Bioorganic & Medicinal Chemistry 20 (2012) 1046–1059

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Pyrrolo- and pyrazolo-[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines as adenosine receptor antagonists

Pier Giovanni Baraldi [a,*], Giulia Saponaro [a], Mojgan Aghazadeh Tabrizi [a], Stefania Baraldi [a], Romeo Romagnoli [a], Allan R. Moorman [c], Katia Varani [b], Pier Andrea Borea [b], Delia Preti [a]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy*
[b] *Dipartimento di Medicina, Clinica e Sperimentale-Sezione di Farmacologia, Università di Ferrara, 44100 Ferrara, Italy*
[c] *King Pharmaceuticals, Inc., Research & Development, 4000 CentreGreen Way, Suite 300, Cary, NC 27513, USA*

ARTICLE INFO

Article history:
Received 12 October 2011
Revised 17 November 2011
Accepted 18 November 2011
Available online 1 December 2011

Keywords:
Adenosine
Pyrazolo-triazolo-pyrimidines
Pyrrolo-triazolo-pyrimidines
Adenosine antagonists

ABSTRACT

The discovery and development of adenosine receptor antagonists have represented for years an attractive field of research from the perspective of identifying new drugs for the treatment of widespread disorders such as inflammation, asthma and Parkinson's disease. The present work can be considered as an extension of our structure-activity relationship studies on the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (PTP) nucleus, extensively investigated by us as a useful template, in particular, for the identification of A_{2A} and A_3 adenosine receptor antagonists. In order to explore the role of the nitrogen at the 7-position, we performed a new synthetic strategy for the preparation of pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives which can be considered as 7-deaza analogues of the parent PTPs. We also synthesised a novel series of pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines as junction isomers of the reference compounds. In both cases we obtained some examples of potent antagonists (K_i in the low nanomolar range) with variable selectivity profiles in relation to the nature of substituents introduced at the C^5-, N^8- and/or N^9-positions. The pyrrolo-triazolo-pyrimidine derivative **9b** appeared to be a potent A_3 adenosine receptor antagonist (K_i = 10 nM) with good selectivity over hA_1 (74-fold) and hA_{2A} (20-fold) adenosine receptors combined with low activity at the hA_{2B} subtype (IC_{50} = 906 nM). Moreover, some examples of high-affinity A_1/A_{2A} dual antagonists have been identified in both series. This work constitutes a new and important contribution for the comprehension of the interaction between PTPs and adenosine receptors.

1. Introduction

Adenosine is an ubiquitous nucleoside essential for the proper functioning of every cell in mammalian species. It is directly linked to energy metabolism through ATP (adenosine triphosphate), ADP (adenosine diphosphate) and AMP (adenosine monophosphate), while at the extracellular level it regulates a wide range of biological functions through activation of specific receptors (adenosine receptors, ARs)[1–3] that belong to the superfamily of G-protein coupled receptors (GPCRs) and are classified as A_1, A_{2A}, A_{2B} and A_3.

The discovery and development of AR antagonists have represented for years an attractive field of research from the perspective of identifying new drugs for the treatment of widespread disorders. A_1 AR antagonists have been studied for the treatment of heart failure with related renal impairment, cystic-fibrosis and asthma.[4] The selective blockade of A_{2A} AR proved to be effective in clinical trials for the treatment of extrapyramidal neurodegenerative disorders such as Parkinson's disease.[5,6] A growing number of A_{2B} AR antagonists are under clinical evaluation in the therapeutic area of respiratory disorders (asthma, COPD).[7] Although very potent and selective A_3 AR antagonists have been reported in the last decade, none of these molecules has yet entered human trials. Nonetheless, it is becoming increasingly apparent that A_3 AR antagonists might be therapeutically useful for the acute treatment of stroke and glaucoma,[8] inflammation,[9–11] and in the development of cerebroprotective,[12,13] antiasthmatic and antiallergic drugs.[14,15] Furthermore, the recent evidence[16–20] of high levels of expression of A_3 ARs in several cell lines has suggested potential applications for selective antagonists in cancer chemotherapy.

The pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (PTP) nucleus has distinguished itself as an attractive scaffold for obtaining adenosine receptor antagonists based upon its strong structural correlation with the non-selective AR antagonist CGS15943 (9-chloro-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine). A wide number of compounds originated from our structure-activity

* Corresponding author. Address: Department of Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17-19, 44121, Ferrara Italy. Tel./fax: +39 0532 455921.
E-mail addresses: baraldi@dns.unife.it, baraldi@unife.it (P.G. Baraldi).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.11.037

optimization work based on the systematic substitution of the C^5-, N^7-, N^8-, C^2- or C^9-positions of PTPs,[21] which allowed us to delineate a structure–activity relationship (SAR) profile mainly concerning the effect of substitutions on subtype selectivity. The 2-furyl ring at the 2-position of the nucleus appeared important for the affinity toward all four AR subtypes. The combined presence of a free amino group at the 5-position and an arylalkyl chain at the N^7-position of PTPs was found essential for both affinity and selectivity at the A_{2A} AR subtype. Two compounds of this family named **SCH-58261** (Fig. 1) and **SCH-63390** (Fig. 1), bearing N^7-arylalkyl functions, proved to be potent and selective A_{2A} AR antagonists both in rat and human models.[7,22] On the other hand, the concurrent presence of a 4-methoxy-phenylcarbamoyl moiety and small alkyl chains (methyl or propyl) at the 5- and 8- positions, respectively, led to the identification of highly potent and selective human A_3 AR antagonists. Of this class, **MRE-3008-F20** (Fig. 1), displays a K_i value of 0.29 nM in binding assays to human A_3 receptors expressed in CHO cells with high selectivity over hA_1 and hA_{2A} ARs (K_i = 1100 and 140 nM, respectively).[23] The isosteric replacement of the phenylcarbamoyl moiety with a salifiable 4-pyridylcarbamoyl moiety improved water solubility and led to the most potent hA_3 antagonist known to date (**MRE-3005-F20**, Fig. 1).[7] Finally, the introduction of substituents at the C^9-position led to the identification of AR antagonists with low selectivity but high potency.[24]

The present work can be considered an extension of our SAR studies on the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus (general structure 1, Fig. 2), in which we focused our attention on the isosteric replacement of the pyrazole ring. Specifically, in order to explore the role of the nitrogen at the 7-position, we performed a new synthetic strategy for the preparation of pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives which can be considered as 7-deaza-analogues of the classical PTPs (Fig. 2A). A series of 8-deaza-PTPs has been claimed recently for their potent and selective activity as A_{2A} AR antagonists.[25] We also synthesised a novel series of N^8/N^9-substituted-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines as junction isomers of the reference compounds (Fig. 2, B and C).

Starting from the data obtained with the previous series of PTPs,[7] we introduced at the N^8 or N^9 positions small alkyl chains, such as a methyl- or a propyl-, or arylalkyl chains, such as a phenylethyl- or a phenylpropyl-function. These modifications allowed us to further explore the contribution of this side of the molecule to the interaction with adenosine receptors. In both series we also studied the 5-position of the tricyclic structure, introducing an amino group, a 4-methoxy-phenylcarbamoyl-, a 4-pyridylcarbamoyl moiety, a morpholine or an *N*-substituted piperazine.

We obtained some examples of potent ARs antagonists (K_i values from binding assays in the low nanomolar range) with variable selectivity profiles in relation to the nature of the substituents introduced at the C^5-, N^8- and N^9-positions.

2. Results and discussion

2.1. Chemistry

2.1.1. Pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines

As depicted in Scheme 1, 1,3-dibenzyl-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,4(3*H*,6*H*)-dione **2**[26] was alkylated with the appropriate alkyl halide to furnish 6-alkyl-derivatives **3a**–**d**. Debenzylation at the 1- and 3-positions with $AlCl_3$ in anhydrous toluene provided derivatives **4a**–**d**. The 2,4-dichloro-6-alkyl-6*H*-pyrrolo[3,4-*d*]pyrimidines **5a**–**d** were obtained by treatment of **4a**–**d** with $POCl_3$ and DBU. Selective substitution of the chlorine atom at the 4-position with furoic acid hydrazide followed by a Dimroth rearrangement led to the desired pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus (**7a**–**d**). Compounds **8a**–**d** were obtained by treating derivatives **7a**–**d** with a saturated solution of ammonia in ethanol. These were converted into the corresponding

SCH-58261[7]
K_i (hA_1) = 549 nM
K_i (hA_{2A}) = 1.1 nM
K_i (hA_{2B}) > 10000 nM
K_i (hA_3) > 10000 nM

SCH-63390[7]
K_i (hA_1) = 350 nM
K_i (hA_{2A}) = 1.2 nM
K_i (hA_{2B}) > 10000 nM
K_i (hA_3) > 10000 nM

MRE-3008-F20[23]
K_i (hA_1) = 1100 nM
K_i (hA_{2A}) = 140 nM
K_i (hA_{2B}) = 2100 nM
K_i (hA_3) = 0.29 nM

MRE-3005-F20[27]
K_i (hA_1) = 250 nM
K_i (hA_{2A}) = 60 nM
K_i (hA_{2B}) = 200 nM
K_i (hA_3) = 0.04 nM

Figure 1. Pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines as A_{2A} and A_3 adenosine receptor antagonists.

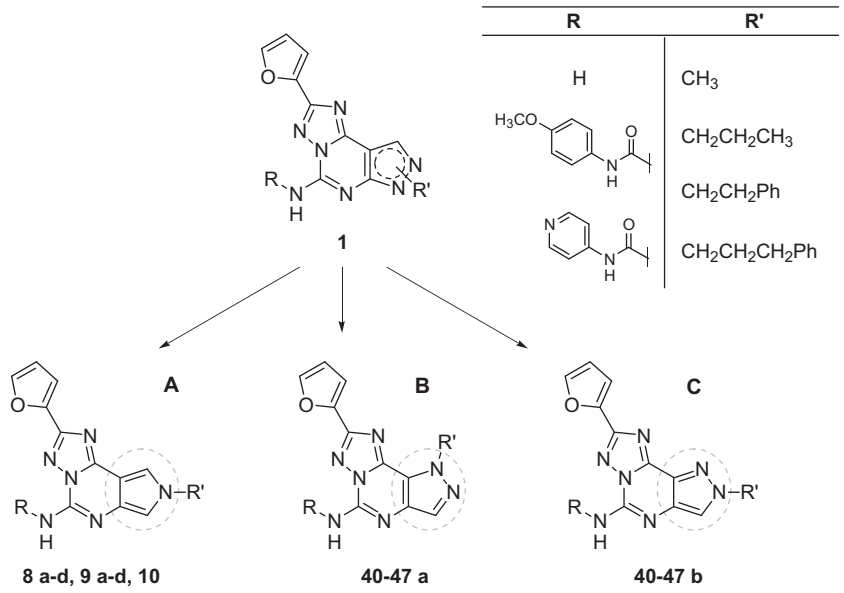
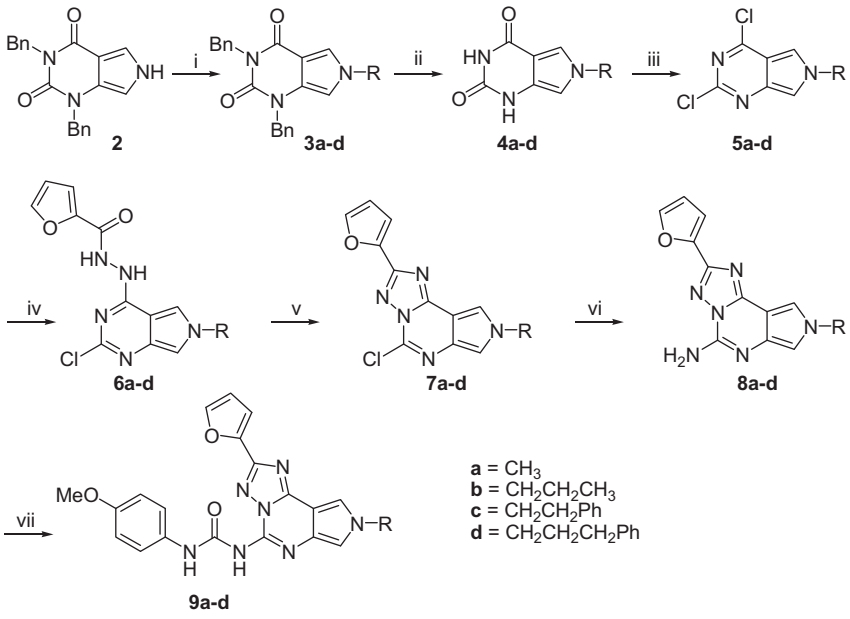
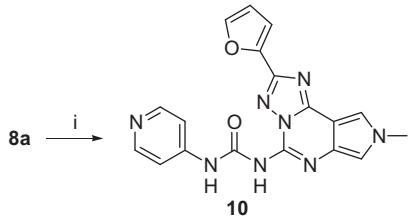


Figure 2. General structures of the newly designed molecules.



Scheme 1. Synthesis of pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines. Reagents and conditions: (i) K_2CO_3, RX, DMF, 40 °C, 4 h; (ii) $AlCl_3$, toluene, rt, 2 h; (iii) $POCl_3$, DBU, 50 °C, 8 h; (iv) 2-furoic acid hydrazide, TEA, 1,4-dioxane, 80–90 °C, 5 h; (v) HMDS, BSA, 120 °C, 18 h; (vi) EtOH satd ammonia soln, 60 °C, 18 h; (vii) 4-OCH_3-phenyl isocyanate, THF, 50 °C, 18 h.



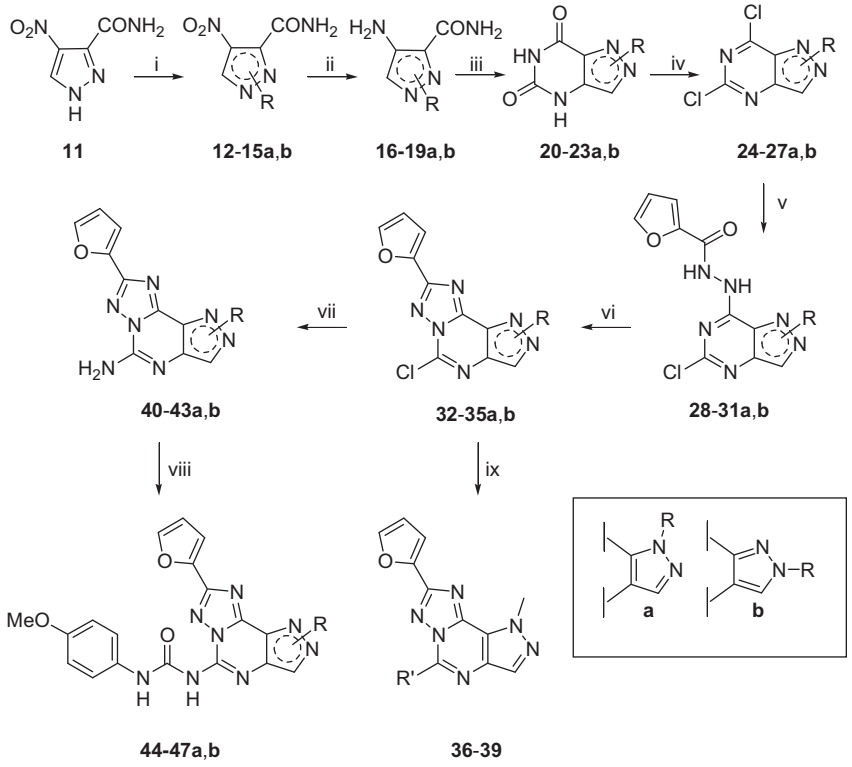
Scheme 2. Synthesis of 5-{[(4-pyridinyl)-carbamoyl]amino}-2-(furan-2-yl) -8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine **10**. Reagents and conditions: (1) 4-pyridine isocyanate, toluene, 5 h, 100 °C.[27]

4-methoxy-phenyl urea derivatives **9a**–**d** by reaction with 4-methoxy-phenylisocyanate.

Compound **10** was prepared according to a similar efficient strategy previously reported[27] and depicted in Scheme 2. Compound **8a** was heated for 5 h in dry toluene with 4-pyridyl isocyanate to give the desired urea derivative **10** (Scheme 2).

2.1.2. Pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines

For the synthesis of these compounds the synthetic strategy depicted in Scheme 3 has been followed. 4-Nitro-1*H*-pyrazole-3-carboxylic acid amide **11**[28] was alkylated with the appropriate alkyl halide in the presence of K_2CO_3 in DMF to give an approximately



Scheme 3. Synthesis of pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines. Reagents and conditions: (i) alkyl halide, K_2CO_3, DMF, rt, 6 h; (ii) H_2, 10% Pd/C, EtOH; (iii) urea, neat, 250 °C; (iv) $POCl_3$, DBU, 80 °C, 8 h; (v) 2-furoic acid hydrazide, TEA, 1,4-dioxane, 80–90 °C, 5 h; (vi) HMDS, BSA, 120 °C, 18 h; (vii) EtOH satd ammonia soln, 60 °C, 18 h; (viii) 4-OCH_3-phenylisocyanate, THF, 50 °C, 18 h; (ix) amines, 2-methoxyethanol, 100 °C, 3 h. R = CH_3 (**12**, **16**, **20**, **24**, **28**, **32**, **40**, **44**); R = $(CH_2)_2CH_3$ (**13**, **17**, **21**, **25**, **29**, **33**, **41**, **45**); R = $(CH_2)_2$Ph (**14**, **18**, **22**, **26**, **30**, **34**, **42**, **46**); R = $(CH_2)_3$Ph (**15**, **19**, **23**, **27**, **31**, **35**, **43**, **47**).

1:1 mixture of the two isomers (**a** and **b**) which were efficiently separated via column chromatography. The nitro group was then reduced by hydrogenation in the presence of a catalytic amount of 10% Pd/C and intermediates **16**–**19a**,**b** were converted into the corresponding 1/2-methyl-1,4-dihydro-pyrazolo[4,3-*d*]pyrimidine-5,7-dione **20**–**23a**,**b** by heating with an excess of urea.

The 5,7-dichloro-1/2-methyl-pyrazolo[4,3-*d*]pyrimidines **24**–**27a**,**b** were obtained by treatment of **20**–**23a**,**b** with $POCl_3$ and DBU. Selective substitution of the chlorine atom at the 7-position with furoic acid hydrazide followed by a Dimroth rearrangement led to the desired pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus (**32**–**35a**,**b**). Compounds **40**–**43a**,**b** were obtained by treating derivatives **32**–**35a**,**b** with a saturated solution of ammonia in ethanol. These were converted into the corresponding 4-methoxy-phenyl urea derivatives **44**–**47a**,**b** by reaction with 4-methoxy-phenylisocyanate. The intermediate 5-chloro-2-(2-furyl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine **32a** was also reacted with different primary and secondary amines to give the final derivatives **36**–**39**.

2.2. Biological activity

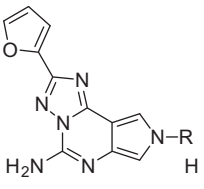
All the synthesized compounds were evaluated in radioligand binding assays to determine their affinities at the human A_1, A_{2A} and A_3 adenosine receptors. Potency of the compounds at the hA_{2B} AR was studied evaluating their capability to inhibit NECA (100 nM) stimulated cAMP production. Affinity data for A_1, A_{2A} and A_3 receptors, expressed as K_i values, and IC_{50} values derived from the cAMP assay carried out for hA_{2B} subtype, are listed in Tables 1 and 2.

2.2.1. Pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines

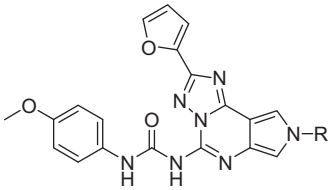
The pyrrolo-triazolo-pyrimidine derivatives **8a-d** (Table 1) bearing a free amino group at the 5- position were substituted at the N^8-position with a methyl-, propyl-, phenylethyl- and phenylpropyl- group, respectively, in accordance with the reference compounds depicted in Figure 1. Both N^8-methyl (**8a**) and N^8-propyl (**8b**) derivatives showed good K_i values for A_{2A} AR (K_i = 20 and 30 nM, respectively) but they also bind to hA_1/hA_3 and block hA_{2B} subtype in the nanomolar range. The N^8-phenylethyl **8c** displayed high and comparable affinity toward the A_1 and A_{2A} receptors (K_i = 4.3 and 3.9 nM, respectively) while a lower affinity/efficacy has been detected at the remaining subtypes (IC_{50} hA_{2B} = 46 and K_i hA_3 = 124 nM). The elongation of the spacer between N^8 and the phenyl ring (**8d**) appeared somewhat detrimental for A_1/A_{2A} affinity. These molecules therefore can be considered quite potent (low nanomolar range) but low selectivity A_1/A_{2A} dual ligands. In this subset, the affinity at the hA_3 receptor decreased with the increase of the steric hindrance around the N^8-position, with the phenylpropyl-derivative **8d** being fivefold less potent than the N^8-methyl analogue **8a**.

As previously noted with the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine series,[7] when the amino group at the 5-position was replaced by a phenylcarbamoyl moiety (derivatives **9a**–**d**), an increase of A_3 affinity was observed resulting in different degrees of selectivity over the other AR subtypes. Both the N^8-methyl (**9a**) and the N^8-propyl (**9b**) derivatives showed high affinity at the human A_3 AR (K_i values of 15 and 10 nM, respectively) with similar patterns of selectivity. Short alkyl chains at the 8-position of the pyrrolo-triazolo-pyrimidine urea derivatives are preferred to N^8-arylalkyl chains (see **9a**,**b** vs **9c**,**d**) for promoting hA_3 selectivity

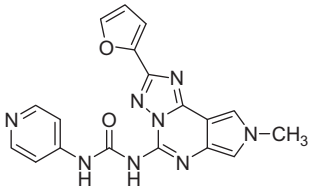
Table 1
Binding (hA_1, hA_{2A} and hA_3) and functional (hA_{2B}) parameters of the synthesized pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines towards adenosine receptors



8a-d



9a-d

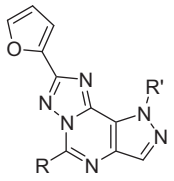


10

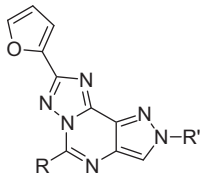
Compd	R	hA_1[a] K_i (nM)	hA_{2A}[b] K_i (nM)	hA_{2B}[c] IC_{50} (nM)	hA_3[d] K_i (nM)
8a	CH_3	100 (83–120)	20 (12–31)	42 (31–57)	50 (41–60)
8b	$(CH_2)_2CH_3$	35 (27–45)	30 (23–38)	90 (81–99)	55 (46–65)
8c	$(CH_2)_2Ph$	4.3 (3.1–6.0)	3.9 (2.5–6.3)	46 (37–56)	124 (96–161)
8d	$(CH_2)_3Ph$	18 (13–23)	50 (41–60)	251 (205–306)	241 (176–330)
9a	CH_3	800 (701–913)	500 (420–595)	838 (713–984)	15 (10–21)
9b	$(CH_2)_2CH_3$	743 (671–821)	200 (166–240)	906 (852–964)	10 (6–17)
9c	$(CH_2)_2Ph$	178 (148–213)	148 (126–173)	740 (722–759)	12 (10–16)
9d	$(CH_2)_3Ph$	210 (162–258)	130 (92–163)	>1000 (12%)	13 (11–19)
10	–	355 (289–437)	>1000 (13%)	>1000 (8%)	111 (74–167)

[a] Displacement of specific [^{3}H]-DPCPX binding to human A_1 receptors expressed in CHO cells (K_i nM).
[b] Displacement of specific [^{3}H]-ZM 241385 binding to human A_{2A} receptors expressed in CHO cells (K_i nM).
[c] cAMP assay in CHO cells expressing hA_{2B} receptors (IC_{50} nM).
[d] Displacement of specific [^{3}H]-MRE3008F20 binding to human A_3 receptors expressed in CHO cells (K_i nM).

Table 2
Binding (hA_1, hA_{2A} and hA_3) and functional (hA_{2B}) parameters of the synthesized pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines towards adenosine receptors



40-47a, **36-39**



40-47b

Compd	R	R'	hA_1[a] K_i (nM)	hA_{2A}[b] K_i (nM)	hA_{2B}[c] IC_{50} (nM)	hA_3[d] K_i (nM)
40a	NH_2	CH_3	10 (8–13)	3.6 (2.5–5.3)	31 (25–38)	749 (662–847)
41a	NH_2	$(CH_2)_2CH_3$	14 (11–20)	12 (8–18)	40 (35–46)	83 (70–99)
42a	NH_2	$(CH_2)_2Ph$	33 (28–38)	42 (37–47)	319 (275–371)	603 (542–670)
43a	NH_2	$(CH_2)_3Ph$	85 (68–105)	122 (86–172)	595 (502–706)	802 (719–895)
44a	(4-OCH_3-Ph)NHCONH	CH_3	129 (97–172)	68 (55–84)	122 (84–177)	61 (42–88)
45a	(4-OCH_3-Ph)NHCONH	$(CH_2)_2CH_3$	61 (44–85)	20 (14–29)	32 (26–45)	161 (132–196)
46a	(4-OCH_3-Ph)NHCONH	$(CH_2)_2Ph$	524 (421–652)	626 (546–717)	772 (660–902)	208 (153–283)
47a	(4-OCH_3-Ph)NHCONH	$(CH_2)_3Ph$	>1000 (38%)	>1000 (32%)	>1000 (32%)	>1000 (25%)
40b	NH_2	CH_3	30 (24–38)	8.1 (6.9–9.7)	33 (28–37)	125 (85–182)
41b	NH_2	$(CH_2)_2CH_3$	22 (18–27)	17 (12–24)	45 (40–51)	432 (363–514)
42b	NH_2	$(CH_2)_2Ph$	4.9 (3.4–7.2)	9.2 (7.9–10.6)	27 (23–32)	315 (255–390)
43b	NH_2	$(CH_2)_3Ph$	7.1 (5.7–8.9)	11 (9–13)	32 (28–37)	526 (482–575)
44b	(4-OCH_3-Ph)NHCONH	CH_3	923 (878–970)	222 (181–273)	580 (453–742)	110 (93–130)
45b	(4-OCH_3-Ph)NHCONH	$(CH_2)_2CH_3$	240 (222–259)	208 (177–245)	863 (801–930)	50 (45–56)
46b	(4-OCH_3-Ph)NHCONH	$(CH_2)_2Ph$	753 (712–798)	672 (621–729)	548 (456–634)	314 (269–358)
47b	(4-OCH_3-Ph)NHCONH	$(CH_2)_3Ph$	>1000 (35%)	>1000 (31%)	>1000 (31%)	815 (722–908)
36	Cyclohexyl-NH	CH_3	12 (8–18)	119 (87–162)	915 (786–1064)	507 (443–582)
37	Morpholine	CH_3	>1000 (6%)	>1000 (4%)	>1000 (4%)	>1000 (1%)
38	4-Me-piperazine	CH_3	>1000 (5%)	>1000 (2%)	>1000 (2%)	>1000 (1%)
39	4-Ph-piperazine	CH_3	>1000 (8%)	>1000 (9%)	>1000 (5%)	>1000 (1%)

[a] Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells (K_i nM, n = 3–6).
[b] Displacement of specific [^{3}H]ZM241385 binding at human A_{2A} receptors expressed in CHO cells (K_i nM, n = 3–6).
[c] Potency (IC_{50}, nM) of examined compounds to inhibit 100 nM NECA stimulation cAMP levels in hA_{2B} CHO cells.
[d] Displacement of specific [^{3}H]MRE3008F20 binding at human A_3 receptors expressed in CHO cells (K_i nM, n = 3–6). For the compounds **47a**, **36**, **37**, **38** and **39** are reported in parentheses the % of inhibition at the 1 μM concentration versus hA_1, A_{2A}, A_{2B} and A_3 adenosine receptors, respectively. The data are expressed as geometric means with 95% or 99% confidence limits in parentheses.

vs hA_1 and hA_{2A} subtypes. The introduction of an N^8-arylalkyl moiety seems indeed related to an increase of A_1/A_{2A} affinity with a resulting decrease of A_3 selectivity. These data are mostly in agreement with previously reported studies on N^8-substituted pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines.[29,30]

The pyridyl-urea derivative **10** was synthesised as this structural modification proved to improve hA_3 affinity and water-solubility when introduced at the same position of the pyrazolo-triazolo-pyrimidine tricycle[27] (see MRE3005-F20, Fig. 1). Quite surprisingly, compound **10** (K_i hA_3 = 111 nM) was found to

be more than 7-fold less potent than the corresponding 4-OCH_3–phenylurea derivative **9a** (K_i hA_3 = 15 nM) in binding the hA_3 receptor.

From the pair-wise comparison between binding affinities of compounds **8**, **9a–d** and **10** with the analogously substituted and previously reported PTPs,[29] some general observation can be derived. The removal of the nitrogen at the 7-position did not significantly affect hA_1 and hA_{2B} affinity/efficacy both for the 5-NH_2 derivatives **8a-d** and the 5-urea derivatives **9a–d**. This would suggest that N^7 is not involved in crucial interaction with these AR subtypes. Conversely, the 5-amino-7-deaza derivatives appeared to be significantly less potent (up to 300-fold[29] in the case of **8d**) than the corresponding N^8-substituted pyrazole-containing tricycles toward the A_{2A} receptor. With respect to the A_3 subtype, the 5-amino-7-deaza derivatives appeared from 6- to 22-fold more potent than the corresponding PTP, while the 5-urea derivatives displayed a lower affinity (see compound **9b** and **10** vs MRE3008-F20 and MRE3005-F20, respectively, Fig. 1). The overall results suggest that the removal of the nitrogen at the 7-position was detrimental to the selectivity of the pyrrole derivatives when compared to the corresponding 7-aza analogues,[7] probably due to a significant alteration of the receptor binding interactions, mainly with the A_{2A} and/or A_3 receptors.

2.2.2. Pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines

With the aim to better investigate the role of the pyrazole ring on the affinity toward ARs we also synthesised a novel series of 8- or 9-substituted pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (see Table 2) as junction isomers of the reference compounds reported in Figure 1. These series allowed us to evaluate the effect of the shift of a pyrazole nitrogen from the 7- to the 9-position of the tricycle.

The 5-amino-N^8-alkyl derivatives (**40–43b**) showed comparable or slightly increased affinity/potency at the A_1, A_{2A} and A_{2B} subtypes in comparison with the corresponding 5-amino-N^8-alkyl pyrrolo-triazolo-pyrimidines (**8a–d**) while the affinity at the A_3 receptor was found to be from 2- to 8-fold lower. Introduction of a 4-methoxy-phenylurea moiety at the 5-position yielded compounds **44–47b**, exerting generally lower affinities if compared to **40–43b** at the A_1 (from 11- to 153-fold) and A_{2A} (from 12- to 90-fold) subtypes. Lower potency at the A_{2B} (from 17- to 37-fold) subtype was also seen. The 4-methoxy-phenylurea moiety produced a significant increase of A_3 affinity (almost ninefold) only in the case of compound **45b** compared to **41b**. The N^8-propyl-substituted **45b** (K_i = 50 nM) exerted twofold higher affinity at A_3 AR compared to the N^8-methyl- derivative **44b** (K_i = 110 nM) while the introduction of N^8-arylalkyl chains (**46–47b**) was somewhat detrimental in terms of A_3 affinity. Comparing the 5-urea-N^8-alkyl-pyrazolo-triazolo-pyrimidine derivatives (**44–47b**) to the corresponding 5-urea-N^8-alkyl-pyrrolo-triazolo-pyrimidines (**9a–d**), the main effect of the introduction of nitrogen at the 9-position was a significantly reduced hA_3 affinity, especially in the case of N^8-arylalkyl substitution (see compounds **46–47b** vs **9c**,**d**). The comparison between **40–47b** and their isomeric N^8-substituted pyrazolo[4,3-*e*]triazolo-pyrimidines[29] showed how the shift of the pyrazole nitrogen from the 7- to the 9-position of the tricycle exerted a remarkable decrease of hA_3 affinity and selectivity of the 5-urea-derivatives (see Fig. 1 compound MRE3008-F20 vs **45b**).

The synthesis of compounds **40–47a** allowed us to evaluate the effect of N^9- vs N^8-substitution. When small alkyl chains were shifted from N^8- to N^9-position, the general effect (excepting **40a** vs **40b** at hA_3) was a random increase of affinity/potency at the four AR subtypes, especially for the 5-urea derivatives (see **44–45a** vs **44–45b**). An opposite trend was instead observed when arylalkyl chains were shifted (see **42–43a** vs **42–43b**). Thus, the increase of the steric hindrance around N^9-position seems poorly tolerated. These data confirm our previous results on C^9-substituted-PTPs,[24] and are in agreement with recent QSAR analyses performed at the A_3 AR.[31] The amino derivatives (**40–43a**) exhibit good affinity toward A_{2A} AR (K_i from 3.6 to 122 nM). Unfortunately, the ligands bind to the A_1 and inhibit the A_{2B} subtypes in the same range of concentration. The conversion of the amino group into the corresponding 4-OCH_3–phenylurea moiety (**44–47a**) did not provide the desired improvement of affinity/selectivity at the A_3 AR in the N^9-substituted series (**40–43a** vs **44–47a**).

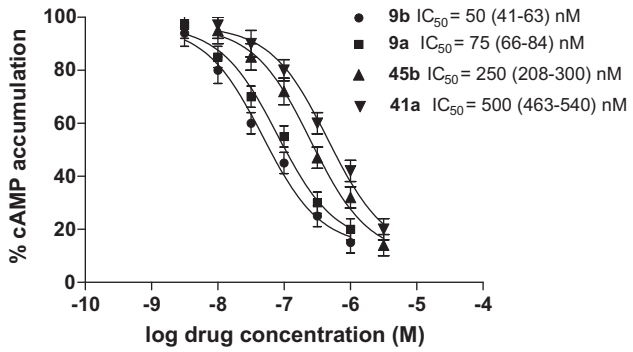


Figure 3. Inhibition curves of cAMP accumulation in hA_3 CHO cells by adenosine antagonists blocking the effect of 100 nM Cl-IB-MECA.

Among compounds **36–39**, in which the chlorine atom at the 5-position of intermediate **32a** (see Scheme 3) was substituted with cyclohexylamine, morpholine, and 4-methyl/phenyl-piperazine, only the cyclohexyl-amino derivative **36** showed some affinity versus ARs, especially for the A_1 receptor (K_i = 12 nM). This would confirm previously reported studies indicating that the amino group at the 5-position is involved in a crucial interaction with ARs as a hydrogen bond donor. Bisubstitution of this nitrogen, by incorporating it in a ring, completely abolished the affinity. Interestingly, compound **36** is characterized by the presence at the 5-position of a cycloalkyl group, which is the typical N^6-substitution of NECA (5′-*N*-ethylcarboxamidoadenosine)-related A_1 AR agonists.[32] This findings would be in agreement with the previously observed parallelism between the C^5-position of PTPs and the C^6-position of NECA-related agonists.[33]

3. Conclusions

Herein, we described a novel synthetic strategy for the preparation of previously unreported pyrrolo- and pyrazolo-[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines that can be, respectively, considered as 7-deaza analogues and junction isomers of A_{2A}/A_3 AR antagonists based on the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus.[7] In both series, we identified examples of potent (nanomolar range) AR antagonists characterized by broad selectivity profiles.

The removal of the nitrogen at the 7-position was found to significantly affect both A_{2A} and A_3 affinities, reflected in a substantial loss of potency/selectivity of the newly examined molecules. The introduction of a 5-phenylcarbamoyl moiety, to promote A_3 affinity and selectivity, appeared effective only in the pyrrole series of which the urea derivatives **9a** and **9b** distinguished as high affinity (K_i = 15 and 10 nM, respectively) and potent (IC_{50} = 75 and 50 nM, respectively, see Fig. 3) hA_3 antagonists.

The comparison between the new N^8-substituted pyrazolo[3,4-*e*]triazolo-pyrimidines (**40–47b**) and their structural isomers N^8-substituted pyrazolo[4,3-*e*]triazolo-pyrimidines[29] showed how the shift of the pyrazole nitrogen from the 7- to the 9-position exerted, as a main effect, a remarkable decrease of hA_3 affinity and selectivity of the 5-urea-derivatives (see Fig. 1 compound MRE3008-F20 vs **45b**).

When small alkyl chains were shifted from the N^8- to N^9-position, the general effect was a random increase of affinity/potency at the four AR subtypes especially for the 5-urea derivatives (see **44**–**45a** vs **44**–**45b**). In contrast, N^9-arylalkyl substitution appeared generally detrimental. Thus, steric hindrance around this position seems poorly tolerated, in accordance with previous findings.[24,31]

The present work can be considered as an innovative and important contribution to the understanding of the SAR of PTPs based AR antagonists.

4. Experimental section

4.1. Chemistry

4.1.1. Materials and methods

Reaction progress and product mixtures were monitored by thin-layer chromatography (TLC) on silica gel (precoated F254 Merck plates) and visualized with aqueous potassium permanganate. ^{1}H NMR data were determined in $CDCl_3$ or DMSO-d_6 solutions with a Varian VXR 200 spectrometer or a Varian Mercury Plus 400 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and J values are given in Hertz. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Light petroleum refers to the fractions boiling at 40–60 °C. Melting points were determined on a Buchi–Tottoli instrument and are uncorrected. Chromatography was performed on Merck 230–400 mesh silica gel. Organic solutions were dried over anhydrous sodium sulfate. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara, and were within ±0.4% of the theoretical values for C, H, and N. All final compounds exhibited a purity of not less than 95%. The mass spectra were obtained on an ESI Micromass ZMD 2000 mass spectrometer in positive scan mode using direct injection of the purified compound solution (MH^+).

4.1.2. General procedure for preparation of 1,3-dibenzyl-6-alkyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-diones (3a–d)

To a solution of 1,3-dibenzyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (**2**,[26] 1.5 mmol) in anhydrous DMF (5 mL), K_2CO_3 was added (4.5 mmol) and the resulting mixture stirred at 40 °C for 10′. After cooling at room temperature, the appropriate alkyl halide (4.5 mmol) was added and the reaction heated at 40 °C for further 4 h. The solvents were removed in vacuo and the residue was suspended in water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified by crystallization or flash chromatography.

4.1.2.1. 1,3-Dibenzyl-6-methyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (3a). Crystallization from Et_2O/petroleum ether. White solid; 97% yield; mp 164–166 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.52–7.22 (m, 10H), 7.18 (d, J = 2.2 Hz, 1H), 6.13 (d, J = 2.4 Hz, 1H), 5.24 (s, 2H), 5.01 (s, 2H), 3.66 (s, 3H). MS (ESI): $[MH]^+$ = 346.4.

4.1.2.2. 1,3-Dibenzyl-6-propyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (3b). Crystallization from Et_2O/petroleum ether. White solid; 89% yield; mp 122 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.51–7.48 (m, 2H), 7.29–7.22 (m, 9H), 6.15 (d, J = 2.4 Hz, 1H), 5.24 (s, 2H), 5.01 (s, 2H), 3.80 (t, J = 7.2 Hz, 2H), 1.77–1.73 (m, J = 7.2 Hz, 2H), 0.86 (t, J = 7.4 Hz, 3H) MS (ESI): $[MH]^+$ = 374.4.

4.1.2.3. 1,3-Dibenzyl-6-phenethyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (3c). Crystallization from Et_2O/petroleum ether. White solid; 76% yield; mp 159–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, J = 2 Hz, 1H), 7.29–7.13 (m, 15H), 6.83 (d, J = 2 Hz, 1H), 5.04 (s, 2H), 4.93 (s, 2H), 4.18 (t, J = 7.6 Hz, 2H), 3.02 (t, J = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 436.8.

4.1.2.4. 1,3-Dibenzyl-6-(3-phenyl-propyl)-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (3d). Column chromatography eluting with a mixture EtOAc/petroleum ether 1:4. White solid; 82% yield; mp 129–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (d, J = 2 Hz, 1H), 7.33–7.12 (m, 15H), 6.86 (d, J = 2 Hz, 1H), 5.08 (s, 2H), 4.99 (s, 2H), 3.97 (t, J = 7.2 Hz, 2H), 2.51–2.44 (m, 2H), 2.03 (m, 2H). MS (ESI): $[MH]^+$ = 450.8.

4.1.3. General procedure for preparation of 6-alkyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-diones (4a–d)

To a solution of the appropriate dibenzyl derivative **3a–d** (4 mmol) in anhydrous toluene (50 mL) was added $AlCl_3$ (28 mmol) and the resulting suspension was stirred at room temperature for 2 h. The solvent was concentrated in vacuo to obtain a red residue that was treated with crushed ice and stirred at 0 °C till the disappearance of the red colour. The aqueous phase was then extracted with EtOAc (3 × 50 mL), the combined organic layers were dried over Na_2SO_4, filtered, and the solvent was evaporated to give a solid residue that was crystallized from EtOAc/Et_2O.

4.1.3.1. 6-Methyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (4a). White solid; 82% yield; mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.36 (br s, 1H), 10.28 (br s, 1H), 7.29 (s, 1H), 6.44 (s, 1H), 3.67 (s, 3H). MS (ESI): $[MH]^+$ = 166.1.

4.1.3.2. 6-Propyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (4b). Pale yellow solid; 87% yield; mp 270 °C dec.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.37 (br s, 1H), 10.27 (br s, 1H), 7.35 (d, J = 2.2 Hz, 1H), 6.47 (d, J = 2.2 Hz, 1H), 3.89 (t, J = 7 Hz, 2H), 1.77–1.66 (m, 2H), 0.79 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 194.1.

4.1.3.3. 6-Phenethyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (4c). White solid; 81% yield; mp 263 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (br s, 1H), 10.28 (br s, 1H), 7.30–7.10 (m, 6H), 6.49 (d, J = 2.4 Hz, 1H), 4.19 (t, J = 7.6 Hz, 2H), 3.03 (t, J = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 256.3.

4.1.3.4. 6-(3-Phenyl-propyl)-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (4d). White solid; 80% yield; mp 225 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (br s, 1H), 10.30 (br s, 1H), 7.38 (d, J = 2 Hz, 1H), 7.30–7.17 (m, 5H), 6.51 (d, J = 2 Hz, 1H), 3.96 (t, J = 6.8 Hz, 2H), 2.52–2.48 (m, 2H), 2.051–2.00 (m, 2H). MS (ESI): $[MH]^+$ = 270.3.

4.1.4. General procedure for preparation of 1-alkyl-4-nitro-1*H*-pyrazole-5-carboxamide and 1-alkyl-4-nitro-1*H*-pyrazole-3-carboxamide (12–15a,b)

To a solution of the nitroamide **11**[28] (1.2 mmol) in anhydrous DMF (36 mL) was added K_2CO_3 (2.3 mmol) and the resulting mixture was stirred for 10′. Then, the appropriate alkyl halide (1.4 mmol) was added and the mixture was stirred for 6 h. The solvent was removed in vacuo and the residue was suspended in water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvents removed under reduced pressure to obtain a crude solid that was purified via column chromatography (gradient from EtOAc/petroleum ether 1:1 to EtOAc) to obtain the desired products.

4.1.4.1. 1-Methyl-4-nitro-1*H*-pyrazole-5-carboxamide (12a). White solid; 40% yield; mp 167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (br s, 1H), 8.34 (br s, 1H), 8.27 (s, 1H), 3.86 (s, 3H).

4.1.4.2. 1-Methyl-4-nitro-1*H*-pyrazole-3-carboxamide (12b). White solid; 38% yield; mp 166 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.03 (br s, 1H), 7.79 (br s, 1H), 3.90 (s, 3H).

4.1.4.3. 1-Propyl-4-nitro-1*H*-pyrazole-5-carboxamide (13a). White solid; 48% yield; mp 98–100 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (br s, 1H), 8.28–8.27 (m, 2H), 4.08 (t, J = 6.8 Hz, 2H), 1.81–1.76 (m, 2H), 0.813 (t, J = 7.6 Hz, 3H).

4.1.4.4. 1-Propyl-4-nitro-1*H*-pyrazole-3-carboxamide (13b). White solid; 43% yield; mp 115–116 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (s, 1H), 7.98 (br s, 1H), 7.73 (br s, 1H), 4.10 (t, J = 6.8 Hz, 2H), 1.83–1.78 (m, 2H), 0.83 (t, J = 7.2 Hz, 3H).

4.1.4.5. 4-Nitro-1-(2-phenylethyl)-1*H*-pyrazole-5-carboxamide (14a). White solid; 25% yield; mp 133 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (br s, 1H), 8.31 (br s, 1H), 8.25 (s, 1H), 7.29–7.16 (m, 5H), 4.38–4.35 (m, 2H), 3.09 (t, J = 7.6 Hz, 2H).

4.1.4.6. 4-Nitro-1-(2-phenylethyl)-1*H*-pyrazole-3-carboxamide (14b). White solid; 50% yield; mp 158–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.00 (br s, 1H), 7.76 (br s, 1H), 7.30–7.18 (m,5H), 4.40 (t, J = 7.2 Hz, 2H), 3.13 (t, J = 7.6 Hz, 2H).

4.1.4.7. 4-Nitro-1-(3-phenylpropyl)-1*H*-pyrazole-5-carboxamide (15a). White solid; 34% yield; mp 93–95 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (br s, 1H), 8.33–8.32 (m, 2H), 7.30–7.19 (m, 5H), 4.16 (t, J = 6.8 Hz, 2H), 2.57 (t, J = 7.2 Hz, 2H), 2.11–2.08 (m, 2H).

4.1.4.8. 4-Nitro-1-(3-phenylpropyl)-1*H*-pyrazole-3-carboxamide (15b). White solid; 57% yield; mp 111–113 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 1H), 8.00 (br s, 1H), 7.77 (br s, 1H), 7.31–7.19 (m, 5H), 4.18 (t, J = 7.2 Hz, 2H), 2.59 (t, J = 7.2 Hz, 2H), 2.15–2.12 (m, 2H).

4.1.5. General procedure for preparation of 4-amino-1-alkyl-1*H*-pyrazole-5-carboxamide and 4-amino-1-alkyl-1*H*-pyrazole-3-carboxamide (16–19a,b)

The appropriate nitroamide **12**–**15a**,**b** (4 mmol) was dissolved in EtOH (200 mL), then Pd on activated charcoal (10%, 150 mg) was added and the mixture was hydrogenated at 50 psi in a Parr apparatus for 5 h at room temperature. The mixture was filtered on Celite and then the solvent was removed under reduced pressure to obtain the crude product which was purified by crystallization from EtOAc/Et_2O.

4.1.5.1. 4-Amino-1-methyl-1*H*-pyrazole-5-carboxamide (16a). White solid; 60% yield; mp 174–175 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.37 (br s, 2H), 7.01 (s, 1H); 4,39 (br s, 2H), 3.89 (s, 3H).

4.1.5.2. 4-Amino-1-methyl-1*H*-pyrazole-3-carboxamide (16b). White solid; 60% yield; mp 171–172 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.10 (br s, 1H), 7.06 (s, 1H), 6.95 (br s, 1H), 4.63 (br s, 2H), 3.72 (s, 3H).

4.1.5.3. 4-Amino-1-propyl-1*H*-pyrazole-5-carboxamide (17a) . White solid; 65% yield; mp 91–92 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.40 (br s, 2H), 7.04 (s, 1H), 4.33–4.24 (br m, 4H), 1.69 (m, 2H), 0.75 (t, J = 7.6 Hz, 3H).

4.1.5.4. 4-Amino-1-propyl-1*H*-pyrazole-3-carboxamide (17b). White solid; 91% yield; mp 115 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.10 (s, 1H), 7.07 (br s, 1H), 6.95 (br s, 1H), 4.62 (br s, 2H), 3.92 (t, J = 6.8 Hz, 2H), 1.79–1.69 (m, 2H), 0.82 (t, J = 7.2 Hz, 3H).

4.1.5.5. 4-Amino-1-(2-phenylethyl)-1*H*-pyrazole-5-carboxamide (18a). White solid; 68% yield; mp 87–88 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.40 (br s, 2H), 7.27–7.15 (m, 5H), 7.05 (s, 1H), 4.53 (t, J = 7.6 Hz, 2H), 4.37 (br s, 2H), 2.94 (t, J = 7.8 Hz, 2H).

4.1.5.6. 4-Amino-1-(2-phenylethyl)-1*H*-pyrazole-3-carboxamide (18b). White solid; 87% yield; mp 82–83 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.29–7.17 (m, 5H), 7.11 (br s, 1H), 7.04 (s, 1H), 6.97 (br s, 1H), 4.61 (br s, 2H), 4.22 (t, J = 7 Hz, 2H), 3.07 (t, J = 7.6 Hz, 2H).

4.1.5.7. 4-Amino-1-(3-phenylpropyl)-1*H*-pyrazole-5-carboxamide (19a). White solid; 87% yield; mp 78 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.42 (br s, 2H), 7.26–7.15 (m, 5H), 7.07 (s, 1H), 4.38–4.31 (m, 4H), 2.51–2.43 (m, 2H), 1.97–1.93 (m, 2H).

4.1.5.8. 4-Amino-1-(3-phenylpropyl)-1*H*-pyrazole-3-carboxamide (19b). White solid; 90% yield; mp 111–112 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.32–7.11 (m, 7H), 6.97 (br s, 1H), 4.65 (br s, 2H), 3.98 (t, J = 6.8 Hz, 2H), 2.58–2.49 (m, 2H), 2.08–2.00 (m, 2H).

4.1.6. General procedure for preparation of 1/2-alkyl-1/2*H*-pyrazolo[4,3-d]pyrimidine-5,7-(4*H*,6*H*)-diones (20–23a,b)

The appropriate aminoamide **16**–**19a**,**b** (7.1 mmol) and urea (33.3 mmol) were finely mixed without solvent in a round bottom flask which was then heated at 200 °C for 2 h. During the reaction course, complete fusion followed by resolidification was observed. The crude product was purified by crystallization from 10% NaOH/acetic acid.

4.1.6.1. 1-Methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (20a). White solid; quantitative yield; mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.15 (br s, 1H), 10.96 (br s, 1H), 8.16 (s, 1H), 4.04 (s, 3H). MS (ESI): $[MH]^+$ = 167.1.

4.1.6.2. 2-Methyl-2*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (20b). White solid; 81% yield; mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (br s, 1H), 10.71 (br s, 1H), 7.64 (s, 1H), 3.94 (s, 3H). MS (ESI): $[MH]^+$ = 167.1.

4.1.6.3. 1-Propyl-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (21a). White solid; quantitative yield; mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 9.34 (br s, 2H), 7.37 (s, 1H), 4.35 (t, J = 6.8 Hz, 2H), 1.81–1.70 (m, 2H), 0.78 (t, J = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 195.1.

4.1.6.4. 2-Propyl-2*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (21b). White solid; 74% yield; mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.48 (br s, 2H), 7.68 (s, 1H), 4.14 (t, J = 6.8 Hz, 2H), 1.85–1.74 (m, 2H), 0.80 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 195.1.

4.1.6.5. 1-(2-Phenylethyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (22a). White solid; quantitative yield; mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.08 (br s, 2H), 7.34 (s, 1H), 7.27–7.10 (m, 5H), 4.62 (t, J = 7.2 Hz, 2H), 3.07 (t, J = 7.4 Hz, 2H). MS (ESI): $[MH]^+$ = 257.1.

4.1.6.6. 2-(2-Phenylethyl)-2*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (22b). White solid; 70% yield; mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.91 (br s, 1H), 10.38 (br s, 1H), 7.58 (s, 1H), 7.32–7.17 (m, 5H), 4.45 (t, J = 7.4 Hz, 2H), 3.14 (t J = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 257.1.

4.1.6.7. 1-(3-Phenylpropyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (23a). White solid; 63% yield; mp 280 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.19 (br s, 1H), 10.02 (br s, 1H),

7.38 (s, 1H), 7.26–7.154 (m, 5H), 4.43 (t, J = 6.8 Hz, 2H), 2.57–2.50 (m, 2H), 2.11–2.04 (m, 2H). MS (ESI): $[MH]^+$ = 271.1.

4.1.6.8. 2-(3-Phenylpropyl)-2*H*-pyrazolo[4,3-*d*]pyrimidine-5,7-(4*H*,6*H*)-dione (23b). White solid; 55% yield; mp >300 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.51 (br s, 2H), 7.71 (s, 1H), 7.28–7.17 (m, 5H), 4.22 (t, J = 6.8 Hz, 2H), 2.58–2.48 (m, 2H), 2.15–2.11 (m, 2H). MS (ESI): $[MH]^+$ = 271.1.

4.1.7. General procedure for preparation of 2,4-dichloro-6-alkyl-6*H*-pyrrolo[3,4-*d*]pyrimidines (5a–d) and 5,7-dichloro-1/2-alkyl-1/2*H*-pyrazolo[4,3-*d*]pyrimidines (24–27a,b)

A mixture of **4a-d** or **20**–**23a**,**b** (6.1 mmol) and phosphorous oxychloride (60 mmol) was heated at 50 °C under an argon atmosphere and DBU (36.6 mmol) was added dropwise under vigorous stirring. The reaction was then heated for further 8 h at 80 °C. After cooling to room temperature, the reaction mixture was slowly poured into cold water and treated with a 50% aqueous solution of NaOH to pH 7. The solution was extracted with Et_2O, the organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure to obtain the desired intermediate. Due to instability, these compounds were used for the next reaction without further purification. The NMR analyses were performed on the crude product.

4.1.7.1. 2,4-Dichloro-6-methyl-6*H*-pyrrolo[3,4-*d*]pyrimidine (5a). Pale yellow solid; 75% yield; crude product; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.24 (s, 1H), 4.07 (s, 3H). MS (ESI): $[MH]^+$ = 202.1.

4.1.7.2. 2,4-Dichloro-6-propyl-6*H*-pyrrolo[3,4-*d*]pyrimidine (5b). Pale yellow solid; 63% yield; crude product; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34 (d, J = 2.2 Hz, 1H), 7.25 (s, 1H), 4.20 (t, J = 7 Hz, 2H), 2.02–1.92 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 230.1.

4.1.7.3. 2,4-Dichloro-6-phenethyl-6*H*-pyrrolo[3,4-*d*]pyrimidine (5c). Pale yellow solid; 73% yield; crude product; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (d, J = 2 Hz, 1H), 7.72 (d, J = 2 Hz, 1H), 7.29–7.19 (m, 5H), 4.59 (t, J = 7.2 Hz, 2H), 3.21 (t, J = 7.6 Hz, 2H). MS (ESI): $[MH]^+$ = 293.1.

4.1.7.4. 2,4-Dichloro-6-(3-phenyl-propyl)-6*H*-pyrrolo[3,4-*d*]pyrimidine (5d). Pale yellow oil; 81% yield; crude product; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.14 (m, 7H), 4.23 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 6.8 Hz, 2H), 2.31–2.28 (m, 2H). MS (ESI): $[MH]^+$ = 307.1.

4.1.7.5. 5,7-Dichloro-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (24a). Pale yellow solid; 63% yield; crude product; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 4.41 (s, 3H).

4.1.7.6. 5,7-Dichloro-2-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidine (24b). Pale yellow solid; 63% yield; crude product; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 4.41 (s, 3H).

4.1.7.7. 5,7-Dichloro-1-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (25a). Pale yellow oil; 57% yield; crude product; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.20 (s, 1H), 4.69 (t, J = 7 Hz, 2H), 2.04–1.92 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H).

4.1.7.8. 5,7-Dichloro-2-propyl-2*H*-pyrazolo[4,3-*d*]pyrimidine (25b). Pale yellow solid; 55% yield; crude product; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.16 (s, 1H), 4.49 (t, J = 7.2 Hz, 2H), 2.11–2.04 (m, 2H), 0.99 (t, J = 7.6 Hz, 3H).

4.1.7.9. 5,7-Dichloro-1-phenethyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (26a). Pale yellow solid; 58% yield; crude product; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.26–7.23 (m, 3H), 7.10–7.07 (m, 2H), 4.94 (t, J = 7.4 Hz, 2H), 3.23 (t, J = 7.6 Hz, 2H).

4.1.7.10. 5,7-Dichloro-2-phenethyl-2*H*-pyrazolo[4,3-*d*]pyrimidine (26b). Pale yellow solid; 50% yield; crude product; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.29–7.26 (m, 3H), 7.08–7.04 (m, 2H), 4.75 (t, J = 7.2 Hz, 2H), 3.34 (t, J = 7 Hz, 2H). MS (ESI): $[MH]^+$ = 292.1.

4.1.7.11. 5,7-Dichloro-1-(3-phenyl-propyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine (27a). Pale yellow solid; 58% yield; crude product; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.27–7.14 (m, 5H), 4.73 (t, J = 7 Hz, 2H), 2.71 (t, J = 7.4 Hz, 2H), 2.33–2.62 (m, 2H).

4.1.7.12. 5,7-Dichloro-2-(3-phenyl-propyl)-2*H*-pyrazolo[4,3-*d*]pyrimidine (27b). Pale yellow solid; 62% yield; crude product; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.30–7.14 (m, 5H), 4.55–4.35 (m, 2H), 2.65 (t, J = 7.4 Hz, 2H), 2.45–2.30 (m, 2H).

4.1.8. General procedure for preparation of furan-2-carboxylic acid *N*′-(2-chloro-6-alkyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazides (6a–d) and furan-2-carboxylic acid *N*′-(5-chloro-1/2-alkyl-1/2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazides (28–31a,b)

To a solution of dichloro derivatives **5a-d** or **24**–**27a**,**b** (0.5 mmol) in anhydrous 1,4-dioxane (4 mL) was added TEA (0.5 mmol) and furan-2-carboxylic acid hydrazide (0.5 mmol). The reaction was heated at 80–90 °C for 5 h. The solvent was removed under reduced pressure to obtain a crude solid that was purified via column chromatography eluting with a mixture of CH_2Cl_2/CH_3OH 9:1.

4.1.8.1. Furan-2-carboxylic acid *N*′-(2-chloro-6-methyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazide (6a). Pale yellow solid; 65% yield; mp 250–251 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (br s, 1H), 10.40 (br s, 1H), 7.89 (s, 1H), 7.77 (t, J = 4.8 Hz, 1H), 7.40 (br s, 1H), 7.23 (d, J = 2.8 Hz, 1H), 6.66–6.65 (m, 1H), 3.45 (s, 3H). MS (ESI): $[MH]^+$ = 292.2.

4.1.8.2. Furan-2-carboxylic acid *N*′-(2-chloro-6-propyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazide (6b). Pale yellow solid; 72% yield; mp 202–203 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.60 (br s, 1H), 9.91 (br s, 1H), 7.96 (s, 1H), 7.52 (s, 1H), 7.31–7.28 (m, 1H), 7.18 (br s, 1H), 6.72–6.71 (m, 1H), 4.17 (m, 2H), 1.81 (m, 2H), 0.88–0.80 (m, 3H). MS (ESI): $[MH]^+$ = 320.2.

4.1.8.3. Furan-2-carboxylic acid *N*′-(2-chloro-6-phenethyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazide (6c). Pale yellow solid; 48% yield; mp 209–210 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.58 (br s, 1H), 10.17 (br s, 1H), 7.95 (s, 1H), 7.45 (s, 1H), 7.27–7.12 (m, 8H), 6.71 (s, 1H), 4.47–4.41 (m, 2H), 3.15–3.12 (m, 1H). MS (ESI): $[MH]^+$ = 382.3.

4.1.8.4. Furan-2-carboxylic acid *N*′-[2-chloro-6-(3-phenyl-propyl)-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl]-hydrazide (6d). Pale yellow solid; 56% yield; mp 204–205 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (br s, 1H), 9.97 (br s, 1H), 7.96 (s, 1H), 7.55 (s, 1H), 7.32–6.99 (m, 7H), 6.71 (s, 1H), 4.22 (t, J = 6.8 Hz, 2H), 2.56 (t, J = 7.2 Hz, 2H), 2.16- 2.12 (m, 2H). MS (ESI): $[MH]^+$ = 396.3.

4.1.8.5. Furan-2-carboxylic acid *N*′-(5-chloro-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (28a). Pale yellow solid; 82% yield; mp 205 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (br s, 1H), 9.98 (br s, 1H), 8.07 (s, 1H), 7.98 (d, J = 1.2 Hz, 1H), 7.33 (d, J = 3.2 Hz, 1H), 6.73–6.72 (m, 1H), 4.29 (s, 3H). MS (ESI): $[MH]^+$ = 293.3.

4.1.8.6. Furan-2-carboxylic acid *N'*-(5-chloro-2-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (28b). White solid; 40% yield; mp 158 °C dec.; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (br s, 1H), 10.61 (br s, 1H), 8.42 (s, 1H), 7.96 (d, J = 1.2 Hz, 1H), 7.29 (d, J = 3.2 Hz, 1H), 6.72–6.70 (m, 1H), 4.19 (s, 3H). MS (ESI): $[MH]^+$ = 293.3.

4.1.8.7. Furan-2-carboxylic acid *N'*-(5-chloro-1-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (29a). White solid; 70% yield; mp 217 °C dec.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.77 (br s, 1H), 9.86 (br s, 1H), 8.11 (s, 1H), 7.98–7.97 (m, 1H), 7.32 (d, J = 3.4 Hz, 1H), 6.74–6.71 (m, 1H), 4.61 (t, J = 7 Hz, 2H), 1.85–1.75 (m, 2H), 0.80 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 321.0.

4.1.8.8. Furan-2-carboxylic acid *N'*-(5-chloro-2-propyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (29b). White solid; 50% yield; mp 164–165 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.66 (br s, 2H), 8.48 (s, 1H), 7.96 (s, 1H), 7.29 (d, J = 3.6 Hz, 1H), 6.72–6.70 (m, 1H), 4.41 (t, J = 7.2 Hz, 2H), 1.97–1.90 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 321.0.

4.1.8.9. Furan-2-carboxylic acid *N'*-(5-chloro-1-phenethyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (30a). White solid; 60% yield; mp 197–198 °C dec.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.78 (br s, 1H), 10.04 (br s, 1H), 8.03 (s, 1H), 7.98 (s, 1H), 7.33 (d, J = 3.4 Hz, 1H), 7.24–7.20 (m, 5H), 6.74–6.72 (m, 1H), 4.94–4.87 (m, 2H), 3.16–3.09 (m, 2H). MS (ESI): $[MH]^+$ = 383.0.

4.1.8.10. Furan-2-carboxylic acid *N'*-(5-chloro-2-phenethyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (30b). White solid; 42% yield; mp 137–138 °C dec.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.66 (br s, 2H), 8.37 (s, 1H),7.96 (s, 1H), 7.32–7.17 (m, 6H), 6.72–6.70 (m, 1H), 4.71 (t, J = 6.8 Hz, 2H), 3.36–3.24 (m, 2H). MS (ESI): $[MH]^+$ = 383.0.

4.1.8.11. Furan-2-carboxylic acid *N'*-[5-chloro-1-(3-phenyl-propyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl]-hydrazide (31a). Pale yellow solid; 50% yield; mp 115–116 °C dec.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.79 (br s, 1H), 9.98 (br s, 1H), 8.10 (s, 1H), 7.99–7.98 (m, 1H), 7.34–7.16 (m, 6H), 6.74–6.71 (m, 1H), 4.70 (t, J = 7 Hz, 2H), 2.59–2.49 (m, 2H), 2.13–2.05 (m, 2H). MS (ESI): $[MH]^+$ = 397.0.

4.1.8.12. Furan-2-carboxylic acid *N'*-[5-chloro-2-(3-phenyl-propyl)-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl]-hydrazide (31b). Pale yellow solid; 56% yield; mp 169–170 °C dec.; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.67 (br s, 2H), 8.51 (s, 1H), 7.97–7.96 (m, 1H), 7.30–7.19 (m, 6H), 6.72–6.70 (m, 1H), 4.49–4.43 (m, 2H), 2.62–2.49 (m, 2H), 2.29–2.21 (m, 2H). MS (ESI): $[MH]^+$ = 397.0.

4.1.9. General procedure for preparation of 5-chloro-2-(furan-2-yl)-8-alkyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (7a–d) and 5-chloro-(2-furan-2-yl)-8/9-alkyl-8/9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (32–35a,b)

The pyrrolo- or pyrazolopyrimidines **6a**–**d** or **28**–**31a**,**b** (0.2 mmol) were suspended in a mixture of hexamethyldisilazane (0.5 mL) and bis(trimethylsilyl)acetamide (0.5 mL) and the reaction was heated at 120 °C for 18 h. The excess of reagents was removed under reduced pressure and the residue was purified via column chromatography eluting with a mixture CH_2Cl_2/CH_3OH 9.5:0.5.

4.1.9.1. 5-Chloro-(2-furan-2-yl)-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (7a). White solid; 75% yield; mp 275 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–8.01 (m, 1H), 7.86 (d, J = 2 Hz, 1H), 7.62 (d, J = 2 Hz, 1H), 7.04–7.03 (m, 1H), 6.76–6.74 (m, 1H), 3.96 (s, 3H). MS (ESI): $[MH]^+$ = 274.2.

4.1.9.2. 5-Chloro-(2-furan-2-yl)-8-propyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (7b). White solid; 63% yield; mp 81–82 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.02 (d, J = 1 Hz, 1H), 7.93 (d, J = 2 Hz, 1H), 7.68 (d, J = 2 Hz, 1H), 7.02–7.01 (m, 1H), 6.76–6.75 (m, 1H), 4.18 (t, J = 7.4 Hz, 2H), 1.91–1.79 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 302.1.

4.1.9.3. 5-Chloro-(2-furan-2-yl)-8-phenethyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (7c). White solid; 60% yield; mp 182–183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–8.01 (m, 1H), 7.87 (d, J = 2 Hz, 1H), 7.63 (d, J = 1.6 Hz, 1H), 7.29–7.20 (m, 5H), 7.02–7.01 (m, 1H), 6.75–6.74 (m, 1H), 4.49 (t, J = 7.2 Hz, 2H), 3.19 (t, J = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 364.0.

4.1.9.4. 5-Chloro-(2-furan-2-yl)-8-(3-phenyl-propyl)-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (7d). White solid; 65% yield; mp 138 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (t, J = 0.8 Hz, 1H), 7.97 (d, J = 2 Hz, 1H), 7.72 (d, J = 2 Hz, 1H), 7.29–7.19 (m, 5H), 7.03 (d, J = 3.2 Hz, 1H), 6.76–6.74 (m, 1H), 4.26 (t, J = 6.8 Hz, 2H), 2.55 (t, J = 8.4 Hz, 2H), 2.18 (m, 2H). MS (ESI): $[MH]^+$ = 379.2.

4.1.9.5. 5-Chloro-(2-furan-2-yl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (32a). White solid; 43% yield; mp 221–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.08–8.07 (m, 1H), 7.10–7.09 (m, 1H), 6.80–6.79 (m, 1H), 4.42 (s, 3H). MS (ESI): $[MH]^+$ = 275.2.

4.1.9.6. 5-Chloro-(2-furan-2-yl)-8-methyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (32b). White solid; 52% yield; mp 238 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.06–8.05 (m, 1H), 7.07–7.06 (m, 1H), 6.78–6.77 (m, 1H), 4.20 (s, 3H). MS (ESI): $[MH]^+$ = 275.2.

4.1.9.7. 5-Chloro-(2-furan-2-yl)-9-propyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (33a). White solid; 76% yield; mp 104–105 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.08–8.06 (m, 1H), 7.10–7.07 (m, 1H), 6.80–6.78 (m, 1H), 4.73 (t, J = 7 Hz, 2H), 2.02–1.99 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 303.0.

4.1.9.8. 5-Chloro-(2-furan-2-yl)-8-propyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (33b). White solid; 46% yield; mp 155 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.71–7.70 (m, 1H), 7.00–6.98 (m, 1H), 6.64–6.62 (m, 1H), 4.41 (t, J = 7.2 Hz, 2H), 2.10–2.06 (m, 2H), 0.97 (t, J = 7.6 Hz, 3H). MS (ESI): $[MH]^+$ = 303.0.

4.1.9.9. 5-Chloro-(2-furan-2-yl)-9-(2-phenylethyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (34a). White solid; 89% yield; mp 178 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.30 (s, 1H), 8.08–8.07 (m, 1H), 7.26–7.21 (m, 5H), 7.11–7.09 (m, 1H), 6.81–6.78 (m, 1H), 4.99 (t, J = 7.2 Hz, 2H), 3.33 (t, J = 7 Hz, 2H). MS (ESI): $[MH]^+$ = 365.0.

4.1.9.10. 5-Chloro-(2-furan-2-yl)-8-(2-phenylethyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (34b). White solid; 57% yield; mp 186 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.71–7.70 (m, 2H), 7.25–7.24 (m, 3H), 7.11–7.02 (m, 2H), 6.99 (m, 1H), 6.61 (m, 1H), 4.66 (t, J = 7.2 Hz, 2H), 3.35 (t, J = 7 Hz, 2H). MS (ESI): $[MH]^+$ = 365.0.

4.1.9.11. 5-Chloro-(2-furan-2-yl)-9-(3-phenylpropyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (35a). White solid;

76% yield; mp 155–156 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.08–8.07 (m, 1H), 7.25–7.15 (m, 5H), 7.10–7.09 (m, 1H), 6.80–6.79 (m, 1H), 4.81 (t, J = 6.8 Hz, 2H), 2.63 (t, J = 7.2 Hz, 2H), 2.33–2.29 (m, 2H). MS (ESI): $[MH]^+$ = 378.9.

4.1.9.12. 5-Chloro-(2-furan-2-yl)-8-(3-phenylpropyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (35b). White solid; 62% yield; mp 173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.89–7.81 (m, 1H), 7.35–7.22 (m, 5H), 7.01–6.99 (m, 1H), 6.75–6.67 (m, 1H), 4.32 (t, J = 6.8 Hz, 2H), 2.54 (t, J = 7.2 Hz, 2H), 2.25–2.19 (m, 2H). MS (ESI): $[MH]^+$ = 378.9.

4.1.10. General procedure for preparation of 5-amino-(2-furan-2-yl)-8-alkyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (8a–d) and 5-amino-(2-furan-2-yl)-8/9-alkyl-8/9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (40–43a,b)

The appropriate pyrrolo- or pyrazolo-triazolo-pyrimidine **7a**–**d** or **32**–**35a**,**b** (0.2 mmol) was dissolved in 20 mL of EtOH previously saturated at 0 °C with ammonia. The mixture was heated in a steel bomb at 60 °C for 18 h. The solvent was removed under reduced pressure and the residue was purified via column chromatography eluting with a mixture CH_2Cl_2/CH_3OH 9.5:0.5.

4.1.10.1. 5-Amino-(2-furan-2-yl)-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (8a). White solid; 80% yield; mp 273 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.92 (d, J = 1 Hz, 1H), 7.55 (d, J = 2.2 Hz, 1H), 7.16 (t, J = 3.4 Hz, 1H), 7.01–7.00 (m, 3H), 6.72 (m, 1H), 3.87 (s, 3H). MS (ESI): $[MH]^+$ = 255.2. Anal. ($C_{12}H_{10}N_6O$) C, H, N.

4.1.10.2. 5-Amino-(2-furan-2-yl)-8-propyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (8b). White solid; 75% yield; mp 176 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.92–7.90 (m, 1H), 7.61 (d, J = 2.2 Hz, 1H), 7.31 (m, 1H), 7.17–7.15 (m, 1H), 7.06 (d, J = 2.2 Hz, 1H), 6.98 (s, 1H), 6.72–6.70 (m, 1H), 4.08 (t, J = 6.8 Hz, 2H), 1.84–1.77 (m, 2H), 0.84 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 283.1. Anal. ($C_{14}H_{14}N_6O$) C, H, N.

4.1.10.3. 5-Amino-(2-furan-2-yl)-8-phenethyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (8c). White solid; 77% yield; mp 149 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91–7.90 (m, 1H), 7.53 (d, J = 2 Hz, 1H), 7.29–7.20 (m, 5H), 7.16–7.15 (m, 1H), 7.05 (d, J = 2 Hz, 1H), 6.99 (br s, 2H), 6.71–6.70 (m, 1H), 4.38 (t, J = 7.2 Hz, 2H), 3.15 (t, J = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 345.4. Anal. ($C_{19}H_{16}N_6O$) C, H, N.

4.1.10.4. 5-Amino-(2-furan-2-yl)-8-(3-phenyl-propyl)-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (8d). White solid; 77% yield; mp 156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (m, 1H), 7.44 (m, 1H), 7.30–7.15 (m, 6H), 6.97 (s, 1H), 6.59 (m, 1H), 6.19 (br s, 2H), 4.08 (m, 2H), 2.61 (m, 2H), 2.23 (m, 2H). MS (ESI): $[MH]^+$ = 359.2. Anal. ($C_{20}H_{18}N_6O$) C, H, N.

4.1.10.5. 5-Amino-(2-furan-2-yl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (40a). White solid; 72% yield; mp 270 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98–7.97 (m, 1H), 7.85 (s, 1H), 7.47 (br s, 2H), 7.27–7.26 (m, 1H), 6.76–6.75 (m, 1H), 4.28 (s, 3H). MS (ESI): $[MH]^+$ = 256.2. Anal. ($C_{11}H_9N_7O$) C, H, N.

4.1.10.6. 5-Amino-(2-furan-2-yl)-8-methyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (40b). White solid; 77% yield; mp 295 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.96 (d, J = 1.2 Hz, 1H), 7.37 (br s, 2H), 7.24 (d, J = 3.6 Hz, 1H), 6.75–6.74 (m, 1H), 4.11 (s, 3H). MS (ESI): $[MH]^+$ = 256.2. Anal. ($C_{11}H_9N_7O$) C, H, N.

4.1.10.7. 5-Amino-(2-furan-2-yl)-9-propyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (41a). White solid; 82% yield; mp 229 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.88 (s, 1H), 7.47 (br s, 2H), 7.26 (d, J = 3.2 Hz, 1H), 6.77–6.74 (m, 1H), 4.58 (t, J = 7 Hz, 2H), 1.99–1.95 (m, 2H), 0.85 (t, J = 7.6 Hz, 3H). MS (ESI): $[MH]^+$ = 284.2. Anal. ($C_{13}H_{13}N_7O$) C, H, N.

4.1.10.8. 5-Amino-(2-furan-2-yl)-8-propyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (41b). White solid; 78% yield; mp 198 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.96–7.95 (m, 1H), 7.36 (br s, 2H), 7.25–7.23 (m, 1H), 6.76–6.73 (m, 1H), 4.32 (t, J = 7 Hz, 2H), 1.94–1.90 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 284.2. Anal. ($C_{13}H_{13}N_7O$) C, H, N.

4.1.10.9. 5-Amino-(2-furan-2-yl)-9-(2-phenylethyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (42a). White solid; 85% yield; mp 239 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.99 (d, J = 1.2 Hz, 1H), 7.85 (s, 1H), 7.47 (br s, 2H), 7.28–7.14 (m, 6H), 6.78–6.76 (m, 1H), 4.84 (t, J = 6.8 Hz, 2H), 3.33–3.24 (m, 2H). MS (ESI): $[MH]^+$ = 346.4. Anal. ($C_{18}H_{15}N_7O$) C, H, N.

4.1.10.10. 5-Amine-(2-furan-2-yl)-8-(2-phenylethyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (42b). White solid; 82% yield; mp 218 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.96 (d, J = 1 Hz, 1H), 7.37 (br s, 2H), 7.27–7.18 (m, 6H), 6.76–6.74 (m, 1H), 4.61 (t, J = 7 Hz, 2H), 3.35–3.25 (m, 2H). MS (ESI): $[MH]^+$ = 346.4. Anal. ($C_{18}H_{15}N_7O$) C, H, N.

4.1.10.11. 5-Amino-(2-furan-2-yl)-9-(3-phenylpropyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (43a). White solid; 78% yield; mp 208 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.99–7.98 (m, 1H), 7.89 (s, 1H), 7.48 (br s, 2H), 7.25–7.15 (m, 6H), 6.78–6.75 (m, 1H), 4.63 (t, J = 7 Hz, 2H), 2.61 (t, J = 7.2 Hz, 2H), 2.32–2.24 (m, 2H). MS (ESI): $[MH]^+$ = 360.4. Anal. ($C_{19}H_{17}N_7O$) C, H, N.

4.1.10.12. 5-Amino-(2-furan-2-yl)-8-(3-phenylpropyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (43b). White solid; 84% yield; mp 162 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.65–7.63 (m, 1H), 7.37–7.16 (m, 6H), 6.62–6.60 (m, 1H), 6.21 (br s, 2H), 4.37 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.8 Hz, 2H), 2.41–2.37 (m, 2H). MS (ESI): $[MH]^+$ = 360.4. Anal. ($C_{19}H_{17}N_7O$) C, H, N.

4.1.11. General procedure for preparation of 5-alkyl-(2-furan-2-yl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (36–39)

The intermediate **32a** (0.5 mmol) was dissolved in 2 mL of 2-methoxyethanol and 1 mL of the appropriate amine was added to the solution. The mixture was heated in a steel bomb at 100 °C for 3 h. The solvent was removed under reduced pressure and the residue was purified via column chromatography eluting with a mixture petroleum ether/EtOAc 1:4.

4.1.11.1. *N*-Cyclohexyl-(2-furan-2-yl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (36). White solid; 76% yield; mp 175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (m, 1H), 7.94 (s, 1H), 7.37 (d, J = 8 Hz, 1H), 7.29–7.28 (m, 1H), 6.76–6.75 (m, 1H), 4.28 (s, 3H), 4.12–3.82 (m, 1H), 1.95–1.13 (m, 10H). MS (ESI): $[MH]^+$ = 338.4. Anal. ($C_{17}H_{19}N_7O$) C, H, N.

4.1.11.2. (2-Furan-2-yl)-9-methyl-5-morpholin-4-yl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (37). White solid; 68% yield; mp 193 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 8.08 (m, 1H), 7.14–7.13 (m, 1H), 6.83–6.82 (m, 1H), 4.38 (s, 3H), 3.33 (m, 4H), 2.98 (m, 4H). MS (ESI): $[MH]^+$ = 326.3. Anal. ($C_{15}H_{15}N_7O_2$) C, H, N.

4.1.11.3. (2-Furan-2-yl)-9-methyl-5-(4-methylpiperazin-1-yl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (38). White solid; 73% yield; mp 159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 8.05–8.04 (m, 1H), 7.11–7.10 (m, 1H), 6.81–6.80 (m, 1H), 4.37 (s, 3H), 3.33 (m, 4H), 3.01 (m, 4H), 2.10 (s, 3H). MS (ESI): $[MH]^+$ = 339.4. Anal. ($C_{16}H_{18}N_8O$) C, H, N.

4.1.11.4. (2-Furan-2-yl)-9-methyl-5-(4-phenylpiperazin-1-yl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (39). White solid; 67% yield; mp 105 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H),8.03–8.02 (m, 1H), 7.23–7.19 (m, 2H), 7.15–7.14 (m, 1H), 6.91–6.89 (m, 2H), 6.80–6.77 (m, 2H), 4.39 (s, 3H), 3.58 (m, 4H), 3.15 (m, 4H). MS (ESI): $[MH]^+$ = 401.4. Anal. ($C_{21}H_{20}N_8O$) C, H, N.

4.1.12. General procedure for preparation of 5-{[(4-methoxy-phenyl)carbamoyl]amino}-8-alkyl-(2-furan-2-yl)-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (9a-d) and 5-{[(4-methoxy-phenyl)carbamoyl]amino}-8/9-alkyl-(2-furan-2-yl)-8/9*H*-pyrazolo [3,4 *e*][1,2,4]triazolo[1,5-*c*]pyrimidine (44–47a,b)

To a solution of the amino derivatives **8a-d** or **40**–**43a**,**b** (0.27 mmol) in anhydrous THF (5 mL) was added 4-methoxy-phenyl-isocyanate (0.54 mmol). The mixture was heated at 50 °C for 18 h. The solvent was removed under reduced pressure and the residue was purified via column chromatography eluting with EtOAc. The resulting solid was further purified by crystallization from CH_3OH.

4.1.12.1. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (9a). White solid; 37% yield; mp 215 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.68 (br s, 1H), 9.18 (br s, 1H), 7.96–7.95 (m, 1H), 7.74 (d, J = 2 Hz, 1H), 7.52 (dd, J = 9.2 Hz, 2H), 7.46 (s, 1H), 7.26 (d, J = 3.2 Hz, 1H), 6.94 (dd, J = 9 Hz, 2H), 6.75–6.73 (m, 1H), 3.95 (s, 3H), 3.76 (s, 3H). MS (ESI): $[MH]^+$ = 404.3. Anal. ($C_{19}H_{17}N_7O_3$) C, H, N.

4.1.12.2. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-propyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (9b). White solid; 40% yield; mp 198 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.72 (br s, 1H), 9.16 (br s, 1H), 7.97–7.96 (m, 1H), 7.82 (m, 1H), 7.55–7.50 (m, 3H), 7.26 (d, J = 3.4 Hz, 1H), 6.95 (d, J = 9.2 Hz, 2H), 6.76–6.73 (m, 1H), 4.18 (t, J = 7.4 Hz, 2H), 3.75 (s, 3H), 1.89–1.85 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 432.1. Anal. ($C_{22}H_{21}N_7O_3$) C, H, N.

4.1.12.3. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-phenylethyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (9c). White solid; 35% yield; mp 182 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.66 (br s, 1H), 9.14 (br s, 1H), 7.96 (s, 1H), 7.74 (d, J = 2 Hz, 1H), 7.54 (d, J = 2 Hz, 1H), 7.51 (dd, J = 9 Hz, 2H), 7.28–7.21 (m, 6H), 6.95 (dd, J = 9 Hz, 2H), 6.76–6.73 (m, 1H), 4.49 (t, J = 7.2 Hz, 2H), 3.76 (s, 3H), 3.20 (t, J = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 494.2. Anal. ($C_{27}H_{23}N_7O_3$) C, H, N.

4.1.12.4. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-phenylpropyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (9d). White solid; 38% yield; mp 176 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.39 (br s, 1H), 9.68 (br s, 1H), 8.26 (s, 1H), 8.03–8.02 (m, 1H), 7.52 (dd, J =9 Hz, 2H), 7.33–7.31 (m, 1H), 7.22–7.19 (m, 6H), 6.95 (dd, J = 9 Hz, 2H), 6.81–6.78 (m, 1H), 4.72 (t, J = 7 Hz, 2H), 3.75 (s, 3H), 2.62 (t, J = 7.2 Hz, 2H), 2.39–2.21 (m, 2H). MS (ESI): $[MH]^+$ = 508.3. Anal. ($C_{28}H_{25}N_7O_3$) C, H, N.

4.1.12.5. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5*c*]pyrimidine (44a). White solid; 55% yield; mp 223 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (br s, 1H), 9.62 (br s, 1H), 8.24 (s, 1H), 8.03–8.02 (m, 1H), 7.51 (dd, J = 8.8 Hz, 2H), 7.37 (d, J = 3.2 Hz, 1H), 6.95 (dd, J = 8.8 Hz, 2H), 6.78–6.78 (m, 1H), 4.36 (s, 3H), 3.75 (s, 3H). MS (ESI): $[MH]^+$ = 405.0. Anal. ($C_{19}H_{16}N_8O_3$) C, H, N.

4.1.12.6. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-methyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (44b). White solid; 61% yield; mp 254 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (br s, 1H), 9.50 (br s, 1H), 8.56 (s, 1H), 8.01–8.00 (m, 1H), 7.50 (dd, J = 9.2 Hz, 2H), 7.34 (d, J = 2.8 Hz, 1H), 6.95 (dd, J = 9.2 Hz, 2H), 6.78–6.77 (m, 1H), 4.20 (s, 3H), 3.75 (s, 3H). MS (ESI): $[MH]^+$ = 405.0. Anal. ($C_{19}H_{16}N_8O_3$) C, H, N.

4.1.12.7. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-9-propyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (45a). White solid; 50% yield; mp 202 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.37 (br s, 1H), 9.61 (br s, 1H), 8.26 (s, 1H), 8.02 (d, J =1 Hz, 1H), 7.52 (dd, J = 8.8 Hz, 2H), 7.36 (d, J = 2.6 Hz, 1H), 6.95 (dd, J = 9 Hz, 2H), 6.78–6.77 (m, 1H), 4.66 (t, J = 6.6 Hz, 2H), 3.75 (s, 3H), 2.08–1.99 (m, 2H), 0.87 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 433.0. Anal. ($C_{21}H_{20}N_8O_3$) C, H, N.

4.1.12.8. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-propyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (45b). White solid; 40% yield; mp 238 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.43 (br s, 1H), 9.58 (br s, 1H), 8.60 (s, 1H), 8.01 (s, 1H), 7.51 (dd, J = 8.8 Hz, 2H), 7.33 (d, J = 3.4 Hz, 1H), 6.95 (dd, J = 9 Hz, 2H), 6.79–6.76 (m, 1H), 4.41 (t, J = 6.8 Hz, 2H), 3.75 (s, 3H), 1.98–1.94 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 433.1. Anal. ($C_{21}H_{20}N_8O_3$) C, H, N.

4.1.12.9. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-9-(2-phenylethyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (46a). White solid; 31% yield; mp 199 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (br s, 1H), 9.55 (br s, 1H), 8.23 (s, 1H), 8.04 (s, 1H), 7.51 (dd, J = 8.8 Hz, 2H), 7.37 (d, J = 3.6 Hz, 1H), 7.24–7.23 (m, 5H), 6.95 (dd, J = 8.8 Hz, 2H), 6.80 (d, J = 1.2 Hz, 1H), 4.92 (t, J = 7 Hz, 2H), 3.75 (s, 3H), 3.34–3.27 (m, 2H). MS (ESI): $[MH]^+$ = 495.5. Anal. ($C_{26}H_{22}N_8O_3$) C, H, N.

4.1.12.10. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-(2-phenylethyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*] pyrimidine (46b). White solid; 30% yield; mp 227 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (br s, 1H), 9.51 (br s, 1H), 8.41 (s, 1H), 8.03 (s, 1H), 7.51 (dd, J = 8.8 Hz, 2H), 7.34 (d, J = 3.6 Hz, 1H), 7.22–7.18 (m, 5H), 6.95 (dd, J = 8.8 Hz, 2H), 6.78 (d, J = 1.2 Hz, 1H), 4.73 (t, J = 7 Hz, 2H), 3.75 (s, 3H), 3.33–3.28 (m, 2H). MS (ESI): $[MH]^+$ = 495.5. Anal. ($C_{26}H_{22}N_8O_3$) C, H, N.

4.1.12.11. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-9-(3-phenylpropyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*] pyrimidine (47a). White solid; 32% yield; mp 206 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.39 (br s, 1H), 9.68 (br s, 1H), 8.26 (s, 1H), 8.03–8.02 (m, 1H), 7.52 (dd, J =9 Hz, 2H), 7.33–7.31 (m, 1H), 7.22–7.19 (m, 5H), 6.95 (dd, J = 9 Hz, 2H), 6.81–6.78 (m, 1H), 4.72 (t, J = 7 Hz, 2H), 3.75 (s, 3H), 2.62 (t, J = 7.2 Hz, 2H), 2.39–2.21 (m, 2H). MS (ESI): $[MH]^+$ = 509.6. Anal. ($C_{27}H_{24}N_8O_3$) C, H, N.

4.1.12.12. 5-{[(4-Methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-(3-phenylpropyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*] pyrimidine (47b). White solid; 35% yield; mp 236 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.41 (br s, 1H), 9.63 (br s, 1H), 8.52 (s, 1H), 8.01(m, 1H), 7.52 (dd, J = 9 Hz, 2H), 7.30–7.29 (m, 1H), 7.22–7.18 (m, 5H), 6.95 (dd, J = 9 Hz, 2H), 6.80 (m, 1H), 4.71 (t, J = 7 Hz, 2H), 3.75 (s, 3H), 2.62 (t, J = 7.2 Hz, 2H), 2.37–2.20 (m, 2H). MS (ESI): $[MH]^+$ = 509.6. Anal. ($C_{27}H_{24}N_8O_3$) C, H, N.

4.1.13. 5-{[(4-Pyridinyl)-carbamoyl]amino}-2-(2-furyl)-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (10)

The compound was prepared as previously reported.[27] White solid; 45% yield; mp 206 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.8 (br s, 1H), 9.69 (br s, 1H),8.45–8.44 (m, 2H), 7.96–7.95 (m, 1H), 7.75 (m, 1H), 7.60–7.58 (m, 2H), 7.48 (m, 1H), 7.25–7.24 (m, 1H), 6.75–6.73 (m, 1H), 3.96 (s, 3H). MS (ESI): $[MH]^+$ = 375.4. Anal. ($C_{18}H_{14}N_8O_2$) C, H, N.

4.2. Biology experiments

4.2.1. CHO membrane preparation

The human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors have been transfected in CHO cells according to the method previously described.[34] The cells were grown adherently and maintained in Dulbecco's modified Eagles medium with nutrient mixture F12 (DMEM/F12) without nucleosides, containing 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 μg/mL), L-glutamine (2 mM) and Geneticin (G418, 0.2 mg/mL) at 37 °C in 5% CO_2/95% air. For membrane preparations, the culture medium was removed and the cells were washed with PBS and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with a Polytron and the homogenate was spun for 10 min at 1000*g*. The supernatant was then centrifuged for 30 min at 100,000*g*. The membrane pellet was suspended in: (a) 50 mM Tris HCl buffer pH 7.4 for A_1 adenosine receptors; (b) 50 mM Tris HCl, 10 mM $MgCl_2$ buffer pH 7.4 for A_{2A} adenosine receptors; (c) 50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA buffer pH 7.4 for A_3 adenosine receptors. The cell suspensions were incubated with 2 IU/mL of adenosine deaminase for 30 min at 37 °C. The membrane preparation was used to perform competition binding experiments.

4.2.2. Human cloned A_1, A_{2A} and A_3 adenosine receptor binding assay

All synthesized compounds have been tested for their affinity at human A_1, A_{2A} and A_3 adenosine receptors. Displacement binding experiments of [^{3}H]-DPCPX (1 nM) to hA_1CHO membranes (50 μg of protein/assay) and at least 6–8 different concentrations of the examined compounds were performed for 120 min at 25 °C. Non-specific binding was determined in the presence of 1 M of DPCPX and this was always $\leqslant$10% of the total binding.[35]

Displacement binding experiments of [^{3}H]-ZM 241385 (2 nM) to hA_{2A}CHO membranes (50 μg of protein/assay) and at least 6–8 different concentrations of the tested ligands were performed for 60 min at 4 °C. Non-specific binding was determined in the presence of 1 μM ZM 241385 and was about 20% of total binding.[36]

Displacement binding experiments of [^{3}H]-MRE-3008-F20 (1 nM) to hA_3CHO membranes (50 μg of protein/assay) and at least 6–8 different concentrations of examined ligands were performed for 120 min at 4 °C. Non-specific binding was defined as binding in the presence of 1 μM MRE-3008-F20 and was about 25% of total binding.[23]

Bound and free radioactivities were separated by filtering the assay mixture through Whatman GF/B glass fibre which was washed three times with ice-cold buffer. Filter bound radioactivity was measured by scintillation spectrometry (Packard 2500-TR) after addition of Aquassure liquid.

4.2.3. Measurement of cyclic AMP levels in CHO cells transfected with human A_{2B} and A_3 adenosine receptors

CHO cells transfected with human A_{2B} or A_3 adenosine receptors were washed with phosphate-buffered saline, diluted trypsin, and centrifuged for 10 min at 200*g*. The pellet containing CHO cells (1×10^6 cells/assay) was suspended in 0.5 mL of incubation mixture (mM): NaCl 15, KCl 0.27, NaH_2PO_4 0.037, $MgSO_4$ 0.1, $CaCl_2$ 0.1, Hepes 0.01, $MgCl_2$ 1, glucose 0.5, pH 7.4 at 37 °C, 2 IU/mL adenosine deaminase and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as phosphodiesterase inhibitor and preincubated for 10 min in a shaking bath at 37 °C. The potencies of novel compounds at hA_{2B} adenosine receptors were determined by antagonism of NECA (100 nM)-induced stimulation of cyclic AMP levels. The potencies of the examined ligands to hA_3 adenosine receptors were determined in the presence of Forskolin (1 μM) by antagonism of the Cl-IB-MECA (100 nM)-induced inhibition of cyclic AMP levels.[37] The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000*g* for 10 min at 4 °C and the supernatant was extracted four times with water saturated diethyl ether. The final aqueous solution was tested for cyclic AMP levels by a competition protein binding assay. Samples of cyclic AMP standard (0–10 pmol) were added to each test tube containing the incubation buffer (trizma base 0.1 M, aminophylline 8.0 mM, 2 mercaptoethanol 6.0 mM, pH 7.4) and [^{3}H] cyclic AMP in a total volume of 0.5 mL. The binding protein previously prepared from beef adrenals, was added to the samples previously incubated at 4 °C for 150 min, and after the addition of charcoal were centrifuged at 2000*g* for 10 min. The clear supernatant was counted in a 2500-TR Packard scintillation counter.

4.2.4. Data analysis

The protein concentration was determined according to a Bio-Rad method[38] with bovine albumin as a standard reference. Inhibitory binding constants, K_i values, were calculated from those of IC_{50} according to the Cheng and Prusoff equation[39] $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where [C*] is the concentration of the radioligand and K_D^* its dissociation constant. A weighted non-linear least-squares curve fitting program LIGAND[40] was also used for computer analysis of inhibition experiments. Potency values (IC_{50}) obtained in cyclic AMP assays were calculated by non-linear regression analysis using the equation for a sigmoid concentration-response curve (Graph PAD Prism, San Diego, CA, USA). Affinity or potency values are expressed as geometric mean, with 95% or 99% confidence limits.

Acknowledgments

We thank King Pharmaceuticals, Inc., Research & Development, 4000 Centre Green Way, Suite 300, Cary, NC 27513, USA, for financial support. We also thank Professor Karl-Norbert Klotz for cDNA encoding the human adenosine receptors.

References and notes

1. Fredholm, B. B.; Arslan, G.; Halldner, L.; Kull, B.; Shulte, G.; Wasserman, W. *Naunyn–Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 364.
2. Murphree, L. J.; Linden, J. *Encyclopaedia Biol. Chem.* **2004**, *1*, 34.
3. Jacobson, K. A.; Knutsen, L. J. S. *Handbook Exp. Pharmacol.* **2001**, *151*, 129.
4. Nadeem, A.; Obiefuna, P. C.; Wilson, C. N.; Mustafa, S. J. *Eur. J. Pharmacol.* **2006**, *551*, 116.
5. Fuxe, K.; Ferre, S.; Canals, M.; Torvinen, M.; Terasmaa, A.; Marcellino, D.; Goldberg, S. R.; Staines, W.; Jacobsen, K. X.; Lluis, C.; Woods, A. S.; Agnati, L. F.; Franco, R. *J. Mol. Neurosci.* **2005**, *26*, 209.
6. Hodgson, R. A.; Bedard, P. J.; Varty, G. B.; Kazdoba, T. M.; Di Paolo, T.; Grzelak, M. E.; Pond, A. J.; Hadjtahar, A.; Belanger, N.; Gregoire, L.; Dare, A.; Neustadt, B. R.; Stamford, A. W.; Hunter, J. C. *Exp. Neurol.* **2010**, *225*, 384.
7. Baraldi, P. G.; Tabrizi, M. A.; Gessi, S.; Borea, P. A. *Chem. Rev.* **2008**, *108*, 238.
8. Stone, T. W. *Adv. Exp. Med. Biol.* **2002**, *513*, 249.
9. Beaven, M. A.; Ramkumar, V.; Hydar, A. *Trends Pharmacol. Sci.* **1994**, *15*, 13.
10. Ramkumar, V.; Stiles, G. L.; Beaven, M. A.; Ali, H. *J. Biol. Chem.* **1993**, *268*, 16887.
11. Akkari, R.; Burbiel, J. C.; Hockemeyer, J.; Mueller, C. E. *Curr. Top. Med. Chem.* **2006**, *6*, 1375.
12. Von Lubitz, D. K. J. E.; Carter, M. F.; Deutsch, S. I.; Lin, R. C. S.; Mastropaola, J.; Meshulam, Y.; Jacobson, K. A. *Eur. J. Pharmacol.* **1995**, *275*, 23.
13. Nieber, K.; Lewerenz, A.; Hentschel, S.; Vissiennon, Z. *Bioforum.* **2002**, *25*, 237.
14. Forsythe, P.; Ennis, M. *Inflamm. Res.* **1999**, *48*, 301.
15. Brown, R. A.; Spina, D.; Page, C. P. *Br. J. Pharmacol.* **2008**, *153*, S446.

16. Brambilla, R.; Cattabeni, F.; Ceruti, S.; Barbieri, D. *Naunyn- Schmiedeberg's Arch. Pharmacol.* **2000**, *361*, 225.
17. Jacobson, K. A.; Moro, S.; Kim, Y. C.; Li, A. *Drug Dev. Res.* **1998**, *45*, 113.
18. Abbracchio, M. P.; Ceruti, S.; Brambilla, R.; Barbiri, D.; Camurri, A.; Franceschi, C.; Giammaroli, A. M.; Jacobson, K. A.; Cattabeni, F.; Malorni, W. *Drug Dev. Res.* **1998**, *45*, 379.
19. Baraldi, P. G.; Tabrizi, M. A.; Romagnoli, R.; Fruttarolo, F.; Merighi, S.; Varani, K.; Gessi, S.; Borea, P. A. *Curr. Med. Chem.* **2005**, *12*, 1319.
20. Merighi, S.; Mirandola, P.; Varani, K.; Gessi, S.; Capitani, S.; Leung, E.; Baraldi, P. G.; Tabrizi, M. A.; Borea, P. A. *Biochem. Pharmacol.* **2003**, *66*, 739.
21. Baraldi, P. G.; Tabrizi, M. A.; Romagnoli, R.; El-Kashef, H.; Preti, D.; Bovero, A.; Fruttarolo, F.; Gordaliza, M.; Borea, P. A. *Curr. Org. Chem.* **2006**, *10*, 259.
22. Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Monopoli, A.; Ongini, E.; Varani, K.; Borea, P. A. *J. Med. Chem.* **2002**, *45*, 115.
23. Varani, K.; Merighi, S.; Gessi, S.; Klotz, K. N.; Leung, E.; Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Borea, P. A. *Mol. Pharmacol.* **2000**, *57*, 968.
24. Baraldi, P. G.; Fruttarolo, F.; Tabrizi, M. A.; Preti, D.; Romagnoli, R.; El-Kashef, H.; Moorman, A.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. *J. Med. Chem.* **2003**, *46*, 1229.
25. Moorman, A. R. US 7723343B2, 2010.
26. Zimmerman, M. N.; Nemeroff, N. H.; Bock, C. W.; Bhat, K. L. *Heterocycles* **2000**, *53*, 205.
27. Maconi, A.; Pastorin, G.; Da Ros, T.; Spalluto, G.; Gao, Z. G.; Jacobson, K. A.; Baraldi, P. G.; Cacciari, B.; Varani, K.; Moro, S.; Borea, P. A. *J. Med. Chem.* **2002**, *45*, 3579.
28. Seneci, P.; Nicola, M.; Inglesi, M.; Vanotti, E.; Resnati, G. *Synth. Commun.* **1999**, *29*, 311.
29. Baraldi, P. G.; Tabrizi, M. A.; Fruttarolo, F.; Bovero, A.; Avitabile, B.; Preti, D.; Romagnoli, R.; Merighi, S.; Gessi, S.; Varani, K.; Borea, P. A. *Drug Dev. Res.* **2003**, *58*, 315.
30. Cheong, S. L.; Federico, S.; Venkatesan, G.; Paira, P.; Shao, Y.-M.; Spalluto, G.; Yap, C. W.; Pastorin, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2898.
31. Pran Kishore, D.; Balakumar, C.; Raghuram Rao, A.; Roy, P. P.; Roy, K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 818.
32. Schenone, S.; Brullo, C.; Musumeci, F.; Bruno, O.; Botta, M. *Curr. Top. Med. Chem.* **2010**, *10*, 878.
33. Baraldi, P. G.; Borea, P. A. *Trends Pharm. Sci.* **2000**, *21*, 456.
34. Klotz, K. N.; Hessling, J.; Hegler, J.; Owman, C.; Kull, B.; Fredholm, B. B.; Lohse, M. J. *Naunyn-Schmied. Arch. Pharm.* **1998**, *357*, 1.
35. Borea, P. A.; Dalpiaz, A.; Varani, K.; Gilli, G. *Life Sci.* **1996**, *59*, 1373.
36. Borea, P. A.; Dalpiaz, A.; Varani, K.; Gessi, S.; Gilli, G. *Biochem. Pharmacol.* **1995**, *49*, 461.
37. Varani, K.; Gessi, S.; Merighi, S.; Vincenzi, F.; Cattabriga, E.; Benini, A.; Klotz, K. N.; Baraldi, P. G.; Tabrizi, M. A.; Mac Lennan, S.; Leung, E.; Borea, P. A. *Biochem. Pharmacol.* **2005**, *70*, 1601.
38. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
39. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
40. Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.