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New Strategies for the Synthesis of A_3 Adenosine Receptor Antagonists

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Abstract—New A_3 adenosine receptor antagonists were synthesized and tested at human adenosine receptor subtypes. An advanced synthetic strategy permitted us to obtain a large amount of the key intermediate **5** that was then submitted to alkylation procedures in order to obtain the derivatives **6**–**8**. These compounds were then functionalised into ureas at the 5-position (compounds **9**–**11**, **18** and **19**) to evaluate their affinity and selectivity versus hA_3 adenosine receptor subtype; in particular, compounds **18** and **19** displayed a value of affinity of 4.9 and 1.3 nM, respectively. Starting from **5**, the synthetic methodologies employed permitted us to perform a rapid and a convenient divergent synthesis. A further improvement allowed the regioselective preparation of the N^8-substituted compound **7**. This method could be used as an helpful general procedure for the design of novel A_3 adenosine receptor antagonists without the difficulty of separating the N^8-substituted pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidines from the corresponding N^7-isomers.

Introduction

Adenosine, an endogenous modulator of a wide range of biological functions in the nervous, cardiovascular,[1] renal, and immune systems, interacts with at least four cell surface receptor subtypes classified as A_1, A_{2A}, A_{2B} and A_3. These receptor subtypes belong to the superfamily of G protein-coupled receptors and have been cloned from several animal species.[2] In particular, the A_3 adenosine receptor subtype, which is distributed in different organs (lung, liver, heart, kidney, and, in low density, in the brain)[3] exerts its action through the modulation of two second messengers systems: stimulation of phospholipases C[4] and D[5] and inhibition of adenylate cyclase.[6] The potential therapeutic applications of activating or antagonizing this receptor subtype have been investigated in recent years and in particular, antagonists for A_3 receptor promise to be useful for the treatment of inflammation and in regulation of cell growth.[7,8]

Recently, our research group reported a large series of pyrazolo-triazolo-pyrimidines bearing substituted phenylcarbamoyl residues at the amino group at the 5-position (Chart 1, compound **a**) as highly potent and selective antagonists of the human A_3 adenosine receptor.[9,10] Among these, Compound **b** (5-[(phenyl)amino]-carbonyl]amino-8-methyl-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidine (Chart 1) showed highly favorable binding affinity and selectivity for the human A_3 adenosine receptor.[11]

Unfortunately, the major problem within this class of compounds is the typical low water solubility that has limited the in vivo pharmacological screening. Starting from these experimental observations, we decided to introduce oxygenated functions like β-hydroxyethyl, acetic, and diethyloxyethyl groups at the 8-position of the pyrazole nitrogen (Fig. 1) with the aim of increasing the water solubility of the final compounds obtained

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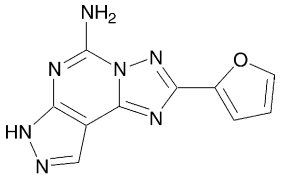
(**10**, **11** and **19**). As in the typical A_3-antagonist structures previously synthesized by our group, the 5-position of the new N^8-substituted-pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine derivatives was functionalized as 3-chlorophenyl or simply phenyl ureas in order to evaluate their affinity and selectivity versus the human A_3 adenosine receptor subtype.

These new derivatives were obtained by direct alkylation of the 2-furan-2-yl-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl amine (**5**) with the appropriate alkyl halides. The tricyclic starting compound **5** was synthesized using an expedited synthetic methodology[12] that permitted us to obtain this important key intermediate in a very good yield. In the past the same intermediate was obtained by a tedious de-*tert*-butylation procedure, performed by the treatment of 2-furan-2-yl-7-*tert*-butyl-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidin-5-yl-amine with 99% formic acid for 48 h.[10]



5

The new synthetic steps employed allowed us to acquire a large amount of **5** in a very short time and the intermediates obtained did not require tedious purifications procedures. However, the alkylation of compound **5** furnished a mixture of the N^7- and N^8-alkylated regioisomers, not easily separated by chromatography. In this paper, we also described a multistep regioselective strategy, reported in the chemistry section, to prepare the N^8-substituted derivative **7** in good yield, devoid of the corresponding N^7-isomer. This synthetic route provides only substituted 3-amino-4-cyano-pyrazoles that are then utilized to obtain tricyclic pyrazolo-triazolo-pyrimidine derivatives functionalized at the 8-position of the pyrazole nitrogen.

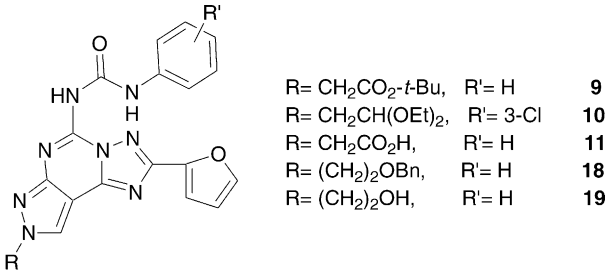


Figure 1. New A_3 adenosine receptor antagonists synthesized.

Chemistry

Synthesis of **5** has been performed using a new synthetic strategy described in the patent literature and depicted in Scheme 1.[12] Starting from diethyl malonate and guanidine hydrochloride, both commercially available, condensation in basic conditions gave the intermediate 2-amino-4,6-dihydroxypyrimidine **1**.[13] The corresponding transformation into **2** was achieved in good yield by treatment with $POCl_3$ and DMF (Vilsmeier's reaction)[14] at refluxing temperature. Compound **2** was then reacted with 2-furoic acid hydrazide. Compound **3** was then cyclized by treatment with hydrazine in ethanol to furnish **4**. Treatment of **4** with DMF, HMDS and BSA at 220 °C (Dimroth-type rearrangement) induced the formation of compound **5** in good yield which was subsequently utilized for alkylation reactions.

The intermediate **5** was functionalised as reported in Scheme 2. Alkylation of **5** with bromoacetaldeyde diethylacetal, 2-iodoethanol and 2-bromoacetic acid *tert*-butyl ester in DMF and 60% NaH gave compounds **6**–**8** which are separated from the N^7-isomers by flash chromatography.

The free amino group at the 5-position of compounds **6** and **8** was converted into the corresponding ureas (**9**–**10**) by treatment with phenylisocyanate or 3-chlorophenylisocyanate and catalytic amount of TEA (Scheme 3). The *tert*-butyl ester at the 7-position of **9** was

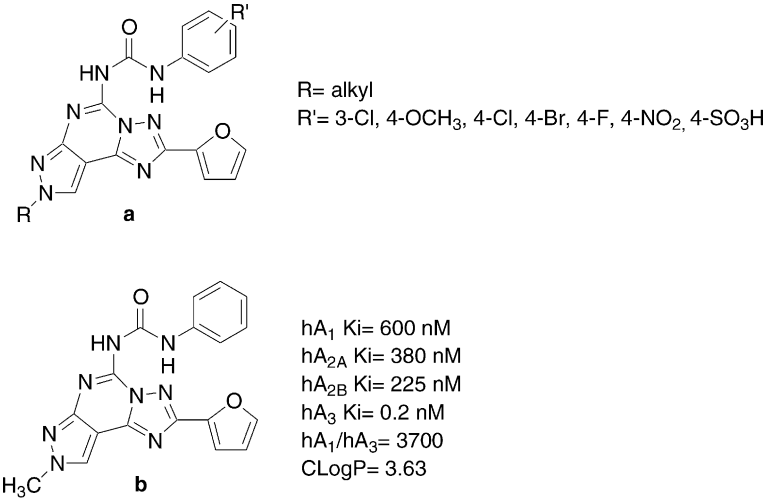
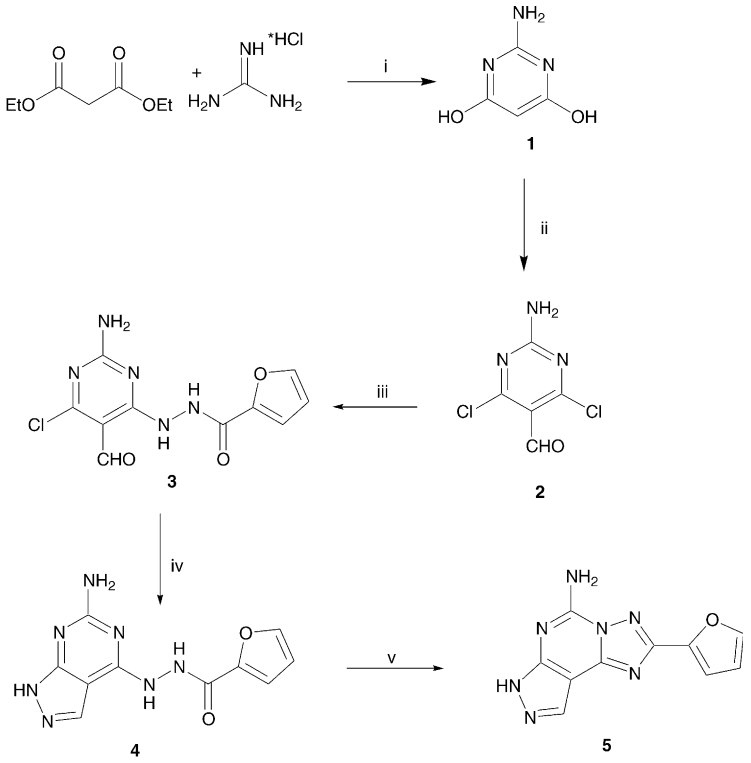


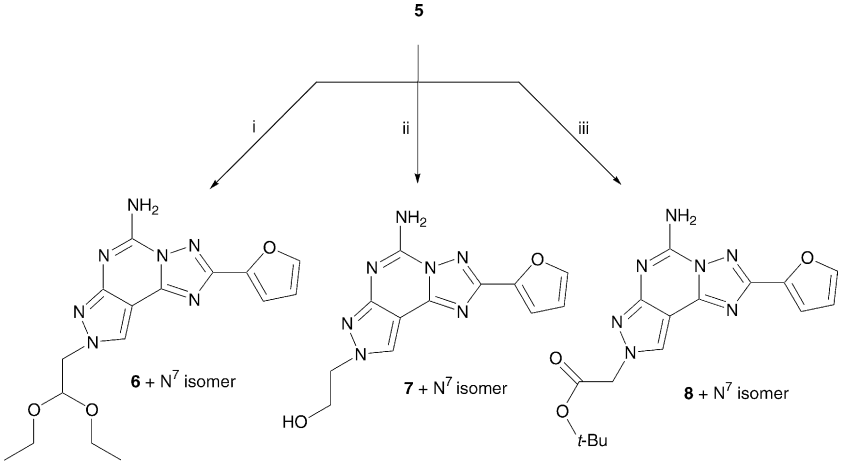
Chart 1. Structure of the pyrazolo-triazolo-pyrimidines **a** and **b** as hA_3 adenosine receptor antagonists.

removed by treatment with TFA at reflux temperature. The free acid function was conceived to increase the water solubility of the final compound **11**.

For the synthesis of compound **7**, was utilized a regioselective synthetic procedure that permitted the preparation only of the N^8-isomer as depicted in Scheme 4. The reaction between benzaldehyde and 2-hydroxyethylhydrazine, commercially available, gave the corresponding hydrazone **12**. Subsequent reaction with ethoxymethylenemalononitrile and hydrolysis with concentrated HCl provided only **13** in good yield. The free hydroxyl group was then protected by treatment with benzylbromide and the free amino group of the compound **14** was transformed into the imidate **15** by refluxing in $HC(OEt)_3$. The pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine **17** was obtained using the synthetic steps already employed for the synthesis of the tricyclic compounds and reported in our previous papers.[9,10] The imidate **15** was reacted with 2-furoic acid hydrazide in refluxing 2-methoxyethanol to provide the pyrazolo[4,3-*e*]pyrimidine intermediate, following the



Scheme 1. Reagents: (i) EtONa, EtOH abs; (ii) $POCl_3$, DMF, Rfx; (iii) 2-furoic acid hydrazide, THF; (iv) hydrazine·H_2O, 2-methoxyethanol; (v) HMDS, BSA, DMF.



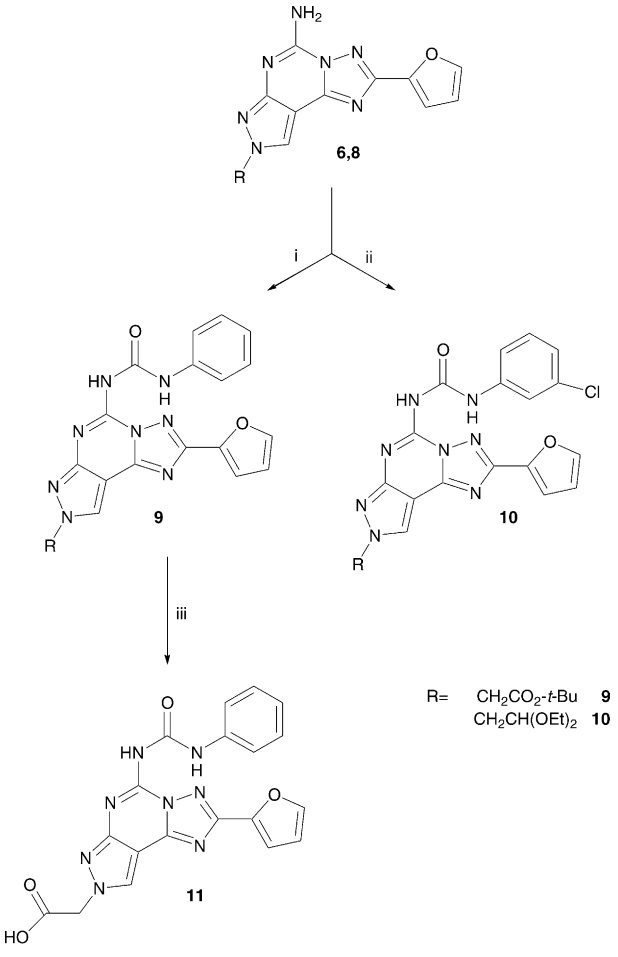
Scheme 2. Reagents: (i) bromacetaldehyde diethylacetal, NaH, 80 °C; (ii) iodoethanol, 60% NaH, rt; (iii) 2-bromoacetic acid *t*-butylester, 60% NaH, 80 °C.

method of Gatta et al.[15] The latter compound was converted through a thermally-induced cyclization in diphenylether to the tricyclic derivative **16** in good yield. Treatment of **16** with dilute hydrochloric acid induced pyrimidine ring opening. The intermediate was then converted into **17** by treatment with an excess of cyanamide in 1-methyl-2-pyrrolidone at 160 °C. The urea derivative **19** was obtained by treatment of **17** with phenyl isocyanate to afford the *O*-protected **18**. The final deprotection of the hydroxylic group provided **19** in a good yield. The *O*-debenzylation at the 8-position, necessary to reduce the reactivity of the hydroxylic group in the side chain, was achieved by treatment with HCO_2NH_4 and 10% Pd/C to afford **7**.

Results and Discussion

The biological results of the synthesized compounds at human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors, expressed as affinity values (K_i), are shown in Table 1.

Alkylation of the key intemediate **5** furnished compounds **6–8** that were also tested in binding experiments, with particular regard to the affinity value versus the A_3 adenosine subtype. We can note that these compounds, displaying a free amino group at the 5-position of the pyrimidine ring, show poor affinity and low selectivity only versus the A_{2A} adenosine receptor subtype. The transformation of the amino function into urea leads to a significant increase of the A_3 interaction and a drastic decrease in A_{2A} interaction (compounds **9–11**). This behavior confirms that functionalization of the free amino group in addition to an alkyl or arylalkyl substituent at the 8-position of the pyrazole nitrogen is necessary for strong interaction with the A_3 adenosine receptor.



Scheme 3. Reagents: (i) phenylisocyanate, TEA, dry CH_3CN; (ii) 3-chlorophenylisocyanate, TEA, dry CH_3CN; (iii) TFA, rt.

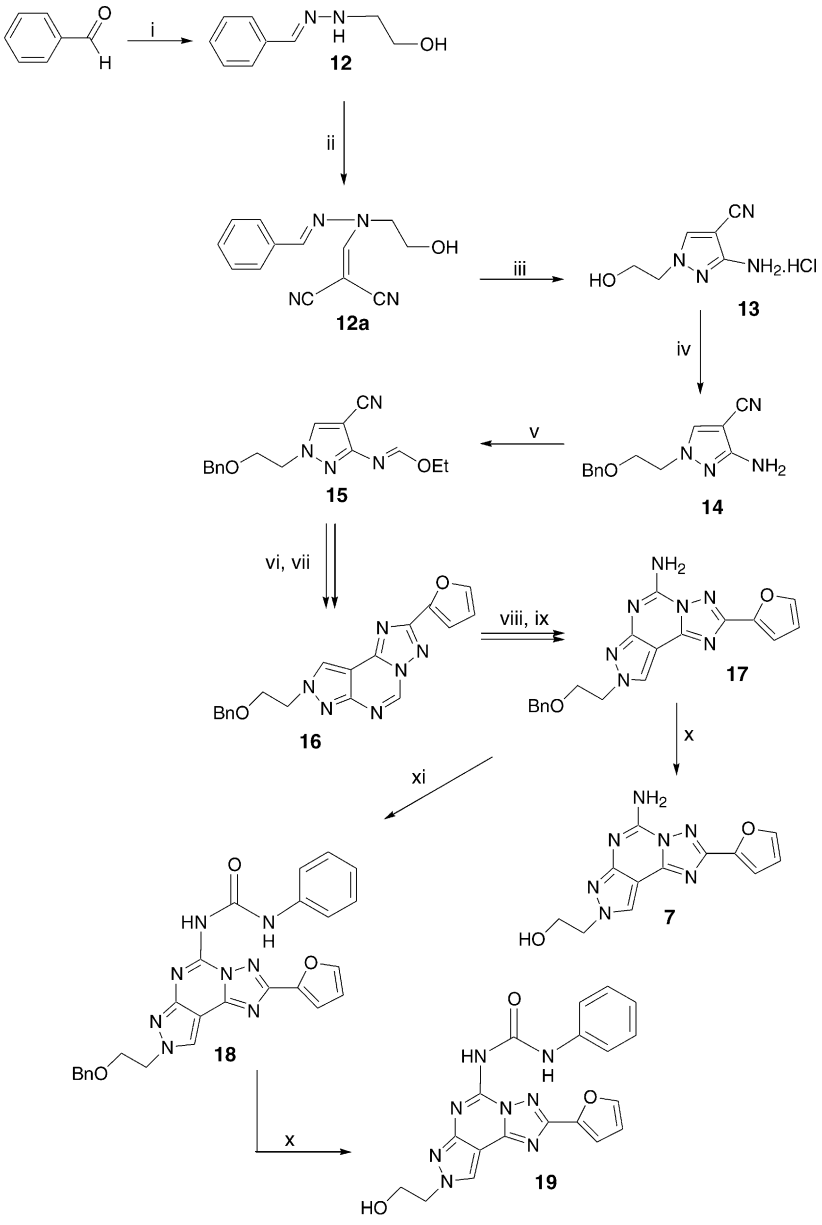
The best results in terms of affinity and selectivity versus the human A_3 adenosine receptor subtype are shown by compound **19,** that display an hydroxyethyl group at the 8-position of the pyrazole nitrogen and a phenylureido function at the 5-position. The corresponding *O*-protected compound **18** also has good affinity versus the hA_3 receptor, but less than the deprotected analogue, probably due to the steric hindrance introduced by the benzyl group; however, it's important to highlight that the presence of this protecting group is favorable for the interaction with the A_1 adenosine receptor subtype (compounds **17** and **18**, K_i $A_1 = 15$ and 32 nM, respectively).

The free carboxylic acid function of **11** was designed to improve the water solubility of the compound by the transformation of the carboxylic group into a salt; the selectivity of this molecule is very good but the affinity versus the A_3 receptor subtype is quite low and less than the *tert*-butyl derivative **9**. The diethyloxyethyl group of **10** also represents a versatile functionality, with the possibility of deprotecting the aldehydic group and coupling it with amines or other nucleophiles to obtain compounds with enhanced water-solubility.

In order to evaluate the increase of the hydrophilic profile of the new derivatives obtained, the hydrophilic/lipophilic balance was determined by the calculation of the ClogP values derived from ChemDraw Ultra analyses, version 6.0.1, Cambridge Software. The ClogP values calculated for **11** and **19** are 3.23 and 2.93, respectively, versus compound **b** that displayed a lipophilic value of 3.63.

The methodology applied to obtain **5** was demonstrated to be a convenient synthetic route: the intermediates are obtained in good yield, minor synthetic steps are employed, and easy purifications are the principals advantages in contrast to the de-*tert*-butylation pathway used in the past by our research group.[10]

Starting from **5**, the synthetic pathway used for the synthesis of the compounds described in this work permitted us to prepare several molecules endowed with adenosine antagonist activity, performing a rapid and a divergent synthesis. Further, the regioselective route to achieve the N^8-substituted tricyclic derivatives, performed for the synthesis of **7**, is an useful and convenient methodology that permits the direct preparation of N^8-functionalized compounds with high affinity and selectivity versus the A_3 adenosine receptor subtype, after the transformation of the free amino group into an urea.



Scheme 4. Reagents: (i) 2-hydroxyethylhydrazine, abs EtOH; (ii) ethoxymethylene-malono nitrile, benzene; (iii) concd HCl; (iv) benzylbromide, 60% NaH; (v) $HC(OEt)_3$, rfx; (vi) 2-furoic acid hydrazide, 2-methoxyethanol; (vii) Ph_2O, 260 °C; (viii) aq HCl 10%; (ix) NH_2CN, pTsOH; (x) HCO_2NH_4, Pd/C 10%; (xi) phenylisocyanate, TEA.

Conclusion

In summary, we have reported new synthetic methodologies to obtain the key intermediate **5** in large amount and an extendible regioselective route to obtain selectively N^8-substituted pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidine-N^5-ureas. The compounds described in this work have an high affinity and selectivity toward the hA_3 adenosine receptor subtype displaying hydrophilic functionalities, an important feature to improved the pharmacological profile and to make in vivo pharmacological screening easier.

Experimental

Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{245} Merck plates) and products visualized with iodine or potassium permanganate solution. ^1H NMR were determined in $CDCl_3$ or DMSO-d_6 solutions with a Bruker AC 200 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and *J* values are given in Hz. Light petroleum ether refers to the fractions boiling at 40–60 °C. Melting points were determined on a Buchi-Tottoli instrument

and are uncorrected. Chromatographies were performed using Merck 60–200 mesh silica gel. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous magnesium sulphate. Elemental analyses were performed by the micro-analytical laboratory of Dipartimento di Chimica, University of Ferrara, and were within ±0.4% of the theoretical values for C, H, and N. Elemental analyses are reported in Table 2.

Furan-2-carboxylic acid *N*-(2-amino-6-chloro-5-formyl-pyrimidin-4-yl)-hydrazide (3)

To a suspension of **2** (1 g, 5.2 mmol) in dry THF (40 mL) was added 2-furoic acid hydrazide (730 mg, 5.72 mmol) and TEA (5.2 mmol). The mixture was heated at reflux for 2 h, then the solvent was removed at reduced pressure. The residue was crystallized from a mixture of methanol and ethyl ether (8:1). The solid formed was filtered off and dried to furnish **3** as a yellow solid (4.2 mmol, 82%). Mp 130 °C; ^{1}H NMR (DMSO-d_6) δ: 6.67 (t, 1H, J = 2), 7.25 (d, 1H, J = 2.1), 7.88 (bs, 2H), 7.91 (d, 1H, J = 2), 9.95 (s, 1H), 10.36 (bs, 1H), 10.69 (bs, 1H).

Furan-2-carboxylic acid N-(6-amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)-hydrazide (4). To a solution of **3** (0.5 g, 1.7 mmol) in 2-methoxyethanol (25 mL) was added hydrazine monohydrate (0.16 mL, 3.4 mmol) and the mixture was stirred at room temperature for 1.5 h. Then the solvent was removed at reduced pressure and the residue was crystallized from a mixture of methanol and ethyl ether (8:1). The solid formed was filtered off and dried to furnish **4** as a yellow solid (1.4 mmol, 83%). Mp 272 °C; ^{1}H NMR (DMSO-d_6) δ: 6.72 (s, 1H), 7.52 (s, 1H), 7.96 (bs, 2H), 8.02 (s, 1H), 8.54 (s, 1H), 10.97 (bs, 1H), 11.38 (bs, 1H), 13.35 (bs, 1H).

2-Furan-2-yl-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl amine (5). To a suspension of **4** (0.5 g, 1.9 mmol) in DMF (12 mL) was added HMDS (4 mL, 19 mmol) and BSA (4.6 mL, 19 mmol). The mixture was heated at 230 °C for 30 min, then the solvent was removed under vacuum. The residue obtained was crystallized from methanol to furnish **5** as yellow solid (1.2 mmol, 67%). Mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ: 6.72 (t, 1H, J = 1.4), 7.20 (d, 1H, J = 1.4), 7.90 (d, 1H, J = 1.5), 7.92 (bs, 2H), 8.18 (s, 1H), 13.30 (bs, 1H).

General procedure for the preparation of compounds 6–8

To a stirred suspension of 60% NaH (1.2 mmol) in dry DMF (15 mL), chilled in an ice-bath, was added **5** (1.2 mmol) in small portions and the mixture was stirred at room temperature for several minutes. Then was added the appropriate alkyl halide (1.2 mmol) in dry DMF (2 mL) and the reaction was heated at 80 °C until the TLC analyses indicated the disappearance of the starting material (14–16 h, 1:1 EtOAc/light petroleum). The solvent was removed at reduced pressure and the residue was purified by flash chromatography (1:1 EtOAc/light petroleum) to afford **6–8** as solids, free of N^7-isomers.

6: 8-(2,2-Diethoxy-ethyl)-2-furan-2-yl-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidin-5-yl amine. White solid, yield 60%, 0.72 mmol; mp 213 °C; ^{1}H NMR (DMSO-d_6) δ: 1.04 (t, 6H, J = 7.6), 3.42 (m, 2H), 3.65 (m, 2H), 4.35 (d, 2H, J = 6), 4.92 (t, 1H, J = 7.4), 6.73 (m, 1H), 7.21 (d, 1H, J = 2), 7.65 (bs, 2H), 7.93 (s, 1H), 8.56 (bs, 1H).

7: 8-(2-Hydroxy-ethyl)-2-furan-2-yl-*8H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidin-5-yl amine. White solid, yield 51%, 0.62 mmol; mp 269 °C; ^{1}H NMR (DMSO-d_6) δ: 3.81 (m, 2H), 4.30 (t, 2H, J = 7.6), 4.90 (bs, 1H), 6.73

Table 1. Biological data of synthesized compounds 6–19

Compd	A_1 K_i (nM)[a]	A_{2A} K_i (nM)[b]	A_{2B} K_i (nM)[c]	A_3 K_i (nM)[d]
6	130 (106–158)	100 (82–120)	376 (332–426)	> 1000 (84%)
7	136 (110–167)	88 (75–104)	71 (54–94)	900 (829–976)
8	> 1000 (72%)	250 (202–301)	> 1000 (80%)	> 1000 (77%)
9	> 1000 (69%)	> 1000 (68%)	> 1000 (81%)	39 (20–80)
10	250 (208–299)	796 (717–883)	876 (831–924)	5.5 (3.2–9.4)
11	> 1000 (76%)	> 1000 (75%)	> 1000 (80%)	62 (50–77)
17	15 (12–19)	14 (7–27)	35 (27–45)	609 (547–678)
18	32 (22–46)	103 (79–134)	202 (152–267)	4.9 (3.4–7.2)
19	776 (731–824)	816 (754–884)	> 1000 (83%)	1.3 (1.0–1.6)

[a]Displacement of [^{3}H]DPCPX binding at human A_1 adenosine receptors expressed in CHO cells.
[b]Displacement of [^{3}H]SCH58261 binding at human A_{2A} adenosine receptors expressed in CHO cells.
[c]Displacement of [^{3}H]DPCPX binding at human A_{2B} adenosine receptors expressed in HEK-293 cells.
[d]Displacement of [^{3}H]MRE 3008F20 binding at human A_3 adenosine receptors expressed in CHO cells.

Table 2. Elemental analyses of the final compounds described 5–19

Compd	Formula	M_r	Calculated			Found		
			% C	% H	% N	% C	% H	% N
5	$C_{10}H_7N_7O$	241.21	49.79	2.93	40.65	49.77	2.92	40.64
6	$C_{16}H_{19}N_7O_3$	357.37	53.77	5.36	27.44	53.76	5.34	27.44
7	$C_{12}H_{11}N_7O_2$	285.26	52.35	4.06	32.87	52.33	4.05	32.86
8	$C_{16}H_{17}N_7O_3$	355.35	54.08	4.82	27.59	54.06	4.80	27.57
9	$C_{23}H_{22}N_8O_4$	474.47	58.22	4.67	23.62	58.21	4.65	23.61
10	$C_{23}H_{23}ClN_8O_4$	510.93	54.07	4.54	21.93	54.05	4.53	21.91
11	$C_{19}H_{14}N_8O_4$	418.37	54.55	3.37	26.78	54.54	3.35	26.76
17	$C_{19}H_{17}N_7O_2$	357.38	60.79	4.56	26.18	60.78	4.55	26.16
18	$C_{26}H_{22}N_8O_3$	494.51	63.15	4.48	22.66	63.13	4.46	22.65
19	$C_{19}H_{16}N_8O_3$	404.38	56.43	3.99	27.71	56.42	3.98	27.69

(t, 1H, $J=7.80$), 7.20 (d, 1H, $J=1.6$), 7.63 (bs, 2H), 7.94 (s, 1H), 8.55 (s, 1H).

8: (5-Amino-2-furan-2-yl-pyrazolo4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-8-yl)-acetic acid *tert*-butyl ester. White solid, yield 52%, 1.04 mmol, mp 194–197 °C; ^{1}H NMR (DMSO-d_6) δ: 1.44 (s, 9H), 5.13 (s, 2H), 6.73 (s, 1H), 7.22 (s, 1H), 7.70 (bs, 2H), 7.94 (s, 1H), 8.59 (s, 1H).

[2-Furan-2-yl-5(3-phenylureido)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidin-8-yl] acetic acid *tert*-butyl ester (9). To a suspension of **8** (0.56 mmol) in dry CH_3CN (10 mL) was added a catalytic amount of TEA and phenylisocyanate (0.89 mmol) and the resultant mixture was heated at 70 °C for 6–8 h. Then the solvent was removed at reduce pressure and the residue was purified by flash chromatography (EtOAc/light petroleum 1:1) to afford **9** as white solid (0.27 mmol, 51%), mp 194–197 °C; ^{1}H NMR (DMSO-d_6) δ: 1.46 (s, 9H), 5.27 (s, 2H), 6.76 (s, 1H), 7.13 (s, 1H), 7.33 (m, 3H), 7.59 (m, 2H), 7.99 (s, 1H), 8.80 (s, 1H), 9.76 (bs, 1H), 10.69 (bs, 1H).

1-(3-Chlorophenyl)-3-[8-(2,2-diethoxyethyl)-2-furan-2-yl-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]urea (10). To a suspension of **6** (0.20 mmol) in dry CH_3CN (15 mL) was added a catalytic amount of TEA and 3-chlorophenylisocyanate (1.1 mmol) and the resultant mixture was heated at 90 °C for 12 h. Then the solvent was removed at reduce pressure and the residue was purified by flash chromatography (EtOAc/light petroleum 4:6) to afford **10** as white solid (0.13 mmol, 65%), mp 187–190 °C; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, 6H, $J=6$), 3.43 (m, 2H), 3.65 (m, 2H), 4.48 (d, 2H, $J=8.1$), 4.97 (t, 1H, $J=6.1$), 6.76 (m, 1H), 7.30 (m, 4H), 7.80 (s, 1H), 8.00 (s, 1H), 8.79 (s, 1H), 9.90 (bs, 1H), 10.67 (bs, 1H).

[2-Furan-2-yl-5-(3-phenylureido)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-8-yl] acetic acid (11). A solution of **9** (70 mg, 0.15 mmol) in TFA (1.5 mL) was stirred at room temperature for 15 min. The solid formed was filtered off, washed with cold water, and recrystallized from a mixture of DMF/water (1:1) to afford **11** as white solid (0.09 mmol, 60%), mp 290 °C, ^{1}H NMR (DMSO-d_6) δ: 5.27 (s, 2H), 6.76 (s, 1H), 7.30 (s, 1H), 7.33 (m, 3H), 7.55 (m, 2H), 7.99 (s, 1H), 8.80 (s, 1H), 9.73 (bs, 1H), 10.70 (bs, 1H), 13.09 (bs, 1H).

2-(*N*′-Benzylidene-hydrazino) ethanol (12). To a solution of benzaldehyde (10 mL, 98 mmol) in abs. EtOH (50 mL) was added 2-hydroxyethylhydrazine (6.67 mL, 98 mmol) in abs. EtOH (10 mL). The solution was heated at reflux for 1 h, then the solvent was removed at reduced pressure to furnish **12** as yellow oil (97.5 mmol, 99%), ^{1}H NMR ($CDCl_3$) δ: 3.37 (t, 2H, $J=6.2$), 3.83 (t, 1H, $J=7$), 3.85 (t, 2H, $J=6.1$), 7.33 (m, 4H), 7.54 (d, 2H, $J=3.9$), 7.65 (s, 1H).

2-[*N*′-Benzylidene-*N*-(2-hydroxyethyl)-hydrazinomethylene]-malononitrile (12a). To a solution of **12** (5 g, 30 mmol) in benzene (20 mL) was added ethoxymethylenemalononitrile (3.72 g, 30 mmol) dissolved in benzene (20 mL). The mixture was heated at reflux for 1 h, then the solvent was removed at reduce pressure to furnish **12a** as yellow solid (12.5 mmol, 41.6%), directly utilized for the next synthetic step. ^{1}H NMR (DMSO-d_6) δ: 3.62 (t, 2H, $J=6.3$), 4.11 (t, 2H, $J=6.9$), 5.12 (t, 1H, $J=6.2$), 7.48 (m, 3H), 7.91 (m, 3H), 8.38 (s, 1H).

3-Amino-1-(2-hydroxyethyl)-1*H*-pyrazole-4-carbonitrile hydrochloride (13). To a solution of **12a** (12.5 mmol) in abs EtOH (20 mL) was added 36% HCl (1.41 mL). The reaction was heated at reflux for 30 min, then the solvent was removed at reduced pressure and the residue was recrystallized from boiling Et_2O to afford **13** as yellow solid (10.5 mmol, 84%), mp 232 °C, IR (KBr): 1516, 1569, 1653, 2222, 3207, 3439 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.64 (t, 2H, $J=6.4$), 3.88 (t, 2H, $J=6.2$), 4.89 (bs, 1H), 5.53 (bs, 2H), 7.35 (bs, 1H), 8.02 (s, 1H).

3-Amino-1-(2-benzyloxy-ethyl)-1*H*-pyrazole-4-carbonitrile (14). To a suspension of 60% NaH (6.7 mmol) in dry DMF (25 mL) at 0 °C was added **13** (3.9 g, 25.4 mmol) in small portions. The mixture was stirred at room temperature for several min and then benzylbromide (26.9 mmol) in dry DMF (8 mL) was added. The resulting mixture was stirred at room temperature for 1 h then the solvent was removed at reduced pressure. To the residue was added water (40 mL) and the aqueous layer was extracted with EtOAc (5×25 mL). The organic layers were dried (Na_2SO_4) and evaporated under vacuum to afford a solid purified by flash chromatography (EtOAc/light petroleum 4:1) to furnish **14** as a pale yellow solid (12.4 mmol, 49.5%), mp 210 °C, ^{1}H NMR (DMSO-d_6) δ: 3.70 (t, 2H, $J=6$), 4.04 (t, 2H, $J=6.1$), 4.44 (s, 2H), 5.57 (bs, 2H), 7.3 (m, 5H), 8.08 (s, 1H).

***N*-[1-(2-Benzyloxy-ethyl)-4-cyano-1*H*-pyrazol-3-yl]-formimidic acid ethyl ester (15).** A solution of **14** (3 g, 12.4 mmol) in $HC(OEt)_3$ (25 mL) was heated at reflux for 16 h. Then the solvent was removed at reduced pressure and the residue was recrystallized from a mixture of light petroleum/Et_2O (1:1) to afford **15** as white solid (9.9 mmol, 79.8%), mp 198 °C, ^{1}H NMR (DMSO-d_6) δ: 1.30 (t, 3H, $J=6.2$), 3.76 (t, 2H, $J=6.6$), 4.26 (m, 4H), 4.46 (s, 2H), 7.26 (m, 5H), 8.27 (s, 1H), 8.42 (s, 1H).

8-(2-Benzyloxy-ethyl)-2-(furan-2-yl)-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (16). To a solution of **15** (2.95 g, 9.9 mmol) in 2-methoxyethanol (35 mL) was added 2-furoic acid hydrazide (9.9 mmol) and the mixture was heated at reflux for 12 h. After cooling, the solvent was removed under reduced pressure and the oily residue was cyclized without other purification in diphenyl ether (50 mL) at 260 °C for 1.5 h. Then the mixture was poured into light petroleum (300 mL) and cooled. The precipitate was filtered off and purified by recrystallization from EtOAc to afford **16** as a white solid (8.3 mmol, 84%), mp 232 °C, ^{1}H NMR (DMSO-d_6) δ: 3.95 (t, 2H, $J=6.3$), 4.49 (s, 2H), 4.67 (t, 2H, $J=6.2$), 6.74 (m, 1H), 7.24 (m, 7H), 7.96 (bs, 1H), 8.93 (s, 1H).

8-(2-Benzyloxy ethyl)-2-(furan-2-yl)-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl amine (17). A solution of **16** (3 g, 8.3 mmol) in aqueous 10% HCl (30 mL) and dioxan (15 mL) was refluxed for 1 h. Then the solution was cooled, basified with 10% NaOH at 0 °C, and extracted with EtOAc (3×50 mL). The organic layers were dried over anhyd. Na_2SO_4 and evaporated under vacuum. The residue obtained was recrystallized from EtOAc to afford the hydrolyzed compound as a solid utilized without other purification for the next step of reaction.

To a solution of the hydrolyzed intermediate (1 g, 2.8 mmol) in *N*-methylpyrrolidone (8 mL) were added cyanamide (16.8 mmol) and *p*-toluenesulphonic acid (4.2 mmol), and the mixture was heated at 160 °C for 4 h. Then cyanamide (16.8 mmol) was added again and the solution was heated overnight. Then the solution was diluted with EtOAc (100 mL) and the precipitate (excess of cyanamide) was filtered off; the filtrate was concentrated under reduced pressure, and washed with water (3×50 mL). The organic layer was dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by chromatography (EtOAc/light petroleum 1:1) to afford **17** as a solid (2.9 mmol, 35% from **16**), mp 181–183 °C, ^{1}H NMR (DMSO-d_6) δ: 3.88 (t, 2H, J = 6.3), 4.48 (s, 4H), 6.58 (bs, 2H), 6.72 (m, 1H), 7.25 (m, 5H), 7.55 (s, 1H), 7.93 (d, 1H, J = 1.4), 8.61 (s, 1H).

1-[8-(2-Benzyloxy-ethyl)-2-furan-2-yl-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-3-phenyl urea (18). To a solution of **17** (260 mg, 0.69 mmol) in dry CH_3CN (10 mL) was added phenylisocyanate (2.6 mmol) and a catalytic amount of TEA. The solution was heated at 80 °C for 24 h, then the solvent was removed at reduced pressure and the oily residue was purified by flash chromatography (EtOAc/light petroleum 1:1) to afford **18** as a white solid (0.30 mmol, 55%), mp 185–187 °C, ^{1}H NMR (DMSO-d_6) δ: 3.94 (t, 2H, J = 6.2), 4.50 (s, 2H), 4.60 (t, 2H, J = 6.5), 6.76 (m, 1H), 7.10 (t, 1H, J = 2.1), 7.25 (m, 8H), 7.42 (m, 2H), 7.98 (s, 1H), 8.83 (s, 1H), 9.71 (bs, 1H), 10.67 (bs, 1H).

1-[8-(2-Hydroxy-ethyl)-2-furan-2-yl-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-3-phenyl urea (19). To a solution of **18** (100 mg, 0.2 mmol) in dry acetone (15 mL) was added HCO_2NH_4 (1.6 mmol) and 10% Pd/C (100 mg).The resulting mixture was heated at reflux for 8 h.The solution was cooled, the catalyst was removed by filtration, and the solvent was removed at reduced pressure. The residue was washed with water (100 mL), the aqueous layer was extracted with EtOAc (3×30 mL) and the recombined organic phases were dried over anhyd. Na_2SO_4 and evaporated under reduced pressure to afford **19** as a white solid (0.17 mmol, 85%), mp 192–194 °C, ^{1}H NMR (DMSO-d_6) δ: 4.01 (t, 2H, J = 6.2), 4.41 (t, 2H, J = 6.3), 5.15 (t, 1H, J = 6.8), 6.75 (bs, 1H), 7.27 (t, 1H, J = 1.5), 7.38 (m, 3H), 7.55 (d, 2H, J = 1.4), 9.97 (s, 1H), 8.71 (d, 1H, J = 1.8), 9.73 (bs, 1H); 10.75 (bs, 1H).

8-(2-Hydroxy-ethyl)-2-furan-2-yl-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl amine (7) (*O*-debenzylation). To a solution of **17** (100 mg, 0.26 mmol) in dry acetone (20 mL) was added HCO_2NH_4 (2.13 mmol) and 10% Pd/C (100 mg) and the resulting mixture was heated at reflux for 8 h. The solution was cooled and the catalyst was removed by filtration. The solvent was evaporated at reduced pressure and the residue was washed with water (100 mL). The aqueous layer was extracted with EtOAc (5×25 mL) and the recombined organic phases were dried over anhyd. Na_2SO_4 and evaporated under reduced pressure to afford **7** as a white solid (0.19 mmol, 75%). Physical properties reported above.

Pharmacology

All synthesized compounds have been tested for their affinity to human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors.

The affinity values were determined by receptor binding assays at human A_1, A_{2A}, A_{2B} and A_3 adenosine receptor subtypes cloned in CHO and HEK-293 cells using [^{3}H]DPCPX, [^{3}H]SCH 58261, [^{3}H]DPCPX and [^{3}H]MRE 3008F20, respectively.

Human cloned adenosine receptor binding assay

The cells were grown adherently and maintained in Dulbecco's modified Eagle's medium with nutrient mixture F12 without nucleosides at 37 °C in 5% CO_2/95% air. The cells were washed with phosphate-buffered saline and scrapped off 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 centrifuged for 30 min at 48,000*g*. The membrane pellet was resuspended in 50 mM Tris–HCl buffer at pH 7.4 for A_1 adenosine receptors, in 50 mM Tris–HCl, 10 mM $MgCl_2$ at pH 7.4 for A_{2A} adenosine receptors, in 50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EDTA at pH 7.4 for A_3 adenosine receptors and were utilized for binding assays. HEK 293 cells transfected with the human recombinant A_{2B} adenosine receptor were obtained from Receptor Biology, Inc. (Beltsville, MD, USA).

Binding of [^{3}H]-DPCPX to CHO cells transfected with the human recombinant A_1 adenosine receptor was performed according to the method previously described by Varani et al.[16] Displacement experiments were performed for 120 min at 25 °C in 200 μL of buffer containing 1 nM [^{3}H]-DPCPX, 20 μL of diluted membranes (50 μg of protein/assay) and at least 6–8 different concentrations of examined compounds. Non-specific binding was determined in the presence of 10 μM of CHA and this is always ≤10% of the total binding.

Binding of [^{3}H]-SCH58261 to CHO cells transfected with the human recombinant A_{2A} adenosine receptors (50 μg of protein/assay) was performed according to Varani et al.[16]

In competition studies, at least 6–8 different concentrations of compounds were used and non-specific binding was determined in the presence of 1 μM SCH58261 for an incubation time of 60 min at the temperature of 25 °C.

Binding of [^{3}H]-DPCPX to HEK 293 cells transfected with the human recombinant A_{2B} adenosine receptors were performed essentially to the method described by Varani et al.[16]

In particular, assays were carried out for 60 min at 25 °C in 100 μL of 50 mM Tris–HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA, 0.1 mM benzamidine pH 7.4, 2 IU/mL adenosine deaminase containing 40 nM [^{3}H]-DPCPX, diluted membranes (20 μg of protein/assay) and at least 6–8 different concentration of tested compounds. Non specific binding was determined in the presence of 100 μM of NECA and was always $\leq$30% of the total binding.

Binding of [^{3}H]MRE3008 F20 to CHO cells transfected with the human recombinant A_3 adenosine receptors was performed according to Varani et al.[16] Competition experiments were carried out in duplicate in a finale volume of 250 μL in test tubes containing 1 nM [^{3}H]MRE3008 F20, 50 mM Tris–HCl buffer, 10 mM $MgCl_2$, pH 7.4 and 100 μL of diluted membranes (50 μg protein/assay) and at least 6–8 different concentrations of examined ligands for 120 min at 4 °C. Non-specific binding was defined as binding in the presence of 1 μM of MRE3008 F20 and was about 25% of total binding.

Bound and free radioactivity were separated by rapid filtration through Whatman GF/B glass-fiber filters which were washed three times with ice cold buffer. The filter bound radioactivity was counted in a Beckman LS-1800 spectrometer (efficiency 55%).

Data analysis

The protein concentration was determined according to a Bio-Rad method[17] with bovine albumin as a standard reference. Inhibitory binding constant, K_i, values were calculated from those of IC_{50} according to Cheng and Prusoff equation.[18] A weighted non linear least-squares curve fitting program LIGAND[19] was used for computer analysis of saturation and inhibition experiments.

Data are expressed as geometric mean, with 95 or 99% confidence limits in parentheses.

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References and Notes

1. Ralevic, V.; Burnstock, G. *Pharmacol. Rev.* **1998**, *50*, 413.
2. Fredholm, B. B.; Arslan, G.; Halldner, L.; Kull, B.; Schulte, G.; Wasserman, W. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 364.
3. Salvatore, C. A.; Jacobson, M. A.; Taylor, H. E.; Linden, J.; Johnson, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10365.
4. Jacobson, K. A.; Suzuki, F. *Drug. Dev. Res.* **1996**, *39*, 289.
5. Abbracchio, M. P.; Brambilla, R.; Kim, H. O.; von Lubitz, D. K.; Jacobson, K. A.; Cattabeni, F. *Mol. Pharmacol.* **1995**, *48*, 1038.
6. Ali, H.; Choi, O. H.; Fraundorfer, P. F.; Yamada, K.; Gonzaga, H. M. S.; Beaven, M. A. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 837.
7. Ramkumar, V.; Stiles, G. L.; Beaven, M. A.; Ali, H. *J. Biol. Chem.* **1993**, *268*, 16887.
8. Brambilla, R.; Cattabeni, F.; Ceruti, S.; Barbieri, D.; Franceschi, C.; Kim, Y.; Jacobson, K. A.; Klotz, K. N.; Lohse, M. J.; Abbracchio, M. P. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *361*, 225.
9. Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Klotz, K. N.; Leung, E.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. *J. Med. Chem.* **1999**, *42*, 4473.
10. Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Moro, S.; Klotz, K. N.; Leung, E.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. *J. Med. Chem.* **2000**, *43*, 4768.
11. Baraldi, P. G.; Cacciari, B.; Moro, S.; Spalluto, G.; Pastorin, G.; Da Ros, T.; Klotz, K. N.; Varani, K.; Gessi, S.; Borea, P. A. *J. Med. Chem.* **2002**, *45*, 770.
12. Schering Corporation, NJ 07033-0530 (US), International Publication Number: WO01/92264 A1, 2001.
13. Dunn, L. D.; Skinner, C. G. *J. Org. Chem.* **1975**, *40*, 3713.
14. Seela, F.; Steker, H. *Helv. Chim. Acta* **1986**, *69*, 1602.
15. Gatta, F.; Del Giudice, M. R.; Borioni, A.; Borea, P. A.; Dionisotti, S.; Ongini, E. *Eur. J. Med. Chem.* **1993**, *28*, 569.
16. 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.
17. Bradfor, M. M. *Anal. Biochem.* **1976**, *72*, 248.
18. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
19. Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.