J. Med. Chem. **1998,** *41,* 668−673

Novel 3-Aralkyl-7-(amino-substituted)-1,2,3-triazolo[4,5-*d*]pyrimidines with High Affinity toward A_1 Adenosine Receptors

Laura Betti,[§] Giuliana Biagi,[†] Gino Giannaccini,[§] Irene Giorgi,[†] Oreste Livi,*[,†] Antonio Lucacchini,[§] Clementina Manera,[†] and Valerio Scartoni[†]

Dipartimento di Scienze Farmaceutiche and Istituto Policattedra di Discipline Biologiche, Facoltà di Farmacia, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy

Received March 4, 1997

Three series of several 1,2,3-triazolo[4,5-*d*]pyrimidine derivatives bearing various amino substituents at the 7 position and one of three lipophilic substituents at the 3 position (benzyl, phenethyl, or 2-chlorobenzyl) were prepared starting from the corresponding 7-chloro compounds, by nucleophilic substitution by the appropriate amine. Radioligand binding assays at bovine brain adenosine A_1 and A_{2A} receptors showed that some compounds possessed a high affinity and selectivity for the A_1 receptor subtype. In particular the biological results suggested the compounds bearing cycloalkylamino (cyclopentyl- and cyclohexylamino) or aralkylamino (α-methylbenzyl- and 1-methyl-2-phenylethylamino or amphetamino) substituents at the 7 position were the most active derivatives. The best lipophilic substituent at the 3 position was the 2-chlorobenzyl (A_1 affinity K_i < 50 nM) followed by the benzyl and then the phenethyl groups. This pattern of structure−activity relationship (SAR) was similar to that previously reported for analogous 1,2,3-triazolopyridazino derivatives (Biagi et al., 1994, 1995, 1996) except for the compounds bearing substituted aromatic amines which presented a generalized and strong decrease of the A_1 receptor affinity. These facts allowed us to attribute to these molecules a binding mode within the A_1 adenosine receptor analogous to that of the corresponding triazolopyridazines.

Introduction

A wide variety of actions of the local modulator adenosine in the nervous, cardiovascular, renal, immune, and other systems is mediated by adenosine receptors.[1] Up to now four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, A_3) have been defined on the basis of pharmacological distinctions and on molecular cloning and characterization.[2−6]

The receptor subtype A_{2A} exhibits high affinity for adenosine in the low-nanomolar range, while the subtype A_{2B} is a low-affinity receptor in the low-micromolar range. Activation of A_1 and A_3 adenosine receptors can lead to an inhibition of adenylate cyclase activity, while on the contrary the cyclase can be stimulated by the activation of A_{2A} and A_{2B} adenosine receptors.

All of the adenosine receptor agonists synthesized thus far are structurally related to adenosine itself.[1,7,8] In fact it is known that compounds which present the ribose moiety mainly intact are agonist,[1] whereas the substitution of the ribose moiety with a lipophilic group leads to products which act as antagonists.[9−12]

Many selective agonists or antagonists have shown promise as potential therapeutic agents for the treatment of cognitive disease, renal failure, Alzheimer's disease, and cardiac arrhythmias (A_1) or Parkinson's disease,[13−15] Huntington's chorea, schizophrenia, myasthenia gravis, and myastenic syndromes (A_{2A}).[16] Few selective and/or high-affinity antagonists for A_{2B} and A_3 receptor subtypes have been reported.[17]

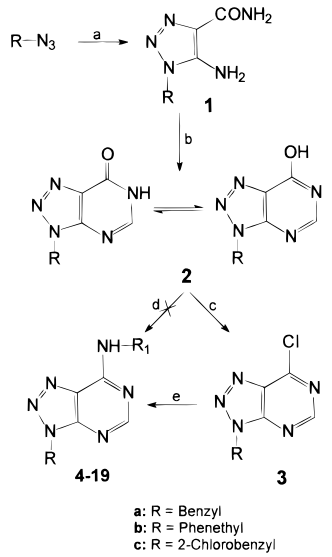
In the past, with the aim of obtaining potent A_1 antagonists, we synthesized several 8-azaadenines (1,2,3-triazolo[4,5-*d*]pyrimidines) easily prepared providing flexibility of substitution at C2 , N^6, and N9 positions.[18−23] This rational study, aimed at discovering the best substituents in the three different positions, led to the preparation, among others, of 2-phenyl-N^6-cyclohexyl-*N*9-benzyl-8-azaadenine (K_i 1.6 nM)[24] and was interpreted as evidence for the presence of three lipophilic pockets in the A_1 receptor site.[25−30] The phenyl group on C2, the substitution of the ribosyl moiety on N9 with a lipophilic group (e.g., benzyl group), and a lipophilic group on N^6 concurred to increase A_1 affinity and selectivity among 8-azaadenines as antagonists. According to this finding we hypothesized the possible dual mode of binding of the bound exogenous molecule inside the A_1 receptor, sterically related by rotation around an ideal C2−N7 axis of the 8-azaadenine nucleus.[25] Other similar results concerning the arrangement of some antagonist molecules in the A_1 receptor subtype have been also reported separately by Müller et al.[31]

Recent studies concerning 7-hydroxy-1,2,3-triazolo-[4,5-*d*]pyridazines, bearing lipophilic substituents in the 1 position and amino substituents in the 4 position of the heterocycle, showed high affinity and selectivity toward the adenosine A_1 receptor.[32−35] As in the N^6-substituted adenosines,[36] the structure−activity relationship (SAR) analysis of these compounds required a hydrogen atom on N^4 together with a lipophilic substituent such as an unsubstituted cycloalkyl, meta- or para-monosubstituted aryl, α-methylbenzyl, or 1-methylphenethyl one. Compounds bearing a chiral substituent on N^4 had shown a stereoselective effect. The *R*

* Author for correspondence and reprint requests.
[†] Dipartimento di Scienze Farmaceutiche.
[§] Istituto Policattedra di Discipline Biologiche.

S0022-2623(97)00133-7 CCC: $15.00
Published on Web 01/30/1998

Scheme 1[a]



[a] (a) $CNCONH_2$; (b) $HCONH_2$; (c) $SOCl_2$, $CHCl_3$, DMF; (d) R_1NH_2, HMDS; (e) R_1NH_2, TEA, EtOH.

configuration both in N^4-α-methylbenzyl and in N^4-1-methyl-2-phenylethyl led to the more effective stereoisomer, as in the selective A_1 agonist *R*-PIA.[36b,c] The best lipophilic substituent at the 1 position was the 2-chlorobenzyl, which assured the greatest affinity compared with the benzyl and phenethyl ones. These results confirmed the presence inside the adenosine A_1 receptor of a lipophilic pocket with well-defined dimensions facing the N1 position of the 1,2,3-triazolo[4,5-*d*]-pyridazine nucleus. To investigate the mode of binding of these compounds with the A_1 receptor, we undertook the synthesis and biological evaluation of new 1,2,3-triazolo[4,5-*d*]pyrimidines (8-azapurines) as analogues of the 1,2,3-triazolo[4,5-*d*]pyridazines quoted above. The comparison of the SAR analysis of such compounds should allow the understanding of the similarities between the possible mode of binding with the receptor.

Chemistry

Synthesis of the 1,2,3-triazolo[4,5-*d*]pyrimidine ring and its 7-amino-substituted derivatives is illustrated in Scheme 1. The appropriate azide, by ionic 1,3-dipolar cycloaddition reaction with cyanacetamide, provided the corresponding 1-substituted-4-carbamoyl-5-amino-1*H*-1,2,3-triazoles **1a**−**c.** These compounds, by heating in an excess of formamide, were cyclized to the triazolopyrimidines **2a**−**c**, which were then converted by thionyl chloride to the corresponding reactive chloro derivatives **3a**−**c**. Then the halogen atom was easily replaced by nucleophilic displacement with primary amines, even if weakly basic, in the presence of triethylamine, to obtain the final products **4**−**19**.

The silylation−amination reaction of aromatic hydroxy *N*-heterocycles[37] using hexamethyldisilazane and 6-hydroxy-8-azahypoxanthine (**2**) gave unsatisfactory results, contrary to the results obtained with 1,2,3-triazolo[4,5-*d*]pyridazines.[33−35]

The choice of amines, used for the nucleophilic displacement reaction, and that of azides, for the cycloaddition reaction, to introduce the lipophilic groups on C7 and C3, respectively, were based in part on the 1,2,3-triazolo[4,5-*d*]pyridazines which had been found previously to be biologically active. In addition some other new derivatives, bearing the same substituents in the 3 position but previously unknown groups in the 7 position, were also prepared.

The 7-(amino-substituted)-3-benzyl-1,2,3-triazolo[4,5-*d*]pyrimidine derivatives are reported in the Supporting Information (Table 1); the 1,2,3-triazole **1a**[38] and the triazolopyrimidine intermediates, 7-hydroxy **2a**[39] and 7-chloro **3a**,[40] have already been described in the literature. The **4a**−**17a** derivatives were obtained according to the general procedure, starting from the chloroazapurine **3a** and the appropriate primary amine; compounds **18a** and **19a** were obtained by reduction of the corresponding nitro derivatives **16a** and **17a** with hydrazine hydrate. 3-Benzyl-substituted compounds (series **a**) **4**−**6**, **8**−**10**, and **12**−**15** were analogues of the triazolopyridazines previously described.

Fewer than in the series **a**, 3-phenethyl-1,2,3-triazolo-[4,5-*d*]pyrimidine derivatives (Table 2 in Supporting Information) were prepared, because of the low biological activity of the 1-phenethyl-substituted triazolopyridazines. The 1-phenethyl-4-carbamoyl-5-amino-1*H*-1,2,3-triazole (**1b**) has been described in the literature,[41] while the 8-azapurines **2b** and **3b** were prepared in the usual manner. The 3-phenethyl-substituted compounds (series **b**) **4**, **6**, **10**, and **11** were analogues of the previously reported triazolopyridazines.[34]

The 3-(2-chlorobenzyl)-1,2,3-triazolo[4,5-*d*]pyrimidines (series **c**), analogous to the 1-(2-chlorobenzyl)-1,2,3-triazolo[4,5-*d*]pyridazines[35] which were previously found to provide the highest affinity, are reported in the Supporting Information (Table 3). Thus, starting from 2-chlorobenzyl azide,[42] according to the usual synthetic route (Scheme 1), the 1,2,3-triazole compound **1c**[43] and the triazolopyrimidine intermediates **2c** and **3c** were prepared in good yield.

The structures of all the newly prepared compounds were confirmed by analytical and spectroscopic data. ^1H NMR data of some selected compounds are reported in the Supporting Information (Table 4).

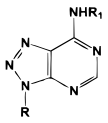
Biological Evaluation

The 7-(amino-substituted)-1,2,3-triazolo[4,5-*d*]pyrimidines were tested in radioligand binding assays for affinity at A_1 and A_{2A} adenosine receptors in bovine brain cortical membranes and in bovine brain striatal membranes, respectively. [^{3}H]-(*R*)-(−)-N^6-(2-Phenylisopropyl)adenosine (*R*-PIA) was used as the A_1 radioligand and [^{3}H]-2-{[[*p*-(2-carboxyethyl)phenyl]ethyl]amino}-5′-(*N*-ethylcarbamoyl) adenosine (CGS 21680) as the A_{2A} radioligand.

Results and Discussion

In Table 1 are reported only the results of the A_1 adenosine receptor binding assay, expressed as inhibition constants (K_i, nM), for the three series [**a**, 3-benzyl; **b**, 3-(2-phenylethyl); **c**, 3-(2-chlorobenzyl)] of 7-(amino-substituted)-1,2,3-triazolo[4,5-*d*]pyrimidine derivatives. In fact the binding assay at adenosine A_{2A} receptors showed that these compounds presented very low inhibition percentages at 1 μM, so that the corresponding K_i values were not calculated for the majority of the compounds. The more effective compound toward A_{2A}

Table 1. A_1 Adenosine Receptor Binding [K_i (nM) ± SEM] of 1,2,3-Triazolo[4,5-*d*]pyrimidines **4a**−**19a**, **4b**−**13b**, and **4c**−**18c**[a]



R_1	R					
						
cyclohexyl	**4a**	121 ± 8.7	**4b**	216 ± 10	**4c**	43 ± 2.5
cyclopentyl	**5a**	127 ± 5.1	**5b**	176 ± 12	**5c**	21 ± 1.5
3-methylcyclohexyl	**6a**	770 ± 40			**6c**	102 ± 4.1
cyclohexylmethyl	**7a**	2790 ± 161			**7c**	4260 ± 147
p-tolyl	**8a**	502 ± 20	**6b**	1670 ± 67	**8c**	119 ± 5.5
m-tolyl	**9a**	1100 ± 38	**7b**	4380 ± 227	**9c**	5880 ± 237
m-nitrophenyl	**10a**	443 ± 20	**8b**	2250 ± 155	**10c**	3300 ± 152
m-chlorophenyl	**11a**	463 ± 29	**9b**	3320 ± 115	**11c**	1830 ± 63
aniline	**12a**	6700 ± 309	**10b**	7200 ± 478	**12c**	> 10000
(*R,S*)-α-methylbenzyl	**13a**	438 ± 18	**11b**	2200 ± 88	**13c**	468 ± 29
(*R,S*)-1-methyl-2-phenylethyl	**14a**	575 ± 33	**12b**	2490 ± 115	**14c**	73 ± 5.0
(*R*)-1-methyl-2-phenylethyl	**15a**	274 ± 9.7			**15c**	39 ± 2.5
p-nitrobenzyl	**16a**	> 10000	**13b**	245 ± 17	**16c**	> 10000
p-nitrophenethyl	**17a**	> 10000			**17c**	> 10000
p-aminobenzyl	**18a**	9500 ± 300			**18c**	> 10000
p-aminophenethyl	**19a**	1540 ± 97				

[a] The tests were carried out dissolving the compounds in DMSO (DMSO/buffer, 2%) unless otherwise indicated. The K_i values are means ± SEM of four separate assays, each performed in triplicate.

receptors was **13a**, which showed an affinity constant of K_i = 1750 nM.

Compounds **4** and **5** had low K_i values with a moderate preference for the cyclopentyl (**5a**−**c**) over the cyclohexyl (**4a**−**c**) group; the most potent compounds were **5c** (K_i = 21 nM) and **4c** (K_i = 43 nM), with a receptor selectivity (K_i A_{2A}/K_i A_1) corresponding to 215 and 230, respectively. A comparison of these results showed that the A_1 affinity increased with changes in the lipophilic substituent at the 3 position of the heterocycle, according to the sequence: 2-chlorobenzyl > benzyl > 2-phenylethyl.

Introduction of a methyl group at the 3 position of the cyclohexyl ring (compounds **6a**,**c**), chosen by analogy to the *m*-toluidino substituent of the active triazolopyridazine derivatives,[34,35] caused a decrease in receptor affinity, which appeared more considerable when the cyclohexyl ring was more distant from the nitrogen atom (compounds **7a**,**c**).

The aralkylamino derivatives with the (*R,S*)-α-methylbenzylamino substituent (**13a**, **11b**, and **13c**) and the (*R,S*)-amphetamino substituent (**14a**, **12b**, and **14c**) presented the same A_1 affinity trend as for compounds **4** and **5** regarding the substituent at the 3 position of the heterocycle. Compared with compounds bearing the α-methylbenzyl group, the 2-chlorobenzyl derivatives bearing the amphetamino substituent (**14c** and **15c**) presented the highest affinity, and the *R* enantiomer (**15c**: K_i = 39 nM) was more potent than the racemic mixture (**14c**: K_i = 73 nM). For **15c** and **14c** the receptor selectivity (K_i A_{2A}/K_i A_1) was >250 and 46, respectively.

The 3-benzyl derivatives **16a**−**19a** and the 3-(2-chlorobenzyl) derivatives **16c**−**18c** possessed low receptor affinity. Instead the 3-(2-phenylethyl) derivative **13b** showed an appreciable affinity (K_i = 245 nM).

Compounds **8a**−**11a**, **6b**−**9b**, and **8c**−**11c**, bearing an aromatic amino substituent at C7, were less potent at A_1 receptors than the corresponding triazolopyridazine derivatives.[32−35] In addition, contrary to these last compounds, the 7-toluidino-substituted triazolopyrimidines showed that the para substitution on the aromatic ring (compounds **8a, 6b**, and **8c**) appeared clearly more effective than substitution at the meta position (compounds **9a**, **7b**, and **9c**). But the contribution to the effectiveness of the molecules shown by the lipophilic substituents on N3 followed the known trend: 2-chlorobenzyl > benzyl > 2-phenylethyl, only for the 7-*p*-toluidino derivatives **8a**, **6b**, and **8c**. Considering the previous triazolopyridazine derivatives, we had preferred, at the start of this work, the meta substitution, so that other new derivatives (**10a**, **11a**, **8b**, **9b, 10c**, and **11c**) with a *m*-nitro- or *m*-chloro-substituted anilino group in the 7 position were prepared. In these cases the best lipophilic substituent in the 3 position of the heterocycle was the benzylic group.

The observed change in affinity among compounds bearing C7 meta-substituted anilino groups would appear to depend on inductive and/or mesomeric effects; in fact in the benzyl (**a**) and 2-phenylethyl (**b**) series the nitro group (**10a** and **8b**) was slightly better than the chloro atom (**11a** and **9b**), which was in turn better than the methyl group (**9a** and **7b**). Finally introduction of a phenylhydrazino substituent in the 7 position (**12a**, **10b**, and **12c**) caused a strong decrease of the receptor binding affinity.

Summary

In conclusion, the best agreement between triazolopyridazines and triazolopyrimidines was found with the cyclohexylamino, cyclopentylamino, and amphetamino groups on C4 and C7, respectively, in the two series **a** and **c**. In addition, we observed the same enantioselective effect regarding the amphetamino derivatives: the *R* stereoisomer (**15a**,**c**) was more active than the racemic mixture (**14a**,**c**). Our data regarding the stereochemical requirement of the site facing the N7 position when engaged by a 1-methyl-2-phenylethyl group were in accordance with the results obtained with *R*-PIA.[36]

The important similarities of stereoselectivity together with those concerned with the compounds bearing cycloalkyl substituents, neglecting some minor behavior differences among less active products, induced us to consider that both triazolopyridazines and triazolopyrimidines could bind to the same site in the A_1 adenosine receptor.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot stage and are uncorrected. IR spectra in Nujol mulls were recorded on a Perkin-Elmer model 1310 spectrometer. ^{1}H NMR spectra were recorded with a Varian CFT-20 spectrometer in δ units from TMS as an internal standard. Mass

spectra were performed with a Hewlett-Packard MS/System 5988. Elemental analyses (C,H,N) were within ±0.4% of the theoretical values and were performed on a Carlo Erba elemental analyzer model 1106 apparatus. Optical rotations were measured with a Violet AA-5 polarimeter.

3-Phenethyl-7-hydroxy-1,2,3-triazolo[4,5-*d*]pyrimidine (2b). A solution of 5.08 g (22.0 mmol) of 1-phenethyl-4-carbamoyl-5-amino-1*H*-1,2,3-triazole (**1b**)[41] in 24 mL of formamide was refluxed for 2 h. After cooling the solution was diluted with H_2O, and the precipitated solid was collected by filtration and washed with H_2O: 4.73 g, yield 89%; mp 262−264 °C (EtOH); MS 241 (M^+), 150 (base peak). Anal. ($C_{12}H_{11}N_5O$) C, H, N.

3-(2-Chlorobenzyl)-7-hydroxy-1,2,3-triazolo[4,5-*d*]pyrimidine (2c). A solution of 1.0 g (3.97 mmol) of 1-(2-chlorobenzyl)-4-carbamoyl-5-amino-1*H*-1,2,3-triazole (**1c**)[43] in 4 mL of formamide was worked up as described for the preparation of **2b**: 1.02 g, 98% yield; mp 282−285 °C (EtOH). Anal. ($C_{11}H_8N_5OCl$) C, H, N.

3-Phenethyl-7-chloro-1,2,3-triazolo[4,5-*d*]pyrimidine (3b). To a suspension of **2b** (2.0 g, 8.3 mmol) in 40 mL of boiling anhydrous $CHCl_3$ were added 1.5 mL of DMF and 7.0 mL of $SOCl_2$. The reaction mixture was refluxed for 2 h, the solvent was evaporated in vacuo (temperature < 35 °C), and the residue, after cooling at 0 °C, was triturated with crushed ice. The solid formed was collected by filtration, dried, and extracted repeatedly with boiling 60−80 °C petroleum ether. The combined extracts were evaporated in vacuo to give **3b** as a white solid: 1.64 g, 76% yield; mp 94−95 °C; MS 259 (M^+), 104 (base peak). Anal. ($C_{12}H_{10}N_5Cl$) C, H, N.

3-(2-Chlorobenzyl)-7-chloro-1,2,3-triazolo[4,5-*d*]pyrimidine (3c). To a suspension of **2c** (2.26 g, 8.67 mmol) in 40 mL of boiling anhydrous $CHCl_3$ were added 1.5 mL of DMF and 8.2 mL of $SOCl_2$. The reaction mixture was worked up as described for the preparation of **3b**: 1.74 g, 72% yield; mp 112−115 °C; MS 226 (M^+), 125 (base peak). Anal. ($C_{11}H_7N_5Cl_2$) C, H, N.

3-Benzyl-7-(cycloalkylamino)-1,2,3-triazolo[4,5-*d*]pyrimidines 4a−7a. A suspension of 3-benzyl-7-chloro-1,2,3-triazolo[4,5-*d*]pyrimidine (**3a**)[40] (0.400 g, 1.63 mmol), TEA (0.25 mL, 1.80 mmol), and 1.80 mmol of the appropriate cycloalkylamine in 10 mL of absolute EtOH was heated under reflux for the time reported in Table 1 (Supporting Information). For compounds **4a**, **5a**, and **6a**, the reaction mixture was evaporated in vacuo and the residue was triturated with H_2O and 10% HCl (pH ≅ 4), collected by filtration, washed with EtOH, and crystallized (Table 1 in Supporting Information). Compound **7a** crystallized from the reaction mixture (Table 1 in Supporting Information).

3-Benzyl-7-(arylamino)-1,2,3-triazolo[4,5-*d*]pyrimidines 8a−12a. A suspension of **3a** (0.400 g, 1.63 mmol), TEA (0.25 mL, 1.80 mmol), and 1.80 mmol of the appropriate arylamine in 10 mL of absolute EtOH was refluxed for the time reported in Table 1 (Supporting Information). The title compounds crystallized from the reaction mixture, were collected by filtration, were washed with H_2O and EtOH, and eventually recrystallized (Table 1 in Supporting Information).

3-Benzyl-7-(aralkylamino)-1,2,3-triazolo[4,5-*d*]pyrimidines 13a−17a. A suspension of **3a** (0.400 g, 1.63 mmol), TEA (0.25 mL, 1.80 mmol, for **13a**−**15a**; 0.50 mL, 3.60 mmol, for **16a** and **17a**, because amino hydrochlorides were used), and 1.80 mmol of the appropriate aralkylamine in 10 mL of absolute EtOH was refluxed for the time reported in Table 1 (Supporting Information). For compounds **13a**−**15a** the reaction mixture was evaporated in vacuo to give an oily residue. For **13a** the residue was dissolved in $CHCl_3$, and the solution, after washing with 10% HCl and H_2O, was evaporated to give **13a** as an oil (77% yield) which was converted to solid hydrochloride (Table 1 in Supporting Information). For **14a** the residue was treated with 10% HCl (pH ≅ 4), and the solid formed was collected by filtration and crystallized (Table 1 in Supporting Information). For **15a** the residue was dissolved in AcOEt/60−80 °C petroleum ether mixture and converted to solid hydrochloride (Table 1 in Supporting Information). Compounds **16a** and **17a** crystallized from the reaction mixture, were collected by filtration, and were triturated with boiling EtOH (Table 1 in Supporting Information).

3-Phenethyl-7-(substituted amino)-1,2,3-triazolo[4,5-*d*]pyrimidines 4b−13b. To a stirred suspension of 3-phenethyl-7-chloro-1,2,3-triazolo[4,5-*d*]pyrimidine (**3b**) (0.400 g, 1.54 mmol) in 10 mL of absolute EtOH were added 0.23 mL (1.70 mmol) of TEA and 1.70 mmol of the suitable amine (for compound **13b**, 3.40 mmol of TEA, because *p*-nitrobenzylamine hydrochloride was employed). The mixture was heated under reflux for the time reported in Table 2 (Supporting Information). After one night the crystallized precipitate was collected by filtration, washed with H_2O and EtOH, and eventually recrystallized (Table 2 in Supporting Information).

3-(2-Chlorobenzyl)-7-(cycloalkylamino)-1,2,3-triazolo[4,5-*d*]pyrimidines 4c−7c. To a stirred suspension of 3-(2-chlorobenzyl)-7-chloro-1,2,3-triazolo[4,5-*d*]pyrimidine (**3c**) (0.400 g, 1.43 mmol) in 10 mL of absolute EtOH were added TEA (0.18 mL, 1.70 mmol) and the suitable amine (1.70 mmol for **4c** and **5c**; 5.10 mmol for **6c** and **7c**), and the mixture was refluxed for the time reported in Table 3 (Supporting Information). For compounds **4c**, **5c**, and **6c** the reaction mixture was evaporated in vacuo, the liquid residue was treated with H_2O and 10% HCl (pH ≅ 3), and the solid formed was collected and crystallized (Table 3 in Supporting Information). Compound **7c** crystallized from reaction mixture and was collected after one night (Table 3 in Supporting Information).

3-(2-Chlorobenzyl)-7-(arylamino)-1,2,3-triazolo[4,5-*d*]pyrimidines 8c−12c. To a stirred suspension of **3c** (0.400 g, 1.43 mmol) in 10 mL of absolute EtOH were added TEA (0.18 mL, 1.70 mmol, for **8c**, **10c**, and **11c**; 0.54 mL, 5.10 mmol, for **9c** and **12c**) and the suitable amine (5.10 mmol for **8c** and **11c**; 3.40 mmol for **9c**, **10c**, and **12c**). The mixture was refluxed for the time reported in Table 3 (Supporting Information). The title compounds crystallized from the reaction mixture and were collected after one night (Table 3 in Supporting Information).

3-(2-Chlorobenzyl)-7-(aralkylamino)-1,2,3-triazolo[4,5-*d*]pyrimidines 13c−17c. To a stirred suspension of **3c** (0.400 g, 1.43 mmol) in 10 mL of absolute EtOH were added TEA (0.18 mL,1.70 mmol, for **13c**, **14c**, and **15c**; 0.54 mL, 5.10 mmol, for **16c** and **17c**) and the suitable amine (5.10 mmol for **13c**; 3.40 mmol for **16c** and **17c** as hydrochlorides; 1.70 mmol for **14c** and **15c**). The mixture was refluxed for the time reported in Table 3 (Supporting Information). Compounds **13c**, **16c**, and **17c** crystallized from the reaction mixture and therefore were collected and recrystallized (Table 3 in Supporting Information). For compounds **14c** and **15c** the reaction mixture was evaporated in vacuo, and the residue was triturated with H_2O and 10% HCl (pH = 3−4); the acid solution was decanted and the residue washed with H_2O and dried. For **14c** the residue was dissolved in AcOEt, 60−80 °C petroleum ether was added, and the mixture cooled at −20 °C; the precipitated solid was collected and recrystallized (Table 3 in Supporting Information). For **15c** the residue was extracted with portions of boiling 60−80 °C petroleum ether, and the combined extracts were evaporated. The oily residue was purified by flash chromatography (230−400 mesh silica gel column, 14 × 2 cm) eluting with 1:3 AcOEt/40−60 °C petroleum ether mixture. Compound **15c** was isolated as a viscous oil which was converted to solid hydrochloride (Table 3 in Supporting Information).

3-Benzyl-7-[(*p*-aminobenzyl)amino]-1,2,3-triazolo[4,5-*d*]pyrimidine (18a) and 3-Benzyl-7-[(*p*-aminophenethyl)amino]-1,2,3-triazolo[4,5-*d*]pyrimidine (19a). To a stirred and boiling solution of 0.70 mmol of the nitro derivative **16a** or **17a** in 5 or 10 mL of EtOH, respectively, was added 50−100 mg of Raney nickel, and successively a solution of 99% hydrazine hydrate (0.15 mL, 3.20 mmol) in 3 mL of EtOH was dripped. The refluxing was continued for 1 h; then the catalyst was filtered off and washed with boiling EtOH. The combined filtrates were concentrated in vacuo until the title compounds crystallized (Table 1 in Supporting Information).

3-(2-Chlorobenzyl)-7-[(*p*-aminobenzyl)amino]-1,2,3-triazolo[4,5-*d*]pyrimidine (18c). A stirred suspension of **16c** (0.505 g, 1.27 mmol) and 99% hydrazine hydrate (0.18 mL, 1.27 mmol) in 10 mL of 1,2-dichloroethane−EtOH (1:1) mixture was heated at 30 °C for 20 min; ≅100 mg of Raney nickel was added and the suspension heated at 40 °C for 10 h. The catalyst was filtered off, and the filtrate was evaporated in vacuo. The crude solid residue (0.340 g) was purified by flash chromatography (230−400 mesh silica gel column, 14 × 2 cm) eluting with AcOEt/40−60 °C petroleum ether (2:3) mixture (Table 3 in Supporting Information).

Biochemical Assays: A_1 Receptor Binding. Bovine cerebral cortex was homogenized in ice-cold 0.32 M sucrose containing protease inhibitors, as previously described.[44] The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatant again centrifuged at 48000*g* for 15 min at 4 °C. The final pellet was dispersed in 10 volumes of fresh buffer, incubated with adenosine deaminase (2 units/mL) to remove endogenous adenosine at 37 °C for 60 min, and then recentrifuged at 48000*g* for 15 min at 4 °C. The pellet was suspended in buffer and used in the binding assay.

The [^{3}H]CHA binding assay was performed in triplicate by incubating aliquots of the membrane fraction (0.2−0.3 mg of protein) at 25 °C for 45 min in 0.5 mL of Tris-HCl, pH = 7.7, containing 2 mM $MgCl_2$, with approximately 1.2 nM [^{3}H]CHA. Nonspecific binding was defined in the presence of 50 μM *R*-PIA. The assay was completed by filtration through Whatman GF/C glass microfiber filters under suction and washing twice with 5 mL of ice-cold buffer.

A_{2A} Receptor Binding. Bovine striatum was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl, pH = 7.5, containing 10 mM $MgCl_2$ and protease inhibitors. The membrane homogenate was centrifuged at 48000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 units/mL of adenosine deaminase and incubated at 37 °C for 30 min. The membrane homogenate was centrifuged, and the final pellet was frozen at −80 °C. Routine assays were performed in triplicate by incubating an aliquot of striatal membranes (0.2−0.3 mg of protein) in 50 mM Tris-HCl, pH = 7.5, containing 10 mM $MgCl_2$ with approximately 5 nM [^{3}H]CGS 21680 in a final volume of 0.5 mL. Incubation was carried out at 25 °C for 90 min. Nonspecific binding was defined in the presence of 50 μM CGS 21680. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer and placed in scintillation vials. The radioactivity was counted in a 4-mL Beckman Ready-Protein scintillation cocktail in a scintillation counter. The compounds were dissolved in DMSO and added to the assay mixture to make a final volume of 0.5 mL. Blank experiments were carried out to determine the effect of the solvent (2%) on the binding. The concentrations of the tested compounds to produce 50% inhibition of specific [^{3}H]CHA or [^{3}H]CGS 21680 binding (IC_{50}) were determined from semilog plots of data from experiments of binding inhibition. The K_i values were calculated from the IC_{50} values using the equation $IC_{50}/(L/K_d)$.[45] For [^{3}H]CHA K_d =10.5 nM and L = 1.2 nM; for [^{3}H]CGS 21680 K_d = 1 nM and L = 5 nM. Protein estimation was based on the method reported,[46] using bovine serum albumin as standard.

Acknowledgment. We wish to thank the Consiglio Nazionale delle Ricerche (CNR) for financial support.

Supporting Information Available: Tables containing physicochemical data of compounds **4a**−**19a** (Table 1), **4b**−**13b** (Table 2), and **4c**−**18c** (Table 3) and ^{1}H NMR spectral data (δ) of some selected compounds (Table 4) (4 pages). See any current masthead page for ordering information.

References

(1) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Adenosine receptors: pharmacology, structure−activity relationship and therapeutical potential. *J. Med. Chem.* **1992**, *35*, 407−422.

(2) Libert, F.; Schiffmann, S. N.; Lefort, A.; Parmentier, M.; Gerard, C.; Dumont, J. E.; Vanderhaegen, J. J.; Vassart, G. The orphan receptor cDNA RDC7 encodes an A1 adenosine receptors. *EMBO J.* **1991**, *10*, 1677−1682.

(3) Maenhaut, C.; Sande, J. V.; Libert, F.; Abramowicz, M.; Parmentier, M.; Vanderhaeghen, J. J.; Dumont, J. E.; Vassart, G.; Schiffmann, S. RDC8 codes for an adenosine A2 receptor with physiological constitutive activity. *Biochem. Biophys. Res. Commun.* **1990,** *173*, 1169−1178.

(4) Stehle, J. H.; Rivkees, S. A.; Lee, J. J.; Weaver, D. R.; Deeds, J. D.; Reppert, S. M. Molecular cloning and expression of the cDMA for a novel A2-adenosine receptor subtype. *Mol. Endocrinol.* **1992**, *6*, 384−393.

(5) Zhou, Q. Y.; Li, C. Y.; Olah, M. E.; Johnson, R. A.; Stiles, G. L.; Civelli, O. Molecular cloning and characterization of an adenosine receptor − the A3 adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7432−7436.

(6) Collis, M. G.; Hourani, S. M. O. Adenosine receptor subtypes. *Trends Pharmacol. Sci.* **1993**, *14*, 360−366.

(7) Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borioni, A.; Viziano, M.; Dionisotti, S.; Ongini, E. Current developments of A_{2a} adenosine receptor agonists. *Curr. Med. Chem.* **1995**, *2*, 707−722.

(8) Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Ji, X.-d.; Olah, M. E.; Stiles, G.; Dionisotti, S.; Zocchi, C.; Ongini, E.; Jacobson, K. A. Novel N^6-(substituted-phenylcarbamoyl)adenosine-5′-uronamides as potent agonist for A_3 adenosine receptors. *J. Med. Chem.* **1996**, *39*, 802−806.

(9) Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W.; Daly, D. T.; Olsson, R. A. N^6-Substituted 9-methyladenines: a new class of adenosine receptor antagonists. *FEBS Lett.* **1987**, *215*, 203−208.

(10) Thompson, R. D.; Secunda, S.; Daly, J. W.; Olsson, R. A. N,^{6}9-Disubstituted adenines: potent, selective antagonists at the A_1 adenosine receptor. *J. Med. Chem.* **1981**, *34*, 2877−2882.

(11) Jacobson, K. A.; Siddiqi, S. M.; Olah, M. E.; Ji, X.-d.; Melman, N.; Bellamkonda, K.; Meshulam, Y.; Stiles, G. L.; Kim, H. O. Structure−activity relationships of 9-alkyladenine and ribose-modified adenosine derivatives at rat A_3 adenosine receptors. *J. Med. Chem.* **1995**, *38*, 1720−1735.

(12) Lohse, M. J.; Klotz, K.-N.; Diekmann, E.; Friedrich, K.; Schwabe, U. 2′,3′-Dideoxy-N^6-cyclohexyladenosine: an adenosine derivative with antagonist properties at adenosine receptors. *Eur. J. Pharmacol.* **1988**, *156*, 157−160.

(13) Jacobson, K. A.; Trivedi, B. K.; Churchill, P. C.; Williams, M. Novel therapeutics acting via purine receptors. *Biochem. Pharmacol.* **1991**, *41*, 1399−1410.

(14) Suzuki, F. Adenosine A_1 antagonists. A new therapeutic approach to cognitive deficits and acute renal failure. *Drug News Perspect.* **1992**, *5*, 587−591.

(15) Kanda, T.; Shiozaki, S.; Shimada, J.; Suzuki, F.; Nakamura, J. KF17837: A novel selective adenosine A_{2a} receptor antagonist with anticataleptic activity. *Eur. J. Pharmacol.* **1994**, *256*, 263−268.

(16) Sebastião, A. M., Ribeiro, J. A. Receptor mediated excitatory actions on the nervous system. *Prog. Neurobiol.* **1996**, *48*, 167−189.

(17) Jiang, J.-l.; van Rhee, A. M.; Melman, N.; Ji, X.-d.; Jacobson, K. A. 6-Phenyl-1,4-dihidropyridine derivatives as potent and selective A_3 adenosine receptor antagonists. *J. Med. Chem.* **1996**, *39*, 4667−4675.

(18) Barili, P. L.; Biagi, G.; Livi, O.; Scartoni, V. A facile "one pot"synthesis of 2,9-disubstituted 8-azapurin-6-ones (3,5-disubstituted 7-hydroxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidines). *J. Heterocycl. Chem.* **1985**, *22*, 1607−1609.

(19) Biagi, G.; Franchi, M.; Livi, O.; Scartoni, V. "One pot"synthesis of 2-substituted 9-(2′-hydroxy-3′-aminopropyl)-8-azahypoxanthines and 8-azaadenines (5-substituted 3-(2′-hydroxy-3′-aminopropyl)-7-amino and 7-hydroxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidines). *J. Heterocycl. Chem.* **1989**, *26*, 39−43.

(20) Barili, P. L.; Biagi, G.; Livi, O.; Scartoni, V. A method for the synthesis of racemic and optically active 2-substituted 9-(2′,3′-dihydroxypropyl)-8-azahypoxanthines and 8-azadenines. *J. Heterocycl. Chem.* **1991**, *28*, 1351−1355.

(21) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V. Synthesis and ADA inhibitory activity of new 2-aryl-8-azadenosines. VIII. *Farmaco* **1992**, *47*, 537−550.

(22) Biagi, G.; Giorgi, I.; Livi, O.; Lucacchini, A.; Scartoni, V. Evaluation of the quantitative contribution of an aryl group on C(2) of 8-azaadenines to binding with adenosine deaminase: a new synthesis of 8-azaadenosines. XI. *Farmaco* **1992**, *47*, 1457−1476.

(23) Biagi, G.; Giorgi, I.; Livi, O.; Martini, C.; Scartoni, V.; Tacchi, P. C2,N9-Disubstituted 8-azapurines: structure−activity relationships in the binding with the A_1 receptor. *Int. J. Pur. Pyrim. Res.* **1991**, *2*, 93−96.

(24) Biagi, G.; Giorgi, I.; Livi, O.; Lucacchini, A.; Scartoni, V. N(9)-Substituted 2-phenyl-N(6)-benzyl-8-azaadenines: A_1 adenosine receptor affinity. A comparison with the corresponding N(6)-substituted 2-phenyl. N(9)-benzyl-8-azaadenines. *Farmaco* **1996**, *51*, 395−399.
(25) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V. Affinity of 8-aza-adenines toward adenosine receptors. Presented in Genova at Januachem 92, XVII Congresso Nazionale della Società Chimica Italiana, 1992; Abstracts, p 62.
(26) Biagi, G.; Giorgi, I.; Livi, O.; Lucacchini, A.; Martini, C.; Scartoni, V.; Tacchi, P. N(6)-Substituted 2-*n*-butyl-9-benzyl-8-azaadenines. Affinity for adenosine A_1 and A_2 receptors. IV. *Farmaco* **1994**, *49*, 183−186.
(27) Biagi, G.; Giorgi, I.; Livi, O.; Lucacchini, A.; Martini, C.; Scartoni, V.; Tacchi, P. N(6)-Substituted 2-phenyl-9-benzyl-8-azaadenines. Affinity for adenosine A_1 and A_2 receptors. A comparison with 2-*n*-butyl analogous derivatives. V. *Farmaco* **1994**, *49*, 187−191.
(28) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V.; Lucacchini, A.; Martini, C.; Tacchi, P. Synthesis of new N^6-substituted 2-phenyl-8-azaadenosines. Their affinity for adenosine A_1 and A_2 receptors. A comparison with the corresponding 2-phenyl-9-benzyl-8-azaadenines. *Farmaco* **1995**, *50*, 13−19.
(29) Biagi, G.; Breschi, C.; Giorgi, I.; Livi, O.; Martini, C.; Scartoni, V.; Scatizzi, R. N(6) or N(9) Substituted 2-phenyl-8-azaadenines: affinity for A_1 adenosine receptors. VII. *Farmaco* **1995**, *50*, 659−667.
(30) Biagi, G.; Giorgi, I.; Livi, O.; Lucacchini, A.; Scartoni, V. Qualitative structure−activity relationships: binding of adenine analogues and A1 adenosine receptors. Presented in Ferrara at II Congresso Congiunto Italiano-Spagnolo di Chimica Farmaceutica, 1995; Abstracts, p 198.
(31) Müller, C. E.; Geis, U.; Grahner, B.; Lanzner, W.; Eger, K. Chiral pyrrolo[2,3-*d*]pyrimidine and pyrimido[4,5-*b*]indole derivatives: structure−activity relationships of potent highly stereoselective A_1-adenosine receptor antagonists. *J. Med. Chem.* **1996**, *39*, 2482−2491.
(32) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V.; Martini, C.; Tacchi, P.; Merlino, S.; Pasero, M. 1,2,3-Triazolo[4,5-d]pyridazines. II. New derivatives tested on adenosine receptors. *Farmaco* **1994**, *49*, 175−181.
(33) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V.; Lucacchini, A.; Senatore, G.; Barili, P. L. 1,2,3-Triazolo[4,5-d]pyridazines. III. Synthesis of new 4-amino derivatives and their affinity toward adenosine receptors. *Farmaco* **1994**, *49*, 357−362.
(34) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V.; Velo, S.; Martini, C.; Senatore, G.; Barili, P. L. 1,2,3-Triazolo[4,5-d]pyridazines. IV. Preparation and adenosine receptor binding of new 4 and/or 7 aminoderivatives. *Farmaco* **1995**, *50*, 99−105.
(35) Biagi, G.; Giorgi, I.; Livi, O.; Manera, C.; Scartoni, V.; Lucacchini, A.; Senatore, G. 1,2,3-Triazolo[4,5-d]pyridazines. V. Preparation and adenosine receptor binding of new 4-amino derivatives. *Farmaco* **1996**, *51*, 601−608.
(36) (a) Trivedi, B. K.; Bridges, A. J.; Patt, W. C.; Priebe, S. R.; Bruns, R. F. N^6,N-Bicycloalkyladenosines with unusually high potency and selectivity for the adenosine A1 receptor. *J. Med. Chem.* **1989**, *32*, 8−11. (b) Kusachi, S.; Thompson, R. D.; Bugni, W. J.; Yamada, N.; Olsson, R. A. *J. Med. Chem.* **1985**, *28*, 1636−1643. (c) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331−346.
(37) Vorbruggen, H.; Krolikiewicz, K. Silylation-amination of hydroxy N-heterocycles. *Chem. Ber.* **1984**, *117*, 1523−1541.
(38) Albert, A. The Dimroth rearrangement. Part XV. Catalysis by methylamine salts. Preparation of 7-methylamino- and 6,7-dihydro-7-imino-*v*-triazolo[4,5-d]pyrimidines via 4-ethoxymethyleneamino-1,2,3-triazoles. *J. Chem. Soc., Perkin Trans. I* **1973**, 2659−2664.
(39) Dornow, A.; Helberg, J. Darstellung und *ortho*-kondensation einiger 4,5-disubstituierter 1,2,3-triazole. *Chem. Ber.* **1960**, *93*, 2001−2010.
(40) Albert, A. 1,2,3,4,6-Penta-azaindenes (8-azapurines). Part V. A comparison of 1,2,3-triazoles and pyrimidines as intermediates for the preparation of 9-substituted 8-azapurines. Rearrangement of 6-mercapto-8-azapurines and of 4-aminotriazoles. *J. Chem. Soc. C* **1969**, 152−160.
(41) Biagi, G.; Giorgi, I.; Livi, O.; Scartoni, V.; Velo, S.; Lucacchini, A.; Senatore, G.; Barili, P. L. 1,2,3-Triazolodiazepines. I. Preparation and benzodiazepine receptor binding of 1-benzyl- and 1-phenethyl-1,2,3-triazole[4,5-b][1,4]diazepines. *J. Heterocycl. Chem.* **1995**, *32*, 169−176.
(42) Abu-Orabi, S. T.; Atfah, A.; Jibril, I.; Al-Sheikh Ali, A.; Marii, F. Effect of solvent and reaction time on the products of the 1,3-dipolar cycloaddition of substituted benzyl azides with di-*tert*-butyl acetylenedicarboxylate. *Gazz. Chim. Ital.* **1992**, *122*, 29−33.
(43) Ried, W.; Laoutidis, J. Synthesis neuer stickstoffreicher heterocyclen. *Chemiker-Ztg.* **1990**, *114*, 246−248.
(44) Martini, C.; Poli, M. G.; Lucacchini, A. Properties of [^{3}H]-N^6-cyclohexyladenosine binding to adenosine receptors in sheep cerebral cortex. *Bull. Mol. Biol. Med.* **1986**, *11*, 1−10.
(45) Cheng, Y. C.; Prusoff, W. H. Relation between inhibition constant K_i and the concentration of inhibitor which causes fifty percent inhibition (I_{50}) of an enzymic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(46) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

JM9701334