Study on Affinity Profile toward Native Human and Bovine Adenosine Receptors of a Series of 1,8-Naphthyridine Derivatives

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A new series of 1,8-naphthyridine derivatives (**29**−**44** and **46**−**52**) bearing various substituents in different positions on the heterocyclic nucleus were synthesized in order to analyze the effects produced on the affinity toward the bovine adenosine receptors. These derivatives represent an extension of our previous work on this class of compounds with high affinity toward A_1 adenosine receptors.[19] The results of radioligand binding assays indicate that a large number of the 1,8-naphthyridine derivatives proved to be A_1 selective, with a high affinity toward bovine adenosine receptors in the low nanomolar range, and one (**29**) in the subnanomolar range. Furthermore, the new series of 1,8-naphthyridine derivatives (**29**−**44** and **46**−**52**), together with the analogous derivatives **1**−**28** previously studied,[19] were tested to evaluate their affinity toward human cortical A_1 receptors and human striatal A_{2A} receptors. The results indicate that all the 1,8-naphthyridine compounds generally possess a higher affinity toward the bovine A_1 receptor compared with the human A_1 receptor. As regards the affinity toward the A_{2A} bovine receptor, only a few compounds possess a moderate affinity, which for some compounds remained approximately the same toward the A_{2A} human receptor. A molecular modeling study of the docking of the 1,8-naphthyridine compounds with both the bovine and the human A_1 adenosine receptors was carried out with the aim of explaining the marked decrease in the affinity toward human A_1 adenosine receptors in comparison with bovine A_1 adenosine receptors. This study indicated that the structural differences, albeit small, of the active sites of the two receptors make differences in the dimensions of the site and this influenced the ability of the title compounds to interact with the two A_1 receptors.

Introduction

Adenosine, which is formed from the purine base adenine and the ribose moiety, is a ubiquitous neuromodulator in both the periphery and the central nervous system. Part of the biological activity of adenosine occurs through the activation of specific cell membrane receptors belonging to the extensive family of G-protein-coupled receptors.[1,2] Currently, four adenosine receptors have been cloned and characterized pharmacologically, namely A_1, A_{2A}, A_{2B}, and A_3. The adenosine receptors are associated with different second messenger systems: A_1 and A_3 mediate adenylate cyclase inhibition, whereas A_{2A} and A_{2B} stimulate adenylate cyclase activity by controlling intracellular cyclic AMP levels.[1] The discovery of adenosine receptor subtypes opened up new avenues for potential drug treatment of a variety of conditions such as asthma, neurodegenerative disorders, psychosis and anxiety, chronic inflammatory diseases, and many other physiopathological states that are believed to be associated with changes in adenosine levels.[3−6] Consequently, selective and potent agonists or antagonists at the human receptor subtypes are needed for therapeutic intervention.

In the past few years, a variety of different classes of heterocyclic compounds have been reported to possess an antagonistic activity at adenosine receptors, including xanthine, adenines, 7-deazaadenines, 7-deaza-8-azapurines,[7−12] pyrazolo[3,4-*c*]quinolines,[13] pyrazolo[1,5-*a*]pyridines,[14] triazoloquinoxaline,[15] triazoloquinazoline (e.g. CGS 15943), pyrazolotriazolopyrimidine,[16,17] and triazinobenzimidazolones.[18] Recently[19] we have undertaken a systematic research program involving the synthesis and testing of a series of 1,8-naphthyridine derivatives (**1**−**28**; see Table 1) bearing a phenyl group at position 2 and various substituents at positions 4 and 7, to evaluate their affinity for the bovine A_1, A_{2A}, and A_3 adenosine receptor subtypes. In binding to bovine brain cortical membranes, most of the compounds showed an affinity for A_1 receptors in the low nanomolar range and two (**15** and **16**) in the subnanomolar range with an interesting degree of A_1 versus A_{2A} and A_3 selectivity. Moreover, the two most potent and selective derivatives in the binding assays, **16** and **21**, were also shown to be full antagonists toward A_1 receptors.

Adenosine receptors from different species show a good amino acid sequence homology (82−93%), the only exception being the A_3 subtype which only exhibits 74% primary sequence homology between rat and human or sheep.[20−22] Although there is only little difference in the A_1 receptor sequence of different species,[11] some species differences in agonist binding have been reported.[23] The bovine A_1 receptor has an affinity for agonist and antagonist ligands that is 10-fold higher than that of

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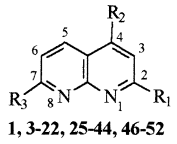
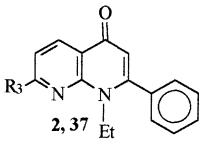
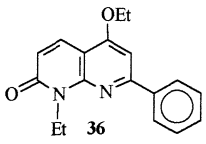
Table 1. Affinity of 1,8-Naphthyridine Derivatives in Radioligand Binding Assays at Bovine Brain A_1, A_{2A} and Human Brain A_1 and A_{2A} Receptors[a,b]



1, 3-22, 25-44, 46-52



2, 37



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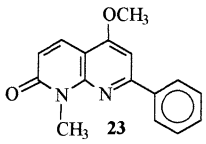

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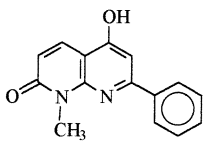

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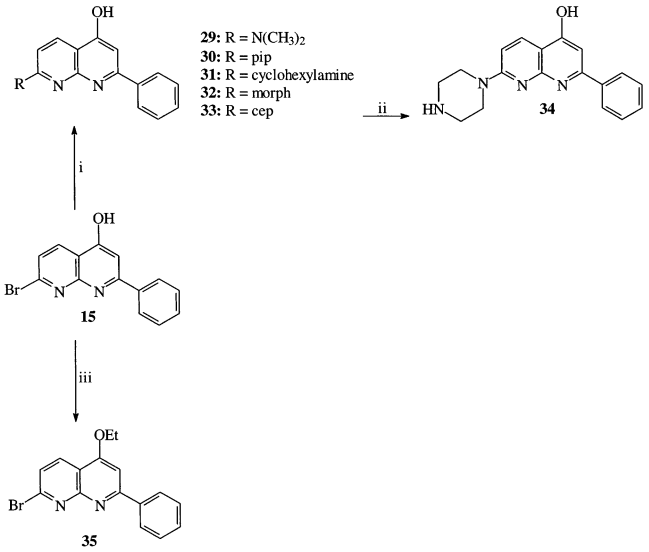
				K_i (nM)					
compd	R_1	R_2	R_3	bA_1	bA_{2A}	bA_{2A}/bA_1	hA_1	hA_{2A}	hA_{2A}/hA_1
1	Ph	OH	CH_3	5.3 ± 0.6	460 ± 38	87	430 ± 35	550 ± 47	1.3
2			CH_3	450 ± 92	> 10000	>22	>10000	>10000	
3	Ph	SH	CH_3	80 ± 7	1250 ± 230	16	2000 ± 150	230 ± 20	0.12
4	Ph	Cl	CH_3	1000 ± 110	>10000	>10	>10000	>10000	
5	Ph	OCH_3	CH_3	6000 ± 600	>10000	>2	>10000	>10000	
6	Ph	OPh	CH_3	810 ± 87	>10000	>12	8800 ± 850	>10000	>1.1
7	Ph	N_3	CH_3	>10000	>10000		>10000	>10000	
8	Ph	NH_2	CH_3	17 ± 4	420 ± 29	25	2000 ± 170	210 ± 15	0.10
9	Ph	$N(CH_3)_2$	CH_3	550 ± 57	7000 ± 630	13	>10000	>10000	
10	Ph	cyclohexylNH	CH_3	28 ± 3	830 ± 79	30	560 ± 52	490 ± 45	0.88
11	Ph	Cep [c]	CH_3	1200 ± 130	>10000	>8	>10000	>10000	
12	Ph	Pipz [d]	CH_3	8700 ± 750	>10000	>1	>10000	>10000	
13	*p*-FPh	OH	CH_3	5.3 ± 0.4	630 ± 65	119	3900 ± 400	440 ± 45	0.11
14	*o*-FPh	OH	CH_3	11 ± 4	470 ± 42	43	490 ± 45	40 ± 5	0.081
15	Ph	OH	Br	0.70 ± 0.05	66 ± 5	94	360 ± 35	100 ± 9	0.28
16	Ph	OH	Cl	0.15 ± 0.01	100 ± 15	670	300 ± 27	450 ± 42	1.5
17	Ph	OH	F	4.1 ± 0.6	170 ± 35	41	1400 ± 150	2700 ± 25	1.9
18	Ph	OH		16 ± 4	>10000	>625	1300 ± 120	2000 ± 180	1.5
19	Ph	OH	OPh	26 ± 4	1900 ± 170	73	>10000	4800 ± 450	< 0.48
20	Ph	OH	OEt	5.2 ± 0.7	>10000	>1920	2600 ± 270	1500 ± 130	0.58
21	Ph	OH	OCH_3	1.6 ± 0.2	1400 ± 140	875	2000 ± 180	490 ± 50	0.25
22	Ph	OH	OH	>10000	>10000		>10000	>10000	
23				1300 ± 280	>10000	>8	>10000	>10000	
24				4900 ± 340	>10000	>2	>10000	6500 ± 620	< 0.65
25	Ph		CH_3	6900 ± 700	>10000	>1	>10000	>10000	
26	*p*-NO_2Ph	OH	CH_3	9.9 ± 0.8	460 ± 37	46	3100 ± 280	640 ± 57	0.21
27	Ph	$NHNH_2$	CH_3	100 ± 11	1800 ± 150	18	>10000	4100 ± 400	< 0.41
28	Ph	OH	NH_2	5.3 ± 0.5	5900 ± 420	1110	3400 ± 350	9000 ± 870	2.6
29	Ph	OH	$N(CH_3)_2$	0.56 ± 0.03	2300 ± 210	4110	980 ± 90	1300 ± 110	1.3
30	Ph	OH	Pip[e]	7.2 ± 0.7	>10000	>1390	7500 ± 720	>10000	>1.3
31	Ph	OH	cyclohexylNH	70 ± 6	2500 ± 230	36	2200 ± 200	1700 ± 160	0.77
32	Ph	OH	Morph[f]	160 ± 15	>10000	>63	>10000	>10000	
33	Ph	OH	Cep[c]	>10000	>10000		>10000	>10000	
34	Ph	OH	Pipz[d]	>10000	>10000		>10000	>10000	
35	Ph	OEt	Br	50 ± 4	2900 ± 250	58	1000 ± 110	970 ± 90	0.97
36				4700 ± 450	7200 ± 650	1.5	>10000	3800 ± 370	< 0.38
37			OEt	48 ± 5	>10000	208	930 ± 90	1400 ± 150	1.5
38	Ph	OEt	OEt	290 ± 30	>10000	>34	>10000	>10000	
39	Ph	OH	CH_3CONH	340 ± 35	>10000	>29	>10000	>10000	
40	Ph	SCH_3	CH_3	380 ± 35	>10000	>26	>10000	210 ± 20	< 0.021
41	*p*-$NH_2C_6H_5$	OH	CH_3	6.8 ± 0.5	500 ± 47	73	910 ± 90	110 ± 10	0.12
42	*p*-$AcNHC_6H_5$	OH	CH_3	1.0 ± 0.2	2300 ± 220	2300	2000 ± 200	1600 ± 150	0.8
43	*m*-$NO_2C_6H_5$	OH	CH_3	15 ± 4	580 ± 60	39	2200 ± 200	3400 ± 350	1.5
44	*m*-$NH_2C_6H_5$	OH	CH_3	23 ± 3	230 ± 20	10	4000 ± 380	1300 ± 110	0.32
46	CH_2Ph	OH	CH_3	1900 ± 200	>10000	>5.3	>10000	>10000	
47	H	OH	CH_3	7000 ± 710	>10000	>1.4	>10000	1800 ± 170	< 0.18
48	H	NH_2	CH_3	>10000	>10000		>10000	>10000	
49	CH_3	OH	OH	>10000	>10000		>10000	>10000	
50	CH_3	OH	NH_2	>10000	>10000		>10000	7500 ± 730	< 0.75
51	$CH_2CH_2CH_3$	OH	CH_3	48 ± 6	900 ± 85	19	1500 ± 140	1000 ± 110	0.66
52	$CH_2CH_2CH_3$	OH	NH_2	210 ± 20	>10000	>48	4100 ± 400	>10000	>2.4
DPCPX				0.25 ± 0.02	200 ± 17	800	6.7 ± 0.5	144	21.5
SCH58261				357 ± 35	2.0 ± 0.1	0.0056	463 ± 45	3.25 ± 0.2	0.0070

[a] Inhibition of specific [^{3}H]CHA binding to bovine and human brain cortical membranes expressed as $K_i \pm$ SEM ($n = 3$) in nM. [b] Inhibition of specific [^{3}H]CGS21680 binding to bovine and human striatal membranes expressed as $K_i \pm$ SEM ($n = 3$) in nM. [c] Cep = ethylcarbethoxypiperazinyl. [d] Pipz = piperazinyl. [e] Pip = piperidinyl. [f] Morph = morpholinyl.

rat and human receptors; in the bovine receptor, the typical A_1 receptor rank order of potency (*R*)-PIA > NECA > (*S*)-PIA is partially altered in that it has a specifically reduced binding affinity for the 5′-substituted adenosine analogues compared with rat and human receptors.[24] Furthermore, N^6-substituted adenosine derivatives, such as (*R*)-PIA, are more potent at bovine than at human or rat A_1 receptors. This phenomenon has been called the "phenyl effect" and is the strongest at the bovine A_1 receptor.[23]

Recently, human recombinant adenosine receptors expressed in mammalian cell lines (CHO, HEK) have often been found application in the screening of new ligands.[25−30] Reevaluation of compounds at all four

Scheme 1[a]



[a] Reagents and conditions: (i) amine, heated in a sealed tube at 140 °C, 48 h; (ii) EtOH, NaOH, reflux, 5 h; (iii) DMSO, NaH, EtI, heated at 80 °C, 24 h.

human cloned adenosine receptor subtypes has shown that ligands which had been considered to be A_1-selective may not be selective for human A_1 adenosine receptors, in some cases due to species differences (e.g. a lower affinity for human than for rat receptors), or due to a high affinity of the compounds for the new A_3 or A_{2B} adenosine receptors.[31,32]

Although methods of purification and characterization of the native human adenosine A_1 and A_{2A} receptors have been reported in the literature,[33,34] no binding tests using these receptors have so far been published for new synthetic compounds structurally different from classical ligands of adenosine receptors.

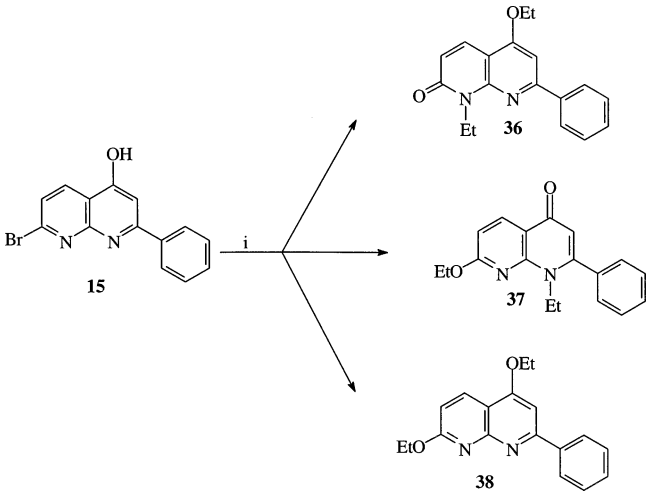
In light of these considerations, we established two aims: (i) extending and completing the analysis of the effects produced on the affinity and the selectivity toward the bovine A_1 adenosine receptor (bA_1AR) by the substitution of the 1,8-naphthyridine nucleus at the 1, 2, 4, and 7 positions, synthesizing for this purpose a new series of compounds which were analogues of those previously studied; (ii) both for this new series of compounds and for the 1,8-naphthyridine derivatives previously reported,[19] evaluating the binding activity at human A_1, A_{2A}, and A_3 receptors, using human cortical (hA_1AR) and striatal ($hA_{2A}AR$) membranes and human cloned receptors (hA_3AR).

Finally, a molecular modeling study of the interaction of the 1,8-naphthyridine compounds with both the bovine and the human A_1 adenosine receptors was carried out.

Chemistry

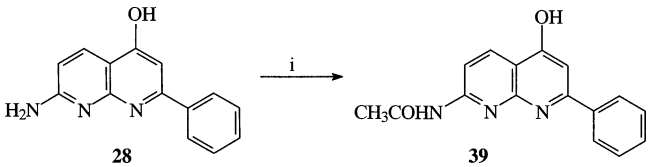
The compounds described in this study are shown in Tables 1, and their methods of synthesis are outlined in Schemes 1−7. Reaction of 7-bromo-4-hydroxy-2-phenyl-1,8-naphthyridine **15**[19] with an excess of the appropriate amine in a sealed tube at 140 °C gave **29**−**33** (Scheme 1). The piperazine derivative **34** was obtained by alkaline hydrolysis of compound **33**. When the naphthyridine **15** was treated with NaH and EtI in DMSO, the 4-ethoxy derivative **35** was obtained. (Scheme 1). Reaction of compounds **15** with EtI and KOH in a hydro-alcoholic solution gave **36**−**38** (Scheme 2).

Scheme 2[a]



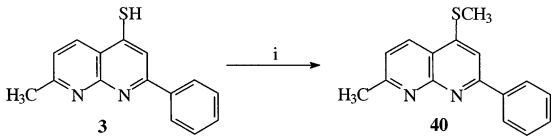
[a] Reagents and conditions: (i) EtOH, KOH, EtI, heated in sealed tube at 80 °C, 72 h.

Scheme 3[a]



[a] Reagents and conditions: (i) acetic anhydride, heated at 100 °C, 4.5 h.

Scheme 4[a]

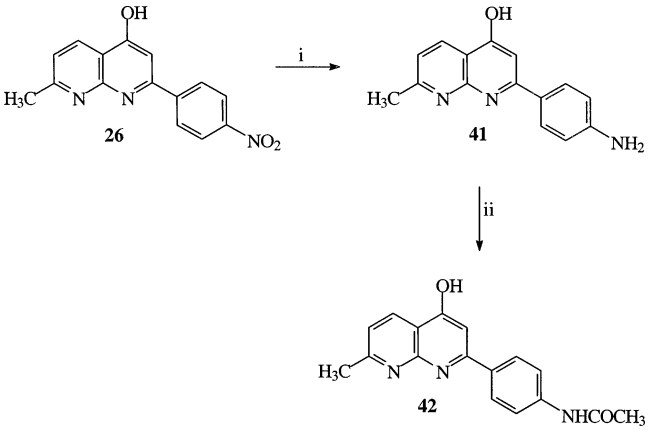


[a] Reagents and conditions: (i) MeOH, Na, CH_3I, 2 h.

The 7-acetylamino derivative **39**[35] was prepared by reaction of the 7-amino derivative **28**[35] with Ac_2O (Scheme 3). The reaction of the 4-mercaptonaphthyridine **3**[19] with MeONa and CH_3I in MeOH at room temperature afforded the corresponding methylthio derivative **40** (Scheme 4). The catalytic reduction of the *p*-nitrophenyl derivative **26**[36] was performed in AcOH, in the presence of Pd/C as a catalyst, to give the *p*-aminophenylnaphthyridine **41**, which by the reaction with Ac_2O gave the corresponding acetamido derivative **42** (Scheme 5). The nitration of 2-phenylnaphthyridine **1**[37] carried out with potassium nitrate in concentrated H_2SO_4 gave the *m*-nitrophenyl derivative **43**, which by catalytic reduction in the presence of Pd/C as a catalyst was converted to the corresponding *m*-aminophenylnaphthyridine **44** (Scheme 6). The condensation of 2-amino-6-methylpyridine with ethyl 4-phenylacetoacetate, prepared as described in the literature,[38] in polyphosphoric acid at 100 °C gave the 4*H*-pyrido-[1,2-*a*]pyrimidin-4-one (**45**), which was converted to the 2-benzyl-1,8-naphthyridine derivative **46** by reflux in Dowtherm A (Scheme 7).

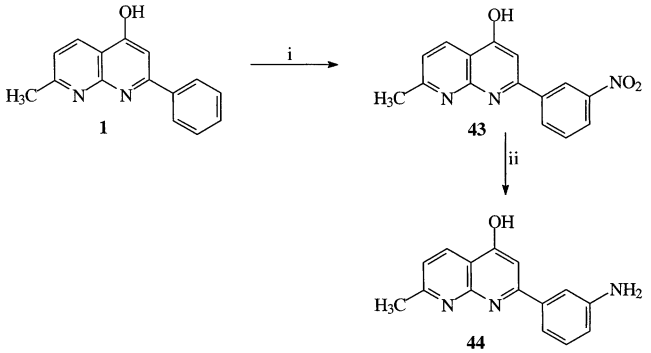
All the compounds synthesized were characterized by elemental analysis, IR, and ^1H NMR.

Scheme 5[a]



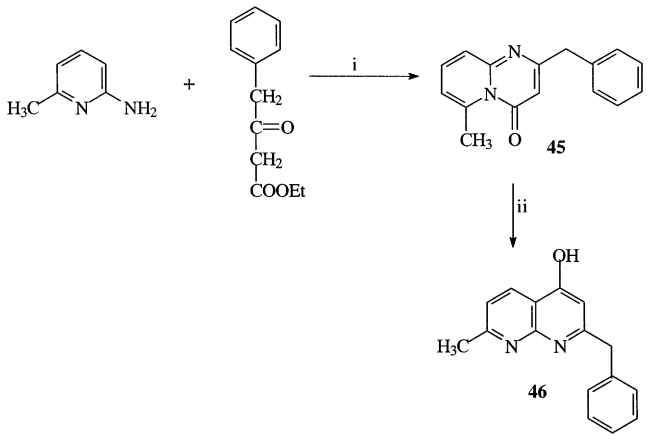
[a] Reagents and conditions: (i) H_2, Pd/C, 3h; (ii) acetic anhydride, reflux, 5 h.

Scheme 6[a]



[a] Reagents and conditions: (i) H_2SO_4, KNO_3, 30 minutes; (ii) H_2, Pd/C, 3 h.

Scheme 7[a]



[a] Reagents and conditions: (i) PPA, heated at 100 °C, 4 h; (ii) Dowtherm A, heated at 220 °C, 5 h.

Results and Discussion

The affinities of 1,8-naphthyridine derivatives **1**−**44** and **46**−**52**[39−41] were determined by measuring their ability to displace the specific binding of agonist [^{3}H]-N^6-(cyclohexyl)-adenosine ([^{3}H]CHA) and [^{3}H]2-{[[p-(2-carboxyethyl)-phenyl]ethyl]amino}-5′-(*N*-ethylcarbamoyl)-adenosine ([^{3}H]CGS21680) from bovine (**29**−**44** and **46**−**52**[39−41]) and human (**1**−**44** and **46**−**52**[39−41]) cortical (A_1) and striatal (A_{2A}) membranes, respectively.[33,34,42,43] Moreover, for some compounds (**10**, **15**, **16**, **22**, **29**, **42**, **50**,[35,41] **51**[40]), the affinity for A_1 receptors was also determined by displacement experiment using the antagonist [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine (DPCPX). These data, plus the receptor affinities for the antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and 5-amino-7-(2-phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH58261), expressed as inhibition constants (K_i, nM), are summarized in Table 1 and Table 2. Finally, the affinity of some 1,8-naphthyridine derivatives (**3**, **8**, **14**, **15**, **40**, and **41**) was determined by measuring their ability to displace the binding of [^{125}I]AB-MECA from human cloned receptors (A_3).

Table 2. Affinity of Several Representative 1,8-Naphthyridine Derivatives in Antagonist Radioligand Binding Assays at A_1 Receptors in Bovine and Human Brain

	A_1 K_i (nM)[a]	
compd	bovine	human
10	39.0 ± 5 (28 ± 3)	448 ± 52 (560 ± 52)
15	1.60 ± 0.5 (0.70 ± 0.05)	336 ± 27 (0.70 ± 0.05)
16	1.62 ± 0.7 (0.15 ± 0.01)	670 ± 58 (300 ± 27)
22	>10000	>10000
29	6.02 ± 0.6 (0.56 ± 0.03)	1370 ± 130 (980 ± 90)
42	1.67 ± 0.5 (1 ± 0.2)	1460 ± 150 (2000 ± 200)
50	>10000	>10000
51	61.5 ± 5 (48 ± 6)	1447 ± 130 (1500 ± 140)

[a] Displacement of [^{3}H]DPCPX from bovine and human brain cortical membranes expressed as K_i ± SEM (n = 3). In parentheses are reported the value of K_i obtained using the agonist [^{3}H]CHA.

For a better understanding of the biological data, it needs to be pointed out that the numbering of the 1,8-naphthyridine nucleus used is the same as that reported in Table 1.

A_1 Receptors. Bovine A_1 Receptor (bA_1AR). The results reported in Table 1 for the new compounds **29**−**44** and **46**−**52**[39−41] show that, as regards the structural modifications introduced at the position 2 (R_1) of the heterocyclic nucleus, the lack of any substituent, or replacing of the phenyl group in this position with a methyl group or an *n*-propyl group reduces the receptor affinity, as can be seen from a comparison of compounds **47**[39] (K_i = 7000 nM) and **51**[40](K_i = 48 nM) versus **1** (K_i = 5.3 nM), a comparison of compound **50**[35,41] (K_i > 10000 nM) and **52**[35] (K_i = 210 nM) versus **28** (K_i = 5.3 nM) and finally that of compound **48**[39] (K_i > 10000 nM) versus **8** (K_i = 17 nM). The introduction of a methylenic group between the naphthyridine nucleus and the phenyl group induces a decrease in the affinity, as can be seen from a comparison of compound **46** (K_i = 1900 nM) versus **1** (K_i = 5.3 nM). Furthermore, the introduction of a substituent on the phenyl group (**41**−**44**) maintains an affinity in the nanomolar range (K_i = 1.0−23 nM), similar to that of the analogous compound **1**, unsubstituted on the phenyl group (K_i = 5.3 nM). In the case of compound **42** (K_i = 1.0 nM) the affinity is higher than that of **1** (K_i = 5.3 nM).

As regards the structural modifications at the position 4 (R_2) of the 1,8-naphthyridine nucleus, the substitution of the mercapto group (**3**, K_i = 80 nM) with a methylthio group was found to produce a decrease in the affinity (**40**, K_i = 380 nM). Analogously, the substitution of the hydroxy group in the same position with the ethoxy group leads to compounds exhibiting a decrease in affinity, as is clear from a comparison of compound **35** (K_i = 50 nM) versus **15** (K_i = 0.70 nM) and compound

38 (K_i = 290 nM) versus **20** (K_i = 5.2 nM). The low affinity of compounds **35** and **38** might also be justified by the fact that the tautomeric structure, in which the oxygen in position 4 is in the quinoid form and the nitrogen in position 1 is protonated, is not possible for these compounds.[19] This quinoid structure was found to be preferred for the 4-OH substituted compounds and proved to be very important for the affinity at the bA_1-AR.[19]

As regards the structural modifications at the position 7 (R_3) of the 1,8-naphthyridine nucleus, the substitution of the methyl group with secondary amines, like $N(CH_3)_2$ or piperidine, was found to lead to compounds **29** and **30**, respectively, with a remarkable affinity at the bA_1-AR (**29**, K_i = 0.56 nM, **30**, K_i = 7.2 nM). The 7-cyclohexylamino derivative **31** showed an appreciable affinity (K_i = 70 nM). On the contrary, the substitution at the same position with a cyclic amine bearing another heteroatom on the ring, like morpholine, caused a decrease in the affinity at the bA_1AR (**32**, K_i = 160 nM), which in some cases was remarkable, as for the ethoxycarbonylpiperazine (**33**) and piperazine groups (**34**), with a K_i > 10000 nM. Furthermore, the substitution of the methyl group with an acetamido group produced a decrease in the affinity, as can be seen from a comparison of compound **39** (K_i = 340 nM) versus **1** (K_i = 5.3 nM).

As regards the compounds which possess a quinoid structure in position 4 or 7, the N8-ethyl-substituted derivative **36**, with the quinoid structure at the position 7, showed a low affinity toward the bA_1AR (K_i = 4700 nM), similar to that of compound **23** (K_i = 1300 nM) previously studied.[19] The 4,7-dihydroxy derivative **49**,[41] which possesses the quinoid structure with the N8 protonated (as demonstrated on the basis of theoretical calculations for the analogous compound **22**[19]) showed a very low affinity (K_i > 10000 nM). Finally, the N1-ethyl-substituted compound **37** showed an intermediate affinity K_i = 48 nM. This last result confirms the importance for the affinity at the bA_1AR of the tautomer in which the oxygen in position 4 is in the quinoid form and the nitrogen in position 1 is protonated.[19]

The results show that some of compounds **29**−**44** and **46**−**52** possess interesting affinity at the bA1AR, in some cases in the low nanomolar range, with a different degree of selectivity at this receptor. In particular compounds **29**, **30**, **41**, and **42** exhibit a remarkable affinity with K_i < 10 nM.

Native Human A_1 Receptor (hA_1AR). To obtain information about the differences between the affinity versus bovine receptors and versus native human receptors, the new series of 1,8-naphthyridine derivatives **29**−**44, 46**−**52**[39−41] and the derivatives **1**−**28** previously studied[19] were tested to evaluate their affinity toward human cortical A_1 receptors. The results shown in Table 1 indicate that all compounds showed a poor affinity at the native hA_1AR. Only compounds **1**, **10**, **14**−**16** possessed an intermediate affinity (K_i = 560−300 nM). Moreover, it is evident that all the compounds exhibit a remarkable decrease in affinity, ranging from 10 to 2000 times, in comparison with the affinity at the bA_1-AR. In particular, compounds **16** and **29**, which showed a high affinity at the bA_1AR (**16**, K_i = 0.15 nM; **29**, K_i = 0.56 nM), possess an intermediate affinity at the hA_1-AR (**16**, K_i = 300 nM; **29**, K_i = 980 nM), with a decrease in the affinity of 2000 and 1750 times, respectively.

To establish whether the reduction in the affinity of the 1,8-naphthyridine derivatives studied for human tissue compared with bovine tissue might reflect a difference in the coupling between receptors and G proteins of different species,[44] we performed competition experiments for certain representative compounds (**10**, **15**, **16**, **22, 29**, **42**, **50**,[35,41] and **51**[40]), using the selective antagonist [^{3}H]DPCPX as the radioligand. The results shown in Table 2 revealed, also in this case, a different level of affinity for bovine and human tissue, confirming the loss of affinity for human tissue compared with bovine tissue, as had been found by using the agonist radioligand, [^{3}H]CHA. Only derivatives **16** and **29** were found to be about 10 times less potent on bovine tissue, using the antagonist instead of the agonist as the radioligand, probably due to a difference in G protein coupling. However, the large differences in affinity between the two species remain incomprehensible, seeing that they cannot be completely explained by the different type of interaction between receptors and G proteins. The discrepancies between the two species might be due to the primary amino acid sequence of the receptor: it is possible that the variation in this sequence might modulate the affinity of the ligand and determine differences in the pharmacological profile of the different species.[1,11]

All compounds exhibit a poor affinity at the native hA_1AR with a remarkable decrease in affinity in comparison with the affinity at the bA_1AR.

A_{2A} Receptors. Bovine A_{2A} Receptor ($bA_{2A}AR$). The data in Table 1 show that the new 1,8-naphthyridines **29**−**44** and **46**−**52**[39−41] possess a very poor affinity at the $bA_{2A}AR$, like the previous series. Only four derivatives (**41**, **43**, **44**, and **51**[40]) possess an intermediate affinity, with a K_i of 230−1000 nM. Five derivatives (**29**, **31**, **35**, **36**, and **42**) showed a moderate affinity in the order of a micromolar concentration (K_i = 2300−7200 nM), whereas other compounds were completely ineffective.

Native Human A_{2A} Receptor ($hA_{2A}AR$). The most effective compound toward the $hA_{2A}AR$ was **14**, with a K_i = 40 nM. A few other compounds (**1**, **3**, **8**, **10**, **13**, **15**, **16**, **21**, **26**, **35**, **40**, and **41**) showed an average affinity (K_i = 970−100 nM). The rest of the compounds possessed a very low affinity (K_i values > 1000 nM).

The results of the binding studies indicate that the affinity at the $hA_{2A}AR$ is not very different from that at the $bA_{2A}AR$. Indeed, the affinity at the native hA_{2A}-AR decreased a little for some compounds, for other compounds it remained approximately the same, whereas for a few compounds there was a little increase. In particular, the 4-methylthio derivative **40**, which was found to be inactive toward the $bA_{2A}AR$ (K_i > 10000 nM), showed an appreciable affinity toward the hA_{2A}-AR (K_i = 210 nM), with the highest increase in affinity compared with the affinity at bovine receptors. Furthermore, the 2-*o*-fluorophenyl-substituted derivative **14** showed the highest affinity at human receptors, with a K_i value of 40 nM and a high increase in comparison with the affinity at bovine receptors (K_i = 470 nM).

The most compounds possess a low affinity both at the $hA_{2A}AR$ and at the $bA_{2A}AR$. Furthermore, the

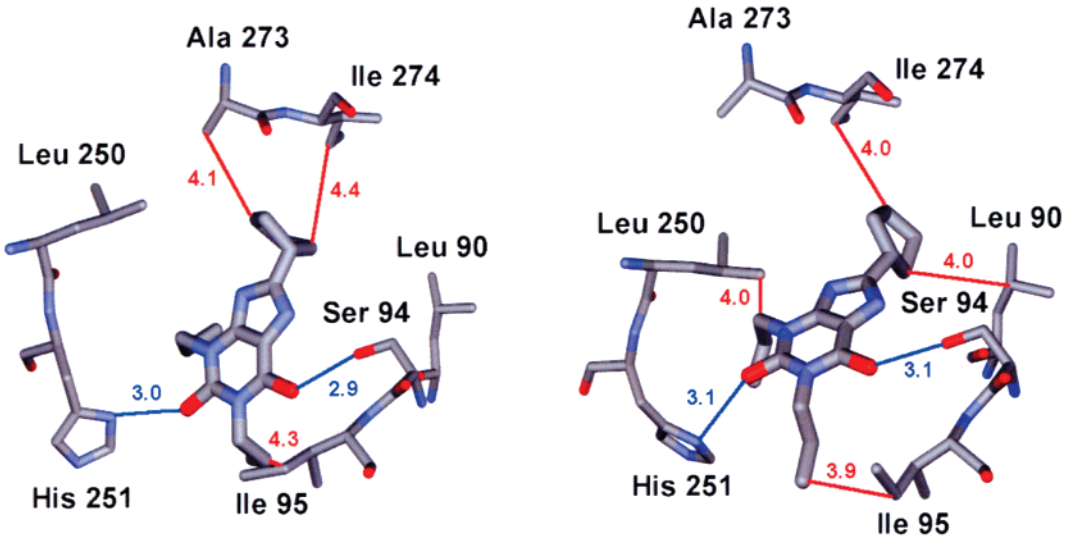


Figure 1. DPCPX docked into the hA_1AR (left) and bA_1AR (right) binding site. Interatomic distances between H-bonded atoms are reported in blue; carbon−carbon distances evidencing lipophilic interactions are reported in red. All distances are in Angstroms.

affinity at the $hA_{2A}AR$ is not very different from that at the $bA_{2A}AR$.

A_3 Receptors. As the analogous 1,8-naphthyridine derivatives previously studied[19] were ineffective at bovine A_3 receptors, we considered it sufficient to evaluate the affinity toward bovine A_3 receptors only for some new compounds (**29**, **30**, **41**, and **42**). The binding assay showed that these compounds presented very low inhibition percentages at a concentration of 10 μM, with the result that the corresponding K_i values were not calculated.

For the compounds which proved to be most effective at the native $hA_{2A}AR$ (**3**, **8**, **14**, **15**, **40**, and **41**), the affinity at the hA_3AR was also evaluated using cloned receptors, but all the tested compounds provided to be ineffective.

Molecular Modeling

With the aim of explaining the marked lowering of the affinity toward the native hA_1AR in comparison with the bA_1AR, a molecular modeling study was carried out in order to evaluate theoretically the interaction of the 1,8-naphthyridine derivatives with the two A_1 adenosine receptors from two species.

The model of the hA_1AR, made up of seven helices, was constructed by following a homology procedure in which the crystallographic structure of bovine rhodopsin[45] was used as a template; the model thus obtained was then optimized so as to interact suitably with the specific ligands CPA and DPCPX on the basis of the available site-directed mutagenesis data.[46] These data indicated that the residues Thr91, Ser94, His278, and Thr277 were important for the activity of the agonists, and the residues Ser94 and His251 were fundamental for the affinity of the antagonists. Therefore, the starting geometries of the complexes between the hA_1AR and the agonist CPA and subsequently the antagonist DPCPX were arranged in such a manner that the ligands could favorably interact with the appropriate residues. The computational procedures are fully described in the Experimental Section.

The model of the bA_1AR was constructed on the basis of the hA_1AR model, following a similar procedure. Figure 1 illustrates DPCPX docking into both the site of the hA_1AR (on the left) and the bA_1AR (on the right) and shows that DPCPX interacts at a similar distance with Ser94 and His251 through its hydroxylic functions by means of H-bonds which appear to be shorter and therefore stronger in the hA_1AR (d = 2.9 and 3.0 Å) than the bA_1AR (d = 3.1 Å). However, a series of lipophilic interactions due to Leu90, Ile95, Leu250, Ala273, and Ile274 are able to better stabilize DPCPX in the bA_1-AR: the cyclophentyl group interacts with Ala273 in hA_1AR (d = 4.1 Å) and Ile274 (d=4.4 Å), while it interacts with Ala273 (d = 4.0 Å) and Leu90 (d = 4.0 Å) in bA_1AR; moreover, in bA_1AR the two *n*-propyl chains are able to interact with Ile95 (d = 3.9 Å) and Leu250 (d = 4.0 Å), while in hA_1AR there is only the interaction with Ile95 (d = 4.3 Å).

Figure 2 shows that the fairly limited structural differences that exist in the transmembranal regions of the two receptors hA_1AR and bA_1AR (only seven residues) are, however, able to induce a clear difference in the 3D arrangement of the seven helices in the two models (the RMSD calculated on the backbone is 3.79 Å). An important point in determining the conformational differences in the models of the hA_1AR and the bA_1AR could be the replacement of the Met82 residue of the hA_1AR with Lys82 in the bA_1AR, which induces a different arrangement of the interhelix H-bonds and therefore helices TM2 and TM3 are closer in the case of the hA_1AR. The RMSD between the α carbons of helix 2 and the corresponding α carbons of helix 3 is 9.8 Å in the case of the hA_1AR and 10.5 Å in the case of the bA_1-AR.

The different arrangement of these two helices also modifies the rest of the structure, and, as a consequence, the binding pocket appears to be narrower in the bA_1-AR than in the hA_1AR. This fact could be the reason for the interaction differences shown in Figure 1.

Some 1,8-naphthyridine derivatives were then docked into the two receptor models; the compounds selected for this purpose were **16**, **22**, **28**, **29**, **50**, **51**, and **52**, which possessed different K_i values toward the hA_1AR and the bA_1AR. The docking procedure was carried out by taking into account the site-directed mutagenesis

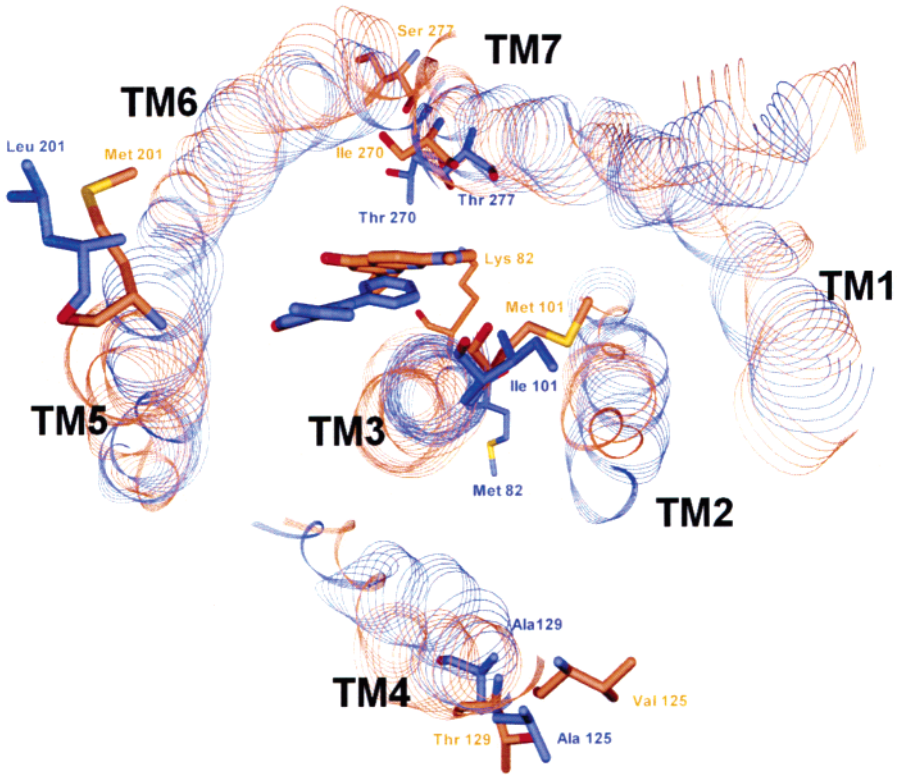


Figure 2. Superimposition of the complexes of DPCPX with hA_1AR (blue) and bA_1AR (orange); the seven not conserved residues of the seven transmembranal helices are shown.

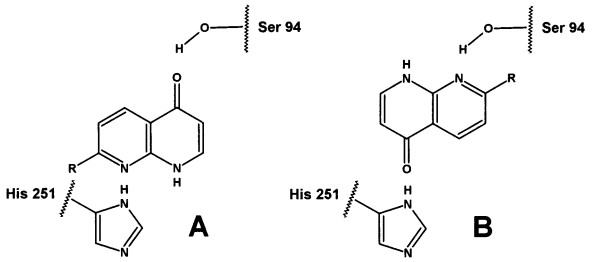


Figure 3. The two possible geometries for the interaction of 1,8-naphthyridine derivatives with A_1 adenosine receptors.

data, as for DPCPX. Therefore, the selected compounds were initially placed in the receptor sites so that they could interact favorably with Ser94 and His251.

The groups of compounds considered capable of interacting at the same time with these two residues were the substituent in position 4 and the nitrogen in position 8, which possess suitable chemical characteristics and a suitable spatial arrangement. Therefore, the two interaction geometries A and B, shown in Figure 3, are to be taken into consideration: in the first one, A, the substituent in position 4 gives an H bond with Ser94 and the nitrogen in position 8 gives an H-bond with His251; in the other one, B, the same groups of the selected compounds give H-bonds with the same residues, but in an inverse manner.

The complexes thus obtained (two for each compound and for each receptor model) were then optimized by means of molecular dynamic simulations followed by energy minimization with the AMBER force field; the computational procedure is fully described in the Experimental Section.

The antagonist−receptor interaction energies were calculated as the sum of the nonbonded terms of the molecular mechanics steric energy referring to the interaction between the atoms of the receptor model and the atoms of the ligand.

Table 3. Interaction Energies (kcal/mol) of hA_1AR and bA_1AR Models and Selected Ligands

compd	A_1bAR[a]	conformer[b]	A_1hAR[a]	conformer[b]	selectivity[c]	ΔE[d]
DPCPX	−30.2	-	−26.7	-	27	3.5
16	−29.7	B	−24.0	B	2000	5.7
22	−27.4	B	−25.2	B	-	-
28	−27.3	B	−22.2	B	640	5.1
29	−30.7	B	−26.3	B	1750	4.4
50	−21.1	B	−24.1	B	-	-
51	−22.6	A	−21.0	B	31	1.6
52	−23.0	B	−21.9	B	20	1.1

[a] Sum of the nonbonded terms of the interaction between the atoms of the receptor model and the atoms of the ligand. [b] Arrangement energetically preferred by the ligand for interaction with the receptor model (see Figure 4). [c] Selectivity of the ligand computed as the ratio between the K_i for hA_1AR and K_i for bA_1AR; the selectivity is not reported in the case of inactive compounds. [d] Energy difference between the interaction energy of the ligand with hA_1AR minus the interaction energy with bA_1AR; the energy difference is not reported in the case of inactive compounds.

All active compounds prefer arrangement B when they are fitted into both the hA_1AR and the bA_1AR. The differences in the interaction energy between the two arrangements, A and B, range from 2 to 6 kcal/mol.

The relative values of the interaction energy are reported in Table 3 and indicate that all active ligands have a more favorable interaction with the model of the bA_1AR, and this is in agreement with the greater affinity of the considered ligands toward the bA_1AR with respect to the hA_1AR; a correlation between these values and the selectivity ratio can also be observed.

In Figure 4 the complex between compound **16**, the most selective one, and the two models of the hA_1AR

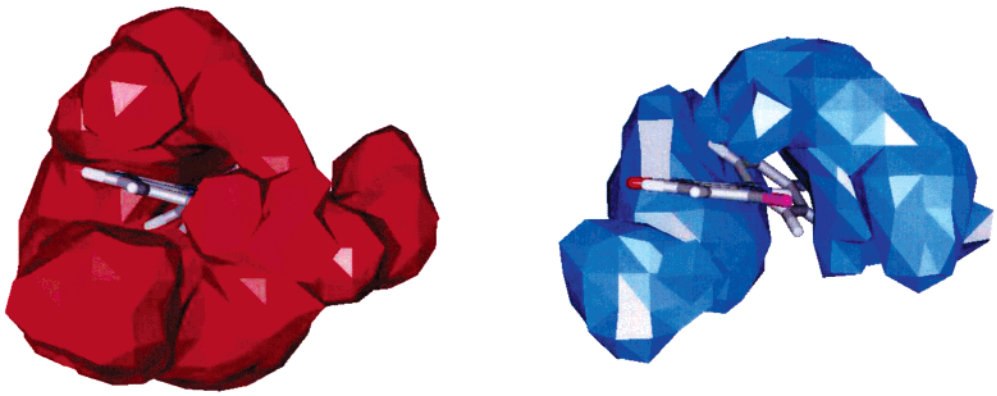


Figure 4. Compound **16** docked into the hA_1AR (left) and bA_1AR (right) binding sites. The volumes of the cavities between **16** and the two receptors are shown.

and bA_1AR receptors is shown. Figure 4 also reports the volumes of the cavities between the ligand and the receptors and is thus able to show the difference in the dimensions of the two binding sites, which could be quite important in determining the selectivity of the 1,8-naphthyridine derivatives considered.

The compounds considered here are less bulky than classic A_1AR antagonists and therefore they can interact more strongly with the sterically restricted site in the bA_1AR, while they cannot occupy so efficiently the larger site of the hA_1AR, as already indicated by the interaction energy values reported in Table 3.

In Figure 5 the details of the interaction of compounds **16** and **29** with the human and bovine A_1AR are presented. In the case of the complex with the bA_1AR, these antagonists are able to give H-bonds with Ser94 and His251 and at the same time their phenyl substituent in position 2 is able to give a hydrophobic interaction with Ile270 and other residues that make up a pocket (Tyr271, Ala273, Ile274). The pocket in the bA_1AR is able to optimally accept the phenyl ring linked in position 2 of the naphthyridine system, and therefore this structural feature of the bA_1AR binding site could explain the higher activity found for compounds that possess only an unsubstituted phenyl ring in position 2 of the naphthyridine system such as, for example, **16** and **29**. In fact, the presence of substituents on this phenyl ring, as, for example, in **13**, **26,** and **43** and the replacement of this phenyl ring with a less bulky group, as in **50**, **51**, and **52**, induces a decrease in the affinity. Moreover, the insertion of a spacer between the naphthyridine system and this phenyl ring, as in **46**, induces the almost complete loss of affinity.

In the case of the hA_1AR, the H-bonds and the hydrophobic interaction are weaker due to the larger dimensions of the site; moreover, in this receptor, Ile270 is substituted by the less hydrophobic Thr270. In particular, the lipophilic pocket in the hA_1AR is larger and the presence of a phenyl ring linked in position 2 is less important for the affinity.

Conclusions

On the basis of the results obtained in a previous paper,[19] a new series of 1,8-naphthyridine derivatives (**29**−**44** and **46**−**52**), with various substituents in different positions of the heterocyclic nucleus, were tested to evaluate their affinity toward bovine A_1 and A_{2A} receptors. Furthermore, the affinity at native human A_1 and A_{2A} receptors and human cloned A_3 receptors of this new series of compounds and of the 1,8-naphthyridine derivatives previously reported,[19] was assessed.

Results showed that also a large number of the new 1,8-naphthyridine derivatives proved to be A_1 bovine adenosine receptor selective with a high affinity toward the same receptor. In particular, compounds **29** and **42** showed a very high selectivity (A_{2A}/A_1 ratio 4110 and 2300, respectively), higher than that of the compounds previously studied.[19] Furthermore, it is possible to assume that the highest affinity at the bA_1AR requires a phenyl group in position 2, a quinoid oxygen in position 4 with the nitrogen in position 1 protonated, and a lipophilic group or an aliphatic amine without a large steric hindrance in position 7a.

As regards the affinity toward the $bA_{2A}AR$, only a few compounds possess a moderate affinity, which for some compounds remained approximately the same as toward the native $hA_{2A}AR$. In some cases, on the contrary, the affinity toward the $hA_{2A}AR$ was found to be higher than both the affinity toward the $bA_{2A}AR$ and toward the hA_1-AR. All the tested compounds proved to be totally inactive toward the cloned hA_3AR.

As regards the affinity toward the hA_1AR, all the 1,8-naphthyridine derivatives generally lost their affinity to an extent that in some cases is truly considerable (more than 1000 times) and decidedly higher than that reported in the literature for agonist and antagonist ligands.[23,24] To verify whether this great species selectivity could be explained in terms of a difference between the binding site of the native hA_1AR and the bA_1AR, these two sites were modeled, taking into account the available mutagenesis data. The interaction energy values of the 1,8-naphthyridine derivatives with the two receptor models were in agreement with their affinities and therefore with the observed species selectivity; the better interaction with the bA_1AR seems to be due to the smaller size of the binding site of this receptor, which allows a particularly good interaction with these 1,8-naphthyridine derivatives. In fact, the molecules of these ligands have a lower hindrance with respect to the classic A_1AR antagonists such as DPCPX, and therefore they can better occupy this site. Figure 6 shows that the volume of DPCPX is larger than that of **16** and that it is mainly due to the precence of the freely rotatable *n*-propyl chains in the structure of DPCPX.

Other differences in the structure of the hA_1AR and the bA_1AR, and in particular in the loop regions, could

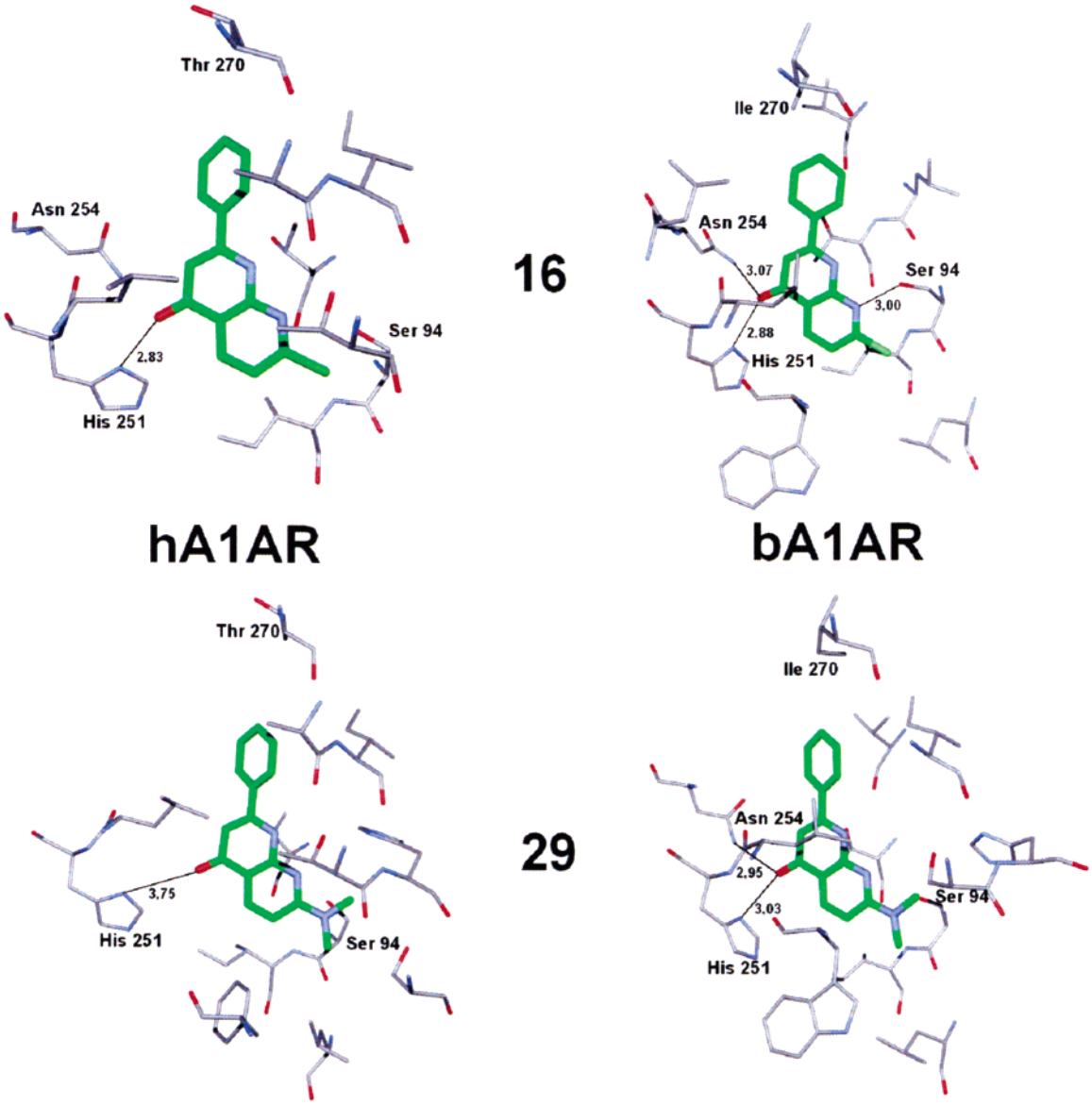


Figure 5. Compounds **16** (up) and **29** (down) docked into the hA_1AR (left) and bA_1AR (right) binding sites.

provide further reasons for the species selectivity, but the results of the molecular modeling study suggest that in the case of the 1,8-naphthyridine derivatives, their interaction with the intrahelical binding site of the two receptors should be surely responsible for this selectivity.

Experimental Section

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. ^{1}H 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.

General Procedure for the Preparation of 7-Substituted-4-hydroxy-2-phenyl-1,8-naphthyridine 29−33. A mixture of 7-bromonaphthyridine **15**[19] (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 48 h. The reaction mixture was treated with H_2O, and the solid was collected by filtration to obtain the title compounds. **7-Dimethylamino-4-hydroxy-2-phenyl-1,8-naphthyridine (29)**: 0.230 g, yield 72%; mp 257−260 °C (crystallized from toluene); MS *m*/*z* 265 (M^+). ^{1}H NMR: δ 9.00 (brs, OH), 8.33 (d, 1H, H_5), 7.50 (m, 5H, Ar), 6.56 (d, 1H, H_6), 6.44 (s, 1H, H_3), 3.15 (s, 6H, $N(CH_3)_2$). Anal. ($C_{16}H_{15}N_3O$) C, H, N. **4-Hydroxy-2-phenyl-7-piperidinyl-1,8-naphthyridine (30)**: 0.240 g, yield 65%; mp 247−249 °C (crystallized from toluene); MS *m*/*z* 305 (M^+). ^{1}H NMR: δ 8.95 (brs, OH), 8.27 (d, 1H, H_5), 7.46 (m, 5H, Ar), 6.63 (d, 1H, H_6), 6.40 (s, 1H, H_3), 3.63 (m, 4H, piperidine), 1.63 (m, 6H, piperidine). Anal. ($C_{20}H_{22}N_3O$) C, H, N. **7-Cyclohexylamino-4-hydroxy-2-phenyl-1,8-naphthyridine (31)**: 0.120 g, yield 31%; mp 156−159 °C (crystallized from toluene); MS *m*/*z* 319 (M^+). ^{1}H NMR: δ 9.20 (brs, OH), 8.21 (d, 1H, H_5), 7.47 (m, 5H, Ar), 6.42 (s, 1H, H_3), 6.33 (d, 1H, H_6), 4.95 (brs, NH), 3.75 (m, 1H, cyclohexylamine), 2.15−1.74 (m, 10H, cyclohexylamine). Anal. ($C_{20}H_{21}N_3O$) C, H, N. **4-Hydroxy-7-morpholinyl-2-phenyl-1,8-naphthyridine (32)**: 0.271 g, yield 74%; mp 258−261 °C (crystallized from toluene); MS *m*/*z* 307 (M^+). ^{1}H NMR: δ 8.68 (brs, OH), 8.34 (d, 1H, H_5), 7.50 (m, 5H, Ar), 6.62 (d, 1H, H_6), 6.43, (s, 1H, H_3), 3.71 (m, 8H, morpholine). Anal. ($C_{18}H_{17}N_3O_2$) C, H, N. **7-(4-Carbethoxypiperazin-1-yl)-4-hydroxy-2-phenyl-1,8-naphthyridine (33)**: 0.315 g, yield 70%; mp 237−239 °C (crystallized from toluene); MS *m*/*z* 378 (M^+). ^{1}H NMR: δ 8.65 (brs, OH), 8.33 (d, 1H, H_5), 7.50 (m, 5H, Ar), 6.53 (d, 1H, H_6), 6.42, (s, 1H, H_3), 4.16 (q, 2H, CH_2), 3.64 (m, 8H, piperazine), 1.28 (t, 3H, CH_3). Anal. ($C_{21}H_{22}N_4O_3$) C, H, N.

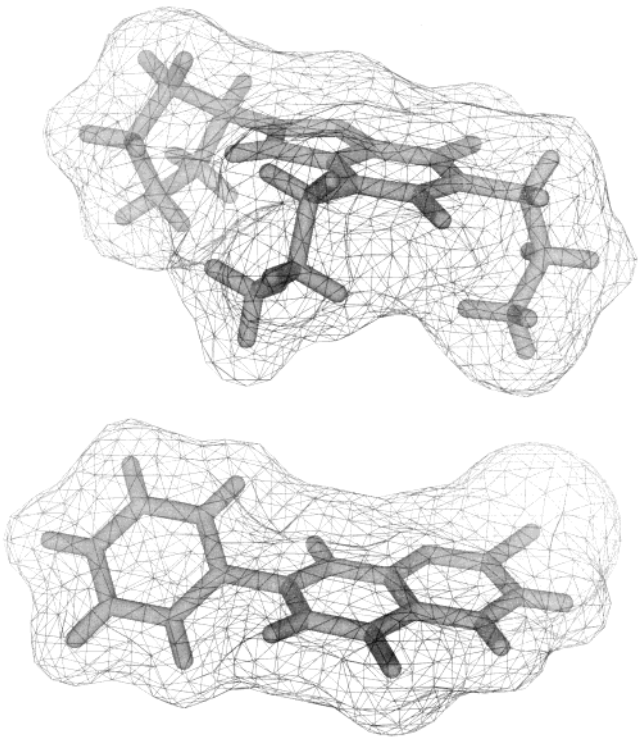


Figure 6. Molecular volumes of DPCPX (up) and **16** (down).

4-Hydroxy-2-phenyl-7-(piperazin-1-yl)-1,8-naphthyridine (34). A suspension of carbethoxypiperazinylnaphthyridine **33** (0.400 g, 1.05 mmol) in EtOH (10 mL) and 10% aqueous NaOH (25 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. The solid residue was purified by crystallization from toluene to obtain **34** (0.075 g, yield 23%): mp 239−241 °C; MS *m*/*z* 306 (M^+). ^{1}H NMR: δ 10.22 (brs, 1H exch.), 9.31 (brs, 1H exch.), 8.05 (d, 1H, H_5), 7.75 (m, 2H, Ar), 7.48 (m, 3H, Ar), 6.82 (d, 1H, H_6), 6.13, (s, 1H, H_3), 3.27 (m, 8H, piperazine). Anal. ($C_{18}H_{18}N_4O$) C, H, N.

7-Bromo-4-ethoxy-2-phenyl-1,8-naphthyridine (35). NaH (0.100 g, 2.17 mmol, 50% in mineral oil) was added to a solution of 7-bromonaphthyridine **15**[19] (0.516 g, 1.70 mmol) in anhydrous DMSO (10 mL), and the mixture was stirred for 1 h at room temperature. Ethyl iodide (0.20 mL), 2.50 mmol) was added to the suspension obtained, and the mixture was heated at 80 °C for 24 h. The reaction mixture was treated with H_2O and filtered. The residual solid was crystallized from petroleum ether 100°-140°C to obtain **35** (0.180 g, yield 32%): mp 182−184 °C; MS *m*/*z* 329 (M^+). ^{1}H NMR: δ 8.31 (d, 1H, H_5), 8.15 (m, 2H, Ar), 7.47 (m, 4H, Ar + H_6), 7.23, (s, 1H, H_3), 4.31 (q, 2H, CH_2), 1.60 (t, 3H, CH_3). Anal. ($C_{16}H_{13}BrN_2O$) C, H, N.

5-Ethoxy-1-ethyl-7-phenyl- (36), 7-ethoxy-1-ethyl-2-phenyl- (37), and 4,7-diethoxy-2-phenyl-1,8-naphthyridine (38). A suspension of bromonaphthyridine **15**[19] (0.500 g, 1.60 mmol) and ethyl iodide (1.95 mL, 2.4 mmol) in EtOH (3.7 mL) and 10% aqueous KOH (7.5 mL) was heated at 100 °C in a sealed tube for 72 h. The reaction mixture was evaporated to dryness under reduced pressure to obtain a residue which was treated with H_2O. The pH was adjusted to 8 with concentrated ammonium hydroxide, and then the mixture was extracted with chloroform. The organic solution was dried ($MgSO_4$), and the solvent was removed under reduced pressure to give an oily mixture of products **36**, **37**, and **38**. Fractionation of the mixture by flash chromatography on silica gel eluting with ethyl acetate:petroleum ether, 4:1, provided the title compounds. **36**: (0.037 g, yield 8%): mp 149−150 °C (EtOH); MS *m*/*z* 294 (M^+). ^{1}H NMR: δ 8.01 (m, 2H, Ar), 7.97 (d, 1H, H_4), 7.44 (m, 3H, Ar), 7.03 (s, 1H, H_6), 6.61 (d, 1H, H_3), 4.66 (q, 2H, CH_2), 4.29 (q, 2H, CH_2), 1.48 (m, 6H, CH_3). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N. **37**: (0.061 g, yield 13%): mp 158−159 °C (petroleum ether 100−140 °C); MS *m*/*z* 294(M^+). ^{1}H NMR δ 8.53 (d, 1H, H_5), 7.43 (m, 5H, Ar), 6.72 (d, 1H, H_6), 6.19 (s, 1H, H_3), 4.45 (q, 2H, CH_2), 4.23 (q, 2H, CH_2), 1.34 (m, 6H, CH_3). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N. **38**: (0.033 g, yield 7%): mp 176−178 °C (EtOH); MS *m*/*z* 294 (M^+). ^{1}H NMR δ 8.60 (d, 1H, H_5), 7.63 (m, 5H, Ar), 7.26 (d, 1H, H_6), 7.04 (s, 1H, H_3), 4.67 (m, 4H, CH_2), 1.56 (m, 6H, CH_3). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

7-Acetamido-4-hydroxy-2-phenyl-1,8-naphthyridine (39). A mixture of 7-aminonaphthyridine **28**[35] (0.200 g, 0.84 mmol) and acetic anhydride (78 mL) was heated at 100 °C for 4.5 h. After cooling, the solid obtained was collected by filtration, washed with H_2O, purified by flash chromatography (ethyl acetate:petroleum ether, 10:1), and recrystallized from EtOH to give **39** (0.085 g, yield 31%): mp >320 °C MS *m*/*z* 279 (M^+). ^{1}H NMR: δ 11.80 (brs, OH), 10.61 (brs, NH), 8.40 (d, 1H, H_5), 8.05 (d, 1H, H_6), 7.78 (m, 2H, Ar), 7.56 (m, 3H, Ar), 6.31, (s, 1H, H_3), 2.19 (s, 3H, CH_3). Anal. ($C_{16}H_{13}N_3O_2$) C, H, N.

7-Methyl-4-methylthio-2-phenyl-1,8-naphthyridine (40). Mercaptonaphthyridine **3**[19] (0.250 g, 0.99 mmol) was added to a solution of sodium (0.027 g, 1.2 g atom) in anhydrous methanol (10 mL), and the mixture was stirred at room temperature for 2 h. Methyl iodide (0.081 g, 0.52 mmol) was then added. After 16 h, the solvent was removed under reduced pressure to obtain a residue which was treated with H_2O and then the mixture was extracted with chloroform. The organic solution was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude solid was purified by crystallization from cyclohexane to give **40** (0.112 g, yield 42%): mp 135−138 °C; MS *m*/*z* 266 (M^+). ^{1}H NMR: δ 8.36 (d, 1H, H_5), 8.32 (m, 2H, Ar), 7.64 (s, 1H, H_3), 7.53 (m, 3H, Ar), 7.35 (d, 1H, H_6), 2.84 (s, 3H, CH_3), 2.73 (s, 3H, CH_3). Anal. ($C_{16}H_{14}N_2S$) C, H, N.

2-(4-Aminophenyl)-4-hydroxy-7-methyl-1,8-naphthyridine (41). A solution of 4-nitrophenylnaphthyridine **26**[36] (0.20 g, 0.71 mmol) in glacial acetic acid (45 mL) was submitted to hydrogenation in the presence of 10% Pd/C (0.02 g) at room pressure and temperature for 3 h. The catalyst was filtered off, and the solvent was evaporated to dryness under reduced pressure to give a residual solid which was crystallized from H_2O to obtain **41** (0.100 g, yield 60%): mp 130−134 °C; MS *m*/*z* 251 (M^+). ^{1}H NMR: δ 11.83 (brs, OH) 8.28 (d, 1H, H_5), 7.57 (d, 2H, Ar), 7.23 (d, 1H, H_6), 6.33 (d, 2H, Ar), 6.24 (s, 1H, H_3), 5.71 (brs, NH_2), 2.59 (s, 3H, CH_3). Anal. ($C_{15}H_{13}N_3O$) C, H, N.

2-(4-Acetamidophenyl)-4-hydroxy-7-methyl-1,8-naphthyridine (42). A mixture of 4-aminophenylnaphthyridine **41** (0.40 g, 1.59 mmol) and acetic anhydride (4.0 mL) was refluxed for 5 h. After cooling, the solid was collected by filtration, washed with H_2O, and crystallized from DMF to obtain **42** (0.15 g, yield 33%): mp > 320 °C MS *m*/*z* 293 (M^+). ^{1}H NMR: δ 12.10 and 10.20 (2 brs, NH and OH), 8.32 (d, 1H, H_5), 7.80 (d, 2H, Ar), 7.70 (d, 2H, Ar), 7.27 (d, 1H, H_6), 6.34 (s, 1H, H_3), 2.60 (s, 3H, CH_3), 2.08 (s, 3H, CH_3). Anal. ($C_{17}H_{15}N_3O_2$) C, H, N.

4-Hydroxy-7-methyl-2-(3-nitrophenyl)-1,8-naphthyridine (43). Potassium nitrate (0.215 g, 2.12 mmol) was added portionwise to an ice-cooled solution of 2-phenylnaphthyridine **1**[37] (0.50 g, 2.12 mmol) in concentrated sulfuric acid (6.5 mL). The reaction mixture was stirred at room temperature for 30 min and then treated with crushed ice and concentrated ammonium hydroxide at pH 8. The solid precipitate was collected by filtration, washed with H_2O, and purified by crystallization from EtOH to obtain **43** (0.10 g, yield 17%): mp 306−310 °C MS *m*/*z* 281 (M^+). ^{1}H NMR: δ 8.67 (s, 1H, Ar), 8.36 (m, 3H, Ar + H_5), 7.82 (m, 1H, Ar), 7.33 (d, 1H, H_6), 6.50 (s, 1H, H_3), 2.62 (s, 3H, CH_3). Anal. ($C_{15}H_{11}N_3O_3$) C, H, N.

2-(3-Aminophenyl)-4-hydroxy-7-methyl-1,8-naphthyridine (44). A solution of 3-nitrophenylnaphthyridine **43** (0.18 g, 0.64 mmol) in glacial acetic acid (40 mL) was submitted to

hydrogenation in the presence of 10% Pd/C (0.02 g) at room pressure and temperature for 3 h. The catalyst was filtered off, and the solvent was evaporated to dryness under reduced pressure to give an oily residue, which was treated with H_2O and concentrated ammonium hydroxide to pH 8. The solid precipitate was collected by filtration, washed with H_2O, and purified by crystallization from EtOH to obtain **44** (0.10 g, yield 62%): mp 239−241 °C MS *m*/*z* 251 (M^+). ^{1}H NMR: δ 12.07 (brs, OH), 8.31 (d, 1H, H_5), 7.27 (d, 1H, H_6), 7.15 (t, 1H, Ar), 6.92 (m, 2H, Ar), 6.71 (m, 1H, Ar), 6.19 (s, 1H, H_3), 5.31 (brs, NH_2), 2.59 (s, 3H, CH_3). Anal. ($C_{15}H_{13}N_3O$) C, H, N.

2-Benzyl-6-methylpyrido[1,2-a]pyrimidin-4(4*H*)-one (45). A mixture of 2-amino-6-methylpyridine (0.90 g, 8.40 mmol) and 4-phenylacetoacetate ethyl ester (2.0 g, 9.7 mmol) with polyphosphoric acid (30 g) was heated under stirring at 100 °C for 4 h. After cooling, the solution obtained was poured into crushed ice and treated with concentrated ammonium hydroxide at pH 5. The suspension obtained was extracted with chloroform, and the combined extracts were dried ($MgSO_4$) and evaporated under pressure to obtain a tarry residue which was purified by flash chromatography (ethyl acetate: petroleum ether, 3:1) to give **45** (0.315 g, yield 15%): mp 117−120 °C MS *m*/*z* 250 (M^+). ^{1}H NMR: δ 7.24 (m, 7H, H_7 +H_9+Ar), 6.48 (m, 1H, H_8), 5.95 (s, 1H, H_3), 3.83 (s, 2H, CH_2), 2.92 (s, 3H, CH_3). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

2-Benzyl-4-hydroxy-7-methyl-1,8-naphthyridine (46). A solution of pyridopyrimidinone **45** (0.130 g, 0.51 mmol) in Dowtherm A (6.0 mL) was heated at 220 °C for 5 h. After cooling, the precipitate was collected, washed with petroleum ether, and crystallized from ethanol to obtain **46** (0.060 g, yield 47%): mp 246−250 °C MS *m*/*z* 250 (M^+). ^{1}H NMR: δ 11.40 (brs, OH), 8.22 (d, 1H, H_5), 7.30 (m, 5H, Ar), 7.18 (d, 1H, H_6), 5.88 (s, 1H, H_3), 3.92 (s, 2H, CH_2), 2.55 (s, 3H, CH_3). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

Biological Methods. Materials. [^{3}H]-CHA, [^{3}H]-DPCPX, [^{3}H]-CGS21680, [^{3}H]-*(R)*-PIA, and [^{125}I]AB-MECA were obtained from PerkinElmer Life Sciences. N^6-(Cyclohexyl)adenosine (CHA), N^6-(cyclopentyl)adenosine (CPA), (*R*)-N^6-(2-phenylisopropyl)adenosine [(*R*)-PIA], (*S*)-PIA, and 5′-*N*-(ethylcarboxamido)adenosine (NECA) were purchased from RBI (Natik, MA). Adenosine deaminase was from Sigma Chemical Co. (St. Louis, MO). All other reagents were from standard commercial sources and of the highest grade commercially available.

Bovine brains were obtained from the local slaughterhouse. Post-mortem human brains were collected at the Department of Pathological Anatomy, University of Pisa, from subjects with no past history of neurological or mental disorders and with no primary or secondary brain diseases. The time between death and tissue dissection/freezing ranged from 18 to 36 h. Immediately after removal from the skull, the cortex and corpus striatus were dissected on ice and frozen in liquid nitrogen and stored at −80 °C until use.

A_1 and A_{2A} Receptor Binding Assay. Displacement of [^{3}H]-CHA (39 Ci/mmol) from A_1 adenosine receptor in cortical membranes and [^{3}H]-CGS21680 (45 Ci/mmol) from A_{2A} adenosine receptor in striatal membranes was performed as previously described.[19] Adenosine A_1 receptor affinities were also determined with [^{3}H]-DPCPX as radioligand. The [^{3}H]-DPCPX (89 Ci/mmol) binding assay was performed in triplicate by incubating aliquots of the membrane fractions (0.05−0.1 mg of protein) at 25 °C for 120 min in 0.5 mL of 50 mM Tris/HCl, pH 7.7 containing 1 mM EDTA, 2 mM $MgCl_2$, with approximately 0.5 nM [^{3}H]-DPCPX. Nonspecific binding was defined in the presence of 10 μM of (*R*)-PIA by filtration through Whatman GF/C glass microfiber filters under suction and washing twice with 5 mL of ice-cold buffer. The filters were treated as reported above for the radioactivity measurement.

A_3 Receptor Binding Assay. Displacement of [^{3}H]-*(R)*-PIA (75 Ci/mmol) from A_3 bovine adenosine receptor was performed as previously described.[19] For hA_3AR we used portions (0.20 g) of CHO cell membranes expressing human A_3 receptor, diluted (1:10) in a pH 7.4 buffer solution (Tris-HCl 50 nM, EDTA 1 mM, $MgCl_2$ 10 mM) and homogenized. Small portions containing about 40 μg of proteins were incubated with 0.2 nM solution of [^{125}I]AB-MECA (2000 Ci/mmol), 20 μL of a 2 U/mL solution of adenosine deaminase, and, in the case of nonspecific binding measurements, also 20 μL of a 50 μM solution of NECA. The resulting mixture was then diluted with the pH 7.4 buffer to a total volume of 100 μL and incubated at 25 °C for 60 min, after which time the samples were rapidly filtered under a vacuum through Whatman GF/C filters, which had been previously treated for 1h at 4 °C with an aqueous solution (1 g/200 mL of polyethyenimine (PEI). The filters were then washed three times with 5 mL of cold buffer and then treated as reported above for the radioactivity measurement.

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 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.[47] The K_d of [^{3}H]-CHA binding to cortex membranes from bovine and human was 1.2 nM and 3.5 nM, respectively. The K_d of [^{3}H]DPCPX binding to cortex membranes from bovine and human was 0.8 nM and 3.9 nM, respectively. The K_d of [^{3}H]CGS 21680 binding to striatal membranes from bovine and human was 10 nM and 12 nM, respectively. Protein concentration was determined in accordance with the method of Lowry as modified by Peterson[48] using bovine serum albumin as the standard.

Computational Details. All the molecular mechanics and molecular Dynamics calculations were performed through the MACROMODEL program[49] by using the AMBER force field.[50] The electrostatic charges were those included in the force field and a distance-dependent dielectric constant of 4.0 was used. In molecular mechanics minimizations (MM) the minimized value was the Conjugated Gradient until a convergence value of 0.1 Kcal/A·mol; in molecular dynamics simulations (MD) the temperature was set at 300 °K and the time step was 1 fs. All graphic manipulations and visualizations were performed by means of the InsightII[51] and WebLabViewe[52] programs.

Modeling of the hA_1AR. The sequence of the seven helices was obtained from GPCRDB[53] which was aligned on the crystallographic structure of bovine rhodopsin[45] archived in PDB[54] as 1F88. The alignment of the two primary sequences was obtained through the CLUSTALW program[55] by using the PAM matrix. 1F88 was used as a template for the modeling of the helices of the hA_1AR according to the following procedure:

1. 50 ps of MD followed by MM with a constraint of 10 kcal/mol on all backbone atoms of the hA_1AR forcing them on the corresponding atoms of 1F88;

2. 50 ps of MD followed by MM with a constraint of 10 kcal/mol on all C α atoms of the hA_1AR forcing them on the corresponding atoms of 1F88;

3. 50 ps of MD followed by MM with a restraint of 5 kcal/mol simulating the intramolecular H-bonds that maintain the structure of each helix plus a constraint of 1kcal/mol maintaining the C α of the hA_1AR in its position;

4. as in the previous step, but reducing the constraint of the C α to 0.1 kcal/mol.

Modeling of the hA_1AR−CPA Complex. The selective agonist CPA was then docked in the receptor model thus obtained. The docking was performed manually in order to respect the information given by mutagenesis for the agonist.[46] In particular, H-bonds of the ligand with Thr91, Ser94, Thr277, and His278 were searched together with a hydrophobic interaction of the cyplopentyl ring with Leu88. Such interactions were not possible simultaneously because the distance between Thr91 (on TM3) and Thr277 (on TM7) was too large. To make all interactions possible, TM3 was rotated by 90° and translated by about 2 Å along its axis in the extracellular direction. The receptor model thus modified

allowed all interactions: Thr91 and Ser94 with the nitrogen atoms of the adenine portion, Thr277 and His278 with the ribose portion, and the Leu88 with the cyclopentyl ring.

The complex thus obtained was then refined by the following procedure:

5. 200 ps of MD followed by MM with a constraint of 50 kcal/mol maintaining the interaction between the receptor and CPA, 10 kcal/mol on all the C α and 5 kcal/mol on all the intramolecular H-bonds of the helices;

6. As previously, but the constraint on C α was reduced to 1 kcal/mol.

7. As previously, but the constraint on all C α was substituted with a constraint of 10 kcal/mol on only the fourteen terminal C α of the receptor model.

8. As previously, but the constraint on the receptor−ligand interactions was reduced to 20 kcal/mol.

9. As previously, but all constraints maintaining the receptor−ligand interactions were removed, so that CPA was allowed to relax completely.

At this point CPA was replaced by DPCPX in order to obtain the model of the hA_1AR suitable for interaction with the antagonists. DPCPX was oriented in accordance with the mutagenesis data available for the antagonists[46] and with the hypothesis of the N6−C8 superimposition;[56] therefore the two xanthinic oxygens were allowed to form H-bonds with Ser94 and His251 in such a manner that a hydrophobic interaction between the cyclopentenic ring and Leu88 was observed. The modeling procedure consisted of three steps very similar to steps 5−9 described above.

Modeling of the bA_1AR and of the Complexes bA_1AR−CPA and bA_1AR-DPCPX. The procedures for the modeling of the bA_1AR model and its complexes with CPA and DPCPX were the same already used for the hA_1AR, but this time the template was the hA_1AR model obtained after the optimization of the complex with CPA. In this case, due to the high homology between the hA_1AR and the bA_1AR, neither translation nor rotation of helices was required.

Modeling of Complexes between the hA_1AR and the bA_1AR with 16, 22, 28, 29, 50, 51, 52. The complexes between the two models of A_1 adenosine receptors with the 1,8-naphthyridine derivatives were manually constructed on the basis of the mutagenesis data available for antagonists. The starting points were the optimized complexes of hA_1AR and bA_1AR with DPCPX in which DPCPX was substituted with compound **16**, **22**, **28**, **29**, **50**, **51**, or **52**. The ligands were considered in their quinoid form, previously found to be largely preferred[19] in the conformation minimized by PM3 calculation. The ligands were oriented in such a manner that the carbonyl oxygen in position 4 and the nitrogen in position 8 could accept an H-bond from Ser94 and His251. Two conformations are possible, and they are shown in Figure 2. Both these conformations were optimized for each ligand and for each receptor model in accordance with the procedure already used for the modeling of the complexes with CPA and DPCPX (steps 5−9).

The ligand−receptor interaction energy was obtained as the sum of all nonbonded terms of the steric energy (electrostatic and van der Waals) between the atoms of the ligand and the atoms of the receptor model calculated by MACROMODEL on the optimized complexes. The volume of the cavities between the ligand and the receptor was calculated by means of the program SURFNET[57] and visualized by means of the program CHIMERA.[58]

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References

(1) Olah, M.; Stiles, G. The role of receptor structure in determining adenosine receptor activity. *Pharmacol. Ther.* **2000**, *85*, 55−75.
(2) Impagnatiello, F.; Bastia, E.; Ongini, E.; Monopoli, A. Adenosine receptors in neurological disorders. *Emerging Ther. Targets* **2000**, *4*, 635−663.
(3) Jacobson, K. A.; Kim, H. O.; Siddiqi, S. M.; Olah, M. E.; Stiles, G. L.; Lubitz, D. A_3-adenosine receptors: design of selective ligands and therapeutic prospects. *Drugs Future* **1995**, *20*, 689−699.
(4) Feoktistov, I.; Polosa, R.; Holgate, S. T.; Biaggioni, I. Adenosine A_{2A} receptors: a novel therapeutic target in asthma? *Trends Pharmacol. Sci.* **1998**, *19*, 148−153.
(5) Müller, C. E.; Stein, B. Adenosine receptor antagonist: structure and potential therapeutic applications. *Curr. Pharm. Des.* **1996**, *2*, 501−530.
(6) Poulsen, S.-A.; Quinn, R. J. Adenosine receptors: new opportunities for future drugs. *Bioorg. Med. Chem.* **1998**, *6*, 619−641.
(7) Betti, L.; Biagi, G.; Giannaccini, G.; Giorgi, I.; Livi, O.; Lucacchini, A.; Manera, C.; Scartoni, V. Novel 3-Aralkyl-7-(amino-substituted)-1,2,3-triazolo[4,5-d]pyrimidines with high affinity towards A_1 adenosine receptors. *J. Med. Chem.* **1998**, *41*, 668−673.
(8) Camaioni, E.; Costanzi, S.; Vittori, S.; Volpini, R.; Klotz, K.-N.; Cristalli, G. New substituted 9-alkylpurines as adenosine receptor ligands. *Bioorg. Med. Chem.* **1998**, *6*, 523−533.
(9) Pfister. J. R.; Belardinelli, L.; Lee, G.; Lum, R. T.; Milner, P.; Stanley, W. C.; Linden, J.; Baker, S. P.; Schreiner, G. J. Synthesis and biological evaluation of the enantiomers of the potent and selective A_1 adenosine antagonists. *J. Med. Chem.* **1997**, *40*, 1773−1778.
(10) Katsushima, T.; Nieves, L.; Wells, J. N. Structure−Activity relationships of 8-cycloalkyl-1,3-dipropylxanthines as antagonists of adenosine receptors. *J. Med. Chem.* **1990**, *33*, 1906−1910.
(11) Müller, C. E. A_1-Adenosine receptor antagonists. *Expert Opin. Ther. Pat.* **1997**, *5*, 419−440.
(12) Poulsen, S.-A.; Quinn, R. J. Synthesis and structure activity relationships of pyrazolo[3,4-d]pyrimidines: potent and selective adenosine A_1 receptor antagonists. *J. Med. Chem.* **1996**, *39*, 4156−4161.
(13) Colotta, V.; Catarzi, D.; Varano, F.; Cecchi, L.; Filacchioni, G.; Martini, C.; Trincavelli, L.; Lucacchini A. Synthesis and structure activity relationships of a new set of 2-arylpyrazolo[3,4-c]-quinoline derivatives as adenosine receptor antagonists. *J. Med. Chem.* **2000**, *43*, 3118−3124.
(14) Kuroda, S.; Akahane, A.; Itani, H.; Nishimura, S.; Durkin, K.; Kinoshita, T.; Tenda, Y.; Sakane, K. Discovery of FR 166124, a novel water-soluble pyrazolo[1,5-a]pyridine adenosine A_1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1979−1984.
(15) Colotta, V.; Catarzi, D.; Varano, F.; Cecchi, L.; Filacchioni, G.; Martini, C.; Trincavelli, L.; Lucacchini, A. 1,2,4-Triazolo[4,3-a]-quinoxalin-1-one: a versatile tool for the synthesis of potent and selective adenosine receptor antagonist. *J. Med. Chem.* **2000**, *43*, 1158−1164.
(16) Baraldi, P. G.; Cacciari, B.; Spalluto, 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]pyrimidines as potent and selective A_{2A} adenosine receptor antagonist. *J. Med. Chem.* **1996**, *39*, 1164−1171.
(17) Ongini, E. SCH 58261: a selective adenosine receptor antagonists. *Drug Dev. Res.* **1997**, *42*, 63−70.
(18) Da Settimo F.; Primofiore, G.; Taliani, S.; Marini A. M.; La Motta, C.; Novellino, E.; Greco, G.; Lavecchia, A.; Trincavelli, L.; Martini, C. 3-Aryl[1, 2, 4]triazino[4,3-a]benzimidazol-4(10H)-ones: a novel class of selective A_1 adenosine receptor antagonists. *J. Med. Chem.* **2001**, *44*, 316−327.
(19) Ferrarini, P. L.; Mori, C.; Manera, C.; Martinelli, A.; Mori, F.; Saccomanni, G.; Barili, P. L.; Betti, L.; Giannacini, G.; Trincavelli, L.; Lucacchini, A. A novel class of high potent and selective A_1 adenosine antagonists: structure-affinity profile of a series of 1,8-naphthyridine derivatives. *J. Med. Chem.* **2000**, *43*, 2814−2823.
(20) Salvatore, C. A.; Jacobson, M. A.; Taylor, H. E.; Linden, J.; Johnson, R. G. Molecular cloning and characterization of the human A_3 adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10365−10369.
(21) Linden, J.; Taylor, H. E.; Robeva, A. S.; Tucker, A. L.; Stehle, J. H.; Rivkees, S. A.; Fink, J. S.; Peppert, S. M. Molecular-cloning and functional expression of sheep A_3 adenosine receptor with widespread tissue distribution. *Mol. Pharmacol.* **1993**, *44*, 524−532.
(22) Ji, X.-D.; von Lubitz, D.; Olah, M.; Stiles, G. L.; Jacobson, K. A. Species differencies in ligand affinity at central A_3-adenosine receptors. *Drug Dev. Res.* **1994**, *33*, 51−59.
(23) Müller, C. E.; Scior, T. Adenosine receptors and their modulators. *Pharm. Act. Helv.* **1993**, *68*, 77−111.
(24) Olah, M.; Ren, H.; Ostrowski, J.; Jacobson, K. A.; Stiles, G. L. Cloning, expression and characterization of the unique bovine A_1 adenosine receptor. *J. Biol. Chem.* **1992**, *267*, 10764−10770.
(25) Klotz, K.-N.; Hessling, J. Owman, C.; Kull, B.; Fredholm, B. B.; Lohse, M. J. Comparative pharmacology of human adenosine receptor subtypes-characterization of stably transfected receptors in CHO cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, *357*, 1−9.

(26) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Monopoli, A.; Ongini, E.; Varani, K.; Borea, P. A. 7-Substituted 5-amino-2-(furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidines as A_{2A} adenosine receptor antagonists: a study on the importance of modifications at the side chain on the activity and solubility. *J. Med. Chem.* **2002**, *45*, 115−126.
(27) Volpini, R.; Costanzi, S.; Lambertucci, C.; Vittori, S.; Klotz, K.-N.; Lorenzen, A.; Cristalli, G. Introduction of alkynyl chains on C-8 of adenosine led to very selective antagonists of the A_3 adenosine receptor. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1931−1934.
(28) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Moro, S.; Klotz, K.-N.; Leung, E.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. Pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine derivatives as highly potent and selective human A_3 adenosine receptor antagonists: influence of the chain at the N^8 pyrazole nitrogen. *J. Med. Chem.* **2000**, *43*, 4768−4780.
(29) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, S.; Klotz, K.-N.; Leung, E.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. Pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidine derivatives as highly potent and selective human A_3 adenosine receptor antagonists. *J. Med. Chem.* **1999**, *42*, 4473−4478.
(30) Baraldi, P. G.; Cacciari, B.; Moro, S.; Romagnoli, R.; Ji, X.-D.; Jacobson K. A.; Gessi, S.; Borea, P. A.; Spalluto, G. Fluorosulfonyl- and bis-(*β*-chloroethyl)amino-phenylamino functionalized pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidine derivatives: irreversible antagonists at the human A_3 adenosine receptor and molecular modeling studies. *J. Med. Chem.* **2001**, *41*, 2735−2742.
(31) Müller, C. E. Adenosine receptor ligands-recent developments. Part I. Agonists. *Curr. Med. Chem.* **2000**, *7*, 1269−1288.
(32) Klotz, K.-N. Adenosine receptors and their ligands. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 382−391.
(33) Nakata, H. Biochemical and immunological characterization of A_1 adenosine receptors purified from human brain membranes. *Eur. J. Biochem.* **1992**, *206*, 171−177.
(34) Ji, X.-D.; Stiles, G. L.; van Galen, P. J. M.; Jacobson, K. A. Characterization of human striatal A_2-adenosine receptors using radioligand binding and photoaffinity labeling. *J. Receptor Res.* **1992**, *12*, 149−169.
(35) Ferrarini, P. L.; Mori, C.; Primofiore, G.; Calzolari, L. One step synthesis of pyrimido[1,2-a][1, 8]naphthyridines, pyrido[1,2-a]-pyrimidinones and 1,8-naphthyridinones. Anthypertensive agents. V. *J. Heterocycl. Chem.* **1990**, *27*, 881−886.
(36) 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.
(37) 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.
(38) Wierenga, W.; Skulnick, H. I. *J. Org. Chem.* **1979**, *44*, 310−311.
(39) Brown, E. V. 1,8-Naphthyridine. I. Derivatives of 2- and 4-Methyl-1,8-naphthyridines. *J. Org. Chem.* **1965**, *30*, 1607−1610.
(40) Ferrarini, P. L.; Mori, C.; Livi, O.; Biagi, G.; Marini, A. M. Synthesis of some Substituted Pyrido[1,2a]pyrimidin-4-ones and 1,8-Naphthyridines. *J. Heterocycl. Chem.* **1983**, *20*, 1053−1057.
(41) Carboni, S.; Da Settimo, A.; Pirisino, G.; Segnini, D. Ricerche nel campo delle naftiridine. Precisazioni sulla reazione 2,6-diamminopiridina e acetoacetato di etile. *Gazz. Chim. Ital.* **1966**, *96*, 103−113.
(42) Martini, C.; Pennacchi, E.; Poli, M. G.; Lucacchini, A. Solubilization of adenisine A_1 binding sites from sheep cortex. *Neurochem. Int.* **1985**, *7*, 1017−1020.
(43) 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.
(44) Nanoff, C.; Mitterauer, T.; Roka, F.; Hohenegger, M.; Freissmuth, M. Species Differences in A_1 Adenosine Receptor/G Protein Complex. *Mol. Pharmacol.* **1995**, *48*, 806−817.
(45) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: a G-protein-coupled receptor. *Science* **2000**, *289*, 739 745.
(46) Townsend-Nicholson, A.; Schofield, P. R. A threonine residue in the seventh transmembrane domain of the human A_1 adenosine receptor mediates specific agonist binding. *J. Biol. Chem.* **1994**, *269*, 2373−2376. (b) Rivkees, S. A.; Ladbury, M. E.; Barbhaiya, H. Identification of domains of the human A_1 adenosine receptor that are important for binding receptor subtype-selective ligands using chimeric A_1/A_{2A} adenosine receptors. *J. Biol. Chem.* **1995**, *270*, 20485−20490. (c) Barbhaiya, H.; McClain, R.; Ijzerman, A.; Rivkees, S. A. Site-directed mutagenesis of the human A_1 adenosine receptor: influences of acidic and hydroxy residues in the first four transmembrane domains on ligand binding. *Mol. Pharm.* **1996**, *50*, 1635−1642. (d) Scott, A.; Rivkees, S. A.; Barbhaiya, H.; Ijzerman, A. Identification of the adenine binding site of the human A_1 adenosine receptor. *J. Biol. Chem.* **1999**, *274*, 3617−3621. (e) Scholl, D. J.; Wells, J. N. Serine and alanine mutagenesis of the nine native cysteines of the human A_1 adenosine receptor. *Biochem. Pharmacol.* **2000**, *60*, 1647−1654.
(47) 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.
(48) 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.
(49) Macromodel ver. 7.0; Schrodinger Inc., **1999**.
(50) Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; Cheatham, T. E. III; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K. M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Tsui, V.; Radmer, R. J.; Duan, Y.; Pitera, J.; Massova, I.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. AMBER 6, 1999, University of California, San Francisco.
(51) Insight II (98) Molecular Modeling System; MSI, 1998.
(52) WebLab ViewerPro 3.7; Molecular Simulations Inc., 2000.
(53) Horn, F.; Weare, J.; Beukers, M. W.; Hörsch, S.; Bairoch, A.; Chen, W.; Edvardsen, Ø.; Campagne, F.; Vriend, G. GPCRDB: an information system for G protein-coupled receptors. *Nucleic Acids Res.* **1998** *26(1)*, 277−281.
(54) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, P. I. N.; Bourne, E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(55) Thompson, J. D.; Higgins, D. G.; Gibson, T. J. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673−4680.
(56) Peet, N. P.; Lentz, N. L.; Meng, E. C.; Dudley, M. W.; Ogden, A. M. L.; Demeter, D. A.; Weintraub, H. J. R.; Bey, P. A novel synthesis of xanthines: support for a new binding mode for xanthines with respect to adenosine at adenosine receptors. *J. Med. Chem.* **1990**, *33*, 3127−3130.
(57) Laskowski, R. A. "SURFNET: A program for visualizing molecular surfaces, cavities, and intermolecular interactions" *J. Mol. Graph.* **1995**, *13*, 323−330.
(58) Huang, C. C.; Couch, G. S.; Pettersen, E. F.; Ferrin, T. E. Chimera: An Extensible Molecular Modeling Application Constructed Using Standard Components. *Pac. Symp. Biocomput.* **1996**, *1*, 724−726.

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