Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine Derivatives: Potent and Selective A_{2A} Adenosine Antagonists

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Received October 10, 1995⊗

A series of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives (**10a**−**o**,**q**,**r**), bearing alkyl and aralkyl chains on positions 7 and 8, were synthesized in the attempt to obtain potent and selective antagonists for the A_{2A} adenosine receptor subtype. The compounds were tested in binding and functional assays to evaluate their potency for the A_{2A} compared with the A_1 adenosine receptor subtype. In binding studies in rat brain membranes, most of the compounds showed affinity for A_{2A} receptors in the low nanomolar range with a different degree of A_{2A} versus A_1 selectivity. Comparison of N^7 (**10a**−**d**,**h**−**o**)- and N^8 (**10e**−**g**)-substituted pyrazolo derivatives indicates that N^7 substitution decreases the A_1 affinity with the concomitant increase of A_{2A} selectivity. Specifically, the introduction of a 3-phenylpropyl group at pyrazolo nitrogen in position 7 (**10l**) increased significantly the A_{2A} selectivity, being 210-fold, while the A_{2A} receptor affinity remained high (K_i = 2.4 nM). With regards to the affinity for A_{2A} receptors, also the compound **10n**, bearing in the 7-position a β-morpholin-4-ylethyl group, deserves attention (K_i = 5.6 nM) even though the A_{2A} selectivity (84-fold) was not as high as that of **10l**. Conversely, the compound **10m** (N^7-4-phenylbutyl derivative) showed a remarkable selectivity (A_1/A_{2A} ratio = 129) associated with lower A_{2A} affinity (K_i = 21 nM). In functional studies, most of the compounds examined reversed 5′-(*N*-ethylcarbamoyl)adenosine-induced inhibition of rabbit platelet aggregation inhibition which is a biological response mediated by the A_{2A} receptor subtype. The compounds are potent and selective A_{2A} antagonists which can be useful to elucidate the pathophysiological role of this adenosine receptor subtype. These compounds deserve to be further developed to assess their potential for treatment of neurodegenerative disorders such as Parkinson's disease.

Introduction

Adenosine modulates a wide range of physiological functions by interacting with specific cell surface receptors which have been classified as A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor subtypes.[1,2] Efforts made in medicinal chemistry over the last 2 decades have led to the discovery of a series of adenosine analogs which possess specific agonist properties at A_1, A_{2A}, or A_3 receptors.[3,4] As for adenosine receptor antagonists, a large number of xanthine derivatives have been synthesized in the attempts to improve both receptor subtype affinity and selectivity of the natural compounds caffeine and theophylline.[3] Combined substitution at the 1-, 3-, and 8-positions of the xanthine moiety led to potent and selective A_1 receptor antagonists. For example, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX)[5] is currently used as a reference compound.

A series of 8-styrylxanthines have been also found to have high affinity and selectivity at A_{2A} receptors.[6,7] Two of these compounds, namely, 7-methyl-8-(3,4-dimethoxystyryl)xanthine (KF 17837)[6] and 8-(3-chlorostyryl)-caffeine (CSC),[7] have been further investigated and found to possess antagonist properties in both *in vitro*[8,9] and *in vivo* studies.[9−11] However, these compounds undergo rapid photoisomerization when diluted and exposed to natural light,[12] and this may limit their use as pharmacological tools.

Non-xanthine heterocyclic compounds, including 5-amino-9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazoline (CGS 15943, **1**)[13] and 4-amino-8-chloro-1-phenyl-[1,2,4]triazolo[4,3-*a*]quinoxaline (CP 66,713),[14] also have high affinity for A_{2A} receptors, but they are unselective, being potent at A_1 receptors as well. Recently, a series of CGS 15943 derivatives, in which the *m*-chlorophenyl group was replaced by a heterocycle ring, such as pyrazole or imidazole, have been described to retain high affinity for adenosine receptors.[15] One of them, 5-amino-8-(4-fluorobenzyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8FB-PTP, **2a**), has high affinity at A_{2A} receptors and shows competitive A_{2A} antagonist properties in *in vitro* functional studies, but its selectivity versus A_1 receptor is still low.[16] Conversely, the corresponding N^7 derivative 5-amino-7-(4-fluorobenzyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (7FB-PTP, **2b**) showed a decrease in the affinity at both receptor subtypes, but it was associated with a concomitant increase of A_{2A} versus A_1 selectivity.[15]

With the aim to enhance the A_{2A} selectivity, we have further investigated the lead compounds **2a**,**b**. In our previous study[17] the importance of the hydrophobic, electronic, and steric parameters of substituents at the

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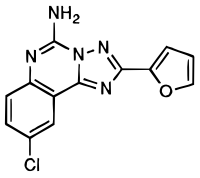
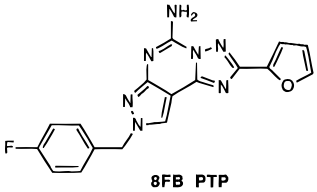
⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1996.

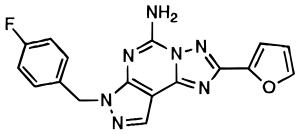
0022-2623/96/1839-1164$12.00/0 © 1996 American Chemical Society



CGS 15943
1



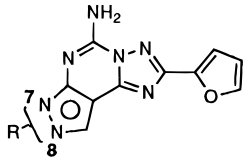
8FB PTP
2a



7FB PTP
2b

pyrazole N^7 or N^8 nitrogens (compounds **10a**−**c**,**e**−**h**) had been evaluated, according to the Topliss operational scheme for aliphatic substituents.[18] The derivatives bearing a substituent in the 7-position showed a better A_{2A} selectivity than the corresponding N^8 derivatives having the same substituent. One of them, namely, SCH 58261 (**10c**), was shown to have high affinity and good selectivity for the A_{2A} receptor subtype and to also possess competitive A_{2A} antagonist properties in *in vitro* functional studies.[19]

On the basis of these interesting results, we have synthesized N^7 derivatives in order to better evaluate the Topliss operational scheme for aliphatic substituents (**10i**,**k**,**q**). Moreover, we focused our studies on the lead compound **10c**, designing the following structural modifications: (i) introduction of different spacers between N^7 nitrogen and phenyl ring (**10l**,**m**,**o**) in order to optimize the length spacer, (ii) increase of the lipophilic nature of the substituent at the N^7 position (**10d**), an essential condition for the interaction with A_{2A} receptors,[17] and (iii) increase of the water solubility (**10d**,**j**,**n**,**r**) of this series of chemical structures.



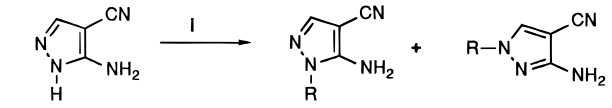
10a-o,q

Chemistry

The preparation of the compounds **10a**−**o** was performed following the general synthetic strategy depicted in Schemes 1−5. Alkylation of 5-amino-4-cyanopyrazole (**3**) with the appropriate alkyl halide in DMF in the presence of anhydrous potassium carbonate led to an ca. 1:1 mixture of N^1 isomers **4a**−**d** and N^2 isomers **4e**−**g**, separable by crystallization or column chromatography (Scheme 1).

In order to improve and facilitate the synthesis of the N^7-substituted derivatives, we decided to synthesize the pyrazole ring using the appropriate hydrazine. This pathway afforded only the N^1 isomer and allowed us to avoid tedious purification procedures. Pyrazoles **4h**−**n** were prepared by reacting (ethoxymethylene)malononitrile with the appropriate hydrazines[20,21] following a well-known procedure[22] (Scheme 2).

Scheme 1[a]

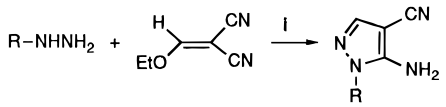


3 **4a-d** **4e-g**

a) R = n-butyl; **b)** R = isopentyl; **c)** R = β-phenylethyl;
d) R = β[(4-isobutyl)phenyl]ethyl; **e)** R = n-butyl
f) R = isopentyl ; **g)** R = β-phenylethyl;

[a] Reagents: (i) RX, K_2CO_3, EtOH, reflux.

Scheme 2[a]

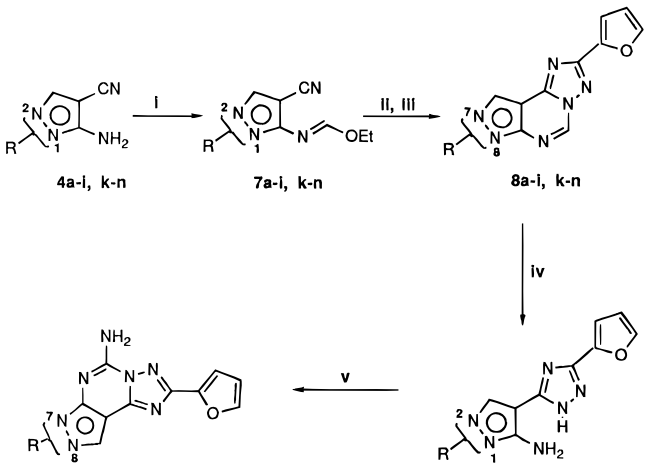


5 **6** **4h-n**

h) R = methyl; **i)** R = phenyl; **j)** R = β-hydroxyethyl;
k) R = t-butyl; **l)** R = 3-phenylpropyl ; **m)** R = 4-phenylbutyl;
n) R = β-(morpholin-4-yl)ethyl

[a] Reagents: (i) EtOH, reflux.

Scheme 3[a]



4a-i, k-n **7a-i, k-n** **8a-i, k-n**

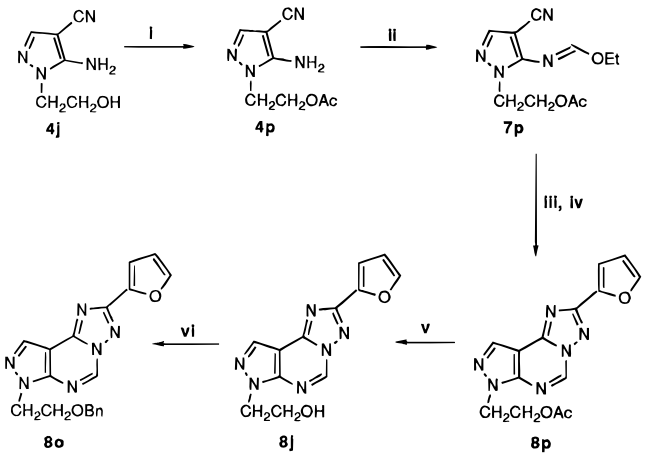
10a-o **9a-o**

a) R = N^1-n-butyl; **b)** R = N^1-isopentyl; **c)** R = N^1-β-phenylethyl;
d) R = N^1-β[(4-isobutyl)phenyl]ethyl; **e)** R = N^2-n-butyl; **f)** R = N^2-isopentyl;
g) R = N^2-β-phenylethyl ; **h)** R = N^1-methyl; **i)** R = N^1-phenyl;
j) R = N^1-β-hydroxyethyl; **k)** R = N^1-t-butyl; **l)** R = N^1-3-phenylpropyl;
m) R = N^1-4-phenylbutyl; **n)** R = N^1-β(morpholin-4-yl)ethyl;
o) R = N^1-β-benzyloxyethyl
* For tricyclic compounds **10a-o**: N^1=N^7 and N^2=N^8

[a] Reagents: (i) $HC(OEt)_3$, reflux; (ii) furoic hydrazide, $MeO(CH_2)_2OH$; (iii) Ph_2O, 260 °C; (iv) 10% HCl, reflux; (v) NH_2CN, 1-methyl-2-pyrrolidone, pTsOH, 140 °C.

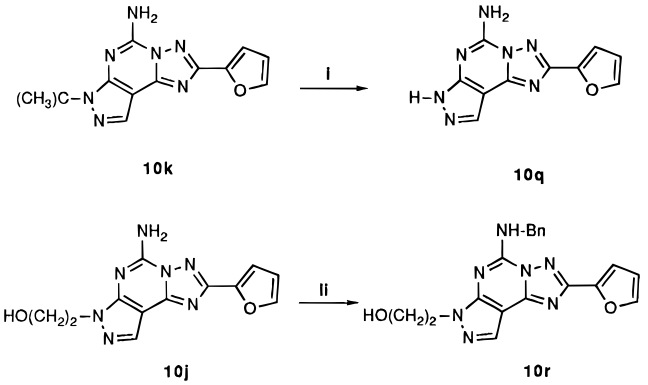
The designed compounds **10a**−**o** were synthesized according to Gatta et al.[15] for the synthesis of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines (Scheme 3). Following the general strategy, the first step involved transformation of pyrazoles **4a**−**i**,**k**−**n** to the corresponding imidates **7a**−**i**,**k**−**n** by refluxing in triethyl orthoformate. This conversion was not possible for **4j** because the hydroxy group prevented a clean reaction. Therefore in order to achieve an improvement in the overall yield, we converted the pyrazole **4j** into the corresponding acetyl derivative **4p** (Scheme 4). Imidates **7a**−**i**,**k**−**n** were reacted with 2-furoic acid hydrazide in refluxing 2-methoxyethanol to provide the pyrazolo[3,4-*d*]pyrimidine intermediates. The latter compounds were converted through a thermally induced

Scheme 4[a]



[a] Reagents: (i) Ac_2O, Py, room temperature; (ii) $HC(OEt)_3$, reflux; (iii) furoic hydrazide, $MeO(CH_2)_2OH$; (iv) Ph_2O, 260 °C; (v) NH_3, MeOH, room temperature; (vi) NaH, BnBr, DMF.

Scheme 5[a]



[a] Reagents: (i) HCO_2H, reflux; (ii) NaH, BnBr, room temperature.

cyclization in diphenyl ether to the derivatives **8a**–**i**,**k**–**n** in good overall yield.

The cyanoaminopyrazole **4p** underwent the same reactions with triethyl orthoformate to afford imidate **7p** and subsequently the tricyclic compound **8p**. The latter was deprotected with methanolic ammonia to give **8j** which was converted to **8o** by benzylation in the presence of sodium hydride (Scheme 3). Benzylation was performed on *β*-hydroxyethyl derivative **8j** instead of the pyrazole **4j** because direct reaction on **4j** with benzyl bromide afforded only the benzylamino derivative. Treatment of **8a**–**o** with dilute hydrochloric acid at reflux temperature induced pyrimidine ring opening to furnish the 5-amino-4-(1*H*-1,2,4-triazol-5-yl)pyrazoles **9a**–**o** in good yield.

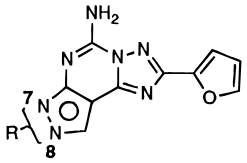
These derivatives were converted into the final compounds **10a**–**o** by reaction with an excess of cyanamide in 1-methyl-2-pyrrolidone at 140 °C. Compound **10k** was transformed into **10q** by refluxing in formic acid, as previously described for the N-di-*tert*-butylation of pyrazole intermediates.[23] In an attempt to prepare directly the benzyloxy derivative **10o**, alkylation of **10j** with benzyl bromide in the presence of sodium hydride at room temperature afforded *N*-benzyl derivative **10r**, as the only product (Scheme 5).

Results and Discussion

The results show that many of the tested compounds have affinity at A_{2A} receptors in the low nanomolar range with different degree of selectivity (Table 1). Most of the compounds examined were able to reverse the platelet aggregation inhibition induced by *N*-ethyl-1′-deoxy-1′-(6-amino-9*H*-purin-9-yl)-*β*-D-ribofuranuronamide (NECA) with potency similar to (**10a**–**c**,**g**,**h**,**j**,**q**) or higher than (i.e., **10e**,**f**,**l**,**n**) that of the reference compound CGS 15943 (Table 1).

The study shows that N^7 substitution (**10a**–**d**,**h**–**o**) on the pyrazole ring retains high affinity at A_{2A} receptors. On the contrary, either substitution at N^8 (**10e**–**g**) or no substitution (**10q**) led to compounds with a good affinity for adenosine receptors but with very low selectivity. Specifically, the introduction of the phenylethyl group at the pyrazole nitrogen in position 7, **10c**, increased significantly A_{2A} selectivity, being about 50- or 100-fold in rat or bovine striatal membranes, respectively.[18] On this basis, further studies were undertaken to investigate the pharmacological characteristics of **10c** as a new prototypic A_{2A} antagonist. The results of *in vitro* functional studies carried out in various models indicate that **10c** is a competitive antagonist for the A_{2A} receptor subtype and has little or no interaction with A_1, A_{2B}, and A_3 receptors.[18] Encouraged by these results, we continued the investigation on the influence of substituents in N^7. For compound **10r**, where the amino group was protected as benzyl derivative, the A_{2A} receptor affinity is low (K_i =166 nM). This confirms that a free amino group is necessary for an efficient receptor interaction. Introduction of an oxygen atom in the substituent chain (**10o**) reduced dramatically the A_{2A} receptor affinity (K_i > 1000 nM) and did not improve water solubility, the latter being a limitation for **10c** and other xanthine and non-xanthine adenosine antagonists.[3] It is likely that the presence of an oxygen atom hinders a correct ligand–receptor interaction. At this time, this problem is under investigation by molecular modeling studies. Instead, replacement of the oxygen atom with a methylene group led to interesting data. For example, A_{2A} affinity of **10m** remained in the nanomolar range (K_i = 21 nM), but A_{2A} selectivity significantly increased when compared with **10c** (129- versus 53-fold). Further shortening of the chain using 3-phenylpropyl, **10l**, led to a compound having higher affinity (K_i = 2.4 nM) and a very good selectivity (A_1/A_{2A} ratio of 210). Thus, it seems that a spacer between the pyrazole and aromatic moiety is necessary to achieve high A_{2A} selectivity, the optimal length being from two to four carbon atoms (**10c**,**d**,**l**–**m**). These data provide support to the concept that the lipophilic nature of the substituent can be important for the interaction with A_{2A} receptors. This is confirmed by the results obtained with (4-isobutylphenyl)ethyl derivative **10d** which has good affinity (K_i = 22 nM) and selectivity (A_1/A_{2A} ratio of >136). In analogy to the compound synthesized at Zeneca, 2-(2-furyl)-5-[(2-morpholinoethyl)amino][1,2,4]-triazolo[1,5-*a*][1,3,5]triazin-7-amine (pIC_{50} value at A_{2A} receptors of 7.6),[24] the introduction of a morpholinoethyl group in the N^7 position led to a compound with an increased affinity (K_i = 5.6 nM) and high A_{2A} selectivity, although lower (84-fold) than **10l**. Very recently, another heterocycle compound, the 4-[2-[[7-amino-2-(2-furyl)[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl]amino]ethyl]-phenol (ZM 241385), has been reported to be a potent (pIC_{50} = 9.5) and selective A_{2A} antagonist.[25] However, the lack of binding data carried out according to

Table 1. Biological Activity of a Series of Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines **10a**−**o**,**q**−**r**



10a-o, q, r

compd	R	binding[a] K_i (nM) A_1	binding[a] K_i (nM) A_{2A}	selectivity, A_1/A_{2A}	platelet[b] aggregation ED_{50} (μM)
1, CGS 15943		6.4 (6.2−6.6)	1.2 (1.1−1.3)	5.3	0.3
2a, 8FB-PTP		3.3 (2.9−3.6)	1.2 (0.9−1.4)	2.8	0.004
2b, 7FB-PTP		189 (164−238)	12.0 (8.6−19.5)	15.8	10.6
DPCPX		1.5 (1.3−1.7)	706 (540−924)	0.0002	>10
10a	N^7-*n*-butyl	236 (486−872)[c]	8.9 (7.9−10)[c]	26.5	0.3
10b	N^7-isopentyl	116 (79−181)[c]	12 (7.6−19)[c]	9.7	0.3
10c	N^7-β-phenylethyl	121 (103−143)[c]	2.3 (2−2.7)[c]	52.6	0.3
10d	N^7-β-(4-isobutylphenyl)ethyl	>3000	22 (12−40)	>136	<1.0
10e	N^8-*n*-butyl	30.4 (21.5−43)[c]	2.4 (2.3−2.5)[c]	12.7	0.04
10f	N^8-isopentyl	5.6 (4.0−7.9)[c]	1.9 (1.4−2.4)[c]	2.9	0.05
10g	N^8-β-phenylethyl	4.7 (4.1−5.5)[c]	1.4 (0.9−2.3)[c]	3.4	0.2
10h	N^7-methyl	651 (486−872)[c]	101 (94−109)[c]	6.4	0.3
10i	N^7-phenyl	119 (74.9−188)	22.7 (14.2−36.3)	5.2	>1
10j	N^7-β-hydroxyethyl	978 (873−1094)	77.7 (55.8−108)	12.6	0.2
10k	N^7-*tert*-butyl	430 (378−488)	260 (205−330)	1.7	>10
10l	N^7-3-phenylpropyl	504 (329−773)	2.4 (1.9−2.9)	210	0.02
10m	N^7-4-phenylbutyl	2705 (1867−3919)	21 (17−26)	129	>1
10n	N^7-β-morpholin-4-ylethyl	471 (385−576)	5.6 (3.0−10.3)	84	0.03
10o	N^7-β-(benzyloxy)ethyl	≫1000	>1000		>10
10q	H	210 (189−234)	22.6 (13.9−36.8)	9.3	0.1
10r	N^7-β-hydroxyethyl 5-aminobenzyl	330 (214−509)	166 (135−203)	2.0	>10

[a] Inhibition of [^{3}H]CHA binding (A_1) in rat whole brain homogenates or [^{3}H]CGS 21680 binding (A_{2A}) in rat striatal homogenates; data are expressed as geometric means, with 95% confidence limits. [b] Dose of antagonist reducing by 50% the inhibitory effect induced by NECA, used at a concentration (0.3 or 1 μM) close to the IC_{50} value, on ADP (5 μM)-induced rabbit platelet aggregation. [c] Binding data from ref 17.

standard criteria does not allow to compare it with compounds previously described.

Conclusions

We have described a series of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives that are potent and selective non-xanthine A_{2A} antagonists. This was achieved as a further optimization of previous work by Gatta et al.[15] who started from CGS 15943 and replaced the chlorophenyl moiety with a substituted pyrazole or imidazole ring. Other studies made using triazole led to potent but unselective compounds.[17] All these data have been of stimulus to make synthetic efforts involving the introduction of alkyl and aralkyl substituents on the pyrazole ring.

Thus the compounds **10a**,**c**,**e**−**g**,**l**,**n** have A_{2A} receptor affinity of <10 nM, and most importantly, A_{2A} versus A_1 selectivity is very high, being greater than 100-fold for **10d**,**m** and 210-fold for **10l**. The prototype compound **10c** has been further characterized in *in vitro* studies and found to possess competitive A_{2A} antagonist properties.[19] *In vivo*, the compound **10c** retains A_{2A} antagonist properties in cardiovascular models[26a] and, like caffeine, produces stimulatory effects on the central nervous system.[26b] Moreover, its tritium-labeled form, [^{3}H]SCH 58261 (**10c**), has been found to specifically label A_{2A} receptors with high affinity (K_D = 0.7 nM) and high specific binding (>90%).[27]

On the basis of the evidence available,[28,29] the compounds deserve to be further developed to assess their potential for treatment of central nervous system disorders, such as neurodegenerative diseases and cognitive deficits.

Experimental Section

Chemistry. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with iodine or aqueous potassium permanganate. Infrared spectra (IR) were measured on a Perkin Elmer 257 instrument. ^{1}H 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 hertz (Hz). 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 with Merck 60−200 mesh silica gel. All products reported showed IR and ^{1}H NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous magnesium 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.

General Procedure for the Preparation of N-Substituted-5-amino-4-cyanopyrazoles 4h−n. The appropriate hydrazine (0.53 mmol) was dissolved in EtOH (5 mL), and (ethoxymethylene)malononitrile (64.8 mg, 0.53 mmol) was added in little portions. Then the mixture was heated at 70 °C for 18 h before evaporating the solvent. The solid residue was purified by chromatography (EtOAc/light petroleum, 1:1) to afford the product as a solid in good yield. The following spectral data are reported as examples.

5-Amino-4-cyano-1-(β-hydroxyethyl)pyrazole (4j): yield 75%, yellow solid; mp 150−151 °C (EtOAc−light petroleum); IR (KBr) 3450−2950, 2210, 1660, 1580, 1440 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.65 (dd, 2H, *J* = 5.7, 5.2), 3.93 (t, 2H, *J* = 5.7),

4.91 (t, 1H, J = 5.2), 6.45 (bs, 2H), 7.52 (s, 1H). Anal. ($C_6H_8N_4O$) C, H, N.

5-Amino-1-*tert*-butyl-4-cyanopyrazole (4k): yield 80%, yellow solid; mp 94−95 °C (EtOAc−light petroleum); IR (KBr) 3350−3150, 2200, 1650, 1470 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.63 (s, 9H), 4.44 (bs, 2H), 7.42 (s, 1H). Anal. ($C_8H_{12}N_4$) C, H, N.

5-Amino-4-cyano-1-(3-phenylpropyl)pyrazole (4l): yield 72%, pale yellow solid; mp 155 °C (EtOAc−Et_2O); IR (KBr) 3355−3160, 2210, 1650, 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.91−2.07 (m, 2H), 2.58 (t, 2H, J = 6), 5.91 (t, 2H, J = 6), 6.47 (bs, 2H), 7.15−7.25 (m, 5H), 7.43 (s, 1H). Anal. ($C_{13}H_{14}N_4$) C, H, N.

5-Amino-4-cyano-1-(4-phenylbutyl)pyrazole (4m): yield 75%, yellow solid; mp 147−149 °C (EtOAc−Et_2O); IR (KBr) 3345−3160, 2220, 1670, 1450 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.55−1.73 (m, 2H), 1.74−1.91 (m, 2H), 2.63 (t, 2H, J = 7), 3.91 (t, 2H, J = 7), 5.39 (bs, 2H), 7.13−7.27 (m, 5H), 7.42 (s, 1H). Anal. ($C_{14}H_{16}N_4$) C, H, N.

5-Amino-1-(*β*-morpholin-4-ylethyl)-4-cyanopyrazole (4n): yield 79%, yellow solid; mp 135−137 °C (EtOAc−Et_2O); IR (KBr) 3360−3158, 2190, 1650, 1450 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.40 (t, 4H, J = 4), 2.58 (t, 2H, J = 8), 3.53 (t, 4H, J = 4), 4.01 (t, 2H, J = 8), 6.62 (bs, 2H), 7.52 (s, 1H). Anal. ($C_{10}H_{15}N_5O$) C, H, N.

1-[*β*-(Acetyloxy)ethyl]-5-amino-4-cyanopyrazole (4p). To a solution of **4j** (0.5 g, 3.28 mmol) in dry pyridine (5 mL) was added acetic anhydride (0.62 mL, 6.6 mmol), and the mixture was heated at 100 °C for 2 h. Then the solvent was removed, and the residue was dissolved in CH_2Cl_2 (10 mL) and washed with water (3 × 10 mL). The organic layer was dried and evaporated to afford **4p** (0.57 g, 90%) as a yellow solid: mp 117−118 °C dec (EtOAc−light petroleum); IR (KBr) 3400−3100, 2200, 1740, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.06 (s, 3H), 4.2 (t, 2H, J = 5.6), 4.4 (t, 2H, J = 5.6), 5.5 (bs, 2H), 7.45 (s, 1H). Anal. ($C_8H_{10}N_4O_2$) C, H, N.

General Procedure for the Preparation of N-Substituted-5-amino-4-cyanopyrazoles 4a−d and N-Substituted-3-amino-4-cyanopyrazoles 4e−g. To a suspension of 4-cyano-5-aminopyrazole (**3**) (1.08 g, 10 mmol) and anhydrous potassium carbonate (1.65 g, 12 mmol) in DMF was added the appropriate alkyl halide (1.2 equiv), and the mixture was stirred at 80 °C for 1−2 h. Then the solvent was removed under reduced pressure, and the two alkylated isomers, N^1 and N^2, usually present in a ratio of 1:1, were separated by flash chromatography (EtOAc/light petroleum, 1:2). The following spectral data are reported as examples.

5-Amino-4-cyano-1-(*β*-phenylethyl)pyrazole (4c): yield 42%, yellow solid; mp 98−100 °C (EtOAc−light petroleum); IR (KBr) 3450−3170, 2200, 1640, 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.07 (t, 2H, J = 6.2), 4.1 (t, 2H, J = 6.2), 4.23 (bs, 2H), 7.17 (s, 1H), 7.0−7.28 (m, 5H). Anal. ($C_{12}H_{12}N_4$) C, H, N.

3-Amino-4-cyano-1-(*β*-phenylethyl)pyrazole (4g): yield 41%, yellow solid; mp 172−173 °C (EtOAc−light petroleum); IR (KBr) 3450−3150, 2200, 1650, 1470 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.04 (t, 2H, J = 6.4), 4.12 (t, 2H, J = 6.4), 5.85 (bs, 2H), 7.21−7.30 (m, 5H), 7.41 (s, 1H). Anal. ($C_{12}H_{12}N_4$) C, H, N.

General Procedure for the Preparation of 1-Substituted-4-cyano-3(or 5)-[(ethoxymethylene)amino]pyrazoles 7a−i,k−p. The mixture of N^1- and N^2-substituted-4-cyano-5-aminopyrazoles **4a−i,k−p** (20 mmol) was dissolved in triethyl orthoformate (40 mL), and the solution was refluxed under nitrogen for 8 h. Then the solvent was removed under reduced pressure, and the oily residue was dissolved in ether and roughly purified on silica gel (EtOAc/light petroleum, 1:9) to afford the corresponding iminoethers. The following spectral data are reported as examples.

1-*n*-Butyl-4-cyano-5-[(ethoxymethylene)amino]pyrazole (7a): yield 87%, pale yellow oil; IR (neat) 2240, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3H, J = 6.2), 1.4 (t, 3H, J = 7), 1.5 (m, 2H), 1.8 (m, 2H), 4.3 (q, 2H, J = 6.2), 4.5 (t, 2H, J = 7), 7.9 (s, 1H), 8.4 (s, 1H). Anal. ($C_{11}H_{16}N_4O$) C, H, N.

4-Cyano-5-[(ethoxymethylene)amino]-1-methylpyrazole (7h): yield 90%, pale yellow oil; IR (neat) 2220, 1650, 1430 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 3H, J = 7), 3.65 (s, 3H), 4.37 (q, 2H, J = 7), 7.89 (s, 1H), 8.45 (s, 1H). Anal. ($C_8H_{10}N_4O$) C, H, N.

4-Cyano-5-[(ethoxymethylene)amino]-1-phenylpyrazole (7i): yield 92%, yellow oil; IR (neat) 2200, 1650, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 7), 3.39 (s, 3H), 4.28 (q, 2H, J = 7), 7.41−7.66 (m, 5H), 8.16 (s, 1H), 8.54 (s, 1H). Anal. ($C_{13}H_{12}N_4O$) C, H, N.

1-*tert*-Butyl-4-cyano-5-[(ethoxymethylene)amino]pyrazole (7k): yield 89%, pale yellow oil; IR (neat) 2210, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7), 1.64 (s, 9H), 4.43 (q, 2H, J = 7), 7.57 (s, 1H), 8.28 (s, 1H). Anal. ($C_{11}H_{16}N_4O$) C, H, N.

4-Cyano-5-[(ethoxymethylene)amino]-1-(3-phenylpropyl)pyrazole (7l): yield 93%, yellow oil; IR (neat) 2210, 1640, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.37 (t, 3H, J = 6), 2.02−2.2 (m, 2H), 2.61 (t, 2H, J = 7.2), 4.07 (t, 2H, J = 7.2), 4.25 (q, 2H, J = 6), 7.1−7.28 (m, 5H), 7.65 (s, 1H), 8.38 (s, 1H). Anal. ($C_{16}H_{18}N_4O$) C, H, N.

4-Cyano-5-[(ethoxymethylene)amino]-1-(4-phenylbutyl)-pyrazole (7m): yield 91%, yellow oil; IR (neat) 2190, 1650, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, J = 6), 1.52−1.67 (m, 2H), 1.72−1.96 (m, 2H), 2.62 (t, 2H, J = 8), 4.08 (t, 2H, J = 8), 4.36 (q, 2H, J = 6), 7.03−7.39 (m, 5H), 7.64 (s, 1H), 8.40 (s, 1H). Anal. ($C_{17}H_{20}N_4O$) C, H, N.

4-Cyano-5-[(ethoxymethylene)amino]-1-(*β*-morpholin-4-ylethyl)pyrazole (7n): yield 91%, yellow oil; IR (neat) 2210, 1660, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7), 2.44−2.49 (m, 4H), 2.75 (t, 2H, J = 6), 3.60−3.65 (m, 4H), 4.16 (t, 2H, J = 6), 4.39 (q, 2H, J = 7), 7.66 (s, 1H), 8.43 (s, 1H). Anal. ($C_{13}H_{19}N_5O_2$) C, H, N.

1-[*β*-(Acetyloxy)ethyl]-4-cyano-5-[(ethoxymethylene)-amino]pyrazole (7p): yield 95%, yellow oil; IR (neat) 2200, 1740, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H, J = 7.2), 2.1 (s, 3H), 4.3−4.5 (m, 6H), 7.68 (s, 1H), 8.44 (s, 1H). Anal. ($C_{11}H_{14}N_4O_3$) C, H, N.

General Procedures for the Preparation of 7- and 8-Substituted-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines 8a−n,p. Iminoethers **7a−i,k−n,p** (20 mmol) were dissolved in 2-methoxyethanol (50 mL), and 2-furoic acid hydrazide (2.5 g, 22 mmol) was added. The mixture was refluxed for 5−10 h; then, after cooling, the solvent was removed under reduced pressure and the dark oily residue dissolved in diphenyl ether (50 mL) and heated at 260 °C using a Dean−Stark trap for the azeotropic elimination of water produced in the reaction. After 1.5 h, the mixture was poured onto hexane (200 mL) and cooled. The precipitate was filtered off and purified by chromatography (EtOAc/hexane, 1:1). In this way, different compounds were obtained. The following spectral data are reported as examples.

7-*n*-Butyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8a): yield 75%, white solid; mp 180−181 °C (EtOAc); IR (KBr) 1520, 1430 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.9 (t, 3H, J = 7.2), 1.3 (m, 2H), 1.9 (m, 2H), 4.5 (t, 2H, J = 7.2), 6.7 (m, 1H), 7.4 (m, 1H), 8.0 (m, 1H), 8.6 (s, 1H), 9.6 (s, 1H). Anal. ($C_{14}H_{14}N_6O$) C, H, N.

7-Isopentyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8b): yield 60%, white solid; mp 165−166 °C (EtOAc−light petroleum); IR (KBr) 1620, 1500, 1430 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0 (d, 6H, J = 6.2), 1.18−1.7 (m, 3H), 4.6 (t, 2H, J = 7.4), 6.6 (m, 1H), 7.3 (m, 1H), 7.7 (m, 1H), 8.8 (s, 1H), 9.1 (s, 1H). Anal. ($C_{15}H_{16}N_6O$) C, H, N.

7-(*β*-Phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidine (8c): yield 20%; mp 174−175 °C (EtOH); IR (KBr) 1660, 1510, 1450 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.23 (t, 2H), 4.74 (t, 2H), 6.75 (s, 1H), 7.14−7.17 (m, 5H), 7.28 (s, 1H), 7.98 (s, 1H), 8.53 (s, 1H), 9.56 (s, 1H). Anal. ($C_{18}H_{14}N_6O$) C, H, N.

8-*n*-Butyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8e): yield 80%, pale yellow solid; mp 245−247 °C (MeOH); IR (KBr) 1610, 1500 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.9 (m, 3H), 1.3 (m, 2H), 1.9 (m, 2H), 4.5 (t, 2H, J = 7.2), 6.2 (m, 1H), 7.3 (m, 1H), 8.0 (m, 1H), 8.9 (s, 1H), 9.4 (s, 1H), 2D NMR (NOESY) N-CH_2 signal (4.5 ppm) shows cross-peak with C9-H signal (8.9 ppm). Anal. ($C_{14}H_{14}N_6O$) C, H, N.

8-Isopentyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8f): yield 67%,pale yellow solid; mp 235−237 °C (MeOH); IR (KBr) 1635, 1510, 1450 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.0 (d, 6H, J = 6.2), 1.5−1.9 (m, 3H), 4.6 (t, 2H, J = 7.4),

6.6 (m, 1H), 7.3 (m, 1H), 7.7 (m, 1H), 8.8 (s, 1H), 9.1 (s, 1H). Anal. ($C_{15}H_{16}N_6O$) C, H, N.

8-(β-Phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8g): yield 60%; mp 268−270 °C (EtOAc−light petroleum); IR (KBr) 1660, 1510, 1450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.32 (t, 2H, $J = 6.7$), 4.72 (t, 2H, $J = 6.7$), 6.73 (s, 1H), 7.23 (m, 5H), 7.95 (s, 1H), 8.8 (s, 1H), 9.41 (s, 1H). Anal. ($C_{18}H_{14}N_6O$) C, H, N.

7-(β-Hydroxyethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8j). This product can also be obtained starting from compound **8p** with the following procedures. To a saturated methanolic solution of ammonia (15 mL) was added at 0 °C compound **8p** (0.5 g, 1.6 mmol). The resulting mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo, and the residue was purified by chromatography (EtOAc/light petroleum, 1:1) to afford the product as a pale yellow solid (0.3 g, 70%): mp 216−217 °C dec; IR (KBr) 3500−3100, 1645, 1450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.89 (m, 2H), 4.94 (bs, 1H), 6.75 (m, 1H), 7.30 (d, 1H, $J = 3.4$), 7.98 (s, 1H), 8.53 (s, 1H), 9.62 (s, 1H). Anal. ($C_{12}H_{10}N_6O_2$) C, H, N.

7-*tert*-Butyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidine (8k): yield 63%, pale yellow solid; mp 162−163 °C (EtOAc); IR (KBr) 1645, 1520, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.87 (s, 9H), 6.62 (m, 1H), 7.28 (d, 1H, $J = 4.2$), 7.65 (d, 1H, $J = 1.2$), 8.35 (s, 1H), 9.09 (s, 1H). Anal. ($C_{14}H_{14}N_6O$) C, H, N.

7-(3-Phenylpropyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8l): yield 68%, pale yellow solid; mp 155 °C (EtOAc); IR (KBr) 1650, 1520, 1445 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.3−2.5 (m, 2H), 2.68 (t, 2H, $J = 8$), 4.58 (t, 2H, $J = 8$), 6.61 (m, 1H), 7.16−7.29 (m, 6H), 7.65 (s, 1H), 8.38 (s, 1H), 9.1 (s, 1H). Anal. ($C_{19}H_{16}N_6O$) C, H, N.

7-(4-Phenylbutyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8m): yield 65%, pale yellow solid; mp 156−158 °C (EtOAc); IR (KBr) 1660, 1520, 1455 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.64−1.68 (m, 2H), 1.99−2.07 (m, 2H), 2.66 (t, 2H, $J = 8$), 4.56 (t, 2H, $J = 8$), 6.58−6.61 (m, 1H), 7.11−7.29 (m, 6H), 7.64 (s, 1H), 8.38 (s, 1H), 9.09 (s, 1H). Anal. ($C_{20}H_{18}N_6O$) C, H, N.

7-(β-Morpholin-4-ylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8n): yield 67%, yellow solid; mp 202−203 °C (EtOAc); IR (KBr) 1660, 1510, 1440 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (t, 4H, $J = 4$), 2.94 (t, 2H, $J = 6$), 3.6 (t, 4H, $J = 4$), 4.66 (t, 2H, $J = 6$), 6.60 (bs, 1H), 7.27 (d, 1H, $J = 4$), 7.65 (s, 1H), 8.37 (s, 1H), 9.11 (s, 1H). Anal. ($C_{16}H_{17}N_7O_2$) C, H, N.

7-[β-(Benzyloxy)ethyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8o). To a suspension of NaH (38 mg, 80% in oil, 1.2 equiv) in dry DMF (20 mL) at 0 °C was added **8j** (0.3 g, 1.1 mmol), and the mixture was allowed to warm at room temperature. When the solution was clear, it was cooled at 0 °C again, benzyl bromide (0.2 mL, 1.2 equiv) was added, and the solution was stirred at room temperature for 18 h. Then the solvent was removed, and the crude oily compound **8o** was used for the next step without any further purifications. An analytical sample was purified by flash chromatography (AcOEt): IR (neat) 1660, 1450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.48 (m, 2H), 4.59 (m, 2H), 5.61 (s, 2H), 6.71 (m, 1H), 7.05 (d, 1H, $J = 3.5$), 7.23 (s, 1H), 7.15−7.48 (m, 5H), 7.9 (s, 1H), 8.5 (s, 1H). Anal. ($C_{19}H_{16}N_6O_2$) C, H, N.

7-[β-(Acetyloxy)ethyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (8p): yield 76%, yellow solid; mp 174−176 °C dec (EtOAc−light petroleum); IR (KBr) 1735, 1645, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.98 (s, 3H), 4.60 (t, 2H, $J = 5.3$), 4.80 (t, 2H, $J = 5.3$), 6.62 (m, 1H), 7.29 (d, 1H, $J = 4.3$), 7.66 (d, 1H, $J = 1.1$), 8.41 (s, 1H), 9.12 (s, 1H). Anal. ($C_{14}H_{12}N_6O_3$) C, H, N.

General Procedures for the Preparation of N-Substituted-4-[3-(2-furyl)-1,2,4-triazol-5-yl]-5-aminopyrazoles 9a−c,h−o and N-Substituted-4-[3-(2-furyl)-1,2,4-triazol-5-yl]-3-aminopyrazoles 9d−g. A solution of the mixture of **8a−o** (10 mmol) in aqueous 10% HCl (50 mL) was refluxed for 3 h. Then the solution was cooled and basified with concentrated ammonium hydroxide at 0 °C. The compounds were extracted with EtOAc (3 × 20 mL); the organic layers were dried with Na_2SO_4 and evaporated under vacuum. The residue was purified by chromatography (EtOAc/light petroleum, 2:1) to afford the desired compound as a solid. The following spectral data are reported as examples.

5-Amino-1-(β-phenylethyl)-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9c): yield 75%, yellow solid; mp 175−176 °C (EtOH); IR (KBr) 3350−3150, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.15 (t, 2H, $J = 6.5$), 4.48 (t, 2H, $J = 6.5$), 5.78 (s, 1H), 6.37 (s, 1H), 7.1 (s, 1H), 7.27−7.28 (m, 5H), 7.82 (s, 1H), 14.51 (bs, 2H). Anal. ($C_{17}H_{16}N_6O$) C, H, N.

3-Amino-1-(β-phenylethyl)-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9g): yield 80%, yellow solid; mp 205−206 °C (EtOH); IR (KBr) 3350−3150, 1625 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.12 (t, 2H, $J = 6.5$), 4.46 (t, 2H, $J = 6.5$), 5.75(s, 1H), 6.34 (s, 1H), 6.63 (s, 1H), 7.01 (s, 1H), 7.21−7.27 (m, 5H), 7.79 (s, 1H), 14.41 (bs, 2H). Anal. ($C_{17}H_{16}N_6O$) C, H, N.

5-Amino-1-methyl-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9h): yield 68%, yellow solid; mp 130−131 °C (EtOAc−light petroleum); IR (KBr) 3280−3150, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.60 (s, 3H), 6.16 (bs, 2H), 6.62 (s, 1H), 6.96 (s, 1H), 7.63 (s, 1H), 7.77 (s, 1H), 13.81 (bs, 1H). Anal. ($C_{10}H_{10}N_6O$) C, H, N.

5-Amino-1-phenyl-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9i): yield 70%, yellow solid; mp 185−186 °C (EtOAc); IR (KBr) 3200−3100, 1640, 1470 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.60 (bs, 2H), 6.65 (m, 1H), 6.70 (bs, 1H), 7.20 (d, 1H, $J = 3.4$), 7.4−7.6 (m, 5H), 7.78 (s, 1H), 7.9 (bs, 1H). Anal. ($C_{15}H_{12}N_6O$) C, H, N.

5-Amino-1-(β-hydroxyethyl)-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9j): yield 78%, yellow solid; mp 218−220 °C (EtOAc); IR (KBr) 3480−3100, 1630, 1450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.20 (bs, 1H), 3.80 (t, 2H, $J = 5.3$), 4.07 (t, 2H, $J = 5.3$), 5.99 (bs, 2H), 6.54 (m, 1H), 6.93 (d, 1H, $J = 4.2$), 7.61 (d, 1H, $J = 1.2$), 7.72 (s, 1H), 13.6 (bs, 1H). Anal. ($C_{11}H_{12}N_6O_2$) C, H, N.

5-Amino-1-*tert*-butyl-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9k): yield 75%, yellow solid; mp 148−150 °C (MeOH); IR (KBr) 3160, 1600, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.6 (m, 9H), 6.1 (s, 1H), 6.6 (s, 1H), 6.9 (m, 1H), 7.7 (s, 1H), 7.8 (s, 1H), 13.9 (bs, 1H). Anal. ($C_{13}H_{16}N_6O$) C, H, N.

5-Amino-1-(3-phenylpropyl)-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9l): yield 78%, pale yellow solid; mp 138−140 °C (EtOH); IR (KBr) 3150, 1610, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04−2.15 (m, 2H), 2.59 (t, 2H, $J = 8$), 3.89 (t, 2H, $J = 8$), 5.13 (bs, 2H), 6.44 (bs, 1H), 6.93 (d, 1H, $J = 2$), 7.09−7.26 (m, 5H), 7.42 (s, 1H), 7.73 (s, 1H), 13.9 (bs, 1H). Anal. ($C_{18}H_{18}N_6O$) C, H, N.

5-Amino-1-(4-phenylbutyl)-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9m): yield 82%, pale yellow oil; IR (neat) 3140, 1600, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.6−1.8 (m, 2H), 1.81−2.0 (m, 2H), 2.64 (t, 2H, $J = 7$), 3.95 (t, 2H, $J = 7$), 5.1 (bs, 2H), 6.55 (d, 1H, $J = 2$), 7.21−7.26 (m, 6H), 7.49 (s, 1H), 7.74 (s, 1H), 9.06 (bs, 1H). Anal. ($C_{19}H_{20}N_6O$) C, H, N.

5-Amino-1-(β-morpholin-4-ylethyl)-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9n): yield 76%, dark yellow oil; IR (neat) 3150, 1620, 1465 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (t, 4H, $J = 4$), 2.70−2.75 (m, 2H), 3.71 (t, 4H, $J = 4$), 4.08−4.17 (m, 2H), 6.33 (bs, 2H), 6.49 (dd, 1H, $J = 2, 4$), 6.99 (d, 2H, $J = 4$), 7.43 (s, 1H), 7.49 (d, 1H, $J = 2$), 7.7 (bs, 1H). Anal. ($C_{15}H_{19}N_7O_2$) C, H, N.

5-Amino-1-[β-(benzyloxy)ethyl]-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazole (9o): yield 70%, yellow solid; mp 167−168 °C (EtOAc); IR (KBr) 3350−3150, 1625, 1500 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.69 (t, 2H, $J = 5.3$), 4.00 (t, 2H, $J = 5.3$), 5.02 (m, 2H), 5.62 (s, 2H), 6.43 (bs, 1H), 6.63 (m, 1H), 7.03 (d, 1H, $J = 4.2$), 7.15−7.35 (m, 6H), 7.8 (s, 1H). Anal. ($C_{18}H_{18}N_6O_2$) C, H, N.

General Procedures for the Preparation of 5-Amino-7(or 8)-substituted-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines 10a−o. To a solution of pyrazole derivatives **9a−o** (10 mmol) in *N*-methylpyrrolidone (40 mL) were added cyanamide (0.42 g, 60 mmol) and *p*-toluenesulfonic acid (2.85 g, 15 mmol), and the mixture was heated at 160 °C for 4 h. Then cyanamide (0.42 g, 60 mmol) was added again, and the solution was heated overnight. Then the solution was diluted with EtOAc (80 mL), and the precipitate (excess of

Table 2. Characterization of Pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidine Derivatives **10a**−**o**,**q**−**r**

compd	yield (%)	mp (°C)	crystallization solvent	MW	formula	anal.
10a	35	157−158	EtOH	297.32	$C_{14}H_{15}N_7O$	C, H, N
10b	50	208−210	EtOH	311.34	$C_{15}H_{17}N_7O$	C, H, N
10c	20	225−226	EtOAc	345.36	$C_{18}H_{15}N_7O$	C, H, N
10d	75	207−210	EtOAc	401.47	$C_{22}H_{23}N_7O$	C, H, N
10e	47	200−203	EtOH	311.34	$C_{15}H_{17}N_7O$	C, H, N
10f	60	212−213	EtOAc	345.36	$C_{18}H_{15}N_7O$	C, H, N
10g	45	183−185	EtOH	297.32	$C_{14}H_{15}N_7O$	C, H, N
10h	30	210−213	EtOH	255.24	$C_{11}H_9N_7O$	C, H, N
10i	42	295−297	EtOH	317.31	$C_{16}H_{11}N_7O$	C, H, N
10j	75	258−260	EtOAc	285.26	$C_{12}H_{11}N_7O_2$	C, H, N
10k	45	238−240	EtOH	297.32	$C_{14}H_{15}N_7O$	C, H, N
10l	65	195−196	EtOH	359.39	$C_{19}H_{17}N_7O$	C, H, N
10m	62	205−208	EtOH	373.42	$C_{20}H_{19}N_7O$	C, H, N
10n	59	260−262	EtOH	354.37	$C_{16}H_{18}N_8O_2$	C, H, N
10o	30	144−145	EtOAc	375.39	$C_{19}H_{17}N_7O_2$	C, H, N
10q	98	248−250	MeOH	241.21	$C_{10}H_7N_7O$	C, H, N
10r	80	187−190	EtOAc	375.39	$C_{19}H_{17}N_7O_2$	C, H, N

cyanamide) was filtered off; the filtrate was concentrated under reduced pressure and washed with water (3 × 30 mL). The organic layer was dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by chromatography (EtOAc/light petroleum, 4:1) to afford the final products **10a**−**o** as solids (Table 2).

5-Amino-7-*n*-butyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10a): white solid; IR (KBr) 3500−3000, 1670, 1640, 1620, 1550, 1440 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.9 (t, 3H), 1.5 (m, 2H), 1.9 (m, 2H), 4.2 (t, 2H), 6.7 (m, 1H), 7.2 (m, 2H), 7.9 (m, 1H), 8.0 (s, 1H), 8.1 (s, 1H).

5-Amino-7-isopentyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10b): white solid; IR (KBr) 3400−2950, 1670, 1645, 1620, 1550, 1440 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.97 (d, 6H, J = 6.4), 1.55 (m, 1H), 1.80 (m, 2H), 4.36 (t, 2H, J = 6.4), 6.1 (bs, 2H), 6.6 (s, 1H), 7.27 (d, 1H, J = 4.2), 7.64 (s, 1H), 8.20 (s, 1H).

5-Amino-7-(*β*-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10c): white solid; IR (KBr) 3500−2950, 1670, 1645, 1620, 1560, 1470 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.21 (t, 2H, J = 6.2), 4.51 (t, 2H, J = 6.2), 6.65 (s, 1H), 7.1−7.44 (m, 6H), 7.78 (s, 1H), 7.89 (bs, 1H), 8.07 (s, 1H).

5-Amino-7-[*β*-(4-isobutylphenyl)ethyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10d): white solid; IR (KBr) 3520−2910, 1650, 1640, 1610, 1555, 1440 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.9 (d, 6H, J = 6), 1.39 (d, 2H, J = 8), 1.65−1.95 (m, 1H), 2.5 (t, 2H, J = 6), 4.07 (t, 2H, J = 6), 6.72 (m, 1H), 6.96 (d, 2H, J = 9), 7.21 (d, 1H, J = 4.2), 7.25 (d, 2H, J = 9), 7.94 (bs, 2H), 8.12 (bs, 1H), 8.13 (s, 1H).

5-Amino-8-*n*-butyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10e): white solid; IR (KBr) 3500−2900, 1685, 1640, 1620, 1550, 1450 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.9 (t, 3H), 1.2 (m, 2H), 1.8 (m, 2H), 4.2 (t, 2H), 6.7 (m, 1H), 7.2 (m, 2H), 7.6 (s, 1H), 8.0 (s, 1H), 8.6 (s, 1H).

5-Amino-8-isopentyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10f): white solid; IR (KBr) 3500−2850, 1670, 1650, 1615, 1560, 1455 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H, J = 6.4), 1.59 (m, 1H), 1.86 (m, 2H), 4.32 (t, 2H, J = 6.4), 6.58 (m, 1H), 6.72 (bs, 2H), 7.21 (d, 1H, J = 4.2), 7.63 (d, 1H, J = 1.2), 8.10 (s, 1H).

5-Amino-8-(*β*-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10g): white solid; IR (KBr) 3500−2900, 1670, 1645, 1620, 1530, 1455 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.21 (t, 2H, J = 6.4), 4.53 (t, 2H, J = 6.4), 6.7 (s, 1H), 7.1−7.4 (m, 6H), 7.65 (bs, 1H), 7.93 (s, 1H), 8.45 (s, 1H).

5-Amino-7-methyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10h): white solid; IR (KBr) 3500−2900, 1670, 1640, 1620, 1550, 1440 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.88 (s, 3H), 6.72 (m, 1H), 7.21 (d, 1H, J = 4.2), 7.94 (bs, 2H), 8.12 (bs, 1H), 8.13 (s, 1H).

5-Amino-7-phenyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10i): white solid; IR (KBr) 3500−2950, 1660, 1650, 1620, 1550, 1455 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.74 (m, 1H), 7.25 (d, 1H, J = 4.2), 7.36−7.4 (m, 1H), 7.51−7.59 (m, 2H), 7.96 (s, 1H), 8.13−8.17 (m, 2H), 8.28 (bs, 2H), 8.44 (s, 1H).

5-Amino-7-(*β*-hydroxyethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10j): white solid; IR (KBr) 3500−2900, 1670, 1640, 1620, 1550, 1455 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.82 (m, 2H), 4.28 (t, 2H, J = 5.2), 4.9 (bs, 1H), 6.73 (m, 1H), 7.22 (d, 1H, J = 4.2), 7.94 (s, 1H), 8.08 (bs, 2H), 8.16 (s, 1H).

5-Amino-7-*tert*-butyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10k): white solid; IR (KBr) 3510−2960, 1670, 1635, 1625, 1560, 1470 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 9H), 6.65 (m, 1H), 7.2 (d, 1H, J = 4.2), 7.82 (bs, 3H), 8.0 (s, 1H).

5-Amino-7-(3-phenylpropyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10l): white solid; IR (KBr) 3500−2950, 1650, 1640, 1625, 1550, 1455 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.15−2.45 (m, 2H), 2.66 (t, 2H, J = 8), 4.37 (t, 2H, J = 8), 6.14 (bs, 2H), 6.60 (dd, 1H, J = 2, 4.2), 7.15−7.27 (m, 6H), 7.63 (s, 1H), 8.2 (s, 1H).

5-Amino-7-(4-phenylbutyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10m): white solid; IR (KBr) 3520−2950, 1655, 1630, 1615, 1550, 1445 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.45−1.6 (m, 2H), 1.7−1.9 (m, 2H), 2.59 (t, 2H, J = 8), 4.27 (t, 2H, J = 8), 6.72 (dd, 1H, J = 2, 4), 7.13−7.23 (m, 6H), 7.94 (s, 1H), 8.09 (bs, 2H), 8.15 (s, 1H).

5-Amino-7-(*β*-morpholin-4-ylethyl)-2-(2-furyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10n): yellow solid; IR (KBr) 3520−2940, 1665, 1640, 1610, 1540, 1445 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.45 (t, 4H, J = 5), 2.75 (t, 2H, J = 7), 3.5 (t, 4H, J = 5), 4.37 (t, 2H, J = 7), 6.74 (dd, 1H, J = 2, 4), 7.22 (d, 1H, J = 4), 7.95 (s, 1H), 7.21 (d, 1H, J = 4), 8.1 (bs, 2H), 8.16 (s, 1H).

5-Amino-7-[*β*-(benzyloxy)ethyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10o): pale yellow solid; IR (KBr) 3500−2800, 1660, 1645, 1620, 1560, 1455 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.95 (t, 2H, J = 5.2), 4.07 (t, 2H, J = 5.2), 5.32 (bs, 2H), 5.62 (s, 2H), 6.52 (m, 1H), 6.98 (d, 1H, J = 4.2), 7.2−7.4 (m, 5H), 7.55 (d, 1H, J = 1.2), 7.82 (s, 1H).

5-Amino-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine (10q). A solution of **10k** (297 mg, 1 mmol) in formic acid (98%, 15 mL) was heated at 100 °C for 4 h. Then the solvent was removed at reduced pressure and the residue purified by chromatography (EtOAc/MeOH, 8:2) to afford **10q** as a yellow solid in quantitative yield: IR (KBr) 3500−2850, 1680, 1655, 1620, 1560, 1455 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.63 (m, 1H), 7.19 (d, 1H, J = 4.2), 7.58 (bs, 1H), 7.70 (d, 1H, J = 1.2), 8.07 (s, 1H), 13.2 (bs, 1H).

5-(Benzylamino)-7-(*β*-hydroxyethyl)-2-(2-furyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (10r). To a suspension of NaH (32 mg, 80% in oil, 1.2 equiv) in dry DMF (20 mL) at 0 °C was added **10j** (0.3 g, 1.05 mmol), and the mixture was allowed to warm to room temperature. When the solution was clear, it was cooled at 0 °C again, benzyl bromide (0.2 mL, 1.2 equiv) was added, and the solution was stirred at room temperature for 18 h. Then the solvent was removed, and the residue was purified by chromatography to afford compound **10r** as a yellow solid (315 mg, 80%): IR (KBr) 3500−2950, 1675, 1655, 1620, 1560, 1455 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.8 (m, 2H), 4.33 (t, 2H, J = 6.2), 4.71 (s, 2H), 4.87 (bs, 1H), 6.73 (m, 1H), 7.1−7.5 (m, 6H), 7.95 (s, 1H), 8.16 (s, 1H), 9.0 (bs, 1H).

Biological Assays: Receptor Binding Assay. The rat brain tissues (whole brain and striatum) were obtained from male Sprague−Dawley rats (Charles-River, Calco, Italy) weighing 150−200 g. A_1 and A_{2A} adenosine receptor binding assays were performed according to Bruns et al.[30] and Jarvis et al.[31] using [^{3}H]-N^6-cyclohexyladenosine ([^{3}H]CHA) and [^{3}H]-2-[[4-(2-carboxyethyl)phenethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine ([^{3}H]CGS 21680) as radioligands, respectively. The IC_{50} values were calculated by probit analysis based on at least six concentrations of each compound. K_i values were calculated from the Cheng−Prushoff[32] equation using 1.0 and 18.5 nM as K_d values in A_1 and A_{2A} binding assays, respectively.

Platelet Aggregation Assay: Antagonism to A_{2A}-mediated antiaggregatory activity was tested in rabbit platelets. Plate-

let aggregation was performed according to the Born turbidimetric technique[33] using a DIC PA-3220 Aggrecorder (Kyoto Dalichi Kagaku Ltd., Japan). Adenosine diphosphate (5 μM) was used as the aggregatory agent.

Citrated blood from New Zealand white male rabbits (Bettinardi Farm, Alzate, Novara, Italy), weighing 3−4 kg, was collected from the left carotid artery during pentobarbital anesthesia (20 mg iv). Platelet-rich plasma preparation and the platelet aggregation assay were performed as previously described.[16] The maximal amplitude of aggregation, recorded 3 min after the addition of ADP, was used for the quantitative evaluation of the aggregation process. Three different concentrations of the test compounds were assayed in duplicate on at least four different animals. The antagonist dose reducing by 50% (ED_{50}) the effect induced by NECA, used at a concentration (0.3−1.0 μM) close to the IC_{50} value, was evaluated by linear regression analysis.

References

(1) Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. Nomenclature and classification of purinoceptors. *Pharmacol. Rev.* **1994**, *46*, 143−156.

(2) (a) Muller, C. E.; Scior, T. Adenosine receptors and their modulators. *Pharm. Acta Helv.* **1993**, *68*, 77−111. (b) Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borioni, A.; Viziano, M.; Dionisotti, S.; Ongini, E. Current developments of A_{2A} adenosine receptor antagonists. *Curr. Med. Chem.* **1995**, *2*, 707−722.

(3) Van Galen, P. J. M.; Stiles, G. L.; Michaels, G. S.; Jacobson, K. A. Adenosine A_1 and A_2 receptors: structure function relationships. *Med. Res. Rev.* **1992**, *12*, 423−471.

(4) Kim, H. O.; Ji, X.; Melman, N.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. Structure-activity relationship of 1,3-dialkylxanthine derivatives at rat A_3 adenosine receptors. *J. Med. Chem.* **1994**, *37*, 3373−3382.

(5) Bruns, R. F.; Fergus, J. K.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. Binding of the A_1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335*, 59−63.

(6) (a) Shimada, J.; Suzuki, K.; Nonaka, H.; Ishii, A.; Ichikawa, S. (E)-1,3-dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthines: potent and selective adenosine A_2 antagonists. *J. Med. Chem.* **1992**, *35*, 2342−2345. (b) Del Giudice, M. R.; Borioni, A.; Mustazza, C.; Gatta, F.; Dionisotti, S.; Zocchi, C.; Ongini, E. (E)-1-(Heterocyclyl or cyclohexyl)-2-[1,3,7-trisubstituted-(xanthin-8-yl)]ethenes as A_{2A} adenosine receptor antagonists. *Eur. J. Med. Chem.* **1996**, in press.

(7) Jacobson, K. A.; Gallo-Rodriguez, C.; Melman, N.; Fischer, B.; Maillard, M.; van Bergen, A.; van Galen, P. J. M.; Karton, Y. Structure-activity relationship of 8-styrylxanthines as A_2-selective antagonists. *J. Med. Chem.* **1993**, *36*, 1333−1342.

(8) Nonaka, H.; Ichimura, M.; Takeda, M.; Nonaka, Y.; Shimada, J.; Suzuki, F.; Yamaguchi, K.; Kase, H. KF 17837 ((E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine), a potent and selective adenosine A_2 receptor antagonist. *Eur. J. Pharmacol.* **1994**, *267*, 335−341.

(9) Jacobson, K. A.; Nikodijevic', O.; Padgett, W. L.; Gallo-Rodriguez, C.; Maillard, M.; Daly, J. W. 8-(3-Chlorostyryl)caffeine (CSC) is a selective A_2-adenosine antagonist in vitro and in vivo. *FEBS Lett.* **1993**, *323*, 141−144.

(10) Jackson, E. K.; Herzer, W. A.; Suzuki, F. KF17837 is an A_2 adenosine receptor antagonist in vivo. *J. Pharmacol. Exp. Ther.* **1993**, *267*, 1304−1310.

(11) Kanda, T.; Shiozaki, S.; Shimada, J.; Suzuki, F.; Nakamura, J. KF 17837: a novel selective adenosine A_{2A} receptor antagonist with anticataleptic activity. *Eur. J. Pharmacol.* **1994**, *256*, 263−268.

(12) Nonaka, Y.; Shimada, J.; Nonaka, H.; Koike, N.; Aoki, N.; Kobayashi, H.; Kase, H.; Yamaguchi, K.; Suzuki, F. Photoisomerization of a potent and selective adenosine A_2 antagonist, (E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine. *J. Med. Chem.* **1993**, *36*, 3731−3733.

(13) Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L. Structure-activity profile of a series of novel triazolo-quinazoline adenosine antagonists. *J. Med. Chem.* **1988**, *31*, 1014−1020.

(14) Sarges, R.; Howard, H. R.; Browne, R. G.; Lebel, L. A.; Seymour, P. A.; Koe, B. K. 4-Amino[1,2,4]triazolo[4,3-a]quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid on set antidepressants. *J. Med. Chem.* **1990**, *33*, 2240−2254.

(15) Gatta, F.; Del Giudice, M. R.; Borioni, A.; Borea, P. A.; Dionisotti, S.; Ongini, E. Synthesis of imidazo[1,2-c]pyrazolo[4,3-e] pyrimidines, pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidines and triazolo[5,1-i]purines: new potent A_2 adenosine receptor antagonists. *Eur. J. Med. Chem.* **1993**, *28*, 569−577.

(16) Dionisotti, S.; Conti, A.; Sandoli, D.; Zocchi, C.; Gatta, F.; Ongini, E. Effects of the new A_2 adenosine receptor antagonist 8FB-PTP, an 8-substituted pyrazolo-triazolo-pyrimidine, on in vitro functional models. *Br. J. Pharmacol.* **1994**, *112*, 659−665.

(17) Baraldi, P. G.; Manfredini, S.; Simoni, D.; Zappaterra, L.; Zocchi, C.; Dionisotti, S.; Ongini, E. Synthesis and activity of new pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine and 1,2,3-triazolo-[4,5-e]1,2,4-triazolo[1,5-c]pyrimidine displaying potent and selective activity as A_{2A} adenosine receptor antagonists. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2539−2544.

(18) Topliss, J. G. Utilization of operational schemes for analog synthesis in drug design. *J. Med. Chem.* **1972**, *15*, 1006−1015.

(19) Zocchi, C.; Ongini, E.; Conti, A.; Monopoli, A.; Negretti, A.; Baraldi, P. G.; Dionisotti, S. The non-xanthine heterocycle SCH 58261 is a new potent and selective A_{2A} adenosine receptor antagonist. *J. Pharmacol. Exp. Ther.* **1996**, in press.

(20) Seidlova, V.; Vejdelek, Z. J.; Rajsner, M.; Jirkovsky, I.; Protiva, M. Neurotropic and psychotic substances. XXXVI. Cycloalkylhydrazines and aralkylhydrazines. *Cesk. Farm.* **1969**, *18*, 190−197.

(21) Bruzzese, T.; Casadio, S.; Coppi, G.; Marazzi-Uberti, E. Synthesis and pharmacological screening of aminoalkyl-hydrazines. *J. Pharm. Sci.* **1965**, *7*, 1056−1057.

(22) Cheng, C. C.; Robins, R. K. Potential purine antagonists. VI. Synthesis of 1-aryl-4-substituted pyrazolo[3,4-*d*]pyridines. *J. Org. Chem.* **1956**, *21*, 1240−125.

(23) Vicentini, C. B.; Veronese, C. A.; Guccione, S.; Guarneri, M.; Manfrini, M.; Giori, P. An efficient procedure for the synthesis of pyrazolo[3,4-d][1,3]thiazin-4-ones. *Heterocycles* **1993**, *36*, 2291−2301.

(24) Jones, G. Azole derivatives as adenosine antagonist. WO 9414812, 1994.

(25) Poucher, S. M.; Keddie, J. R.; Singh, P.; Stoggall, S. M.; Caulkett, P. W. R.; Jones, G.; Collis, M. G. The in vitro pharmacology of ZM 241385, a potent, non-xanthine, A_{2A} selective adenosine receptor antagonist. *Br. J. Pharmacol.* **1995**, *115*, 1096−1102.

(26) (a) Monopoli, A.; Casati, C.; Forlani, A.; Marzanatti, M.; Ongini, E. The in vivo cardiovascular pharmacology of the selective A_{2A} adenosine antagonist SCH 58261 in the rat and rabbit. Proceeding of British Pharmacology Society, Brighton, Dec. 12−15, 1995; p 152. (b) Bertorelli, R.; Ferri, N.; Adami, M.; Ongini, E. Effects of selective agonists and antagonists for A_1 or A_{2A} adenosine receptors on the sleep-waking cycle in rats. Submitted for publication.

(27) Zocchi, C.; Ongini, E.; Ferrara, S.; Baraldi, P. G.; Dionisotti, S. Binding of the radioligand [^{3}H]-SCH 58261, a new non-xanthine A_{2A} adenosine receptor antagonist, to rat striatal membranes. *Br. J. Pharmacol.* **1996**, in press.

(28) Fuxe, K.; Ferré, S.; Snaprud, P.; Von Euler, G.; Johansson, B.; Fredholm, B. Antagonistic A_{2A}/D_2 receptor interactions in the central nervous system. *Drug Dev. Res.* **1993**, *28*, 374−380.

(29) Freedholm, B. B. Adenosine, adenosine receptors and the actions of caffeine. *Pharmacol. Toxicol.* **1995**, *76*, 93−101.

(30) Bruns, R. F.; Daly, J. W.; Snyder, S. H. Adenosine receptors in brain membranes: binding of N -cyclohexyl [^{3}H] adenosine and 1,3-diethyl-8[^{3}H]phenylxanthine. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5547−5551.

(31) Jarvis, M. F.; Schulz, R.; Hutchison, A. J.; Do, U. H.; Sille, M. A.; Williams, M. [^{3}H]CGS 21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888−893.

(32) Cheng, Y. C.; Prushoff, H. R. Relationships between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(33) Born, G. V. R.; Cross, M. J. The aggregation of blood platelets. *J. Physiol.* **1963**, *168*, 178−195.

JM950746L