A Novel Class of Highly Potent and Selective A_1 Adenosine Antagonists: Structure−Affinity Profile of a Series of 1,8-Naphthyridine Derivatives

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A series of 1,8-naphthyridine derivatives (**12**−**36**), bearing a phenyl group in position 2 and various substituents in positions 4 and 7, were synthesized in an attempt to obtain potent, selective antagonists for the A_1 adenosine receptor subtype. The compounds were tested to evaluate their affinity for A_1 compared with A_{2A} and A_3 adenosine receptor subtypes. In binding studies in bovine brain cortical membranes, most of the compounds showed an affinity for A_1 receptors in the low nanomolar range and two in the subnanomolar range with an interesting degree of A_1 versus A_{2A} and A_3 selectivity. Comparison of the 4-substituted derivatives indicated that 4-OH substitution, with a 4-quinoid structure, causes an increase in the A_1 and A_{2A} affinity and generally also in A_1 selectivity. The kind of substitution in position 7 can greatly modulate the affinity: the most interesting substituents in this position seemed to be electron-withdrawing groups; in particular the 7-chloronaphthyridine **25d** showed a remarkable selectivity (A_{2A}/A_1 ratio of 670, A_3/A_1 ratio of 14 000) associated with a higher A_1 affinity (K_i = 0.15 nM). NMR studies on these compounds **12**−**36** indicated that the 4-OH-substituted ones prefer the tautomer in which the oxygen in position 4 is in the quinoid form and the nitrogen in position 1 is protonated. Theoretical calculations are in agreement with the NMR data.

Introduction

Adenosine exhibits a wide variety of physiological actions, including central nervous system depression, lowering of blood pressure, and inhibition of lipolysis and platelet aggregation, which are mediated by membrane receptors. Four distinct subtypes of adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3, have been identified and cloned for several species including humans. The adenosine receptors are associated with a variety of second-messenger systems. Among these, the A_1 and A_3 receptor subtypes are coupled to inhibition of adenylyl cyclase, inhibition or stimulation of phosphoinositol turnover, and activation of guanylyl cyclase, whereas the activation of A_{2A} and A_{2B} receptor subtypes stimulates adenylyl cyclase.[1−9]

Intensive efforts have been made over the past few years with the aim of synthesizing novel compounds, either xanthine or non-xanthine, and highly selective ligands as agonists or antagonists have been developed. A number of classes of tri- and bicyclic non-xanthine antagonists have been reported. Among these, some pyrazolotriazolopyrimidine **1**,[10−12] triazoloquinazoline **2**,[13,14] and furobenzopyranone **3**[15] derivatives, pyranopyrazoles **4**,[16] tetrahydrobenzothiophenones **5**,[17,18] flavonoid derivatives **6**,[15,18] and other bicyclic compounds, including dipyridamole **7**,[15] pyridopyrimidinone **8**,[15] isoquinoline (X = CH), and quinazoline (X = N) **9**,[19] have been found to be potent and selective adenosine A_1, A_{2A}, or A_3 antagonists (Figure 1). Recently two novel classes of adenosine receptor antagonists, 1,8-naphthyridines **10** and pyridopyrimidines **11**, have also been identified (Figure 1). These compounds exhibit affinities for A_1 and A_{2A} of rat brain in the micromolar range.[20]

In light of these considerations, we have now undertaken a systematic research program involving the preparation and testing of a variety of 1,8-naphthyridine derivatives with different substituents in the 1-, 2-, 4-, and 7-positions, to study the influence of these substituents on the binding affinity toward the A_1, A_{2A}, and A_3 receptors.

Chemistry

The compounds described in this study are shown in Tables 1−3, and their synthetic methods are outlined in Schemes 1−4. Alkylation in position 1 of **12**[21] was achieved by means of EtI and KOH in a hydro-alcoholic solution to give **13** (Scheme 1). When the 4-hydroxy-7-methyl-2-phenyl-1,8-naphthyridine **12**[21] was refluxed in toluene with Lawesson's reagent, the corresponding mercapto derivative **14** was obtained. Treatment of the 4-chloronaphthyridine **15**, prepared with $POCl_3$ as described in the literature,[21] with MeONa in MeOH under reflux led to the methoxy derivative **16**. Compound **17** was obtained when **15**[21] was allowed to react with PhONa in DMF at reflux temperature (Scheme 1).

Selective reduction of the 4-azido derivative **18**,[21] obtained by reaction of **15** with NaN_3 as described in the literature,[21] was performed in MeOH, in the pres-

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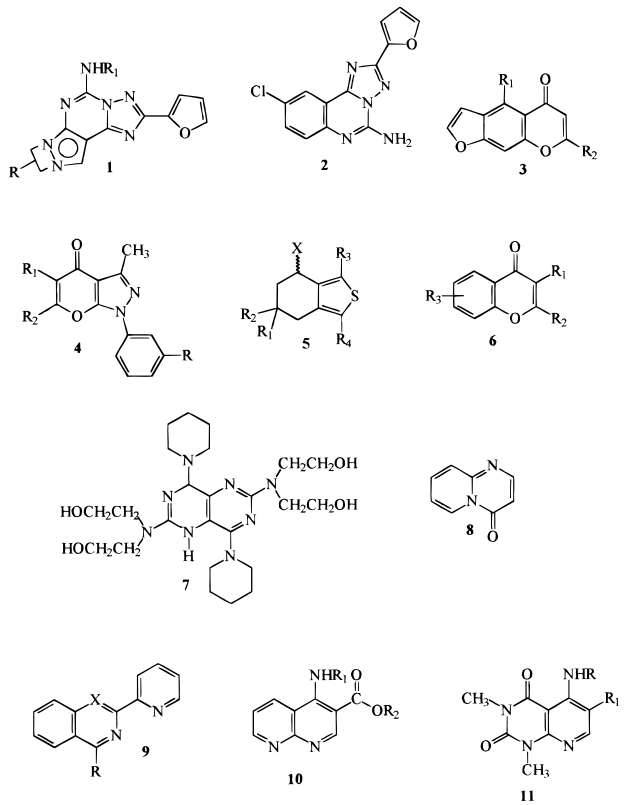
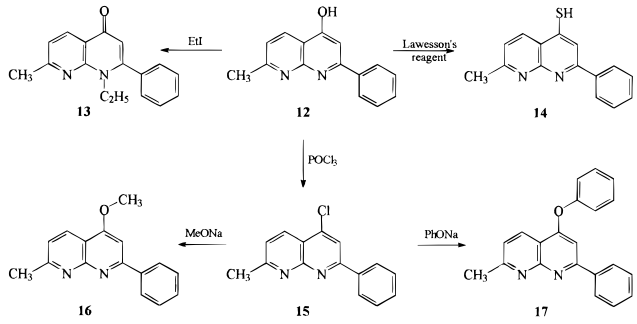


Figure 1. Bi- and tricyclic substances that have an affinity at A_1, A_{2A}, and A_3 adenosine receptors.

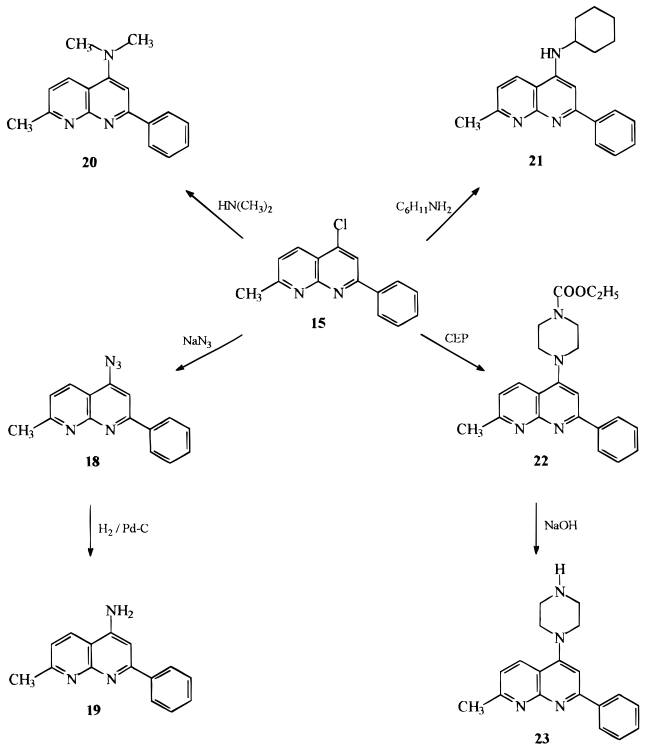
Scheme 1



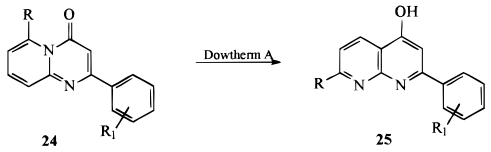
ence of Pd/C as a catalyst, to give the 4-amino derivative **19** (Scheme 2). Reaction of 4-chloro-7-methyl-2-phenyl-1,8-naphthyridine **15**[21] with an excess of the appropriate amine in a sealed tube at 140 °C gave **20**−**22** (Scheme 2). The base **23** was then obtained by alkaline hydrolysis of compound **22** (Scheme 2). The isomerization of 4*H*-pyrido[1,2-*a*]pyrimidinones **24a**−**e**[22] to the corresponding 1,8-naphthyridines **25a**−**e** was carried out in Dowtherm A under reflux conditions as previously reported by us [21,23] (Scheme 3).

Dehalogenation of **25c** with Raney Ni in refluxing dioxane led to 4-hydroxy-2-phenyl-1,8-naphthyridine **26** (Scheme 4). Treatment of the 7-bromo derivative **25c** with EtONa in absolute EtOH or MeONa in MeOH under reflux gave the 7-ethoxynaphthyridine **28** or 7-methoxynaphthyridine **29,** respectively (Scheme 4). The 7-phenoxy derivative **27** was synthesized by reaction of **25c** with PhONa in DMF at 80 °C. Demethylation of **29** was performed with 48% hydrobromic acid in acetic acid to give the dihydroxynaphthyridine **30**, which was also prepared, in low yield, by alkaline hydrolysis of **25c** with 5 N NaOH. The dihydroxynaphthyridine **30**

Scheme 2



Scheme 3



Comp	R	R_1
a	CH_3	*p*-F
b	CH_3	*o*-F
c	Br	H
d	Cl	H
e	F	H

was then alkylated with MeI and KOH in a hydroalcoholic solution to give **31**, which by demethylation performed with 48% hydrobromic acid in acetic acid gave **32** (Scheme 4).

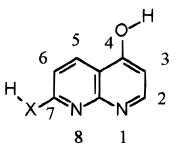
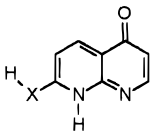
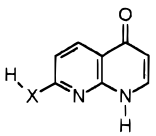
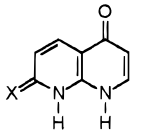
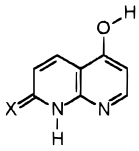
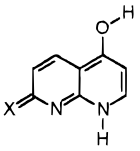
All the compounds synthesized were characterized by elemental analysis, IR, and ^{1}H NMR. Some compounds were also characterized by ^{13}C NMR.

NMR Spectroscopy

In this series of 1,8-naphthyridine derivatives, compounds **12**−**23**, **25**−**30**, and **33**−**36** present different heteroatoms, O, N, or S, in the 4-position of the nucleus, whereas compounds **30** and **31** present an oxygen atom in the 5-position. As the chemical shift of H_x (H_3 or H_6) also depends on the heteroatom present in the 4- or 5-position, we examined the three homogeneous classes of products separately.

In case of the 4-amino derivatives, compounds **20**, **22**, and **23** show the chemical shift of H_x ranging at δ 7.20−7.63, and compounds **19**, **21**, and **35** show the chemical shift of H_x ranging at δ 6.90−7.15. In the case of the mercapto derivative **14**, the chemical shift of H_x is at δ 7.53.

Table 1. Model Compounds of **30** and **36**:[a] Relative Energy Values (kcal/mol) of the Six Possible Tautomers Calculated through Different Methods

X	method	 **8** **1**					
O	6-31G*	10.20	21.12	0.75	5.53	0.00	22.47
O	6-31G*/MP2	7.62	21.58	1.33	7.61	0.00	21.55
O	PM3	6.3	17.5	3.0	8.6	0.0	17.5
O	PM3/6-31G*	9.27	22.13	0.00	5.71	1.16	22.36
NH	6-31G*	11.24	17.79	0.00	21.29	16.35	39.05
NH	PM3	4.03	10.67	0.00	16.25	7.36	24.75

[a] For the sake of clarity we use the same ring numeration for the model compounds as for the corresponding unsimplified compounds which possess a phenyl ring linked in position 2.

Scheme 4

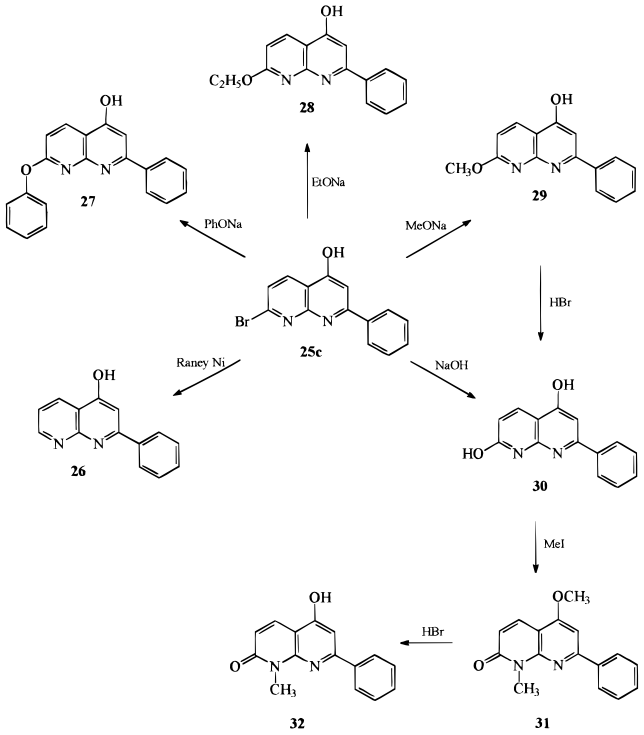
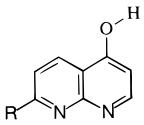
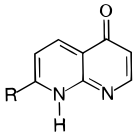
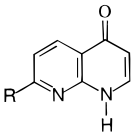


Table 2. RHF 6-31G* SCF Relative Energies of the Tautomers of the Model Compounds of **12**, **25d**, **29**, **30**, and **36**

R			
CH_3	10.96	14.78	0.00
Cl	10.29	18.98	0.00
OCH_3	9.35	22.54	0.00
OH	10.20[a]	21.12[a]	0.75[a]
NH_2	11.24[a]	17.79[a]	0.00[a]

[a] See also Table 1.

To explain the affinity of 4- or 5-oxy derivatives (**12**, **13**, **16**, **17**, **25**−**32**, **34**, **36**) at the adenosine receptors, it was necessary to establish their structure, which was determined on the basis of their NMR spectra. The ^{1}H NMR spectrum of the quinoid compound **13** shows a singlet due to H_x at δ 5.95 and a narrow multiplet due to a phenyl group at δ 7.54, whereas the ^{1}H NMR spectrum of the aromatic compound **16** shows a singlet due to H_x at δ 7.56 and two multiplets at δ 8.30 (2H) and 7.50 (3H) due to the phenyl group. The ^{1}H NMR spectra of compounds **17** and **30**−**32** exhibit a chemical shift of H_x and of the phenyl group analogous to those of **16**. All the ^{1}H NMR spectra of the other compounds (**12**, **25**−**29**, **34**, **36**) show a singlet due to H_x ranging at δ 6.12−6.48 and two multiplets due to the phenyl group ranging at δ 7.29−8.11 and 7.59−8.34, respectively; this behavior is close to that of compound **13**. In light of these results the tautomeric form B (Figure 2) was assigned to compounds **12**, **25**−**29**, **34**, and **36**, whereas the tautomeric form A was assigned to compounds **30** and **32**.

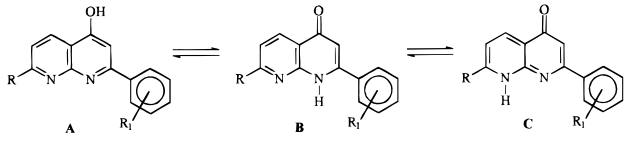


Figure 2. Tautomeric forms of 4-hydroxy-1,8-naphthyridine derivatives.

The ^{13}C NMR spectra of compounds **12**−**14**, **16**, **25a**, and **29**−**31** show a chemical shift due to C_x (C_3 or C_6), confirming the above results obtained with ^{1}H NMR. In fact, the ^{13}C NMR spectrum of quinoid compound **13** shows a signal due to C_x at δ 112.38, whereas the 4-methoxy derivatives **16** and **31** show the C_x signal at δ 98.46 and 97.63, respectively. The ^{13}C NMR spectra of compounds **12**, **25a,** and **29**, with the C_x signal ranging at δ 108.50−108.99, close to that of compound **13**, confirmed the quinoid structure B of these compounds. Moreover, for compound **30** the phenolic structure A was also confirmed by ^{13}C NMR, because the C_x signal at δ 101.13 is close to that of compound **31**.

Theoretical Calculations

Compounds **12**, **25**−**30**, **32**, **34,** and **36**, which possess an OH group capable of lacking a proton in position 4 of the 1,8-naphthyridine system, can exist in three tautomeric forms, as shown in Figure 2; in the case of compounds **30** and **36**, in which also the substituent in position 7 is a group able to lack a proton (OH in **30** and NH_2 in **36**), the number of possible tautomers rises to six (Table 1). NMR studies indicated that these compounds prefer the quinoid form but were not able to specify which tautomer was preferred. Therefore theoretical quantum mechanics calculations were performed in order to study the tautomeric equilibrium in detail and, in addition, to explore the possibility that this equilibrium could furnish an explanation for the

Table 3. Affinity of 1,8-Naphthyridine Derivatives in Radioligand Binding Assays at Bovine Brain A_1, A_{2A}, and Rat Testis A_3 Receptors[a−c]

	K_i (nM)			selectivity		
compd	A_1[a]	A_{2A}[b]	A_3[c,d]	A_{2A}/A_1	A_3/A_1	A_3/A_{2A}
12	5.3 ± 0.6	460 ± 38	8800 ± 740	87	1660	19
13	450 ± 92	>10000	>10000	>22	>22	
14	80 ± 7	1250 ± 230	>10000	16	>125	>8
15	1000 ± 110	>10000	>10000	>10	>10	
16	6000 ± 600	>10000	>10000	>2	>2	
17	810 ± 87	>10000	>10000	>12	>12	
18	>10000	>10000	>10000			
19	17 ± 4	420 ± 29	>10000	25	>590	>24
20	550 ± 57	7000 ± 630	>10000	13	>18	>1
21	28 ± 3	830 ± 79	>10000	30	>360	>12
22	1200 ± 130	>10000	>10000	>8	>8	
23	8700 ± 750	>10000	>10000	>1	>1	
25a	5.3 ± 0.4	630± 65	6100 ± 510	119	1150	10
25b	11± 4	470 ± 42	4600 ± 480	43	418	10
25c	0.70 ± 0.05	66 ± 5	2100 ± 170	94	3000	32
25d	0.15 ± 0.01	100 ± 15	2100 ± 180	670	14000	21
25e	4.1 ± 0.6	170 ± 35	>10000	41	>2440	>59
26	16 ± 4	>10000	3000 ± 290	>625	188	>0.3
27	26± 4	1900 ± 170	>10000	73	>385	>5
28	5.2 ± 0.7	>10000	>10000	>1920	>1920	
29	1.6 ± 0.2	1400 ± 140	>10000	875	>6250	>7
30	>10000	>10000	>10000			
31	1300 ± 280	>10000	>10000	>8	>8	
32	4900 ± 340	>10000	>10000	>2	>2	
33	6900 ± 700	>10000	>10000	>1	>1	
34	9.9 ± 0.8	460 ± 37	>10000	46	>1010	>22
35	100 ± 11	1800 ± 150	>10000	18	>100	>5
36	5.3 ± 0.5	5900 ± 420	>10000	1110	>1890	>2
CHA	1.3 ± 0.1	750 ± 65	25 ± 1	577	19	0.03
CPA	0.3 ± 0.14	390 ± 81	26 ± 7[e]	1300	87	0.07
(*R*)-PIA	0.6 ± 0.2	750 ± 84	53 ± 4[e]	1250	88	0.07
(*S*)-PIA	12 ± 4	1800 ± 580	241 ± 12[e]	150	20	0.13
NECA	14 ± 4	16 ± 3	49 ± 3[e]	1.1	3.5	3.1
XAC	1.3 ± 0.1	63 ± 6	418 ± 80	48	323	6.7

[a] Inhibition of specific [^{3}H]CHA binding to bovine brain cortical membranes expressed as K_i ± SEM (n = 3) in nM. [b] Inhibition of specific [^{3}H]CGS21680 binding to bovine striatal membranes expressed as K_i ± SEM (n = 3) in nM. [c] Inhibition of specific [^{3}H](*R*)-PIA binding to rat testis membranes in the presence of 150 nM DPCPX expressed as K_i ± SEM (n = 3) in nM. [d] Similar results were obtained in A_3 bovine testis membranes. [e] Ref 33. Each value represents the average of three experiments.

differences in the biopharmacological properties of the 4-OH-substituted compounds.

These calculations were performed in vacuo at the Hartree−Fock SCF level by using a 6-31G* basis set, and a full geometry optimization was carried on for each tautomer using the Gaussian94W program.[29] However the molecular dimension of the compounds made the computing time too expensive; therefore the calculations were made on simplified model compounds in which the phenyl ring linked at position 2 of the 1,8-naphthyridine system was substituted by a hydrogen atom; this approximation should be more reliable than that of simplifying the calculation by reducing the basis set or by using semiempirical methods. The compounds taken into consideration were **12**, **25d**, **29,** and **36**, which possessed a high affinity for the A_1 receptor, and **30**, which, on the contrary, is totally inactive. The relative energies of the three tautomers indicated in Figure 2 are reported for all model compounds considered in Table 2.

As regards the model compounds of **30** and **36**, all six possible tautomers were considered and the complete results are reported in Table 1, together with the relative energy values obtained using different calculation methods. In particular, for the tautomers of the model compound of **30**, the contribution of the electron correlation was evaluated at the MP2 level on the RHF/6-31G* minimized structures (Table 1: 6-31G*/MP2); results indicated that the electron correlation increases the stability of the tautomer already preferred at the HF level (Table 1: 6-31G*). Moreover, calculations at the PM3 semiempirical level were performed on both model compounds of **30** and **36** (Table 1: PM3), and results showed that this semiempirical method could be used, even if only for a qualitative evaluation of the relative stability of the tautomers; on the contrary, the calculation of the relative energies at the 6-31G* level on the geometries optimized at the PM3 level does not give good results (Table 1: PM3/6-31G*).

The results reported in Tables 1 and 2 clearly show that the 4-OH-substituted compounds prefer the tautomer in which the substituent in position 4 of the naphthyridine system is in the quinoid form and the nitrogen in position 1 is protonated. However in the case of compound **30**, the preferred tautomer is the one in which the oxygen in position 7 is in the quinoid form and the nitrogen in position 8 is protonated. These results are thus in agreement with the NMR data and, moreover, could suggest an explanation for the lower activity of compound **30** in comparison with the other 4-OH-substituted compounds, because of the preference of this compound to exist in a different tautomeric form with respect to the other ones.

Biological Evaluation

Radioligand Binding Assays. The 1,8-naphthyridines **12**−**36** were tested for their ability to displace the specific binding of [^{3}H]N^6-cyclohexyladenosine ([^{3}H]-CHA) and [^{3}H]2-[[[*p*-(2-carboxyethyl)phenyl]ethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine ([^{3}H]CGS21680) to

bovine cortical (A_1) and striatal (A_{2A}) membranes, respectively.[30,31] Affinity for A_3 adenosine receptors was determined in competition assays of [^{3}H](*R*)-(−)-N^6-(2-phenylisopropyl)adenosine ([^{3}H](*R*)-PIA) on rat testis membranes in the presence of the A_1-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).[32,33]

A_1, A_{2A}, and A_3 Adenosine Receptors. Table 3 reports the results of the A_1, A_{2A}, and A_3 adenosine receptors binding assay, expressed as inhibition constants (K_i, nM), for compounds **12**−**36**. All the compounds were more potent at A_1 receptors, less potent at A_{2A} receptors, and substantially inactive at rat A_3 receptors. Similar results were obtained in A_3 bovine testis membranes.

Variation of substituents in compounds **12**−**36** yielded different A_1 affinities. The lowest levels of A_1 affinity were displayed by derivatives **18** and **30** with K_i values > 10 000 nM. The K_i values of compounds **15**, **16**, **22**, **23**, and **31**−**33** ranged from 8700 to 1000 nM, confirming the binding data reported for 1,8-naphthyridine derivatives.[20]

Compounds **17** (K_i = 810 nM), **20** (K_i = 550 nM), **13** (K_i = 450 nM), and **35** (K_i = 100 nM) possessed an average receptor affinity; instead compounds **14**, **21**, and **27** showed an appreciable affinity with K_i values ranging from 80 to 26 nM. Compounds **19** (K_i = 17 nM), **26** (K_i = 16 nM), **25b** (K_i = 11 nM), and **34** (K_i = 9.9 nM) showed a reduction in K_i values, similar to that of (*S*)-PIA and NECA. Derivatives **12**, **25a**,**e**, **28**, and **36** showed a high affinity with K_i = 5.3, 5.3, 4.1, 5.2, and 5.3 nM, respectively.

Compound **29** showed an affinity constant with K_i = 1.6 nM, similar to the N^6-substituted A_1-selective agonist analogues, CHA (K_i = 1.3 nM). The compounds substituted with Br (**25c**) or Cl (**25d**) in the 7-position show a substantial increase in potency, with K_i values in the subnanomolar range (**25c**: K_i = 0.7 nM; **25d**: K_i = 0.15 nM), similar to, or lower than, that of (*R*)-PIA (K_i = 0.6 nM).

Derivatives **13**−**18**, **22**, **23**, **26**, **27**, **29**−**33**, and **36** showed a poor affinity at A_{2A} receptors, confirming the binding data reported for 1,8-naphthyridine derivatives;[20] the affinity of compounds **12**, **19**, **21**, **25a**,**b**,**d**,**e**, and **34** ranged from 830 to 100 nM. The most effective compound toward A_{2A} receptors was **25c**, which showed an affinity constant of 66 nM.

The binding assay at adenosine A_3 receptors showed that these compounds, **12**−**36**, presented very low inhibition percentages at a concentration of 1 μM, with the result that the corresponding K_i values were not calculated for the majority of the compounds.

Functional Assay at A_1 Receptors. Two potent, selective A_1 compounds (**29**: K_i = 1.6 nM; **25d**: K_i = 0.15 nM) were also tested for their ability to reverse the inhibition of forskolin-stimulated adenylyl cyclase activity induced by the agonist CHA (100 nM).

In rat cerebral cortex membranes, the A_1 adenosine agonist CHA induced a maximal inhibition of adenylyl cyclase activity of 15−20% of total activity, under conditions of stimulation (typically 3−4-fold) in the presence of 0.1 mM forskolin, with an IC_{50} value of 1.4 ± 0.7 nM.[33] The inhibition effect of CHA (100 nM) on adenylyl cyclase activity was antagonized completely and in a concentration-dependent manner by derivatives **29** and **25d**, with IC_{50} values of 0.47 and 0.034 nM, respectively (Figure 3). Moreover, in agreement with radioligand binding data, derivative **25d** was 10-fold more potent than derivative **29** in counteracting the CHA functional response. **29** (5 nM) and **25d** (0.5 nM) also caused a parallel rightward shift of the CHA inhibitory concentration−response curve, suggesting that both compounds showed full antagonist properties (results not shown).

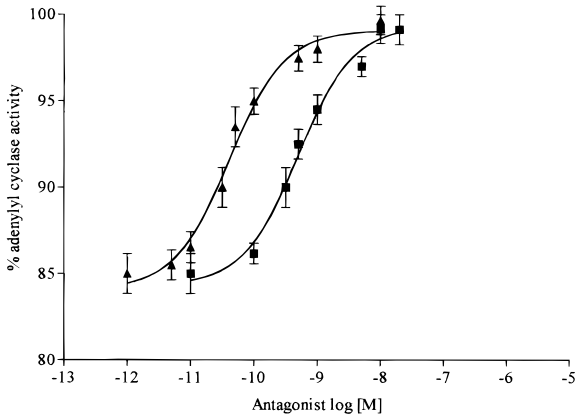


Figure 3. Concentration-dependent reversal of CHA adenylyl cyclase activity inhibition by **29** (■) and **25d** (▲) derivatives. The enzyme activity was assayed at the concentrations of the antagonists indicated in the presence of 100 nM CHA and 0.1 mM forskolin as described in Biological Methods (Experimental Section). Each data point is expressed as a percentage of adenylyl cyclase activity and represents the mean ± SEM of at least three independent experiments.

Discussion

For a better understanding of the biological data, it needs to be pointed out that the numbering of the 1,8-naphthyridine nucleus used in this discussion is the same used in the NMR spectroscopy and theoretical calculations sections.

In a recent paper, a series of 1,8-naphthyridines **10** had been found to have a micromolar affinity at A_1 and A_{2A} adenosine receptors and to be slightly A_1-selective.[20] With the aim of enhancing both the affinity and selectivity, we focused our studies on a series of 1,8-naphthyridine derivatives with the following characteristics: (i) a methyl group in position 7; (ii) a variously substituted nitrogen function in position 4, present in several derivatives of types **1**, **2**, **7**, **10**, and **11**; (iii) a substituent in position 2 with a lipophilic nature, through the introduction of a phenyl group, present in some compounds of types **3**, **4**, and **6**.

The results reported in Table 3 for compounds **18**−**23** and **35** show that some of these compounds have an affinity at A_1 and A_{2A} receptors in the low nanomolar range with a different degree of selectivity toward A_1 receptors. In particular, compound **19** exhibits a remarkable affinity at A_1 receptors (K_i = 17 nM), when compared with compound **10**[20] (generally >1000-fold) with a better selectivity at A_1 receptors. Introduction of an SH group (compound **14**) in position 4 resulted in a small decrease in the affinity and selectivity (K_i = 80 nM, A_{2A}/A_1 ratio of 16). Replacement of the SH group with an OH group (compound **12**) led to a better affinity and selectivity (K_i = 5.3 nM, A_{2A}/A_1 ratio of 87).

We therefore focused our studies on the lead compound **12**, designing the following structural modifications: (i) introduction of a substituent on the phenyl group; (ii) substitution of the methyl group in position 7 with various different groups. With the introduction of *o*-F-, *p*-F-, and *p*-NO_2-phenyl groups in position 2, both the affinity and the selectivity remained in the same nanomolar range when compared with compound **12**.

The substitution of the methyl group in position 7 with an electron-withdrawing group generally resulted in an increase in both the affinity and the selectivity and gave compounds with a subnanomolar affinity at the A_1 receptor (**25c**,**d**). The substitution of the methyl group in position 7 with an electron-withdrawing group led to compounds with a very good affinity at A_1 adenosine receptors; the order of the A_1 affinity of the substituents is, in the case of the halogens, Cl > Br > F and, in the case of the alkoxy derivatives, CH_3O > C_2H_5O > C_6H_5O.

These modifications thus generally caused a remarkable increase in affinity at A_1 and A_{2A} receptors (Table 3), especially in the case of the compounds with a heteroatom in position 4 bearing an acidic hydrogen atom (**12**, **14**, **19**, **21**, **25**−**29**, **34**−**36**). To explain these very good results, we think that a very important factor for the affinity of these 4-hydroxy-substituted compounds could be the tautomeric equilibrium between forms A and B (Figure 2): all compounds which exhibit a remarkable affinity at A_1 receptors and an interesting affinity also at A_{2A} receptors prefer the quinoid tautomeric structure B, with the N_1 protonated (**12**, **25**−**29**, **34**, **36**) rather than the phenolic structure A, as shown by NMR data and theoretical calculations, whereas compounds which exhibit a lower affinity, analogous to that reported for 1,8-naphthyridine derivatives **10**,[20] cannot exist in form B.

Compounds **30**−**32** deserve particular attention. Unlike the compounds with tautomeric form B, these compounds with the quinoid structure in position 2 showed a decrease in the affinity at both receptor subtypes. Compounds **31** and **32**, with a methyl group in position 1, showed a low affinity. Compound **30**, which possesses the same quinoid structure as **31** and **32**, with the N_8 protonated, as demonstrated on the basis of theoretical calculations, dramatically reduced the affinity.

Among all the 1,8-naphthyridine derivatives tested, only six derivatives also possess a moderate affinity at A_3 receptors, in the order of a micromolar concentration, whereas the others are completely ineffective.

The results of the binding studies indicate that all the compounds are A_1–selective. In particular, compounds **25d** (7-Cl), **26** (7-H), **28** (7-EtO), **29** (7-MeO), and **36** (7-NH_2) showed a very high selectivity (A_{2A}/A_1 ratio ranging between 625 and 1920). Moreover, the two most potent and selective derivatives in the binding assays, **25d** and **29**, were also shown to be full antagonists.

Conclusions

The modifications carried out on compounds **10**[20] led to compounds having a higher affinity, up to the subnanomolar range (0.15 nM), and a remarkable selectivity (A_{2A}/A_1 ratio of 1920 and A_3/A_1 ratio of 14 000). The introduction of a phenyl group in position 2 and removal of the substituent in position 3 resulted in an increase in the affinity and selectivity compared with those observed for 1,8-naphthyridine derivatives **10**.[20] On the basis of NMR data, theoretical calculations, and biological data, it may be deduced that the presence of a hydroxy group in position 4, which can lead to a quinoid structure, seems to be important. The most favorable substituents in position 7 seem to be, at this time, electron-withdrawing groups.

The field of 1,8-naphthyridine derivatives deserves to be further developed, to optimize their affinity at the A_1, A_{2A}, and A_3 adenosine receptor subtypes.

Experimental Section

A. Chemistry. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra in Nujol mulls were recorded on an ATI Mattson Genesis Series FTIR spectrometer. ^1H and ^{13}C NMR spectra were recorded with a Bruker AC-200 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.

7-Methyl-2-phenyl-1,8-naphthyridin-4(1*H*)-one (12).[21] ^1H NMR: δ 11.18 (brs, OH), 8.34 (d, 1H, H_5), 7.84 (m, 2H, Ar), 7.35 (m, 3H, Ar), 7.27 (d, 1H, H_6), 6.36 (s, 1H, H_3), 2.59 (s, 3H, CH_3). ^{13}C NMR: δ 176.02 (C_4), 162.59 (C_7), 150.92 (C_2), 150.92 (C_{8a}), 134.66 (C_5), 133.64 ($C_{1'}$), 130.53 ($C_{4'}$), 128.85 ($C_{3'}$), 127.66 ($C_{2'}$), 120.12 (C_6), 117.25 (C_{4a}), 108.51 (C_3), 24.33 (7_{st}).

1-Ethyl-7-methyl-2-phenyl-1,8-naphthyridin-4(1*H*)-one (13). A solution of hydroxynaphthyridine **12** (0.500 g, 2.12 mmol) and C_2H_5I (2.5 mL, 31.2 mmol) in 3 N aqueous KOH (5 mL) and C_2H_5OH (5 mL) was heated in a sealed tube at 100 °C for 24 h. The reaction mixture was treated with H_2O, the pH was adjusted to 8 with concentrated NH_4OH, and then the mixture was extracted with $CHCl_3$. The organic solution was dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude solid, purified by flash chromatography (ethyl acetate), followed by crystallization from petroleum ether 100−140 °C, gave **13** (0.083 g, yield 15%): mp 125−126 °C; MS *m*/*z* 264 (M^+). ^1H NMR: δ 8.41 (d, 1H, H_5), 7.54 (m, 5H, Ar), 7.35 (d, 1H, H_6), 5.95 (s, 1H, H_3), 4.22 (q, 2H, NCH_2), 2.62 (s, 3H, CH_3), 1.09 (t, 3H, CH_3). ^{13}C NMR: δ 175.71 (C_4), 162.05 (C_7), 154.54 (C_2), 149.39 (C_{8a}), 135.32 ($C_{1'}$), 135.23 (C_5), 129.45 ($C_{4'}$), 128.69 ($C_{3'}$), 128.16 ($C_{2'}$), 120.02 (C_6), 118.76 (C_{4a}), 112.38 (C_3), 41.28−14.16 (1_{st}), 24.91 (7_{st}). Anal. ($C_{17}H_{16}N_2O$) C, H, N.

4-Mercapto-7-methyl-2-phenyl-1,8-naphthyridine (14). A solution of hydroxynaphthyridine **12** (0.236 g, 1.0 mmol) and Lawesson's reagent (0.242 g, 0.6 mmol) in toluene (40 mL) was refluxed for 6 h. The solvent was removed under reduced pressure to obtain the title compound which, purified by crystallization from EtOH−H_2O, gave **14** (0,206 g, yield 82%): mp 204−205 °C; MS *m*/*z* 252 (M^+). ^1H NMR: δ 13.30 (brs SH), 8.83 (d, 1H, H_5), 7.89 (m, 2H, Ar), 7.54 (m, 3H, Ar), 7.53 (s, 1H, H_3), 7.40 (d, 1H, H_6), 2.62 (s, 3H, CH_3). ^{13}C NMR: δ 163.81 (C_4), 160.17 (C_7), 146.89 (C_2), 145.59 (C_{8a}), 137.65 (C_5), 132.51 ($C_{1'}$), 131.01 ($C_{4'}$), 129.01 ($C_{3'}$), 128.10 ($C_{2'}$), 124.51 (C_{4a}), 123.51 (C_6), 122.22 (C_3), 24.30 (7_{st}). Anal. ($C_{15}H_{12}N_2S$) C, H, N.

4-Methoxy-7-methyl-2-phenyl-1,8-naphthyridine (16). Chloronaphthyridine **15** (0.254 g, 1.0 mmol) was added to a solution of sodium (0.046 g, 2 g atom) in anhydrous MeOH (50 mL), and the mixture was refluxed for 24 h. The resulting solution was evaporated to dryness under reduced pressure to obtain a residue which was treated with H_2O and 3 N aqueous HCl to adjust the pH to 8. The solid was then collected by filtration, washed with H_2O and crystallized from toluene-petroleum ether 60−80 °C to give **16** (0.149 g, yield 59%): mp 101−102 °C; MS *m*/*z* 250 (M^+). ^1H NMR: δ 8.36 (d, 1H, H_5), 8.30 (m, 2H, Ar), 7.56 (s, 1H, H_3), 7.50 (m, 3H, Ar), 7.39 (d,

1H, H_6), 4.13 (s, 3H, OCH_3), 2.66 (s, 3H, CH_3). ^{13}C NMR: δ 163.08 (C_4), 162.55 (C_7), 159.94 (C_2), 156.17 (C_{8a}), 138.95 ($C_{1'}$), 131.03 (C_5), 129.91 ($C_{4'}$), 128.67 ($C_{3'}$), 127.52 ($C_{2'}$), 121.62 (C_6), 112.28 (C_{4a}), 98.46 (C_3), 56.59 (4_{st}), 24.97 (7_{st}). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

7-Methyl-4-phenoxy-2-phenyl-1,8-naphthyridine (17). NaH (0.170 g, 3.5 mmol, 50% in mineral oil) was added to a solution of phenol (0.197 g, 2.1 mmol) in anhydrous DMF (10 mL), and the mixture was stirred for 30 min at room temperature. Chloronaphthyridine **15** (0.254 g, 1.0 mmol) was added to the suspension obtained, and the mixture was heated at 80 °C for 6 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue was treated with H_2O and filtered. The residual solid was crystallized from petroleum ether 60−80 °C to obtain **17** (0.240 g, yield 77%): mp 154−155 °C; MS *m/z* 312 (M^+). ^1H NMR: δ 8.52 (d, 1H, H_5), 8.05 (m, 2H, Ar), 7.37 (s, 5H, OPh), 7.24 (m, 3H, Ar), 7.22 (d, 1H, H_6), 7.06 (s, 1H, H_3), 2.83 (s, 3H, CH_3). Anal. ($C_{21}H_{16}N_2O$) C, H, N.

4-Amino-7-methyl-2-phenyl-1,8-naphthyridine (19). A solution of azidonaphthyridine **18** (0.261 g, 1.0 mmol) in anhydrous MeOH (25 mL) was submitted to hydrogenation in the presence of 10% Pd/C (0.03 g) at room pressure and temperature for 6 h. The catalyst was filtered off and the solvent evaporated to dryness under reduced pressure to give a residue, which was purified by flash chromatography (ethyl acetate:diethylamine, 10:0.5) and recrystallized from ethyl acetate to give **19** (0.052 g, yield 22%): mp 268−270 °C; MS *m/z* 235 (M^+). ^1H NMR: δ 8.51 (d, 1H, H_5), 8.09 (m, 2H, Ar), 7.50 (m, 3H, Ar), 7.27 (d, 1H, H_6), 7.12 (s, 1H, H_3), 7.07 (brs, NH_2), 2.62 (s, 3H, CH_3). ^{13}C NMR: δ 161.65 (C_4), 158.53 (C_7), 156.68 (C_2), 153.64 (C_{8a}), 139.91 ($C_{1'}$), 131.91 ($C_{4'}$), 129.22 (C_5), 128.60 ($C_{3'}$), 126.92 ($C_{2'}$), 119.48 (C_6), 109.97 (C_{4a}), 99.16 (C_3), 24.88 (7_{st}). Anal. ($C_{15}H_{13}N_3$) C, H, N.

General Procedure for the Preparation of 4-Dimethylamino- (20), 4-Cyclohexylamino- (21), and 4-(4-Carbethoxypiperazin-1-yl)-7-methyl-2-phenyl-1,8-naphthyridine (22). A mixture of chloronaphthyridine **15** (0.300 g, 1.2 mmol) and an excess of the suitable amine (2 mL) was heated at 140 °C in a sealed tube for 24 h. The reaction mixture was treated with H_2O, and the solid was collected by filtration to obtain the title compounds. **20**: 0.270 g, yield 86%; mp 147−149 °C (crystallized from toluene); MS *m/z* 263 (M^+). ^1H NMR: δ 8.22 (d, 1H, H_5), 8.16 (m, 2H, Ar), 7.40 (m, 3H, Ar), 7.27 (d, 1H, H_6), 7.20 (s, 1H, H_3), 3.08 (s, 6H, $N(CH_3)_2$), 2.76 (s, 3H, CH_3). Anal. ($C_{17}H_{17}N_3$) C, H, N. **21**: 0.250 g, yield 66%; mp 235−237 °C (crystallized from benzene); MS *m/z* 317 (M^+). ^1H NMR: δ 8.15 (m, 2H, Ar), 7.93 (d, 1H, H_5), 7.42 (m, 3H, Ar), 7.13 (d, 1H, H_6), 6.90 (s, 1H, H_3), 4.90 (brs, NH), 3.65 (m, 1H, cyclohexylamine), 2.74 (s, 3H, CH_3), 1.74, 1.35 (m, 10H, cyclohexylamine). Anal. ($C_{21}H_{23}N_3$) C, H, N. **22**: 0.412 g, yield 95%; mp 134−136 °C (crystallized from petroleum ether 100−140 °C); MS *m/z* 376 (M^+). ^1H NMR: δ 8.33 (m, 2H, Ar), 8.30 (d, 1H, H_5), 7.56 (m, 3H, Ar), 7.46 (d, 1H, H_6), 7.43 (s, 1H, H_3), 4.23 (q, 2H, OCH_2), 3.83, 3.26 (m, 8H, piperazine), 2.83 (s, 3H, CH_3), 1.33 (t, 3H, CH_3). Anal. ($C_{22}H_{24}N_4O_2$) C, H, N.

7-Methyl-2-phenyl-4-(piperazin-1-yl)-1,8-naphthyridine (23). A suspension of carbethoxypiperazinylnaphthyridine **22** (0.364 g, 1.0 mmol) in EtOH (10 mL) and 5 N aqueous NaOH (10 mL) was refluxed for 5 h. The organic solvent was evaporated from the reaction mixture under reduced pressure and the pH was adjusted to 8 with 3 N aqueous HCl. The suspension obtained was extracted three times with chloroform, and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure and recrystallized from toluene to obtain **23** (0.279 g, yield 92%): mp 187−189 °C. ^1H NMR: δ 10.17 (brs, NH), 8.80 (d, 1H, H_5), 8.21 (m, 2H, Ar), 7.71 (d, 1H, H_6), 7.63 (s, 1H, H_3), 7.62 (m, 3H, Ar), 3.39, 3.69 (m, 8H, piperazine), 2.84 (s, 3H, CH_3). ^{13}C NMR: δ 163.13 (C_4), 159.09 (C_7), 157.63 (C_2), 149.69 (C_{8a}), 138.96 ($C_{1'}$), 134.25 (C_5), 131.75 ($C_{4'}$), 129.02 ($C_{3'}$), 128.67 ($C_{2'}$), 122.29 (C_6), 113.49 (C_{4a}), 107.05 (C_3), 48.38−42.21 (4_{st}), 22.72 (7_{st}). Anal. ($C_{19}H_{20}N_4$) C, H, N.

General Procedure for the Preparation of 4-Hydroxy-7-methyl-2-(4-fluorophenyl)-1,8-naphthyridine (25a) and 4-Hydroxy-7-methyl-2-(2-fluorophenyl)-1,8-naphthyridine (25b). A solution of appropriate pyridopyrimidinone **24** (1.0 mmol) in Dowtherm A (10 mL) was heated at 240 °C for 4 h. After cooling, the precipitate was collected and washed with petroleum ether to obtain the title naphthyridines. **25a**: 0.20 g, yield 81%; mp 291−293 °C (crystallized from 2-propanol−water); MS *m/z* 386 (M^+). ^1H NMR: δ 12.30 (brs, OH), 8.34 (d, 1H, H_5), 7.98 (m, 2H, Ar), 7.34 (m, 2H, Ar), 7.28 (d, 1H, H_6), 6.35 (s, 1H, H_3), 2.60 (s, 3H, CH_3). ^{13}C NMR: δ 177.15 (C_4), 163.43 ($C_{4'}$), 162.60 (C_7), 150.87 (C_2), 149.89 (C_{8a}), 134.64 (C_5), 130.14 ($C_{2'}$), 130.05 (C_1), 120.16 (C_6), 117.18 (C_{4a}), 115.78 ($C_{3'}$), 108.50 (C_3), 24.30 (7_{st}). Anal. ($C_{15}H_{11}N_2OF$) C, H, N. **25b**: 0.140 g, yield 55%; mp 256−258 °C (crystallized from toluene); MS *m/z* 386 (M^+). ^1H NMR: δ 11.35 (brs, OH), 8.30 (d, 1H, H_5), 7.75−7.30 (m, 4H, Ar), 7.25 (d, 1H, H_6), 6.15 (s, 1H, H_3), 2.65 (s, 3H, CH_3). Anal. ($C_{15}H_{11}N_2OF$) C, H, N.

General Procedure for the Preparation of 7-Bromo- (25c), 7-Chloro- (25d), and 7-Fluoro-4-hydroxy-2-phenyl-1,8-naphthyridine (25e). A solution of appropriate pyridopyrimidinone **24** (1.0 mmol) in Dowtherm A (10 mL) was heated at 220 °C for 10 min. The solution was allowed to rest at room temperature and after 24 h the precipitate formed was collected by filtration and washed with petroleum ether to obtain the title compounds. **25c**: 0.27 g, yield 90%; mp 237−238 °C (crystallized from toluene); MS *m/z* 300 (M^+). ^1H NMR: δ 11.18 (brs, OH), 8.30 (d, 1H, H_5), 7.81 (m, 2H, Ar), 7.53 (m, 3H, Ar), 7.50 (d, 1H, H_6), 6.43 (s, 1H, H_3). Anal. ($C_{14}H_9N_2OBr$) C, H, N. **25d**: 0.16 g, yield 61%; mp 293−295 °C (crystallized from DMF−water); MS *m/z* 256 (M^+). ^1H NMR: δ 11.10 (brs, OH), 8.45 (d, 1H, H_5), 7.80 (m, 2H, Ar), 7.45 (m, 3H, Ar), 7.40 (d, 1H, H_6), 6.42 (s, 1H, H_3). Anal. ($C_{14}H_9N_2OCl$) C, H, N. **25e**: 0.18 g, yield 76%; mp >300 °C (crystallized from acetic acid−water); MS *m/z* 240 (M^+). ^1H NMR: δ 11.30 (brs, OH), 8.58 (dd, 1H, H_5), 7.76 (m, 2H, Ar), 7.52 (m, 3H, Ar), 7.10 (dd, 1H, H_6), 6.42 (s, 1H, H_3). Anal. ($C_{14}H_9N_2OF$) C, H, N.

4-Hydroxy-2-phenyl-1,8-naphthyridine (26). A suspension of bromonaphthyridine **25c** (0.300 g, 1.0 mmol) and $NaHCO_3$ (0.300 g) in dioxane (15 mL) was heated at 95 °C, then Raney Ni (3.0 g) was added and the suspension was refluxed for 3 h. The reaction mixture was filtered to separate the catalyst, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (ethyl acetate:petroleum ether, 3:1) and recrystallized from toluene to obtain **26** (0.166 g, yield 75%): mp 225−227 °C; MS *m/z* 222 (M^+). ^1H NMR: δ 11.20 (brs, OH), 8.75 (m, 1H, H_7), 8.45 (dd, 1H, H_5), 7.81 (m, 2H, Ar), 7.53 (m, 1H, H_6), 7.48 (m, 3H, Ar), 6.38 (s, 1H, H_3). Anal. ($C_{14}H_{10}N_2O$) C, H, N.

4-Hydroxy-7-phenoxy-2-phenyl-1,8-naphthyridine (27). A solution of NaH (0.194 g, 4 mmol, 50% in mineral oil), phenol (0.376 g, 4.0 mmol) and bromonaphthyridine **25c** (0.300 g, 1.0 mmol) in anhydrous DMF (10 mL) was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure and H_2O was added. The precipitate formed was collected by filtration, purified by flash chromatography (ethyl acetate: petroleum ether, 3:1) and recrystallized from ethanol to obtain **27** (0.157 g, yield 50%): mp 245−247 °C; MS *m/z* 314 (M^+). ^1H NMR: δ 11.15 (brs, OH), 8.44 (d, 1H, H_5), 7.73 (m, 2H, Ar), 7.46 (s, 5H, OPh), 7.29 (m, 3H, Ar), 6.93 (d, 1H, H_6), 6.33 (s, 1H, H_3). Anal. ($C_{20}H_{14}N_2O_2$) C, H, N.

General Procedure for the Preparation of 7-Ethoxy-4-hydroxy-2-phenyl-1,8-naphthyridine (28) and 4-Hydroxy-7-methoxy-2-phenyl-1,8-naphthyridine (29). Bromonaphthyridine **25c** (0,300 g, 1.0 mmol) was added to a solution of sodium (0.230 g, 10 gatom) in anhydrous EtOH or MeOH (20 mL) and refluxed for 18 h. The reaction mixture was evaporated to dryness under reduced pressure; the residue was treated with H_2O and 3 N aqueous HCl until pH = 6. The solid was collected by filtration and purified by flash chromatography (ethyl acetate:petroleum ether, 3:1) to obtain the title compounds. **28**: 0.171 g, yield 64%; mp 223−225 °C (crystallized from toluene); MS *m/z* 266 (M^+). ^1H NMR: δ 8.98

(brs, OH), 8.40 (d, 1H, H_5), 7.59 (m, 2H, Ar), 7.46 (m, 3H, Ar), 6.65 (d, 1H, H_6), 6.48 (s, 1H, H_3), 4.38 (q, 2H, OCH_2), 1.39 (t, 3H, CH_3). Anal. ($C_{16}H_{14}N_2O_2$) C, H, N. **29**: 0.130 g, yield 52%; mp 244−246 °C (crystallized from toluene); MS *m*/*z* 252 (M^+). ^{1}H NMR: δ 11.80 (brs, OH), 8.31 (d, 1H, H_5), 7.80 (m, 2H, Ar), 7.53 (m, 3H, Ar), 6.81 (d, 1H, H_6), 6.35 (s, 1H, H_3), 4.00 (s, 3H, OCH_3). ^{13}C NMR: δ 176.28 (C_4), 165.14 (C_7), 150.69 (C_2), 150.47 (C_{8a}), 137.17 (C_5), 133.85 ($C_{1'}$), 130.33 ($C_{4'}$), 128.73 ($C_{3'}$), 127.70 ($C_{2'}$), 114.24 (C_{4a}), 108.99 (C_3), 108.66 (C_6), 63.95 (7_{st}). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

4,7-Dihydroxy-2-phenyl-1,8-naphthyridine (30). Method a: A solution of bromonaphthyridine **25c** (0.300 g, 1.0 mmol) in 5 N aqueous NaOH (20 mL) was refluxed for 12 h. The reaction mixture was concentrated and HCl was added until pH = 7. The solid obtained, collected by filtration, was triturated with concentrated HCl, filtered and washed with H_2O to obtain **30** (0.088 g, yield 37%). **Method b:** A solution of methoxynaphthyridine **29** (0.252 g, 1.0 mmol) and 1 mL of 48% HBr in glacial AcOH (2 mL) was refluxed for 4 h. The reaction mixture was evaporated to dryness under reduced pressure and the solid obtained was triturated with $(CH_3)_2$-CO, filtered and purified by crystallization from AcOH to obtain **30** (0.149 g, yield 63%): mp >300 °C; MS *m*/*z* 238 (M^+). ^{1}H NMR: δ 8.08 (m, 2H, Ar), 8.02 (d, 1H, H_5), 7.50 (m, 3H, Ar), 7.20 (s, 1H, H_3), 6.53 (d, 1H, H_6). ^{13}C NMR: δ 162.72 (C_4), 162.53 (C_7), 157.00 (C_2), 151.03 (C_{8a}), 138.17 ($C_{1'}$), 131.82 (C_5), 129.82 ($C_{4'}$), 128.87 ($C_{3'}$), 126.81 ($C_{2'}$), 119.10 (C_6), 104.09 (C_{4a}), 101.13 (C_3), 28.01 (7_{st}). Anal. ($C_{14}H_{10}N_2O_2$) C, H, N.

5-Methoxy-1-methyl-7-phenyl-1,8-naphthyridin-2(1*H*)-one (31). A suspension of NaH (0.122 g, 2.54 mmol, 50% in mineral oil), dihydroxynaphthyridine **30** (0.405 g, 1.7 mmol) in anhydrous DMSO (6 mL) was allowed to react in a sealed tube. After standing at room temperature for 1 h, CH_3I (0.42 mL, 6.8 mmol) was added and the mixture was heated at 90 °C for 24 h. The suspension was treated with H_2O and the precipitate formed was collected by filtration and purified by crystallization with petroleum ether 100−140 °C to obtain **31**: (0.094 g, yield 21%); mp 192−194 °C; MS *m*/*z* 266 (M^+). ^{1}H NMR: δ 8.25 (m, 2H, Ar), 7.96 (d, 1H, H_4), 7.52 (m, 3H, Ar), 7.45 (s, 1H, H_6), 6.55 (d, 1H, H_3), 4.09 (s, 3H, OCH_3), 3.71 (s, 3H, NCH_3). ^{13}C NMR: δ 163.42 (C_5), 162.31 (C_2), 157.78 (C_7), 150.41 (C_{8a}), 138.11 ($C_{1'}$), 131.25 (C_4), 130.07 ($C_{4'}$), 128.74 ($C_{3'}$), 127.27 ($C_{2'}$), 119.83 (C_3), 104.21 (C_{4a}), 97.63 (C_6), 56.75 (5_{st}), 28.07 (1_{st}). Anal. ($C_{16}H_{14}N_2O_2$) C, H, N.

5-Hydroxy-1-methyl-7-phenyl-1,8-naphthyridin-2(1*H*)-one (32). A solution of **31** (0.125 g, 0.47 mmol) and 1 mL of 48% HBr in glacial ACOH (1 mL) was refluxed for 4 h. The mixture was poured over crushed ice and alkalinized with concentrated NH_4OH. The precipitate formed was collected by filtration, washed with H_2O and purified by crystallization from EtOH to obtain **32** (0.040 g, yield 34%): mp 303−305 °C; MS *m*/*z* 252 (M^+). ^{1}H NMR: δ 11.23 (brs, OH), 8.00 (m, 2H, Ar), 7.98 (d, 1H, H_4), 7.46 (m, 3H, Ar), 7.18 (s, 1H, H_6), 6.60 (d, 1H, H_3), 3.72 (s, 3H, NCH_3). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

7-Methyl-2-phenyl-1,8-naphthyridine (33).[21] ^{1}H NMR: δ 8.44 (d, 1H, H_5), 8.30 (m, 2H, Ar), 8.13 (d, 1H, H_4), 8.12 (d, 1H, H_3), 7.54 (m, 3H, Ar), 7.45 (d, 1H, H_6), 2.72 (s, 3H, CH_3).

4-Hydroxy-7-methyl-2-(4-nitrophenyl)-1,8-naphthyridine (34).[23] ^{1}H NMR: δ 11.80 (brs, OH), 8.36 (d, 1H, H_5), 8.34 (m, 2H, Ar), 8.11 (m, 2H, Ar), 7.33 (d, 1H, H_6), 6.46 (s, 1H, H_3), 2.62 (s, 3H, CH_3).

4-Hydrazino-7-methyl-2-phenyl-1,8-naphthyridine (35).[28] ^{1}H NMR: δ 8.05 (d, 1H, H_5), 8.01 (m, 2H, Ar), 7.40 (m, 3H, Ar), 7.15 (s, 1H, H_3), 7.03 (d, 1H, H_6), 3.90 (brs, 3H, $NHNH_2$), 2.62 (s, 3H, CH_3).

7-Amino-2-phenyl-1,8-naphthyridin-4(1*H*)-one (36).[24] ^{1}H NMR: δ 11.50 (brs, OH), 7.97 (d, 1H, H_5), 7.69 (m, 2H, Ar), 7.48 (m, 3H, Ar), 6.67 (brs, 2H, NH_2), 6.46 (d, 1H, H_6), 6.12 (s, 1H, H_3).

B. Biological Methods. Materials. [^{3}H](*R*)-PIA was from Amersham Corp. (Little Chalfont, Buckinghamshire, U.K.), while [^{3}H]CHA and [^{3}H]CGS21680 were obtained from NEN (Boston, MA). CHA, CPA, (*R*)-PIA, (*S*)-PIA, NECA and XAC were purchased from RBI (Natik, MA). Adenosine deaminase was from Sigma Chemical Co. (St. Louis, MO). Bacitracin, benzamidine and PMSF were products of Fluka Chemie AG (Buchs, Switzerland). All other reagents were from standard commercial sources and of the highest grade commercially available.

A_1 Receptor Binding Assay. Bovine brains were obtained from the local slaughterhouse. The cerebral cortex was dissected from bovine brain and immediately homogenized in 10 volumes of ice-cold 0.32 M sucrose containing protease inhibitors (200 μg/mL bacitracin, 160 μg/mL benzamidine and 20 μg/mL soybean trypsin inhibitor) with an Ultra-Turrax homogenizer. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the resulting supernatant again centrifuged at 48000*g* for 15 min at 4 °C. The pellet was resuspended in 10 volumes of ice-cold buffer A (2 mM $MgCl_2$ and 50 mM Tris/HCl, pH 7.7) containing protease inhibitors (as above) and adenosine deaminase (2 units/ml) to remove endogenous adenosine. After incubation for 30 min at 37 °C, the suspension was centrifuged at 48000*g* for 15 min at 4 °C. The final pellet was stored in aliquots at −80 °C until the time of assay.

The [^{3}H]CHA (39 Ci/mmol) binding assay was performed in triplicate by incubating aliquots of the membrane fractions (0.2−0.3 mg of protein) at 25 °C for 45 min in 0.5 mL of buffer A, with approximately 1 nM [^{3}H]CHA. Nonspecific binding was defined in the presence of 10 μM of (*R*)-PIA. Binding reactions were terminated by filtration through Whatman CG/C glass microfiber filters under suction, washing twice with 5 mL of ice-cold buffer, and introduction into scintillation vials. The radioactivity was counted in 4 mL of scintillation cocktail in a scintillation counter.

A_{2A} Receptor Binding Assay. The striatum was dissected from bovine brain and homogenized in 20 volumes of ice-cold 10 mM $MgCl_2$ and 50 mM Tris/HCl buffer, pH 7.4 (buffer B), containing protease inhibitors and resuspended in 20 volumes of buffer B containing protease inhibitors (as above) and adenosine deaminase (2 units/mL). Incubation was carried out for 30 min at 37 °C. The membrane suspension was recentrifuged, and the final pellet was frozen in aliquots at −80 °C until the time of assay.

Routine [^{3}H]CGS21680 (45 Ci/mmol) binding assays were performed in triplicate by incubating an aliquot of striatal membranes (0.2−0.3 mg of protein) in buffer B with approximately 5 nM [^{3}H]CGS21680 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 10 μM NECA. 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. The radioactivity was counted as above.

A_3 Receptor Binding Assay. A_3 receptor binding sites were studied in rat testis membranes. Fresh testicular tissue from Sprague−Dawley rats was dissected free of epididymis and membranes were prepared as described.[32]

Binding of [^{3}H](*R*)-PIA (37 Ci/mmol) to membranes was measured in the presence of DPCPX (150 nM) as described.[32] Briefly, rat testis membranes (0.1−0.2 mg of protein) and [^{3}H]-(*R*)-PIA 4 nM were incubated in 0.5 mL total volume of 50 mM Tris/HCl (pH 7.4), 1 mM EDTA, 10 mM $MgCl_2$ buffer in the presence of 150 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) to block A_1 adenosine receptors. Nonspecific binding was determined in the presence of 15 μM (*R*)-PIA. 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. The radioactivity was counted as above.

Compounds were routinely 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. At least six different concentrations spanning 3 orders of magnitude, adjusted approximately for the IC_{50} of each compound, were used. IC_{50} values, computer-generated using a nonlinear formula on a computer program (GraphPad, San Diego, CA), were converted to K_i

values, knowing the K_d values of radioligands in these different tissues and using the Cheng and Prusoff equation:[34] $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ligand concentration and K_d its dissociation constant. The K_d of [^{3}H]CHA binding to bovine cortex membranes was 1.2 nM, the K_d of [^{3}H]CGS21680 binding to bovine striatal membranes was 10 nM, and the K_d of [^{3}H](*R*)-PIA binding to rat testis membranes was 36 nM. Protein concentration was determined in accordance with the method of Lowry as modified by Peterson,[35] using bovine serum albumin as the standard.

Adenylyl Cyclase Assay. The cerebral cortex was obtained from male Sprague−Dawley rats sacrificed by cervical dislocation. Fresh tissue was suspended in 50 volumes of ice-cold buffer containing 0.32 M sucrose, 200 μg/mL bacitracin, 160 μg/mL benzamidine, 20 μg/mL trypsin inhibitor, 0.1 mM PMSF and 10 mM HEPES/NaOH, pH 7.4, and homogenized with 12 strokes of a Teflon homogenizer at 4 °C. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was collected and centrifuged at 48000*g* for 30 min at 4 °C. The pellet was resuspended in 10 volumes of an ice-cold hypotonic buffer containing all the constituents of the homogenization buffer except sucrose and incubated with adenosine deaminase (2 units/ml) for 30 min at 30 °C. The membrane suspension was centrifuged at 48000*g* for 15 min at 4 °C. This centrifugation step was repeated twice. The final pellet was resuspended in 50 mM HEPES/NaOH, pH 7.4, and used in adenylyl cyclase assays.

Adenylyl cyclase activity was measured by monitoring the conversion of [α-^{32}P]ATP to [α-^{32}P]cAMP, using a previously reported method [36] The method involved addition of [α-^{32}P]-ATP to membranes in the presence of forskolin to stimulate adenylyl cyclase and papaverine as a phosphodiesterase inhibitor. Briefly, enzyme activity was routinely assayed in a 100-μL reaction mixture containing 50 mM HEPES/NaOH buffer, pH 7.4, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mg/mL creatine phosphokinase, 0.1 mg/mL bacitracin, 0.5 mg/mL creatine phosphate, 0.1 mM ATP, 0.05 mM cAMP, 15 units/mL myokinase, 2 units/mL adenosine deaminase, 10 μM GTP, 1 μCi [α-^{32}P]ATP, 0.2 mM papaverine and 0.1 mM forskolin. The incubation was started by the addition of membranes (10−20 μg of proteins) and carried out for 15 min at 23 °C. The reaction was terminated by placing assay tubes in an ice bath and adding 0.5 mL of a stop solution containing 120 mM Zn$(C_2H_3O_2)_2$/[^{3}H]cAMP (10000−20000 cpm/sample) and then 0.6 mL of 144 mM Na_2CO_3. The total radiolabeled cAMP was isolated on columns of Dowex 50 ion-exchange resin and alumina as described.[36]

The antagonist behavior of some compounds was examined for their ability to completely reverse the inhibition of forskolin-stimulated adenylyl cyclase activity induced by the A_1-selective agonist CHA. Experiments were performed evaluating the effects of multiple antagonist concentrations (10^{-12}−10^{-7} nM for **25d** and 10^{-11}−10^{-8} for **29**) on the inhibition of adenylyl cyclase activity induced by 100 nM CHA. The compounds tested were dissolved in DMSO and then diluted with 50 mM HEPES/NaOH buffer, pH 7.4, so the final DMSO concentration never exceeded 1%. The data were analyzed as competition curves and by nonlinear regression analysis for models of one or two noninteracting sites (GraphPad).

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References

(1) Jacobson, K. A., Daly, J. W., Manganiello, V., Eds. *Purine in Cellular Signaling − Targets for New Drugs*; Springer-Verlag: New York, 1990.

(2) Van Galen, P. J. M.; Stiles, G. L.; Michaels, G.; Jacobson, K. A.; Adenosine A_1 and A_2 Receptors: Structure−Function Relationships. *Med. Res. Rev.* **1992**, *12*, 423−471.

(3) Jacobson, K. A.; Van Galen, P. J. M.; Williams, M. Adenosine Receptors − Pharmacology, Structure Activity Relationships and Therapeutic Potential. *J. Med. Chem.* **1992**, *35*, 407−422.

(4) von Lubitz, D. K. J. E.; Jacobson, K. A. Behavioral Effects of Adenosine Receptor Stimulation. In *Adenosine and Adenine Nucleotides*: *From Molecular Biology to Integrative Physiology*; Belardinelli, L., Pelleg, A., Eds.; Kluwer: Norwell, MA, 1995; pp 489−498.

(5) Jacobson, M. Cloning and Expression of Human Adenosine Receptor Subtypes. In *Adenosine and Adenine Nucleotides*: *From Molecular Biology to Integrative Physiology*; Belardinelli, L., Pelleg, A., Eds.; Kluwer: Norwell, MA, 1995; pp 5−13.

(6) Daly, J. W.; Jacobson, K. A. Adenosine Receptors: Selective Agonists and Antagonists. In *Adenosine and Adenine Nucleotides*: *From Molecular Biology to Integrative Physiology*; Belardinelli, L., Pelleg, A., Eds.; Kluwer: Norwell, MA, 1995; pp 157−166.

(7) Linden, J.; Jacobson, M. A.; Hutchins, C.; Williams, M. Adenosine Receptors. In *Handbook of Receptors Channels*; Peroutka, S. J., Eds.; CRC Press: Boca Raton, FL, 1994; Vol. 1, pp 29−44.

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

(9) Ukena, D.; Olsson, R. A.; Daly, J. W. Definition of subclasses of adenosine receptors associated with adenylate cyclase: interaction of adenosine analogues with inhibitory A_1 receptors and stimulatory A_2 receptors. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 365−376.

(10) Baraldi, P. G.; Cacciari, B.; Spallutto, G.; Pineda de las Infantas y Villatoro, M. J.; Zocchi, C.; Dionisotti, S.; Ongini, E. Pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine Derivatives: Potent and Selective A_{2A} Adenosine Antagonists. *J. Med. Chem.* **1996**, *39*, 1164−1171.

(11) Zocchi, C.; Ongini, E.; Conti, A.; Monopoli, A.; Negretti, A.; Baraldi, P. G.; Dionisotti, S. The non xanthine heterocyclic compound SCH 58261 is a new potent and selective A_{2A} adenosine receptor antagonist. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 398−404.

(12) Baraldi, P. G.; Cacciari, B.; Spallutto, G.; Bergonzoni, M.; Dionisotti, S.; Ongini, E.; Varani, K.; Borea, P. A. Design, Synthesis and Biological Evaluation of a Second Generation of Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines as a Potent and selective A_{2A} adenosine receptor antagonist. *J. Med. Chem.* **1998**, *41*, 2126−2133.

(13) Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L.; Stone, G. A.; Desai, M.; Williams, M. Structure−Activity Profile of a Series of Novel Triazoloquinazoline Adenosine Antagonists. *J. Med. Chem.* **1988**, *31*, 1014−1020.

(14) Suzuki, F.; Shimada, J.; Mizumoto, H.; Karasawa, A.; Kubo, K.; Nonaka, H.; Ishii, A.; Kawakita, T. Adenosine A_1 Antagonists. 2. Structure−Activity Relationships on Diuretic Activities and Protective Effects against Acute Renal Failure. *J. Med. Chem.* **1992**, *35*, 3066−3075.

(15) Karton, Y.; Jang, J.; Ji, X. D.; Melman, N.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. Synthesis and Biological Activities of Flavonoid Derivatives as A_3 Adenosine Receptor Antagonists. *J. Med. Chem.* **1996**, *39*, 2293−2301.

(16) Colotta, V.; Catarzi, D.; Varano, F.; Melani, F.; Filacchioni, G.; Cecchi, L.; Trincavelli, L.; Martini, C.; Lucacchini, A. Synthesis and A_1 and A_{2A} adenosine binding activity of some pyrano[2,3-*c*]pyrazol-4-ones. *Farmaco* **1998**, *53*, 189−196.

(17) van Rhee, A. M.; Siddiqi, S. M.; Melman, N.; Shi, D.; Padget, W. L.; Daly, J. W.; Jacobson, K. A. Tetrahydrobenzothiophenone Derivatives as a Novel Class of Adenosine Receptor Antagonists. *J. Med. Chem.* **1996**, *39*, 398−406.

(18) Ji, X.; Melman, N.; Jacobson, K. A. Interactions of Flavonoids and Other Phytochemicals with Adenosine Receptors. *J. Med. Chem.* **1996**, *39*, 781−788.

(19) van Muijlwik-Koezen, J. E.; Timmerman, H.; Link, R.; van der Goot, H.; Ijzerman, A. A novel class of Adenosine A_3 Receptor Ligands. 2. Structure Affinity Profile of a Series of Isoquinoline and Quinazoline Compounds. *J. Med. Chem.* **1998**, *41*, 3994−4000.

(20) Müller, C. E.; Grahner, B.; Heber, D. Amino-substituted 1,8-naphthyridines and pyrido[2,3-*d*]pyrimidines: new compounds with affinity for A_1- and A_2-adenosine receptors. *Pharmazie* **1994**, *49*, 878−880.

(21) Carboni, S.; Da Settimo, A.; Ferrarini, P. L.; Livi, O. Preparazione e studio farmacologico di alcuni derivati 1,2,3-triazol-1,8-naftiridinici. *Farmaco Ed. Sci.* **1978**, *33*, 315−323.

(22) Ferrarini, P. L.; Mori, C.; Manera, C.; Mori, F.; Calderone, C.; Martinotti, E. Condensation of Substituted 2-aminopyridine with β-Ketocarboxylic esters: 4H-Pyrido[1,2-*a*]pyrimidin-4-ones and Pyridin-2-ones. *J. Heterocycl. Chem.*, in press.

(23) Ferrarini, P. L.; Mori, C.; Livi, O.; Biagi, G.; Marini, A. M. Synthesis of some Substituted Pyrido[1,2-*a*]pyrimidin-4-ones and 1,8-naphthyridines. *J. Heterocycl. Chem.* **1983**, *20*, 1053−1057.

(24) Ferrarini, P. L.; Mori, C.; Primofiore, G.; Calzolari, L. One Step Synthesis of Pyrimido[1,2-*a*][1,8]naphthyridinones, Pyrido[1,2-*a*]pyrimidinones and 1,8-Naphthyridinones. Antihypertensive Agents. V. *J. Heterocycl. Chem*. **1990**, *27*, 881−886.
(25) Carboni, S.; Da Settimo, A.; Pirisino, G.; Segnini, D. Ricerche nel campo delle naftiridine. Precisazioni sulla reazione tra 2,6-diaminopiridina e acetoacetato di etile. *Gaz. Chim. Ital*. **1966**, *96*, 103−113.
(26) Brown, E. V. 1,8-Naphthyridine. I. Derivatives of 2- and 4-Methyl-1,8-naphthyridines. *J. Org. Chem*. **1965**, *30*, 1607−1610.
(27) Da Settimo, A.; Ferrarini, P. L.; Mori, C.; Primofiore, G.; Subissi, A. Synthesis of 1,8-naphthyridine derivatives. Potential antihypertensive agents. II. *Farmaco Ed. Sci*. **1986**, *41*, 827−838.
(28) Ferrarini, P. L.; Livi, O.; Menichetti, V. M. Preparazione ed esame farmacologico di alcuni derivati 1,8-naftiridin-4-idrazonici. *Farmaco Ed. Sci*. **1979**, *34*, 165−169.
(29) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raaghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, K. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, B. J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94 w*, version 4.0; Gaussian, Inc.: Pittsburgh, PA, 1995.
(30) Martini, C.; Pennacchi, E.; Poli, M. G.; Lucacchini, A. Solubilization of adenosine A_1 binding sites from sheep cortex. *Neurochem. Int*. **1985**, *7*, 1017−1020.
(31) Mazzoni, M. R.; Martini, C.; Lucacchini, A. Regulation of agonist binding to A_{2A} adenosine receptor: effects of guanine nucleotides (GDP[S] and GTP[S]) and Mg^{2+} ion. *Biochim. Biophys. Acta* **1993**, *1220*, 76−84.
(32) Mazzoni, M. R.; Buffoni, R. S.; Giusti, L.; Lucacchini, A. (Characterization of a Low Affinity Binding Site for N^6-substituted Adenosine Derivatives in Rat Testis Membranes. *J. Recept. Sign. Transd. Res*. **1995**, *15*, 905−929.
(33) Franchetti, P.; Cappellacci, L.; Marchetti, S.; Trincavelli, L.; Martini, C.; Mazzoni, M. R.; Lucacchini, A.; Grifantini, M. 2′C-Methyl Analogues of Selective Adenosine Receptor Agonists: Synthesis and Binding Studies. *J. Med. Chem*. **1998**, *41*, 1708−1715.
(34) Cheng, Y. C.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibition which Causes 50 Percent Inhibition (IC_{50}) of an Enzyme Reaction. *Biochem. Pharmacol*. **1973**, *22*, 3099−3108.
(35) Peterson, G. L. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal. Biochem*. **1977**, *83*, 356−366.
(36) Johnson, R. G.; Aklvarez, R.; Salomon, Y. Determination of adenylyl cyclase catalytic activity using single and double column procedures. *Methods Enzymol*. **1994**, *238*, 31−56.

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