001–1009.
[19] B.J. Vidal, C. Esteve Trias, V. Segarra Matamoros, E. Raviña Rubira, F. Fernández Gonzalez, M.I. Loza García, F. Sanz Carreras, WO Patent 03/000694; 2003.
[20] F. Fernández, O. Caamaño, M.I. Nieto, C. López, X. García-Mera, A. Stefanachi, O. Nicolotti, M.I. Loza, J. Brea, C. Esteve, V. Segarra, B. Vidal, A. Carotti, Bioorg. Med. Chem. 17 (2009) 3618–3629.
[21] B. Grahner, S. Winiwarter, W. Lanzner, C.E. Müller, J. Med. Chem. 37 (1994) 1526–1534.
[22] A. Carotti, M.I. Cadavid, N.B. Centeno, C. Esteve, M.I. Loza, A. Martínez, R. Nieto, E. Raviña, F. Sanz, V. Segarra, E. Sotelo, A. Stefanachi, B. Vidal, J. Med. Chem. 49 (2006) 282–299.
[23] A. Carotti, A. Stefanachi, E. Raviña, E. Sotelo, M.I. Loza, M.I. Cadavid, N.B. Centeno, O. Nicolotti, Eur. J. Med. Chem. 39 (2004) 879–887.
[24] C. Esteve, A. Nueda, J.L. Díaz, J. Beleta, A. Cárdenas, E. Lozoya, M.I. Cadavid, M.I. Loza, H. Ryder, B. Vidal, Bioorg. Med. Chem. Lett. 16 (2006) 3642–3645.

[25] A. Stefanachi, J.M. Brea, M.I. Cadavid, N.B. Centeno, C. Esteve, M.I. Loza, A. Martínez, R. Nieto, E. Raviña, F. Sanz, V. Segarra, E. Sotelo, B. Vidal, A. Carotti, Bioorg. Med. Chem. 16 (2008) 2852–2869.
[26] (a) S. Senda, A. Suzui, M. Honda, H. Fujimura, Chem. Pharm. Bull. 6 (1958) 482–487;
(b) V. Papesch, R.M.G.D. Dobson, J. Org. Chem. 28 (1963) 1329–1331;
(c) A. Stefanachi, F. Leonetti, A. Cappa, A. Carotti, Tetrahedron Lett. 44 (2003) 2121–2123.
[27] A. Stefanachi, O. Nicolotti, F. Leonetti, S. Cellamare, F. Campagna, M.I. Loza, J.M. Brea, F. Mazza, E. Gavuzzo, A. Carotti, Bioorg. Med. Chem. 16 (2008) 9780–9789.
[28] L. Ya, D.C.G. Bertarelli, A.M. Hayallah, H. Meyer, K.-N. Klotz, C.E. Müller, J. Med. Chem. 49 (2006) 4384–4391.
[29] L. Yan, C.E. Müller, J. Med. Chem. 47 (2004) 1031–1043.
[30] P. Majer, R.S. Randad, J. Org. Chem. 59 (1994) 1937–1938.
[31] M. Prats, C. Galvez, Heterocycles 34 (1991) 149–156.