Design, Synthesis, and Biological Evaluation of C^9- and C^2-Substituted Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines as New A_{2A} and A_3 Adenosine Receptors Antagonists

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In the past few years, our group has been involved in the development of A_{2A} and A_3 adenosine receptor antagonists which led to the synthesis of SCH58261 (5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine, **61**), potent and very selective at the A_{2A} receptor subtype, and N^8-substituted-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines-N^5-urea or amide (MRE series, **b**), very selective at the human A_3 adenosine receptor subtype. We now describe a large series of C^9- and C^2-substituted pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines to represent an extension of structure−activity relationship work on this class of tricyclic compounds. The introduction of a substituent at 9 position of the tricyclic antagonistic structure led to retention of receptor affinity but a loss of selectivity in respect to the lead compounds **b**, N^8-substituted-pirazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines-N^5-urea or -amide. The substitution of the furanyl moiety of compound **61**, necessary for receptor binding, with a phenyl or a substituted aromatic ring (compounds **5a**−**d**, **6**−**8**), caused a complete loss of the affinity at all the adenosine receptor subtypes, demonstrating that the furanyl ring is a necessary structural element to guarantee interaction with the adenosine receptor surface. The introduction of an ethoxy group at the ortho position of the aromatic ring to mimic the oxygen of the furan (compound **5c**, 5-amino-7-(2-phenylethyl)-2-(2-ethoxyphenyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) did not enhance affinity. The introduction of the cycloaminomethyl function by Mannich reaction at the 5′ position of the furanyl ring of **61** and the C^9-substituted compound **41** (5-amino-8-methyl-9-methylsulfanyl-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) resulted in complete water solubility but a loss of receptor affinity. We can conclude that modifications or substitutions at the furanyl ring are not allowed and the introduction of a substituent at the 9-position of the core pyrazolo-triazolo-pyrimidine structure caused a severe loss of selectivity, probably due to an increased steric hindrance of the radical introduced.

Introduction

Adenosine, an endogenous modulator of a wide range of biological functions in the nervous, cardiovascular,[1a] 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 super family of G-protein-coupled receptors and have been cloned from several animal species (Fredholm et al., 2000).[1b]

In the past 10 years, great efforts by medicinal chemists and pharmacologists have been devoted to the design of potent and selective antagonists for A_{2A} and A_3 receptors. Thus, the pyrazolotriazolopyrimidines SCH 58261 (5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine, **61**), SCH 63390[2] (5-amino-7-(3-phenylpropyl)-2-(2-furyl)pyrazolo-[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine), and related compounds which possess hydrophilic groups at the para and ortho positions of the aromatic ring have been found to be potent and selective adenosine A_{2A} antagonists,[3] and SCH 58261 is widely used as a tool for characterizing the adenosine A_{2A} receptor subtype.[4,5] At the same time different classes of compounds have been reported to be selective A_3 receptor antagonists (eight classes with nonxanthine structure, including dihydropyridine and pyridine analogues, flavonoid, isoquinoline and triazoloquinazoline derivatives, triazolonaphthiridine and thiazolopyrimidine analogues).[6−13] The best results in terms of A_3-antagonism were obtained with the synthesis of 5-*N*-(substituted phenylcarbamoyl)amino-8-substituted-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidines, where the best substitution on the phenyl ring of the phenyl carbamoyl moiety was a methoxy at the para position or a chlorine atom at the meta position (MRE series).[14−17]

Starting from these observations, and on the basis of data derived from an enlarged series of SCH 58261 (**61**) analogues and MRE series analogues previously re-

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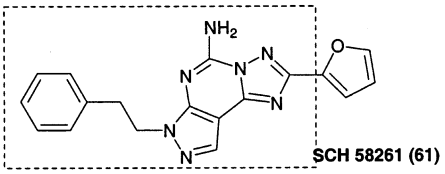
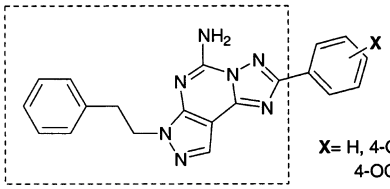
ported (compounds of general formula **b**), we synthesized new molecules structurally modified in different positions with respect to the lead compounds **61** and **b** structure in order to evaluate the change in affinity and selectivity of the new compounds obtained and provide a better understanding of the important features about the associated structure−activity relationships (SAR).

In particular, we investigated two positions of the tricyclic pyrazolotriazolopyrimidine structure: the 2- and the 9-position. For all the A_{2A} adenosine receptor antagonists, the furanyl group at the 2-position was shown to be important for the binding activity of the molecule. Substitution of this heterocycle with other heterocyclic rings, e.g., thiophene or tetrahydrofuranyl[18] led to a severe loss of affinity of the compound for this relevant adenosine receptor. We tried to introduce in the same position a phenyl or an aromatic ring substituted in the para position with suitable substitution groups with electron negative centers to interact with the adenosine receptors (e.g., halogens, free hydroxyl group, amide, and free carboxylic acid functions; compounds of general formula **a**, see below). The ortho position of the aromatic ring was also functionalized with an ethoxy group in an attempt to mimic the oxygen in the furanyl ring. Structure−activity relationship study was evaluated based on the lead compound **61** to appreciate the change in receptor affinity and selectivity.



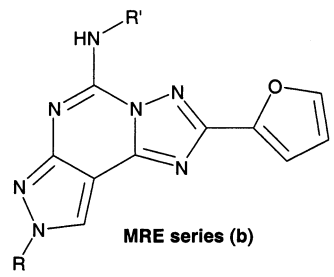
SCH 58261 (61)



X= H, 4-OH, 4-Cl, 2-OEt, 4-OCH_2CO_2Et, 4-OCH_2CO_2H, 4-$OCH_2CONHPh$-p-I

(a)

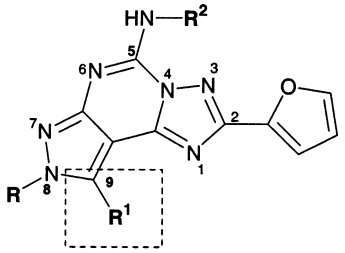
We have also investigated the effects of various substituents at the 9-position of the antagonist pyrazolotriazolo-pyrimidine structure. To evaluate the change in affinity and selectivity versus A_{2A} and A_3 adenosine receptor subtypes, all the other structural requirements necessary for antagonism and present in the lead compounds **61** and **b**, such as planar structure, alkyl or arylalkyl substituents on the pyrazole ring, free exocyclic amino group for A_{2A}-antagonists, and transformation of the free amino group into an amide or urea function for A_3-antagonists, were maintained.



MRE series (b)

R= alkyl, R'= (substituted)phenyl urea or amide

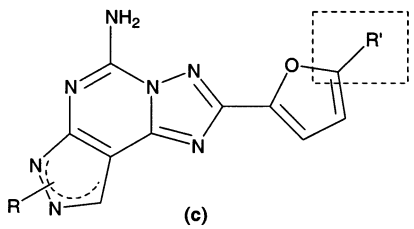
Table 1. C^9-Substituted Compounds Synthesized



compd	R	R^1	R^2
38	CH_3	$NHCH_2CH_3$	H
39	CH_3	NH-Ph-4-OMe	H
40	CH_3	*N*-Me-piperazine	H
41	CH_3	SCH_3	H
42	CH_3	$S(CH_2)_2CH_3$	H
43	$Ph(CH_2)_3$	SCH_3	H
44	CH_3	$NHCH_2CH_3$	CONHPh-4-OMe
45	CH_3	*N*-Me-piperazine	CONHPh-4-OMe
46	CH_3	SCH_3	CONHPh-4-OMe
47	CH_3	SCH_3	$COCH_2$Ph-4-OMe
48	CH_3	SCH_3	$COCH_2$Ph-4-isobutyl
49	CH_3	SCH_3	$COCH_2$Ph-3,4-Medioxy
50	CH_3	$NHCH_2CH_3$	$COCH_2$Ph-3,4-Medioxy
52	CH_3	NH−Ph-4-OH	H
53	CH_3	$NHCH_2CH_3$·HCl	H
54	CH_3	*N*-Me-piperazine · 2HCl	H
55	CH_3	$NHCH_2CH_3$·HCl	CONHPh-4-OMe

All new compounds synthesized are pyrazolotriazolopyrimidines, N^8-substituted principally with small alkyl groups. Compounds with substituents introduced at the 9 position were examined for steric hindrance and hydrophilic/lipophilic balance: they are cycloalkyl, alkyl, or amine functions bound to the tricyclic core by thioether or amine functions (Table 1). The principal aim of these innovative modifications was to evaluate the influence of these variations on receptor interaction. All compounds synthesized were evaluated in binding assays on all four adenosine receptor subtypes, paying particular attention to the results obtained from the interaction with A_{2A} and A_3 receptor subtypes.

The principal difficulty in evaluating the adenosine tricyclic antagonists previously reported by our group were their very lipophilic nature. Starting from these observations, we introduced, by Mannich reaction, another structural modification at the 5′-position of the furanyl ring of the lead compound **61** and C^9-substituted compound **41**, which showed the best results in terms of receptor affinity. The aim of this modification was to improve the water solubility of the new compounds



(c)

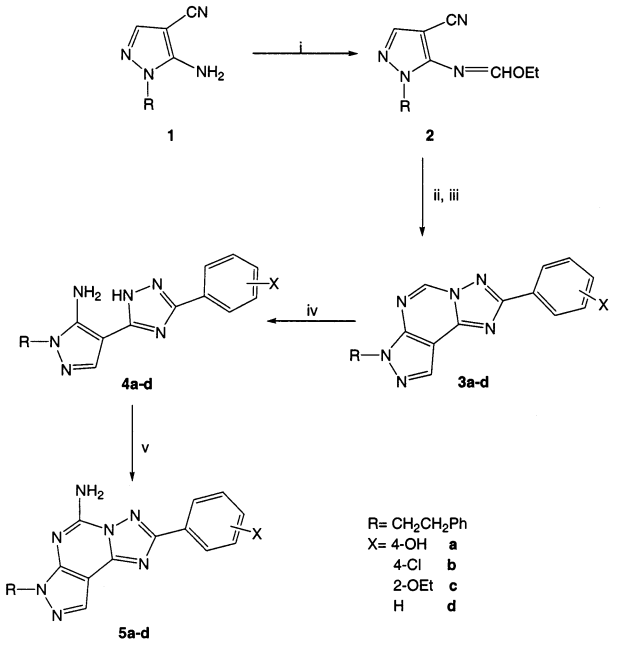
R= N^7 $(CH_2)_2Ph$, R'= H **61**

R= N^8 CH_3, R'= H **C^9-substituted compound 41**

R= N^7 $(CH_2)_2Ph$ or N^8 CH_3 **compounds 51, 57, 58**

R'= CH_2-*N*-methylpiperazine, CH_2-morpholine

Scheme 1[a]



[a] Reagents: (i) triethyl orthoformate, reflux; (ii) (substituted)-benzoic acid hydrazides, 2-methoxyethanol; (iii) Ph_2O, 260 °C; (iv) 10% HCl; (v) cyanamide, 1-methyl-2-pyrrolidone, *p*-TsOH, 140 °C.

obtained and to further evaluate the change in terms of affinity and selectivity of the molecules (compounds of general formula **c**). The amines used for this type of reaction were morpholine and *N*-methylpiperazine. Treatment with hydrochloric acid solution to form the corresponding salt was effected to increase the water solubility and to make pharmacological testing easier.

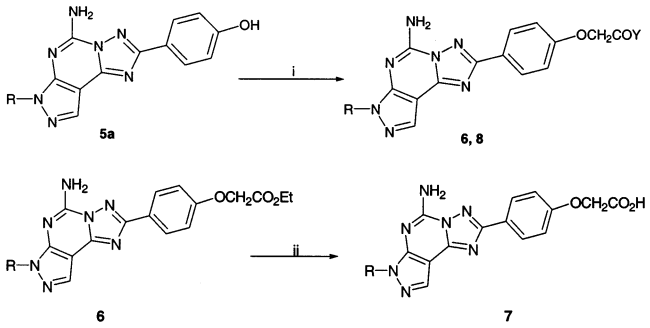
The purposes of this research effort are to better understand what structural modifications introduced on the tricyclic antagonist core pyrazolo-triazolo-pyrimidine play an important role on ligand−receptor interaction. In this way, we can determine what position of the heterocyclic structure should not be modified and, on the contrary, what position is susceptible to modifications or functionalizations, to develop new drugs which target A_{2A} and A_3 adenosine receptor subtypes.

Chemistry

The general synthesis of 2-aryl-pyrazolo-triazolo-pyrimidines is depicted in Scheme 1. The synthetic steps for the synthesis of this new class of compounds are the same utilized for the synthesis of **61** and analogues according to Gatta et al.,[19] except for the (substituted)-hydrazide utilized. The 4-cyano-5-amino-1-(2-phenylethyl)pyrazole[20] **1** was transformed into the corresponding imidate **2** by refluxing in triethyl orthoformate. The imidate was then reacted with benzoic acid hydrazide, 4-hydroxybenzoic acid hydrazide, 4-chlorobenzoic acid hydrazide (commercially available), or 2-ethoxybenzoic acid hydrazide[21] in refluxing 2-methoxyethanol to provide the pyrazolo[4,3-*e*]pyrimidine intermediates. The latter compounds were converted through a thermally induced cyclization in diphenyl ether to the tricyclic derivatives **3a**−**d** in good yield.

Treatment of **3a**−**d** with dilute hydrochloric acid induced pyrimidine ring opening to furnish the amines **4a**−**d** in quantitative yield. These derivatives were converted into the final compounds **5a**−**d** by reaction with an excess of cyanamide in 1-methyl-2-pyrrolidone at 140 °C.

Scheme 2[a]



[a] Reagents: (i) 2-chloroacetyl chloride, 2-chloro-*N*-(4-iodophenyl)acetamide, K_2CO_3, DMF, rt; (ii) 10% HCl, dioxane, 60 °C.

The tricyclic compound **5a** was functionalized at the phenolic hydroxyl group by treatment with 2-chloroacetyl chloride or 2-chloro-*N*-(4-iodophenyl)acetamide[22] in DMF to obtain the derivatives **6** and **8**, respectively. Finally, derivative **6** was converted into **7** by treatment with aqueous HCl in dioxane (Scheme 2).

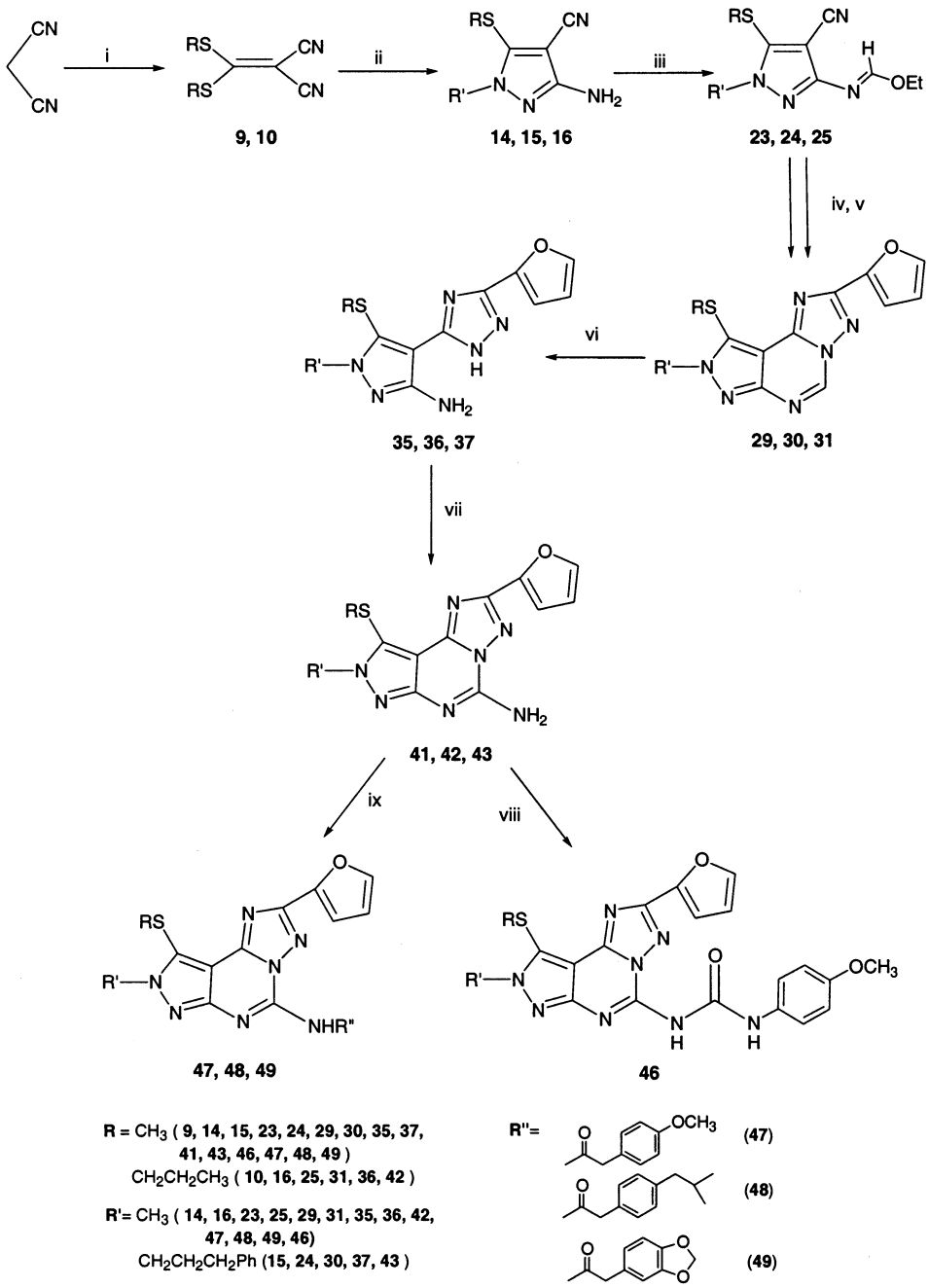
The synthesis of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine derivatives substituted at the 9-position is depicted in Schemes 3−6. The reaction between malononitrile, carbon disulfide, and methyl iodide in the presence of K_2CO_3 and DMF, gave the 2-(bis(methylsulfanyl)methylene)malononitrile **9**.[23] Reaction with methylhydrazine or 3-phenylpropylhydrazine in ethanol gave the amino cyanopyrazoles **14** and **15** (Scheme 3).

It's interesting to note that the only isomer obtained is the 3-amino-4-cyanopyrazole, confirmed by NOESY analyses.[23]

Transformation of pyrazoles **14** and **15** to the corresponding imidates **23** and **24** was achieved by refluxing in triethyl orthoformate according to the procedure of Gatta et al.[19] The imidates were reacted with 2-furoic acid hydrazide in refluxing 2-methoxyethanol to provide the pyrazolo[4,3-*e*]pyrimidine intermediates. The latter compounds were converted to the derivatives **29** and **30**, in good overall yield, through a thermal cyclization in diphenyl ether. Treatment of **29** and **30** 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 **35** and **37** in a very good yield. These derivatives were converted into the final compounds **41** and **43** by reaction with an excess of cyanamide in 1-methyl-2-pyrrolidone at 140 °C. The reaction between malononitrile, carbon disulfide, and 1-bromopropane gave the unsaturated intermediate **10** which was submitted to the same synthetic steps to obtain the final compound **42**.

Compound **9**, by reaction with amines IV, V, and VI in ethanol, furnished the intermediates **11**, **12**, and **13**, as depicted in Scheme 4. Compounds **11**−**13** are then converted into aminocyanopyrazoles by cyclization with methylhydrazine in ethanol at reflux. The synthesis of the final compounds **38**−**40** was performed using the same synthetic methodology employed for compounds **41** and **43**. The pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives **38**, **40**, and **41** were converted into the corresponding ureas (**44**, **45**, and **46**) by reaction

Scheme 3[a]



[a] Reagents: (i) CS_2, CH_3I; (ii) (aryl)alkylhydrazine, reflux; (iii) $HC(OEt)_3$, reflux; (iv) 2-furoic acid hydrazide, $MeO(CH_2)_2OH$; (v) Ph_2O, 260 °C; (vi) 10% HCl; (vii) NH_2CN, *p*-TsOH, 1-methyl-2-pyrrolidone, 140 °C; (viii) 4-methoxyphenyl isocyanate, TEA; (ix) acyl chlorides.

with 4-methoxyphenyl isocyanate and a catalytic amount of TEA (Schemes 3 and 4). The tricyclic compounds **38** and **41** were converted into compounds **47**−**50** (Schemes 3 and 4) by reaction with acyl chlorides in benzene and DMF as solvents. This permitted the introduction of an amide function in the 5-position to evaluate the changes in affinity and selectivity at the A_3 adenosine receptor subtype.

Derivative **39** was converted into **52** by treatment with acetic acid and iodic acid (Scheme 5). The free hydroxyl group was introduced to increase the water solubility of the final compound.

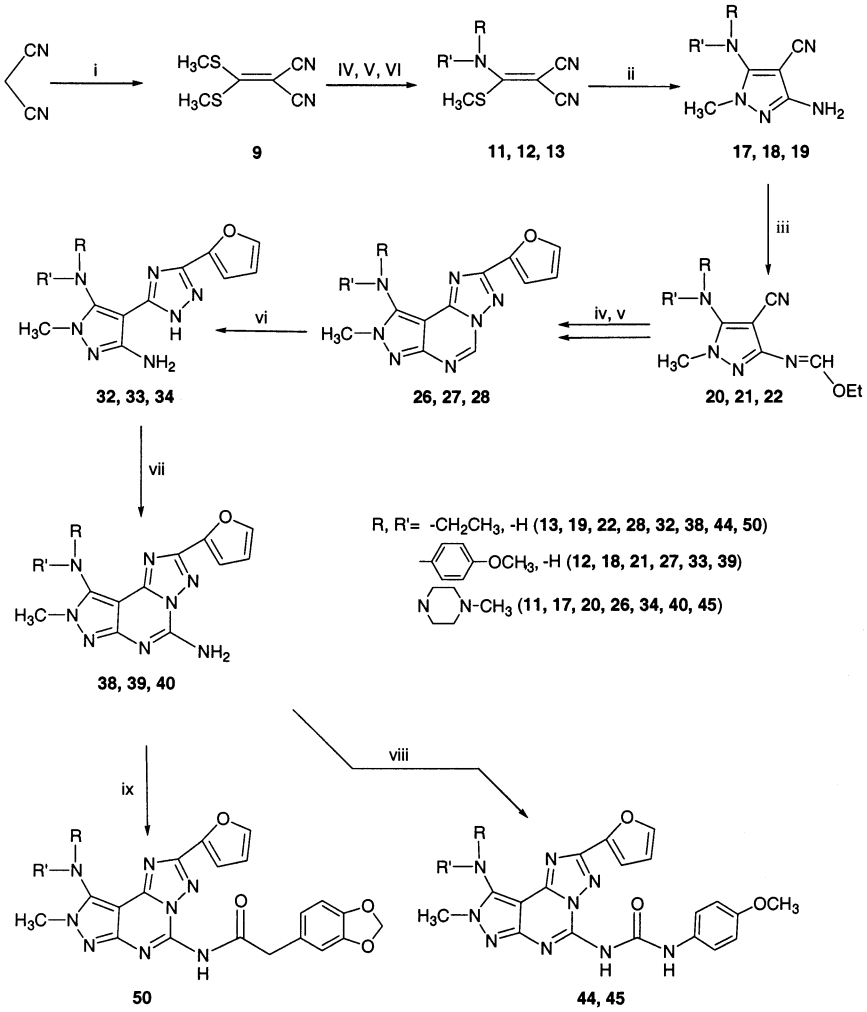
The final compounds which own a free amino function were transformed into a salt by treatment with a saturated methanolic solution of hydrochloric acid. For the modifications at the 5′ position of the furanyl ring of **61** and **41**, we performed the Mannich reaction, as depicted in Scheme 6. The appropriate tricyclic derivatives were reacted with *N*-methylpiperazine or morpholine and 36% aqueous formaldehyde in glacial acetic acid to give the target compounds **51** and **57**−**58** in 20−30% yield.

The treatment of compounds **57**, **58**, and **61** with a saturated solution of hydrochloric acid in methanol gave the salts **56**, **59**, and **60** which displayed significant water solubility.

Results and Discussion

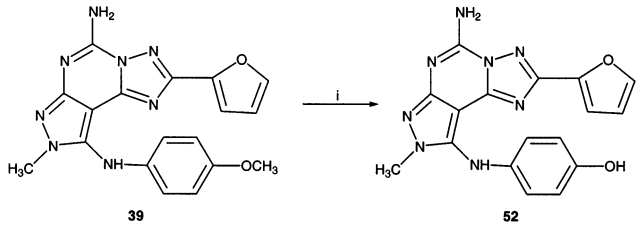
Tables 2, 3, and 5 show the receptor affinity profiles of structurally modified compounds described in this work. The affinity values were determined by receptor binding assays at human A_1, A_{2A}, A_{2B}, and A_3 adenosine

Scheme 4[a]



[a] IV: *N*-methylpiperazine; V: 4-methoxyaniline; VI: ethylamine. Reagents: (i) CS_2, CH_3I; (ii) (aryl)alkylhydrazine, reflux; (iii) $HC(OEt)_3$, reflux; (iv) 2-furoic acid hydrazide, $MeO(CH_2)_2OH$; (v) Ph_2O, 260 °C; (vi) 10% HCl; (vii) NH_2CN, *p*-TsOH, 1-methyl-2-pyrrolidone, 140 °C; (viii) 4-methoxyphenyl isocyanate, TEA; (ix) acyl chlorides.

Scheme 5[a]



[a] Reagents: (i) acetic acid, iodic acid, reflux.

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.

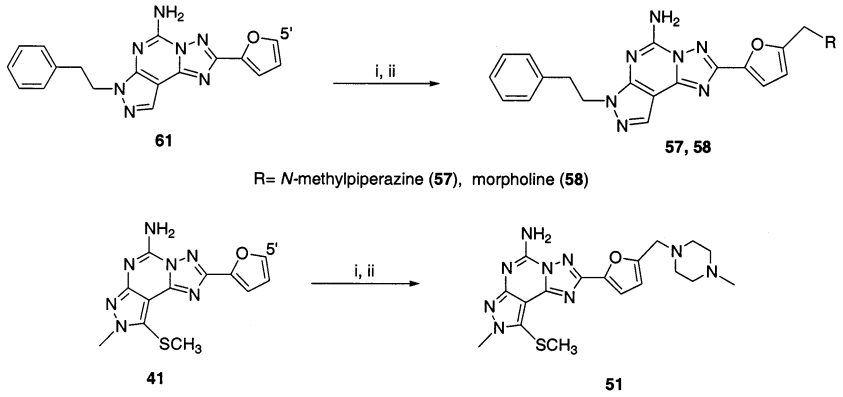
The biological results of the new series of compounds **5a**−**8** modified in the 2-position are shown in Table 2.

In general we observe that substitution of the furanyl moiety with a (substituted)aromatic function or a phenyl ring causes a complete loss of affinity at the A_{2A} adenosine receptor subtype with respect to the lead compound **61**. This provides supportive evidence that the furanyl ring at the 2-position of the tricyclic structure is a necessary element to guarantee the activity of the molecule, probably because in this heterocycle is present an oxygen atom that produces a favorable electronic condition for the interaction with the adenosine receptor.

The introduction of an ethoxy group at the 2′-position of the aromatic ring, to imitate the oxygen of the furan, proved unsuccessful for A_{2A} interaction. The introduction of an ester, acid, or amide function in the para-position, introduced to promote the formation of a hydrogen bond between the molecule and the adenosine receptor surface, also proved fruitless. Only compound **5c** shows a poor affinity for A_3 adenosine receptor subtype, but with complete selectivity. The biological results of the compounds modified at 9-position are showed in Table 3.

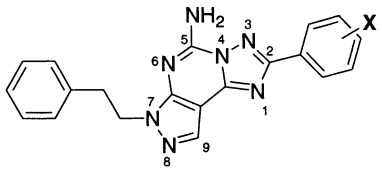
As showed in the table, the introduction of a substituent at the 9-position instead of a hydrogen leads to a loss of selectivity that is present in the lead compound **61** and in compounds **b**, but the receptor affinity is maintained. In general the methylthio group at the 9-position is the best tolerated. A methyl group at 8-position is better tolerated than a phenylpropyl substituent by A_{2A} adenosine receptor subtype. Compound **41**, which has a free amino group at the 5-position, shows good affinity for the A_{2A} adenosine receptor but, unfortunately, low selectivity. Transformation of the amino group into urea (compound **46**) or amide

Scheme 6[a]



[a] Reagents: (i) 36% aqueous formaldehyde, glacial acetic acid, (ii) *N*-methylpiperazine or morpholine.

Table 2. Biological Results of Compounds **5a**−**8**



compd	X	A_1 K_i nM	A_{2A} K_i nM	A_{2B} K_i nM	A_3 K_i nM
61[a]	-	121	2.3	>1000	>1000
5a[b]	4-OH	>1000	>1000	>1000-	>1000
5b[b]	4-Cl	>1000	>1000	>1000	>1000
5c[b]	2-OEt	>1000	>1000	>1000	348 (267−453)
5d[b]	H	235 (181−305)	>1000	>1000	>1000
6[b]	4-OCH_2CO_2Et	>1000	>1000	>1000	>1000
7[b]	4-OCH_2CO_2H	>1000	>1000	>1000	>1000
8[b]	4-$OCH_2CONHPh$-4′-I	>1000	>1000	>1000	>1000

[a] Displacement of [^{3}H]CHA binding (A_1) at rat cortical membrane, displacement of [^{3}H]CGS 21680 binding (A_{2A}) at rat striatal membranes, and displacement of [^{125}I]AB MECA binding at human A_3 adenosine receptors expressed in HEK-293 cells. [b] Displacement of [^{3}H]DPCPX binding (A_1, A_{2B}) at human A_1 and A_{2B} adenosine receptors expressed in CHO and HEK-293 cells, displacement of [^{3}H]SCH58261 binding (A_{2A}) at human A_{2A}adenosine receptors expressed in CHO cells, and displacement of [^{3}H]MRE 3008F20 binding at human A_3 adenosine receptors expressed in CHO cells.

functions (compounds **47**, **48**, and **49**) preserves A_{2A} affinity, but interaction with the A_3 adenosine receptor subtype decreases. In this case we can say that the presence of a substituent at the 9-position does not permit the A_3-interaction like that obtained by similar functionalization of the free amino group at the 5-position of the simpler pyrazolotriazolopyrimidine structure.[17]

The substitution of the methylthio group by a thiopropyl group at the 9-position maintains the A_{2A} affinity and increases the selectivity (compound **42**). Introduction of a phenylpropyl group at N^8-position, instead of a methyl group (compound **43**), leads to a decrease of A_{2A} interaction. In contrast, compound **38**, which possess an ethylamino group at the 9-position, shows good affinity for the A_{2A} adenosine receptor subtype. Functionalization of the amino group into a *p*-methoxyphenylurea causes a decrease of A_{2A} affinity, but a good increase in A_3 affinity (compound **44**). The interaction with A_3 receptor is also increased by the salification of this molecule (compound **55**), probably due to increased water solubility. However, selectivity of this compound versus the other adenosine receptor subtypes is very low.

The introduction of hindered amino functions at 9-position, such as *p*-methoxyphenylamino, *N*-methylpiperazine, or *p*-hydroxyphenylamino (compounds **39**, **40**, and **52**), was ineffectual for A_{2A}-interaction. Functionalization of the amino group of compound **40** into urea was also negative for A_3 interaction, possibly due to an important steric interaction of the radicals introduced (compound **45**).

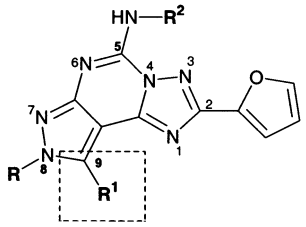
The synthesized compounds were also tested to verify the antagonist behavior. In particular, the most interesting of the examined compounds (**41**, **42**, **46**, **49**, **53**, and **55**) were evaluated for blocking cAMP generation after typical agonist-modulation of A_{2A} and A_3 receptors (Table 4).

The compounds **41** and **42** that show an high affinity versus A_{2A} adenosine receptors are also the most potent antagonists revealing IC_{50} values in the nanomolar range (6.1−14.8 nM). The compound **55** with high affinity versus A_3 adenosine receptors is also the most potent A_3 antagonist. All these data suggest that the potency of the antagonists in the cAMP assays strictly correlated with the affinity values observed in binding assays.

We can conclude that modifications at the 9-position maintain antagonistic activity, particularly with small groups introduced, like methylthio or ethylamino radicals, but lead to a significant loss of selectivity.

The compound **61** and compound **41**, which displayed a better antagonistic activity on A_{2A} adenosine receptor subtype, were modified at 5′-position of the furanyl ring

Table 3. Biological Results of Compounds **38**−**55**



compd	R	R^1	R^2	A_1 K_i nM [a]	A_{2A} K_i nM [b]	A_{2B} K_i nM [c]	A_3 K_i nM [d]
38	CH_3	$NHCH_2CH_3$	H	50 (41−61)	10 (7−14)	81 (70−94)	225 (177 (288)
39	CH_3	NH-Ph-p-OMe	H	260 (201−332)	>1000	>1000	>1000
40	CH_3	*N*-Me-piperazine	H	30 (22−41)	156 (123−198)	35 (27−45)	>1000
41	CH_3	SCH_3	H	8.4 (4.9−14.5)	1.2 (0.8−1.8)	10.3 (7.9−13.4)	35 (27−45)
42	CH_3	$S(CH_2)_2CH_3$	H	9 (7−11)	2.1 (1.5−3.0)	69 (55−87)	224 (186−269)
43	$Ph(CH_2)_3$	SCH_3	H	175 (134−229)	22 (12−43)	31 (25−38)	>1000
44	CH_3	$NHCH_2CH_3$	CONHPh-4-OMe	150 (132−169)	21 (16−27)	37 (27−42)	14 (10−21)
45	CH_3	*N*-Me-piperazine	CONHPh-4-OMe	316 (268−372)	>1000	26 (17−38)	>1000
46	CH_3	SCH_3	CONHPh-4-OMe	70 (53−90)	3.1 (1.3−5.2)	24 (17−35)	212 (162−278)
47	CH_3	SCH_3	$COCH_2$Ph-4-OMe	80 (63−100)	15 (10−22)	45 (34−56)	>1000
48	CH_3	SCH_3	$COCH_2$Ph-4-isobutyl	780 (700−820)	50 (41−60)	180 (140−233)	>1000
49	CH_3	SCH_3	$COCH_2$Ph-3,4-Medioxy	70 (61−80)	4.1 (1.9−7.0)	30 (22−41)	110 (59−165)
50	CH_3	$NHCH_2CH_3$	$COCH_2$Ph-3,4-Medioxy	136 (110−168)	61 (50−74)	65 (56−75)	183 (157−213)
52	CH_3	NH−Ph-p-OH	H	666 (593−748)	>1000	>1000	308 (250−380)
53	CH_3	$NHCH_2CH_3$·HCl	H	41 (30−54)	10 (7−14)	36 (25−52)	25 (17−35)
54	CH_3	*N*-Me-piperazine·2HCl	H	48 (36−65)	135 (97−188)	12 (8−18)	>1000
55	CH_3	$NHCH_2CH_3$·HCl	CONHPh-4-OMe	100 (83−120)	16 (13−20)	23 (18−29)	9 (6−12)

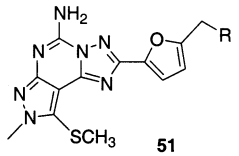
[a] Displacement of [^{3}H]DPCPX binding at human A_1 adenosine receptors expressed in CHO cells. [b] Displacement of [^{3}H]SCH58261 binding at human A_{2A} adenosine receptors expressed in CHO cells. [c] Displacement of [^{3}H]DPCPX binding at human A_{2B} adenosine receptors expressed in HEK-293 cells.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

Table 4. Functional Assay. Effect of Selected Antagonists on Agonist-Mediated CAMP Production[c]

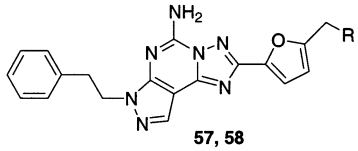
compound	hA_{2A} receptor[a] IC_{50} (nM)	hA_3 receptor[b] IC_{50} (nM)
41	6.1 (4.2−8.8)	188 (166−213)
42	14.8 (10.2−21.5)	852 (764−951)
46	17.5 (13.4−22.9)	813 (740−892)
49	21.5 (15.8−29.2)	466 (414−525)
53	48 (40−59)	103 (79−134)
55	61 (48−77)	45 (30−66)

[a] hA_{2A} adenosine receptors were stimulated with 100 nM NECA [b] hA_3 adenosine receptors were inhibited with 100 nM Cl−IB-MECA [c] Values are the means of at least three experiments and in parentheses the 95% confidence limits are shown.

Table 5. Biological Results of 5′-Substituted Compounds



51



57, 58

compd	R	A_1 K_i nM[a]	A_{2A} K_i nM[b]	A_{2B} K_i nM[c]	A_3 K_i nM[d]
51	*N*-methylpiperazine	>1000	>1000	>1000	>1000
57	*N*-methylpiperazine	>1000	>1000	>1000	>1000
58	morpholine	>1000	>1000	>1000	>1000

[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.

by Mannich reaction, introducing cycloaminomethyl functions. These functions, easily salified, increased water solubility, but a complete loss of affinity at all the adenosine receptors subtypes (see **Table 5**) was observed. Also for compounds **5a**−**8** we can confirm that the furanyl moiety is a necessary element for receptor interaction and that no modifications are allowed.

Conclusion

In the present study we have described the affinity at adenosine receptor subtypes of a new series of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine compounds, structurally modified with respect to lead compounds previously synthesized in our laboratory. These lead molecules are very potent and selective antagonists on A_{2A} and A_3 adenosine receptor subtypes. Modifications at the 2-position and at the 5′-position (Mannich reaction) of **61** have decreased affinity at the A_{2A} receptor subtype, probably due to an increased steric hindrance by the (substituted)aromatic rings introduced. Modifications at 9-position of the tricyclic structure of the adenosine antagonists allowed the affinity at adenosine receptor subtypes to be maintained, but the loss of selectivity was more or less complete. When a small substitution group is at N^8 and 9 positions, compound such as **41**, showed the highest A_{2A} binding affinity.

Whereas, compounds with small alkyl amino substitution group at the 9 position together with an ureidic function at 5 position displayed the highest A_3 subtype selective binding (compound **44**).

These structural modifications introduced to the tricyclic antagonistic structure allowed us to obtain important information about the SAR of pyrazolo-triazolo-pyrimidines: (1) the furanyl ring is an indispensable structural element for adenosine receptor binding, and (2) increased steric hindrance leads to a decrease in receptor affinity.

Experimental Section

General. 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. ^{1}H NMR were determined in $CDCl_3$ or DMSO-d_6 solutions with a Bruker AC 200 spectrometer, peaks 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. Chromatography was performed using Merk 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 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 7-(2-Phenylethyl)-2-(substituted phenyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines 3a−d. The imino ether **2** (3 g, 15 mmol) dissolved in 2-methoxyethanol (35 mL) was added to the selected hydrazide (15 mmol), and the mixture was heated at reflux for 12 h. After being cooled, 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 crystallization from EtOAc to afford the compounds as solids.

7-(2-Phenylethyl)-2-(4-hydroxidphenyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (3a). Yield 50%; yellow solid; mp 246−247 °C; ^{1}H NMR (DMSO-d_6) δ 3.32 (t, 2H, J = 8), 4.73 (t, 2H, J = 8), 6.94 (d, 2H, J = 8), 7.13 (m, 5H), 8.07 (d, 2H, J = 8), 8.51 (s, 1H), 9.52 (s, 1H), 10.22 (bs, 1H).

7-(2-Phenylethyl)-2-(4-chlorophenyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (3b). Yield 76%; yellow solid; mp 237−239 °C; ^{1}H NMR (DMSO-d_6) δ 3.25 (t, 2H, J = 8), 4.76 (t, 2H, J = 8), 7.17 (m, 5H), 7.67 (d, 2H, J = 8), 7.96 (m, 1H), 8.25 (d, 1H, J = 8), 8.56 (s, 1H), 9.60 (s, 1H).

7-(2-Phenylethyl)-2-(2-ethoxyphenyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (3c). Yield 80%, yellow oil used for the next step of reaction without other purifications.

7-(2-Phenylethyl)-2-phenylpyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidine (3d). Yield 75%, orange oil used for the next step of reaction without purifications.

General Procedure for the Preparation of 5-Amino-1-(2-phenylethyl)-4-[3-(substituted-phenyl)-1,2,4-triazolo-5-yl]pyrazoles 4a−d. A solution of the mixture of **3a−d** (10 mmol) in aqueous 10% HCl (20 mL) and dioxane (25 mL) was refluxed for 45 min. Then the solution was cooled and basified with 10% NaOH at 0 °C. The compounds were extracted with EtOAc (3 × 50 mL); the organic layers were dried over Na_2SO_4 and evaporated under vacuum. The residues obtained were recrystallized from EtOAc to afford the desired compounds as solids.

5-Amino-1-(2-phenylethyl)-4-[3-(4-hydroxyphenyl)-1,2,4-triazolo-5-yl]pyrazole (4a). Yield 86%; yellow solid; mp 168−170 °C; ^{1}H NMR (DMSO-d_6) δ 3.03 (t, 2H, J = 9), 4.26 (t, 2H, J = 8), 5.48 (bs, 2H), 6.98 (d, 2H, J = 8), 7.24 (m, 5H), 8.11 (d, 2H, J = 8), 8.21 (s, 1H).

5-Amino-1-(2-phenylethyl)-4-[3-(4-chlorophenyl)-1,2,4-triazolo-5-yl]pyrazole (4b). Yield 90%; yellow solid; mp 202−204 °C; ^{1}H NMR (DMSO-d_6) δ 3.04 (t, 2H, J = 8), 4.19 (t, 2H, J = 8), 6.27 (bs, 2H), 7.27 (m, 5H), 7.23 (d, 2H, J = 8), 7.69 (s, 1H), 8.11 (d, 2H, J = 8), 13.81 (s, 1H).

5-Amino-1-(2-phenylethyl)-4-[3-(2-ethoxyphenyl)-1,2,4-triazolo-5-yl]pyrazole (4c). Yield 83%, orange solid, used for the next step of reaction without purifications.

5-Amino-1-(2-phenylethyl)-4-[3-(2-phenyl)-1,2,4-triazolo-5-yl]pyrazole (4d). Yield 72%, yellow solid, used for the next step of reaction without purifications.

General Procedure for the Preparation of 5-Amino-7-(2-phenylethyl)-2-(substituted phenyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines 5a−d. To a solution of tricycles derivatives **4a−d** (1.5 mmol) in *N*-methylpyrrolidone (5 mL) were added cyanamide (380 mg, 6 molar equiv) and *p*-toluenesulfonic acid (1.5 molar equiv), and the mixture was heated at 160 °C for 4 h. Then cyanamide (6 molar equiv) 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 the final products **5a−d** as solids.

5-Amino-7-(2-phenylethyl)-2-(4-hydroxyphenyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (5a). Yield 32%; pale yellow solid; mp 296−297 °C; ^{1}H NMR (DMSO-d_6) δ 3.18 (t, 2H, J = 8), 4.49 (t, 2H, J = 8), 6.90 (d, 2H, J = 8), 7.16 (m, 5H), 8.02 (bs, 2H), 8.08 (d, 2H, J = 8), 8.16 (s, 1H), 9.97 (s, 1H). Anal. ($C_{20}H_{17}N_7O$) C, H, N.

5-Amino-7-(2-phenylethyl)-2-(4-chlorophenyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (5b). Yield 48%; pale yellow solid; mp 272−273 °C; ^{1}H NMR (DMSO-d_6) δ 3.19 (t, 2H, J = 8), 4.49 (t, 2H, J = 8), 7.21 (m, 5H), 7.65 (d, 2H, J = 8), 8.09 (bs, 2H), 8.20 (d, 2H, J = 8), 8.24 (s, 1H). Anal. ($C_{20}H_{16}ClN_7$) C, H, N.

5-Amino-7-(2-phenylethyl)-2-(2-ethoxyphenyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (5c). Yield 38%; white solid; mp 159−160 °C; ^{1}H NMR (DMSO-d_6) δ 1.33 (t, 3H, J = 8), 3.19 (t, 2H, J = 8), 4.11 (q, 2H, J = 8), 4.54 (t, 2H, J = 8), 7.10 (m, 2H), 7.19 (m, 5H); 7.44 (m, 1H), 7.84 (d, 1H, J = 8), 7.92 (bs, 2H), 8.16 (s, 1H). Anal. ($C_{22}H_{21}N_7O$) C, H, N.

5-Amino-7-(2-phenylethyl)-2-phenyl-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (5d). Yield 42%; pale yellow solid; mp 240−242 °C; NMR (DMSO-d_6) δ 3.21 (t, 2H, J = 8), 4.52 (t, 2H, J = 8), 5.90 (bs, 2H), 7.22 (m, 6H), 7.60 (m, 2H), 8.27 (m, 3H). Anal. ($C_{20}H_{17}N_7$) C, H, N.

Procedure for the Preparation of 2-[4-(5-Amino-7-(2-phenylethyl)-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)phenoxy]acetic Acid Ethyl Ester 6. To a solution of compound **5a** (100 mg, 0.27 mmol) in dry DMF (10 mL) was added anhyd K_2CO_3 (44.7 mg, 0.32 mmol), and the resulting mixture was stirred at room temperature for 10 min.

Then was added 2-chloroethyl acetate (34 μL, 0.32 mmol), and the solution was stirred at the same conditions for 12 h. The solvent was removed under reduced pressure, and to the residue was added water (30 mL).The aqueous phase was extracted with EtOAc (3 × 30 mL), and the organic phases were dried (Na_2SO_4) and evaporated to dryness under vacuum. The oily residue was recrystallized from EtOAc to furnish the desired compound as solid.

Yield 63%; white solid; mp 205−206 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, J = 8), 3.20 (t, 2H, J = 8), 4.21 (q, 2H, J = 6), 4.49 (t, 2H, J = 8), 4.88 (s, 2H), 7.08 (bs, 2H), 7.16 (m, 9H), 8.16 (s, 1H). Anal. ($C_{24}H_{23}N_7O_3$) C, H, N.

Procedure for the Preparation of 2-[4-(5-Amino-7-(2-phenylethyl)-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)phenoxy]acetic Acid 7. A solution of the compound **6** (50 mg, 0.11 mmol) in aqueous 10% HCl (3 mL) and dioxane (5 mL) was heated at 60 °C for 3 h. Then the solution was cooled and basified with 10% NaOH at 0 °C. The solid formed was filtered off and washed with cold water to afford the desired compound.

Yield 94%; white solid; mp 250−251 °C; ^{1}H NMR (DMSO-d_6) δ 3.18 (t, 2H, J = 8), 4.49 8t, 2H, J = 8), 4.75 (s, 2H), 7.18 (m, 9H), 8.02 (bs, 2H), 8.17 (s, 1H), 11.03 (bs, 1H). Anal. ($C_{22}H_{19}N_7O_3$) C, H, N.

Procedure for the Preparation of 2-[4-(5-Amino-7-(2-phenylethyl)-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)phenoxy]-*N*-(4-iodophenyl)acetamide 8. To a solution of compound **5a** (100 mg, 0.27 mmol) in DMF (10 mL) was added K_2CO_3 (44.7 mg, 0.32 mmol), and the mixture was stirred at room temperature for 10 min. Then was added 2-chloro-*N*-(4-iodophenyl)acetamide (95.5 mg, 0.32 mmol) dissolved in DMF (3 mL), and the resulting mixture was heated at 50 °C for 5 h. The solvent was removed under reduced pressure, and the residue obtained was washed with water (30 mL) and extracted with EtOAc (3 × 35 mL). The organic

layer was dried (Na_2SO_4) and evaporated under vacuum to afford a residue that was crystallized from a mixture of EtOAc/CH_3OH to furnish the desired compound.

Yield 71%; white solid; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.14 (t, 2H, J = 8), 4.49 (t, 2H, J = 8), 4.80 (s, 2H), 7.19 (m, 7H), 7.52 (d, 2H, J = 8), 7.69 (d, 2H, J = 8), 8.15 (bs, 2H), 8.17 (m, 3H), 10.25 (bs, 1H). Anal. ($C_{28}H_{23}IN_8O_2$) C, H, N.

General Procedure for the Preparation of 2-(Bis-(methylsulfanyl)methylene)malononitrile 9 and 2-(Bis-(propylsulfanyl)methylene)malononitrile 10. To a solution of malononitrile (8 g, 0.12 mol) in DMF (40 mL) was added an excess of anhydrous K_2CO_3 (12 g) and, after 10 min, CS_2 in small amount (7.2 mL, 0.12 mol). After 10 min was added a catalytic amount of tetrabutylammonium bromide and methyl iodide or propyl bromide (0.12 mol) in small portions, and the mixture was stirred at room temperature for 30 min, heated at 50 °C for 2 h, and finally stirred at room temperature overnight. The residue was washed with water (100 mL), and the solid formed was collected by filtration and washed with cold water.

2-(Bis(methylsulfanyl)methylene)malononitrile (9). Yield 83%; yellow solid; mp 79−81 °C; IR (KBr): 2190, 2210 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.7 (s, 6H).

2-(Bis(propylsulfanyl)methylene)malononitrile (10). Yield 70%; yellow solid; mp 85−86; IR(KBr) 1456, 2218, 2967 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 6H, J = 7.3), 1.75 (m, 4H, J = 7.3), 3.2 (t, 4H, J = 7.3).

General Procedure for the Preparation of 1*H*-1-Substituted-3-amino-4-cyano-5-methylsulfanylpyrazoles 14 and 15. To a solution of compound **9** (5 g, 29 mmol) in absol EtOH (50 mL) was added the appropriate hydrazine (29 mmol), and then the mixture was refluxed for 6 h. The solvent was removed at reduced pressure, and the residue was washed with water (50 mL) and extracted with EtOAc (3 × 35 mL). The organic phases were dried (Na_2SO_4) and evaporated, and the residue obtained was purified by chromatography (EtOAc/light petroleum, 7:3) to afford the products as solids in good yield.

1*H*-1-Methyl-3-amino-4-cyano-5-methylsulfanylpyrazole (14). Yield 95%; yellow solid; mp 120 °C; ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H), 3.70 (s, 3H), 4.17 (bs, 2H).

1*H*-1-(3-Phenylpropyl)-3-amino-4-cyano-5-methylsulfanylpyrazole (15). Yield 60%; yellow solid; mp 110−113 °C; ^{1}H NMR ($CDCl_3$) δ 2.11 (m, 2H), 2.53 (s, 3H), 2.63 (t, 2H, J = 7.3), 4.05 (t, 2H, J = 7.3), 4.1 (bs, 2H), 7.2 (m, 5H).

Preparation of 1*H*-1-Methyl-3-amino-4-cyano-5-propylsulfanylpyrazole 16. To a solution of **26** (6 g, 26 mmol) in absol EtOH (70 mL) was added methylhydrazine (26 mmol), and then the mixture was refluxed for 4 h before evaporating the solvent. The residue was crystallized from a mixture of Et_2O/light petroleum (1:1).

Yield 83%; pale yellow solid; mp 56−58 °C; ^{1}H NMR ($CDCl_3$) δ 1.02 (t, 3H, J = 7.3), 1.63 (m, 2H), 2.92 (t, 2H, J = 7.3), 3.74 (s, 3H), 4.11 (bs, 2H).

General Procedure for the Preparation of 2-Substituted-methylsulfanylmethylenemalononitrile 11−13. To a solution of compound **9** (7 g, 41 mmol) in absol EtOH (80 mL) was added the appropriate amine (41 mmol), and the mixture was heated at reflux for 4−6 h. Then the solvent was evaporated under reduced pressure, and the residue was crystallized from EtOH to afford the product as a solid.

2-[(4-Methyl-1-pyperazinyl)methylsulfanylmethylene]-malononitrile (11). Yield 80%; yellow solid, mp 115−116 °C; ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 3H), 2.44 (t, 4H, J = 5), 2.52 (s, 3H), 3.72 (t, 4H, J = 5).

2-[(4-Methoxyphenylamino)methylsulfanylmethylene]-malononitrile (12). Yield 70%; green solid; mp 150−152 °C; ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H), 3.76 (s, 3H), 6.9 (d, 2H, J = 6), 7.2 (d, 2H, J = 6), 10.37 (bs, 1H).

2-[(ethylamino)methylsulfanylmethylene]malononitrile (13). Yield 61%; yellow solid; mp 74°C; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 7), 2.55 (s, 3H), 3.5 (m, 2H, J = 7), 8.68 (bs, 1H).

General Procedure for the Preparation of 1*H*-1-Methyl-3-amino-4-cyano-5-substituted Pyrazoles 17−19. To a solution of compounds **11−13** (35 mmol) in absol EtOH (50 mL) was added methylhydrazine (1.5 molar equiv), and the mixture was heated at reflux 18 h. The solvent was evaporated under reduced pressure, and the residue was recrystallized from absol EtOH.

1*H*-1-Methyl-3-amino-4-cyano-5-(4-methylpiperazin-1-yl)pyrazole (17). Yield 78%; yellow solid; mp 202−203 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H), 2.52 (t, 4H, J = 5), 3.2 (t, 4H, J = 5), 3.48 (s, 3H), 3.97 (bs, 2H).

1*H*-1-Methyl-3-amino-4-cyano-5-(4-methoxyphenylamino)pyrazole (18). Yield 44%; pale yellow solid; mp 144°C; ^{1}H NMR (DMSO-d_6) δ 3.4 (s, 3H), 3.67 (s, 3H), 6.45 (s, 2H), 6.76 (d, 2H, J = 9), 7.42 (d, 2H, J = 9), 8.15 (s, 1H).

1*H*-1-Methyl-3-amino-4-cyano-5-ethylaminopyrazole (19). Yield 79%; yellow solid, mp 120 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H, J = 8), 3.3 (m, 2H, J = 8), 3.35 (s, 3H), 5.03 (bs, 2H), 6.3 (bs, 1H).

General Procedure for the Preparation of 1*H*-1-Methyl-3-[(ethoxymethylene)amino]-4-cyano-5-substituted Pyrazoles 20−22. The 3-amino-4-cyanopyrazoles **17−19** (29 mmol) were dissolved in triethyl orthoformate (80 mL), and the solution was refluxed under nitrogen for 12 h. Then the solvent was removed under reduced pressure, and the oily residue was recrystallized from absol EtOH to afford the corresponding imino ethers **20−22**.

1*H*-Methyl-3-[(ethoxymethylene)amino]-4-cyano-5-(4-methylpiperazin-1-yl)pyrazole (20). Yield 70%; yellow crystals, mp 108−110 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (t, 3H, J = 7), 2.22 (s, 3H), 2.45 (t, 4H, J = 5), 3.14 (t, 4H, J = 5), 3.55 (s, 3H), 4.25 (q, 2H, J = 7), 8.23 (s, 1H).

1*H*-Methyl-3-[(ethoxymethylene)amino]-4-cyano-5-(4-methoxyphenylamino)pyrazole (21). Yield 60%; yellow solid; mp 125−128 °C; ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3H, J = 7), 3.54 (s, 3H); 3.69 (s, 3H), 4.30 (q, 2H, J = 7), 6.84 (m, 3H), 7.43 (d, 2H, J = 9), 8.46 (s, 1H).

1*H*-Methyl-3-[(ethoxymethylene)amino]-4-cyano-5-ethylaminopyrazole (22). Yield 76%; yellow solid; mp 110−111 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 6.5), 1.28 (t, 3H, J = 7), 3.34 (m, 2H), 3.42 (s, 3H), 4.22 (q, 2H, J = 7), 6.62 (bs, 1H), 8.17 (s, 1H).

General Procedure for the Preparation of 1*H*-1-methyl/(3-phenylpropyl)-3-[(ethoxymethylene)amino]-4-cyano-5-substituted Pyrazoles 23−25. The 3-amino-4-cyanopyrazoles **14−16** (23 mmol) were dissolved in triethyl orthoformate (80 mL), and the solution was refluxed under nitrogen for 12 h. Then the solvent was removed under reduced pressure, and the oily residue was recrystallized from absol EtOH to afford the corresponding imino ethers **23−25**.

1*H*-1-Methyl-3-[(ethoxymethylene)amino]-4-cyano-5-methylsulfanylpyrazole (23). Yield 94%; yellow crystals; mp 35−36°C; ^{1}H NMR (DMSO-d_6) δ 1.29 (t, 3H, J = 7), 2.54 (s, 3H); 3.81 (s, 3H), 4.26 (q, 2H, J = 7), 8.30 (s, 1H).

1*H*-1-(3-Phenylpropyl)-3-[(ethoxymethylene)amino]-4-cyano-5-methylsulfanylpyrazole (24). Yield 74%; orange oil; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7), 2.15 (m, 2H, J = 7), 2.55 (s, 3H), 2.61 (t, 2H, J = 7.5), 4.00 (t, 2H, J = 7.5), 4.22 (q, 2H, J = 7), 7.20 (m, 5H), 8.35 (s, 1H).

1*H*-1-Methyl-3-[(ethoxymethylene)amino]-4-cyano-5-propylsulfanylpyrazole (25). Yield 94%; orange oil; ^{1}H NMR (DMSO-d_6) δ 1.02 (t, 3H, J = 7), 1.37 (t, 3H, J = 7), 1.63 (m, 2H, J = 7), 2.94 (t, 2H, J = 7), 3.84 (s, 3H), 4.37 (q, 2H, J = 7), 8.20 (s, 1H).

General Procedure for the Preparation of 8-Methyl-2-(2-furyl)-9-substituted-pyrazolo[4,3-*e*]-1,2,4-triazolo-[1,5-*c*]pyrimidines 26−28. Imino ethers **20−22** (18 mmol) were dissolved in 2-methoxyethanol (70 mL), and 2-furoic acid hydrazide (18 mmol) was added. The mixture was refluxed for 12 h, then, 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 crystallization from a mixture of CH_2Cl_2/light petroleum.

8-Methyl-2-(2-furyl)-9-(4-methylpiperazin-1-yl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (26). Yield 50%; yellow solid; mp 223−225 °C; ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 2.56 (m, 4H), 3.37 (m, 4H), 3.97 (s, 3H), 6.70 (m, 1H), 7.20 (m, 1H), 7.95 (m, 1H), 9.32 (s, 1H).

8-Methyl-2-(2-furyl)-9-(4-methoxyphenylamino)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (27). Yield 35%; yellow solid; mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3H), 3.96 (s, 3H), 6.74 (m, 1H), 6.85 (d, 2H, J = 8.6), 6.96 (d, 1H, J = 3.5), 7.60 (d, 2H, J = 8.6), 7.97 (d, 1H, J = 1), 8.40 (s, 1H), 9.52 (s, 1H).

8-Methyl-2-(2-furyl)-9-(ethylamino)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (28). Yield 67%; yellow solid; mp 277−280 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, J = 7), 3.78 (s, 3H), 4.00 (q, 2H), 6.70 (m, 2H), 7.13 (d, 1H, J = 3), 7.92 (s, 1H), 9.14 (s, 1H).

General Procedure for the Preparation of 8-Substituted-2-(2-furyl)-9-substituted-pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidines 29−31. Imino ethers **23**−**25** (18 mmol) were dissolved in 2-methoxyethanol (70 mL), and 2-furoic acid hydrazide (18 mmol) was added. The mixture was refluxed for 12 h, then, 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 crystallization from EtOAc.

8-Methyl-2-(2-furyl)-9-methylsulfanylpyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine (29). Yield 30%; white solid; mp 140−142 °C; ^{1}H NMR (DMSO-d_6) δ 2.82 (s, 3H), 4.16 (s, 3H), 6.74 (d, 1H, J = 3.5), 7.25 (d, 1H, J = 3.4), 7.97 (s, 1H), 9.42 (s, 1H).

8-(3-Phenylpropyl)-2-(2-furyl)-9-methylsulfanylpyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine (30). Yield 22%; yellow solid; mp 154−157 °C; ^{1}H NMR ($CDCl_3$) δ 2.30 (m, 2H), 2.67 (t, 2H, J = 7), 2.80 (s, 3H), 4.53 (t, 2H, J = 7), 6.62 (d, 1H, J = 2), 7.20 (m, 5H), 7.35 (d, 1H, J = 2), 7.65 (s, 1H), 9.07 (s, 1H).

8-Methyl-2-(2-furyl)-9-propylsulfanyl-pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine (31). Yield 35%; yellow solid; mp 130−132 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7), 1.45 (m, 2H, J = 7), 3.31 (t, 2H, J = 7), 4.10 (s, 3H), 6.75 (d, 1H, J = 2), 7.24 (d, 1H, J = 3), 7.98 (s, 1H), 9.43 (s, 1H).

General Procedure for the Preparation of 3-Amino-1*H*-1-substituted-5-substituted-4-[3-(2-furyl)-1,2,4-triazol-5-yl]pyrazoles 32−37. A solution of the mixture of **26**−**31** (7 mmol) in aqueous 10% HCl (20 mL) and dioxane (15 mL) was refluxed for 1 h. Then the solution was cooled and basified with 10% NaOH at 0 °C. The compounds were extracted with EtOAc (3 × 50 mL); the organic layers were dried over Na_2SO_4 and evaporated under vacuum. The residues obtained were recrystallized from EtOAc to afford the desired compounds as solids.

3-Amino-1*H*-1-methyl-5-(ethylamino)-4-[3-(2-furyl)1,2,4-triazol-5-yl]pyrazole (32). Yield 94%; yellow solid; mp 230 °C; ^{1}H NMR (DMSO-d_6) δ 1.05 (t, 3H, J = 7), 3.07 (m, 2H), 3.43 (s, 3H), 5.13 (bs, 2H), 6.57 (s, 1H), 6.87 (s, 1H), 7.87 (s, 1H), 13.30 (bs, 1H).

3-Amino-1*H*-1-methyl-5-(4-methoxyphenylamino)-4-[3-(2-furyl)1,2,4-triazol-5-yl]pyrazole (33). Yield 90%; yellow oil; used without further purifications.

3-Amino-1*H*-1-methyl-5-[4-(methylpiperazin-1-yl)]-4-[3-(2-furyl)1,2,4-triazol-5-yl]pyrazole (34). Yield 62%; yellow solid; mp 207−209 °C; ^{1}H NMR (DMSO-d_6) δ 2.21 (s, 3H), 2.41 (m, 4H), 3.11 (m, 4H), 3.48 (s, 3H), 5.10 (bs, 2H), 6.65 (m, 1H), 7.87 (d, 1H, J = 3), 7.85 (s, 1H), 13.50 (bs, 1H).

3-Amino-1*H*-1-methyl-5-methylsulfanyl-4-[3-(2-furyl)1,2,4-triazol-5-yl]pyrazole (35). Yield 84%; yellow solid; mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 3.86 (s, 3H), 5.15 (bs, 2H), 6.50 (m, 1H), 7.00 (d, 1H, J = 3), 7.53 (s, 1H), 12.00 (bs, 1H).

3-Amino-1*H*-1-methyl-5-propylsulfanyl-4-[3-(2-furyl)1,2,4-triazol-5-yl]pyrazole (36). Yield 67%; yellow solid; mp 154−155 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7), 1.42 (m, 2H, J = 7), 2.81 (t, 2H, J = 7), 3.75 (s, 3H), 5.56 (bs, 2H), 6.66 (s, 1H), 7.00 (s, 1H), 7.85 (s, 1H), 13.60 (bs, 1H).

3-Amino-1*H*-1-(3-phenylpropyl)-5-methylsulfanyl-4-[3-(2-furyl)1,2,4-triazol-5-yl]pyrazole (37). Yield 80%; yellow solid; mp 205−207°C; ^{1}H NMR (DMSO-d_6) δ 2.20 (m, 2H, J = 7), 2.67 (s, 3H), 4.21 (t, 2H, J = 7), 4.41 (t, 2H, J = 7), 5.80 (bs, 2H), 6.56 (s, 1H), 7.25 (m, 5H), 7.36 (s, 1H), 7.58 (s, 1H), 13.50 (bs, 1H).

General Procedure for the Preparation of 5-Amino-8-alkyl/arylalkyl-9-substituted-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines 38−43. To a solution of pyrazole derivatives **32**−**37** (3 mmol) in *N*-methylpyrrolidone (20 mL) were added cyanamide (18 mmol) and *p*-toluenesulfonic acid (6 mmol), and the mixture was heated at 160 °C for 4 h. Then cyanamide (18 mmol) was added again, and the solution was heated at 160 °C for additional 18 h. 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 the final products **38**−**43** as solids.

5-Amino-8-methyl-9-(ethylamino)-2-(2-furyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (38). Yield 42%; white crystals; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, 3H, J = 7), 3.64 (s, 3H), 3.99 (q, 2H, J = 7), 6.39 (bs, 2H), 6.70 (s, 1H), 7.08 (s, 1H), 7.35 (bs, 2H), 7.91 (s, 1H). Anal. ($C_{13}H_{14}N_8O$) C, H, N.

5-Amino-8-methyl-9-(4-methoxyphenylamino)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidine (39). Yield 35%; white crystals; mp 230 °C; ^{1}H NMR (DMSO-d_6) δ 3.71 (s, 3H), 3.76 (s, 3H), 6.73 (m, 1H), 6.85 (d, 2H, J = 9), 7.22 (d, 1H, J = 3), 7.58 (d, 2H, J = 9), 7.94 (bs, 2H), 8.02 (bs, 2H). Anal. ($C_{18}H_{16}N_8O_2$) C, H, N.

5-Amino-8-methyl-9-(4-methylpiperazin-1-yl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidine (40). Yield 33%; white crystals; mp 276−278 °C; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 2.50 (m, 4H), 3.31 (m, 4H), 3.79 (s, 3H), 6.70 (m, 1H), 7.12 (d, 1H, J = 4), 7.54 (bs, 2H), 7.92 (s, 1H). Anal. ($C_{16}H_{19}N_9O$) C, H, N.

5-Amino-8-methyl-9-methylsulfanyl-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (41). Yield 83%; white crystals; mp 298 °C; ^{1}H NMR (DMSO-d_6) δ 2.77 (s, 3H), 4.01 (s, 3H), 6.76 (s, 1H), 7.22 (d, 1H, J = 3), 7.70 (bs, 2H), 7.95 (s, 1H). Anal. ($C_{12}H_{11}N_7OS$) C, H, N.

5-Amino-8-methyl-9-propylsulfanyl-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (42). Yield 31%; yellow solid; mp 298−300 °C; ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 3H, J = 7), 1.47 (m, 2H, J = 7), 3.32 (t, 2H, J = 7), 4.02 (s, 3H), 6.72 (s, 1H), 7.20 (s, 1H), 7.90 (s, 1H), 7.91 (bs, 1H). Anal. ($C_{14}H_{15}N_7OS$) C, H, N.

5-Amino-8-(3-phenylpropyl)-9-methylsulfanyl-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (43). Yield 23%; white crystals; mp 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 2.27 (m, 2H), 2.69 (t, 2H, J = 7), 2.76 (s, 3H), 4.33 (t, 2H, J = 7), 5.95 (bs, 2H), 6.60 (s, 1H), 7.24 (m, 5H), 7.32 (s, 1H), 7.63 (s, 1H). Anal. ($C_{20}H_{19}N_7OS$) C, H, N.

General Procedure for the Preparation of 1-[2-(2-Furyl)-8-methyl-9-substituted-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidin-5-yl]-3-(4-methoxyphenyl)urea 44−46. To a solution of compounds **38**, **40**, and **41** (1.7 mmol) in dioxane (20 mL) were added 4-methoxyphenyl isocyanate (7.47 mmol, 4.5 molar equiv) and a catalytic amount of TEA, and the solution was heated at reflux for 18 h. The solvent was removed under reduced pressure, and the residue was purified by chromatography (EtOAc/light petroleum 1:1) to afford the final products **44**−**46** as solids.

1-[2-(2-Furyl)-8-methyl-9-(ethylamino)-8*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-3-(4-methoxyphenyl)urea (44). Yield 60%; white solid; mp >300 °C; ^{1}H

NMR (DMSO-d_6) δ 1.22 (t, 3H, $J = 6$), 3.74 (d, 6H), 4.00 (m, 2H), 6.66 (t, 1H; $J = 4$), 6.71 (m, 1H), 6.93 (d, 2H, $J = 8$), 7.18 (d, 1H, $J = 2$), 7.45 (d, 2H, $J = 8$), 7.95 (m, 1H), 9.23 (bs, 1H), 10.73 (bs, 1H). Anal. ($C_{21}H_{21}N_9O_3$) C, H, N.

1-[2-(2-Furyl)-8-methyl-9-(4-methyl-pyperazin-1-yl)-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidin-5-yl]-3-(4-methoxyphenyl)urea (45). Yield 50%; white solid; mp 199−200 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 2.76 (m, 4H), 3.42 (m, 4H), 3.75 (s, 3H), 3.91 (s, 3H), 6.76 (s, 1H), 6.95 (d, 2H, $J = 9$), 7.28 (s, 1H), 7.45 (d, 2H, $J = 9$), 7.98 (s, 1H), 9.55 (bs, 1H), 10.59 (s, 1H). Anal. ($C_{24}H_{26}N_{10}O_3$) C, H, N.

1-[2-(2-Furyl)-8-methyl-9-methylsulfanyl-8*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-3-(4-methoxyphenyl)urea (46). Yield 30%; white solid; mp 195−197 °C; ^{1}H NMR (DMSO-d_6) δ 2.81 (s, 3H), 3.75 (s, 3H), 4.10 (s, 3H), 6.75 (m, 1H), 6.95 (d, 2H, $J = 10$), 7.30 (d, 1H, $J = 2$), 7.46 (d, 2H, $J = 10$), 7.99 (bs, 1H), 9.60 (bs, 1H), 10.50 (bs, 1H). Anal. ($C_{20}H_{18}N_8O_3S$) C, H, N.

General Procedure for the Preparation of *N*-[2-(2-Furyl)-8-methyl-9-methylsulfanyl/ethylamino-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-2-substituted phenyl)acetamide 47−50. To a solution of compounds **38** and **41** (100 mg, 0.335 mmol) in dioxane (15 mL) were added the appropriate freshly prepared acyl chloride (0.4 mmol) and pyridine (0.335 mmol), and the mixture was refluxed under argon for 8 h. Then the solvent was removed under reduced pressure, and the residue was purified by chromatography (EtOAc/light petroleum 1:1) to afford the final products **47**−**50**.

***N*-[2-(2-Furyl)-8-methyl-9-methylsulfanyl-8*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-2-(4-methoxyphenyl)acetamide (47).** Yield 40%; white solid; mp 199−200 °C; ^{1}H NMR (DMSO-d_6) δ 2.81 (s, 3H), 3.74 (s, 3H), 3.93 (s, 2H), 4.12 (s, 3H), 6.76 (m, 1H), 6.90 (d, 2H, $J = 8$), 7.26 (m, 1H), 7.30 (d, 2H, $J = 8$), 8.00 (m, 1H), 10.37 (bs, 1H). Anal. ($C_{21}H_{19}N_7O_3S$) C, H, N.

***N*-[2-(2-Furyl)-8-methyl-9-methylsulfanyl-8*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-2-(4-isobutyl)-acetamide (48).** Yield 15%; white solid; mp 106−107 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (m, 6H), 1.79 (m, 1H), 2.41 (d, 2H, $J = 8$), 2.81 (s, 3H), 3.16 (d, 2H, $J = 3$), 4.12 (s, 3H), 6.76 (m, 1H), 7.13 (d, 2H, $J = 8$), 7.22 (m, 1H), 7.37 (d, 2H, $J = 8$), 7.98 (m, 1H), 10.98 (bs, 1H). Anal. ($C_{24}H_{25}N_7O_2S$) C, H, N.

***N*-[2-(2-Furyl)-8-methyl-9-methylsulfanyl-8*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-2-(3,4-methylendioxyphenyl)acetamide (49).** Yield 43%; white solid; mp 209−210 °C; ^{1}H NMR (DMSO-d_6) δ 2.81 (s, 3H), 3.90 (s, 2H), 4.12 (s, 3H), 6.00 (s, 2H), 6.76 (m, 1H), 6.87 (m, 2H), 6.99 (s, 1H), 7.27 (d, 1H, $J = 2$), 7.99 (bs, 1H), 11.03 (bs, 1H). Anal. ($C_{21}H_{17}N_7O_4S$) C, H, N.

***N*-[2-(2-Furyl)-8-methyl-9-(ethylamino)-8*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl]-2-(3,4-methylendioxy phenyl)acetamide (50).** Yield 13%; white solid; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.07 (t, 3H, $J = 7$), 3.50 (s, 3H), 3.67 (s, 2H), 4.00 (m, 2H), 5.87 (s, 1H), 6.30 (d, 2H, $J = 8$), 6.47 (s, 1H), 6.60 (d, 2H, $J = 8$), 6.70 (m, 1H, $J = 2$), 7.14 (d, 1H, $J = 2$), 7.94 (s, 1H), 10.50 (bs, 1H). Anal. ($C_{22}H_{20}N_8O_4$) C, H, N.

Procedure for the Preparation of 5-Amino-8-methyl-9-(4-hydroxyphenylamino)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine 52. A solution of compound **39** (60 mg, 0.16 mmol) in acetic acid (0.6 mL) and HI (0.4 mL) was refluxed for 5 h. Then acetic acid (0.6 mL) and HI (0.4 mL) were added again, and the solution was heated at reflux for additional 5 h. The mixture was diluted with water (8 mL), and the aqueous layer was extracted with EtOAc (3 × 50 mL). The organic phases were dried (Na_2SO_4) and evaporated to obtain a residue crystallized from absol EtOH to afford the product **52**.

Yield 70%; pale yellow solid; mp>300 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 6.72 (m, 3H), 7.20 (d, 1H, $J = 3$), 7.45 (d, 2H, $J = 8$), 7.70 (s, 1H), 7.94 (s, 1H), 8.04 (bs, 2H), 8.86 (s, 1H). Anal. ($C_{17}H_{14}N_8O_2$) C, H, N.

General Procedure for the Preparation of Hydrochloric Salts 53−55. The compounds **38**, **40**, and **44** (0.28 mmol) were dissolved in a methanolic satured solution of hydrochloric acid (0.57 mmol), and the solution was stirred at 0 °C for 10 min and then at room temperature for 10 min. The solvent was removed at reduced pressure, and the residue was coevaporated with dry Et_2O and purified by crystallization from absol EtOH to afford the desired products **53**−**55**.

5-Amino-8-methyl-9-(ethylamino)-2-(2-furyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine Hydrochloride (53). Yield 95%; white solid; mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 1.23 (t, 3H, $J = 7$), 3.72 (s, 3H), 4.00 (q, 2H, $J = 7$), 6.10 (bs, 3H), 6.72 (m, 1H), 7.16 (d, 1H, $J = 4$), 7.96 (s, 1H), 8.49 (bs, 2H). Anal. ($C_{13}H_{15}ClN_8O$) C, H, N.

5-Amino-8-methyl-9-(4-methylpiperazin-1-yl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine Dihydrochloride (54). Yield 90%; white solid; mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 2.45 (s, 3H), 2.50 (m, 4H), 3.62 (m, 4H), 3.85 (s, 3H), 6.70 (s, 1H), 7.35 (s, 1H), 7.59 (bs, 2H), 7.94 (s, 1H), 9.00 (bs, 1H), 11.4 (bs, 1H). Anal. ($C_{16}H_{21}Cl_2N_9O$) C, H, N.

1-(2-(2-Furyl)-8-methyl-9-(ethylamino)-8*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-yl)-3-(4-methoxyphenyl)urea Hydrochloride (55). Yield 93%; white solid; mp >300 °C. ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, $J = 6$), 3.73 (d, 6H), 4.00 (m, 2H, $J = 6$), 4.47 (bs, 2H), 6.72(m, 1H), 6.95-(d, 2H, $J = 8$), 7.19 (m, 1H), 7.45 (d, 2H, $J = 8$), 7.95 (s, 1H), 10.68 (bs, 1H). Anal. ($C_{21}H_{22}ClN_9O_3$) C, H, N.

General Procedure for the Preparation of 5-Amino-8-methyl-9-methylsulfanyl-2-[5-(4-methylpiperazin-1-yl-methylene)-2-furyl]pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine 51 and 5-Amino-7-(2-phenylethyl)-2-[5-(4-methylpiperazin/morpholin-1-yl-methylene)-2-furyl]pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines 57, 58. To a solution of compounds **41** and **61** (180 mg, 0.6 mmol) in acetic acid (20 mL) were added *N*-methylpiperazine or morpholine (1.62 mmol) and 36% formaldehyde (0.96 mmol), and the mixture was heated at reflux until the disappearance of starting material. The solvent was removed under reduced pressure, and the residue was purified by chromatography (CH_2Cl_2/MeOH 9:1) to afford the desired compounds as solid.

8-Methyl-2-[5-(4-methylpiperazin-1-yl-methyl)furan-2-yl]-9-methylsulfanyl-8*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-ylamine (51). Yield 20%; white solid; mp 258−260 °C; ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 3H), 2.50 (bs, 4H), 2.76 (s, 3H), 3.34 (bs, 4H), 3.57 (s, 2H), 4.01 (s, 3H), 6.54 (d, 1H, $J = 3$), 7.15 (d, 1H, $J = 3$), 7.72 (s, 2H). Anal. ($C_{18}H_{23}N_9OS$) C, H, N.

2-[5-(4-Methyl-piperazin-1-ylmethyl)furan-2-yl]-7-phenethyl-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-ylamine (57). Yield 22%; pale yellow solid; mp 206−207 °C; ^{1}H NMR (DMSO-d_6) δ 2.13 (s, 3H), 2.42 (bs, 4H), 3.21 (t, 2H, $J = 8$), 3.34 (bs, 4H), 3.55 (s, 2H), 4.48 (t, 2H, $J = 8$), 6.55 (d, 1H, $J = 4$), 7.18 (bs, 5H), 7.24 (d, 1H, $J = 4$), 8.08 (bs, 2H), 8.16 (s, 1H). Anal. ($C_{24}H_{27}N_9O$) C, H, N.

2-(5-Morpholin-4-yl-methyl-furan-2-yl)-7-phenethyl-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidin-5-ylamine (58). Yield 20%; pale yellow solid; mp 200−202 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (bs, 4H), 3.17 (t, 2H, $J = 8$), 3.34 (s, 2H), 3.57 (bs, 4H), 4.48 (t, 2H, $J = 8$), 6.57 (d, 1H, $J = 4$), 7.21 (m, 5H), 7.23 (d, 1H, $J = 4$), 8.09 (bs, 2H), 8.18 (s, 1H). Anal. ($C_{23}H_{24}N_8O_2$) C, H, N.

General Procedure for the Preparation of Hydrochloric Salts 56, 59, 60. The compounds **51**, **57**, **58** (0.24 mmol) were dissolved in a methanolic satured solution of hydrochloric acid (0.48 mmol), and the solution was stirred at 0 °C for several minutes and then at room temperature for 10 min. The solvent was removed at reduced pressure, and the residue was coevaporated with dry Et_2O and purified by crystallization from EtOH to afford the desired products as hydrochloric salts.

5-Amino-8-methyl-9-methylsulfanyl-2-[5-(4-methylpyperazin-1-ylmethylene)-2-furyl]pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine Hydrochloride (56). Yield 76%; white solid; mp 255−256 °C; ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H), 2.75 (s, 3H), 2.79 (s, 3H), 3.30 (m, 4H), 3.66 (m, 4H), 4.01

(s, 2H), 6.91 (d, 1H, $J = 4$), 7.26 (d, 1H, $J = 4$), 7.72 (bs, 2H), 11.71 (bs, 1H). Anal. ($C_{18}H_{24}ClN_9OS$) C, H, N.

5-Amino-7-(2-phenylethyl)-2-[5-(4-methylpiperazin-1-ylmethylene)-2-furyl]pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine Hydrochloride (59). Yield 81%; white solid; mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 2.24 (s, 3H), 2.51 (bs, 4H), 3.26 (t, 2H, $J = 4$), 3.41 (bs, 4H), 3.62 (s, 2H), 4.52 (t, 2H, $J = 8$), 6.62 (d, 1H, $J = 4$), 7.28 (bs, 5H), 7.31 (d, 1H, $J = 4$), 8.15 (bs, 2H), 8.22 (s, 1H), 10.81 (bs, 1H). Anal. ($C_{24}H_{28}ClN_9O$) C, H, N.

5-Amino-7-(2-phenylethyl)-2-[5-(4-morpholin-1-yl-methylene)-2-furyl]pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine Hydrochloride (60). Yield 78%; pale yellow solid; mp 215−216 °C; ^{1}H NMR (DMSO-d_6) δ 2.53 (bs, 4H), 3.23 (t, 2H, $J = 8$), 3.61 (s, 2H), 3.65 (bs, 4H), 4.53 (t, 2H, $J = 8$), 7.01 (d, 1H, $J = 4$), 7.23 (m, 5H), 7.37 (d, 1H, $J = 4$), 8.24 (bs, 2H), 8.31 (s, 1H), 10.22 (bs, 1H). Anal. ($C_{23}H_{25}ClN_8O_2$) C, H, N.

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

Human Cloned Adenosine Receptor Binding Assay. The expression of the human A_1, A_{2A}, and A_3 receptors in CHO cells has been previously described.[25] 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 $MgCl_2$, 1mM 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).

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. Nonspecific 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 nonspecific 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 ≤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 final 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. Nonspecific 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[26] with bovine albumin as a standard reference. Inhibitory binding constant, K_i, values were calculated from those of IC_{50} according to Cheng & Prusoff equation[27] ($K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where [C*] is the concentration of the radioligand and K_D* its dissociation constant. A weighted non linear least-squares curve fitting program LIGAND[28] was used for computer analysis of saturation and inhibition experiments.

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

Measurement of Cyclic AMP Levels in CHO Cells Transfected with Human A_{2A} or A_3 Adenosine Receptors. CHO cells transfected with human A_{2A} or A_3 adenosine receptors were washed with phosphate-buffered saline, diluted with trypsin, and centrifuged for 10 min at 200*g*. The pellet containing CHO cells (1×10^6 cells/assay) was suspended in 0.5 mL of incubation mixture (mM): NaCl 15, KCl 0.27, NaH_2PO_4 0.037, $MgSO_4$ 0.1, $CaCl_2$ 0.1, Hepes 0.01, $MgCl_2$ 1, glucose 0.5, pH 7.4 at 37 °C, 2 IU/mL adenosine deaminase and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20−1724) as phosphodiesterase inhibitor and preincubated for 10 min in a shaking bath at 37 °C. The potency of antagonists (IC_{50}, nM) on A_{2A} adenosine receptors were determined by antagonism of NECA (100 nM)-induced stimulation of cyclic AMP levels. The potency of antagonists (IC_{50}, nM) on A_3 adenosine receptors was determined by antagonism of Cl−IB-MECA (100 nM)-induced inhibition of cyclic AMP levels. The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000*g* for 10 min at 4 °C, and the supernatant was extracted four times with water saturated diethyl ether. The final aqueous solution was tested for cyclic AMP levels by a competition protein binding assay. Samples of cyclic AMP standard (0−10 pmol) were added to each test tube containing the incubation buffer (trizma base 0.1 M, aminophyllin 8.0 mM, 2 mercaptoethanol 6.0 mM, pH 7.4) and [^{3}H] cyclic AMP in a total volume of 0.5 mL. The binding protein, previously prepared from beef adrenals, was added to the samples previously incubated at 4 °C for 150 min, and after the addition of charcoal were centrifuged at 2000*g* for 10 min. The clear supernatant was counted in a Beckman scintillation counter.

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