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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4604–4610
Bioorganic & Medicinal Chemistry Letters

1,8-Naphthyridin-4-one derivatives as new ligands of A_{2A} adenosine receptors

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Received 30 March 2005; revised 23 June 2005; accepted 23 June 2005
Available online 15 August 2005

Abstract—A series of 1,8-naphthyridine derivatives bearing various substituents in position 3, 4, and 7 of the heterocyclic nucleus have been synthesized and evaluated for their affinity at the bovine and human adenosine receptors. The new compounds were found to lack the affinity toward A_1AR, whereas many of them are able to acquire an interesting affinity and selectivity for the A_{2A}AR.

Adenosine is probably the most important neuromodulator in the central and peripheral nervous systems;[1] its formation usually increases under metabolically favorable conditions.[2] This nucleoside modulates its effects through the activation of four subtype receptors located on cell membranes, known as A_1, A_{2A}, A_{2B}, and A_3.[3] These are members of the seven-strong transmembrane spanning G-protein-coupled receptors family. Adenosine receptors are associated with different second messenger systems: in particular, the A_{2A} adenosine receptor (A_{2A}AR) appears to be almost exclusively linked with the stimulation of adenylyl cyclase activity. Its presence in the CNS is abundant in discrete brain regions such as the striatum,[4] where an intricate functional interaction with dopamine receptor signalling occurs. The blockade of the A_{2A}AR is reported to produce direct effects on D_2 receptors;[5] consequently, there is the possibility of using A_{2A}AR antagonists as novel pharmacological tools in the treatment of acute or chronic neurological disorders, such as stroke or Parkinson's disease.[6] In the peripheral system, the A_{2A}AR is present on various tissues[7–9] and then A_{2A}AR agonists can be used to inhibit platelet aggregation in thrombosis, in ischemia,[10,11] and to determine strong anti-inflammatory and immunosuppressive effects.[12]

In view of all these pharmacological profiles of the A_{2A}AR, much effort has been directed in the last few years toward the synthesis of selective A_{2A}AR ligands. As part of our research aimed at finding new AR selective antagonists, we recently reported the synthesis and the binding activity at bovine and native human adenosine receptors (A_1, A_{2A}, and A_3) of 1,8-naphthyridine derivatives bearing various substituents at the positions 2, 4, and 7 of the heterocyclic nucleus.[13,14] The binding results showed that a large part of the new 1,8-naphthyridine derivatives proved to be A_1 bovine adenosine receptor selective, with a high affinity in the low nanomolar range; on the contrary, all the 1,8-naphthyridine derivatives generally lost their affinity for the hA_1AR, in some cases to a considerable extent (more than 1000 times).[14] As regards the affinity for the bA_{2A}AR, 1,8-naphthyridine derivatives generally possess a moderate affinity, and this remained approximately the same as for the native hA_{2A}AR.[14] In the light of these results, it appeared to be of interest to introduce structural modifications into the 1,8-naphthyridine derivatives studied so far, in order to further investigate the SAR in this class of compounds. For these reasons, we synthesized 1,8-naphthyridine derivatives and 1,8-naphthyridin-4-one derivatives of general structures **1**–**3** bearing various substituents at the positions 3, 4,

Keywords: 1,8-Naphthyridine; Adenosine; Adenosine receptors.
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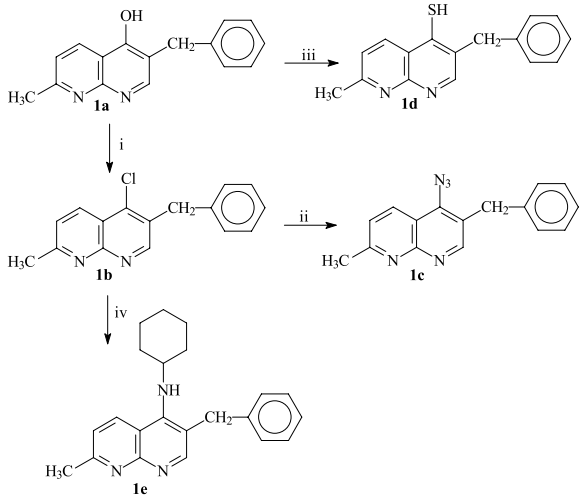
doi:10.1016/j.bmcl.2005.06.064

and 7 of the heterocyclic nucleus. It needs to be pointed out that the numbering of the 1,8-naphthyridine nucleus used is the same as that reported for the previous series.[13,14] At the position 3 of the naphthyridine nucleus, compounds of type **1** are characterized by the presence of a lipophilic substituent, such as a benzylic or *p*-chlorobenzylic group, while compounds of types **2** and **3** are characterized by the presence of an ester group or an aliphatic or aromatic carboxamide group; this last group is considered to be an important structural requirement shown by other classes of adenosine receptor ligands, such as pyrazolotriazolopyrimidine derivatives[15,16] and triazoloquinazoline derivatives.[17] The new compounds (**1**–**3**) were found to lack the affinity towards A_1AR, whereas they are able to acquire an interesting affinity and selectivity for the $A_{2A}AR$, therefore, in order to better rationalize the experimental observations about the quite different affinity of compounds of types **1**–**3** for A_1AR and $A_{2A}AR$, a molecular modeling study was carried out.

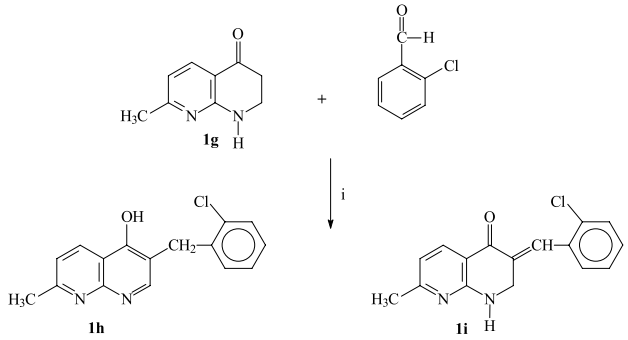
The synthesis of compounds of types **1**–**3** is outlined in Schemes 1–6.

The treatment of 3-benzyl-4-hydroxy-7-methyl-1,8-naphthyridine **1a**[18] with phosphoryl chloride gave the 4-chloro derivative **1b**, which by reaction with NaN_3 led to the azido derivative **1c**. When **1a** was refluxed in toluene with Lawesson's reagent, the corresponding mercapto derivative **1d** was obtained. Finally, the treatment of **1b** with an excess of cyclohexylamine in a sealed tube at 120 °C gave the 1,8-naphthyridine **1e** (Scheme 1).

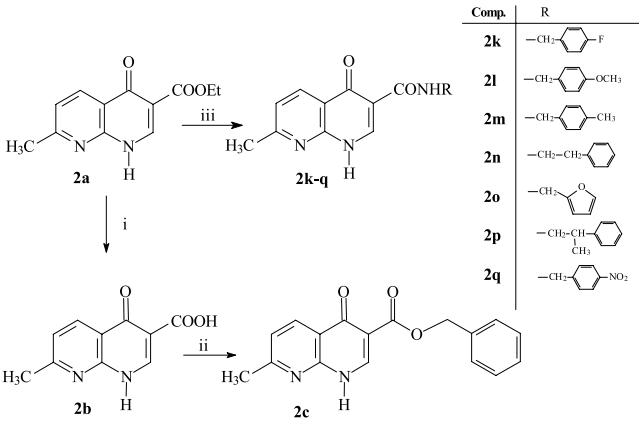
As reported in Scheme 2, the 7-methyl-2,3-dihydro-1,8-naphthyridin-4(1*H*)-one (**1g**),[19] by reaction with *o*-chlorobenzaldehyde and anhydrous hydrogen chloride, provided the 3-benzyl and 3-benzylidene derivatives **1h** and **1i**, respectively, which were separated by flash chromatography.



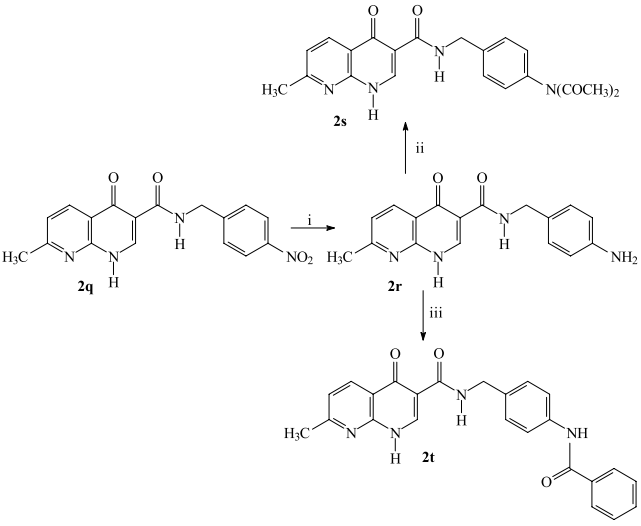
Scheme 1. Reagents and conditions: (i) $POCl_3$, 80 °C, 4 h; (ii) NaN_3, DMF, 130 °C, 5 min; (iii) Lawesson's reagent, toluene, reflux, 4 h; (iv) cyclohexylamine, 120 °C, 24 h.



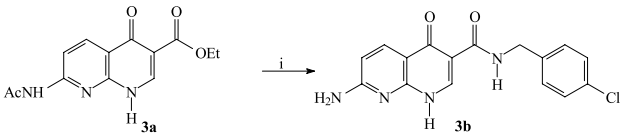
Scheme 2. Reagents: (i) anhydrous HCl, ethanol.



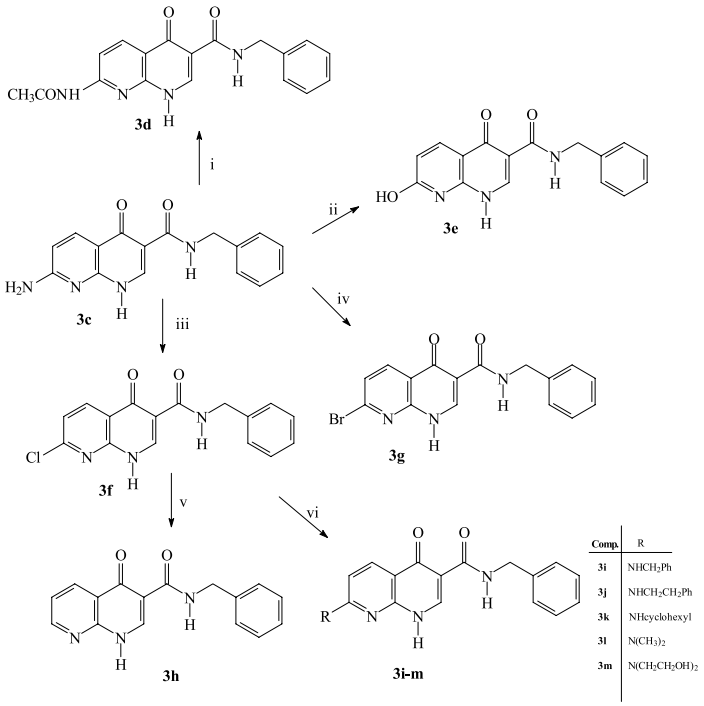
Scheme 3. Reagents: (i) 10% NaOH; (ii) $C_6H_5CH_2OH$, $NaHCO_3$, anhydrous DMF; (iii) RNH_2.



Scheme 4. Reagents: (i) H_2, Pd/C, glacial AcOH; (ii) Ac_2O; (iii) C_6H_5COCl.



Scheme 5. Reagents: (i) *p*-$ClC_6H_4CH_2NH_2$.



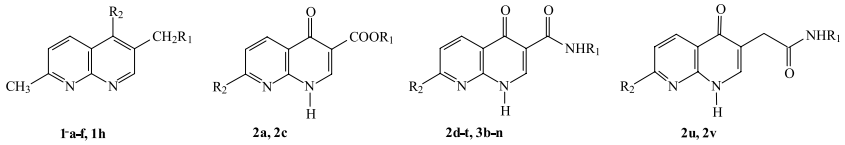
Scheme 6. Reagents: (i) Ac_2O; (ii) $NaNO_2$, H_2SO_4; (iii) $NaNO_2$, HCl; (iv) $NaNO_2$, HBr; (v) H_2, Pd/C; (vi) alkyl or cycloalkyl amines.

The reaction of the 7-methyl-1,8-naphthyridin-4(1*H*)-on-3-carboxylic acid ethyl ester (**2a**)[20] with aqueous 10% sodium hydroxide at reflux provided the 3-carboxylic acid derivative **2b**, which was transformed into the benzyl ester **2c** by treatment with benzylalcohol and sodium hydrogencarbonate in anhydrous DMF (Scheme 3). Following the synthetic route described in the literature for compounds **2d**–**k**,[21] the heating of ester **2a** with the appropriate amine in a sealed tube provided the new carboxamide derivatives **2k**–**q** (Scheme 3). Selective reduction of the nitro derivative **2q** was performed in glacial acetic acid, in the presence of Pd/C as a catalyst, to give the amino derivative **2r** (Scheme 4), which, by reaction with Ac_2O or benzoylchloride, afforded the imide derivative **2s** and the amide derivative **2t**, respectively (Scheme 4). The heating of the 7-acetamido-1,8-naphthyridin-4(1*H*)-on-3-carboxylic acid ethyl ester **3a**[22] with *p*-chlorobenzylamine in a sealed tube provided the carboxamide derivative **3b**. Under these conditions, also the hydrolysis of the acetamido group takes place (Scheme 5). As described in Scheme 6, the treatment of the 7-amino derivative **3c**[21] with Ac_2O afforded the 7-acetamido derivative **3d**. Diazotization of compound **3c**, carried out in aqueous 96% sulfuric acid at −12 °C during the addition of $NaNO_2$, and then at 40 °C for 4 h, gave the 7-hydroxy derivative **3e**. On the contrary, the diazotization of **3c** performed in aqueous 37% hydrochloric acid, or in aqueous 48% hydrobromic acid at −5 °C during the addition of $NaNO_2$, and then at 40 °C for 3 h, afforded the 7-chloronaphthyridine **3f** or 7-bromonaphthyridine **3g**, respectively. Dehalogenation of **3f** was performed with H_2 in the presence of Pd/C as a catalyst to give the derivative **3h**. Finally, the reaction of the 7-chloronaphthyridine **3f** with an excess of the appropriate amine in a sealed tube at 120 °C for 24 h gave **3i**–**m** (Scheme 6).

The affinities of 1,8-naphthyridine derivatives **1a**–**e**,**f**,[23]**h**, **2a**,**c**,**d**–**j**,[21]**k**–**t**,**u**–**v**,[21] and **3b**–**m**, were determined by measuring their ability to displace the specific binding of the agonists [^{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]CGS21 680) from bovine (**1a**–**f**,**h**, **2a**,**c**–**v**, and **3b**–**n**) and human (**1a**–**f**,**h**, **2a**,**c**–**p**,**u**,**v**, **3b** and **3c**) cortical (A_1) and striatal (A_{2A}) membranes, respectively.[24–27] Moreover, for some compounds (**2d**,**g**,**l**, and **2o**), the affinity at the human cloned A_{2B} receptor was also determined by measuring their ability to inhibit NECA-mediated cAMP accumulation.[28] Finally, the affinity of only the 1,8-naphthyridine derivative **2d** was determined by measuring its ability to displace the binding of [^{125}I]AB-MECA from human cloned receptors (A_3).[13] These data, plus the receptor affinity for the antagonist 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.

The data show that the new naphthyridine derivatives generally exhibit a higher affinity for the A_{2A} adenosine receptor than for the A_1 adenosine receptor. The only exceptions are the **1f**, **3i**, and **3j** derivatives, which display a modest affinity for the bA_1 receptor, and are completely ineffective towards the bA_{2A} receptor. As regards the affinity at the hA_{2B}AR and the hA_3AR, these compounds present very low inhibition percentages at the concentration used in the experiments (10 μM for the A_{2B} receptor and 1 μM for the A_3 receptor) with the result that the corresponding K_i values were not calculated. Furthermore, the results of the binding studies indicate that the affinity at the hA_1AR is not very different from that found at the bA_1AR; likewise, the affinity

Table 1. Affinity of 1,8-naphthyridine derivatives **1a**–**f**,**h**, **2a**,**c**,**d**–**v**, **3b**–**n** in radioligand binding assays at bovine brain A_1, A_{2A}, and human brain A_1 and A_{2A}receptors[a,b]



Compound	K_i (nM)					
	R_1	R_2	bA_1	bA_{2A}	hA_1	hA_{2A}
1a	Ph	OH	>10,000	>10,000	4180 ± 217	3330 ± 188
1b	Ph	Cl	>10,000	>10,000	>10,000	>10,000
1c	Ph	N_3	3340 ± 179	3360 ± 184	>10,000	5360 ± 303
1d	Ph	SH	6330 ± 364	>10,000	8720 ± 470	9140 ± 532
1e	Ph	CyclohexylNH	>10,000	>10,000	>10,000	>10,000
1f	Ph	$NHNH_2$	220 ± 10	720 ± 38	>10,000	>10,000
1h	*o*-ClPh	OH	8730 ± 450	>10,000	4160 ± 220	980 ± 51
2a	CH_3CH_2	CH_3	>10,000	>10,000	—	—
2c	$PhCH_2$	CH_3	>10,000	>10,000	—	—
2d	$PhCH_2$	CH_3	280 ± 11	50 ± 3	630 ± 30	30 ± 2
2e	*p*-$ClPhCH_2$	CH_3	1740 ± 58	210 ± 14	1630 ± 64	150 ± 6
2f	Morph	CH_3	>10,000	8660 ± 440	>10,000	>10,000
2g	N-CH_3pipz	CH_3	>10,000	2868 ± 130	>10,000	1750 ± 76
2h	Cyclohexyl	CH_3	>10,000	930 ± 50	>10,000	830 ± 47
2i	4-CH_3-cyclohexyl	CH_3	6000 ± 295	390 ± 17	5600 ± 88	360 ± 14
2j	Isopentyl	CH_3	>10,000	1430 ± 70	>10,000	640 ± 32
2k	*p*-$FPhCH_2$	CH_3	1770 ± 88	200 ± 13	1650 ± 70	115 ± 12
2l	*p*-OCH_3PhCH_2	CH_3	>10,000	740 ± 50	4370 ± 290	2405 ± 175
2m	*p*-CH_3PhCH_2	CH_3	2230 ± 147	185 ± 11	1580 ± 117	159 ± 100
2n	$PhCH_2CH_2$	CH_3	680 ± 25	140 ± 8	730 ± 41	150 ± 17
2o	Furfuryl	CH_3	1350 ± 76	45 ± 2	300 ± 20	35 ± 3
2p	$PhCH(CH_3)$	CH_3	418 ± 20	120 ± 6	717 ± 37	115 ± 8
2q	*p*-NO_2PhCH_2	CH_3	1634 ± 88	99 ± 5	—	—
2r	*p*-NH_2PhCH_2	CH_3	2864 ± 150	146 ± 7	—	—
2s	*p*-$N(COCH_3)_2PhCH_2$	CH_3	>10,000	1644 ± 85	—	—
2t	*p*-(NHCOPh)$PhCH_2$	CH_3	7590 ± 410	588 ± 29	—	—
2u	Cyclohexyl	CH_3	>10,000	>10,000	>10,000	7140 ± 415
2v	$PhCH_2$	CH_3	>10,000	>10,000	>10,000	870 ± 50
3b	*p*-$ClPhCH_2$	NH_2	4490 ± 240	540 ± 37	6240 ± 341	500 ± 25
3c	$PhCH_2$	NH_2	970 ± 50	300 ± 13	630 ± 26	160 ± 8
3d	$PhCH_2$	$NHCOCH_3$	>10,000	6780 ± 355	—	—
3e	$PhCH_2$	OH	>10,000	>10,000	—	—
3f	$PhCH_2$	Cl	586 ± 32	67 ± 4	—	—
3g	$PhCH_2$	Br	5319 ± 300	3543 ± 158	—	—
3h	$PhCH_2$	H	2748 ± 135	1169 ± 60	—	—
3i	$PhCH_2$	$NHCH_2Ph$	677 ± 26	>10,000	—	—
3j	$PhCH_2$	$NHCH_2CH_2Ph$	977 ± 51	>10,000	—	—
3k	$PhCH_2$	CyclohexylNH	>10,000	>10,000	—	—
3l	$PhCH_2$	$N(CH_3)_2$	122 ± 6	57 ± 3	—	—
3m	$PhCH_2$	$N(CH_2CH_2OH)_2$	>10,000	>10,000	—	—
SCH58621			357 ± 35	2.2 ± 0.3	463 ± 45	2.5 ± 0.4

[a] Inhibition of specific [^{3}H]CHA binding to bovine and human brain cortical membranes expressed as K_i ± SEM (n = 3) in nM.
[b] Inhibition of specific [^{3}H]CGS21680 binding to bovine and human striatal membranes expressed as K_i ± SEM (n = 3) in nM.

at the hA_{2A}AR is very similar to that found at the bA_{2A}AR.

Compounds **2d**,**o**, **3f** and **3l** show a significant affinity at the A_{2A}AR, with K_i values in the range of 30.0–67.0 nM, while compounds **2e**,**k**,**m**,**n**,**p**,**q**, and **2r** display an average A_{2A}AR affinity, with K_i values in the range of 99.0–300.0 nM. The other compounds show a moderate affinity, or are completely ineffective.

The results obtained indicate that the structural requirements for a good affinity at the A_{2A}AR in this series of naphthyridine derivatives appear to be the presence of a polar substituent at the position 3 of the heterocyclic nucleus, instead of a lipophilic group, as confirmed by the comparison of **1a** with **2d**. In particular, the presence of an aromatic carboxamide, such as benzylamide or furfurylamide, seems to be necessary. Furthermore, the presence of a methylene spacer between the naphthyridine nucleus and the carboxamide group leads to a

marked decrease in the affinity, as can be seen from a comparison of **2d** with **2v**. Lastly, in order to obtain a good affinity at the A_{2A}AR, a lipophilic group, or an aliphatic amine without a large steric hindrance, seems to be necessary at the position 7 of the naphthyridine nucleus as is clear from a comparison of compound **2d** versus **3e** and compound **3l** versus **3j**.

Models of the bA_1AR and the hA_1AR had been recently developed through a homology procedure, which used the bovine rhodopsin as a template;[14] specific ligands were then docked into the models and so obtained complexes were optimized in such a manner they were in agreement with site-directed mutagenesis data.

Subsequently, a molecular model of the hA_{2A}AR was built[29] through a similar procedure but the initial hA_1AR model, the one not completely refined through MD procedure, was used as a template so that the manual refinement carried out on the A_1AR model in order to adjust the structure on the experimental data of site directed mutagenesis was identical in both A_1AR and A_{2A}AR. Successively, the structure of the complex with the specific ligand CGS21680 was optimized, in order to respect the information given by mutagenesis[30a–30f] for agonists and antagonists.

The final model of the hA_{2A}AR was used to perform a manual docking procedure of the compounds **1b**,**f**, **2c**,**d**,**h**, **3e**,**f**,**h**, and **3l**, availing the arrangement into the bA_1AR of some compounds previously studied,[14] considering all the orientations that allow the interactions suggested by the mutagenesis data as starting points for the complexes construction. During the MD simulation was applied distance constraints between the residue Ser94 and the naphthyridine N1, and between His251 and the oxygen at the position 4 of the ligands, but the constraints were gradually relaxed during the simulation, and removed in the last step of minimization.

The docking was studied in the hA_{2A}AR because of the lack of the primary structure of the bA_{2A}AR, that prevents the construction of its molecular model, but the affinity data for the bA_{2A}AR were used, because they were more complete and homogeneous; on the other hand, the available data indicated the same affinity profile of these antagonists in both the bA_{2A}AR and the hA_{2A}AR.

For each of the considered antagonists **1b**,**f**, **2c**,**d**,**h**, **3e**,**f**,**h**, and **3l**, the complexes with the bA_1AR and the hA_{2A}AR were optimized through MacroModel[31] program after a computational procedure of 200 ps of Molecular Dynamics at 300 °K followed by PR Conjugate Gradient (PRCG) minimization, using the Amber[32] forcefield with the derivative convergence criterion at a value of 0.05 kJ/A-mol.

The receptor–ligand interaction energies of the optimized complexes were calculated by MacroModel 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.

All compounds **1b**,**f**, **2c**,**d**,**h**, **3e**,**f**,**h**, and **3l** occupied approximately the same binding site of the bA_1AR as the 1,8-naphthyridine derivatives previously studied,[14] also if these first ones arranged deeper into the receptor. As already reported,[14] the binding of the previously studied 1,8-naphthyridines (and also of other potent A_1AR antagonists like DCPCX), had appeared to occur in a region between TM3, TM6, and TM7, where a series of lipophilic interactions were able to better stabilize these antagonists in the bA_1AR than in the hA_1AR. A graphical comparison between the computational studies carried out on the already published 1,8-naphthyridin-2,4,7-trisubstituted derivatives[14] and the new 1,8-naphthyridines substituted at 3, 4, and 7 positions show that the increasing of the molecular size provokes a progressive shift of the ligand towards the TM3 and a loss of interaction with the residues of the TM6, such as His251 (see Fig. 1).

Between the two compounds of type **1**, only **1f** can interact effectively in the binding site of the bA_1AR with His251 (Fig. 2), because of the hydrazine on the naphthyridine core which bends this residue forwards: the ligand stretches parallel to TM3, in a narrow longitudinal cavity delimited by Ile95, Leu98, Phe242, and Trp247, and as a result naphthyridine N1 can interact with Ser94 through a hydrogen bond. In the bA_1AR, the binding cavity is smaller because of the presence of the nonpreserved Pro86 in TM3 and Pro191 in TM5, which changes the folding of the helices and allows the interhelix hydrogen bonding between TM3 and TM5 (Gln92-Trp188 and Gln92-Asn184). In the hA_{2A}AR, where the two prolines are a valine and a leucine, the

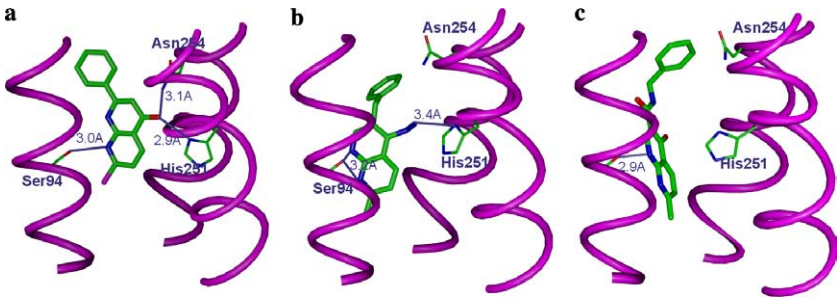


Figure 1. Docking of the 7-chloro-4-hydroxy-2-phenyl-1,8-naphthyridine previously reported[14] (a), compound **1f** (b), and compound **2d** (c) in the A_1AR.

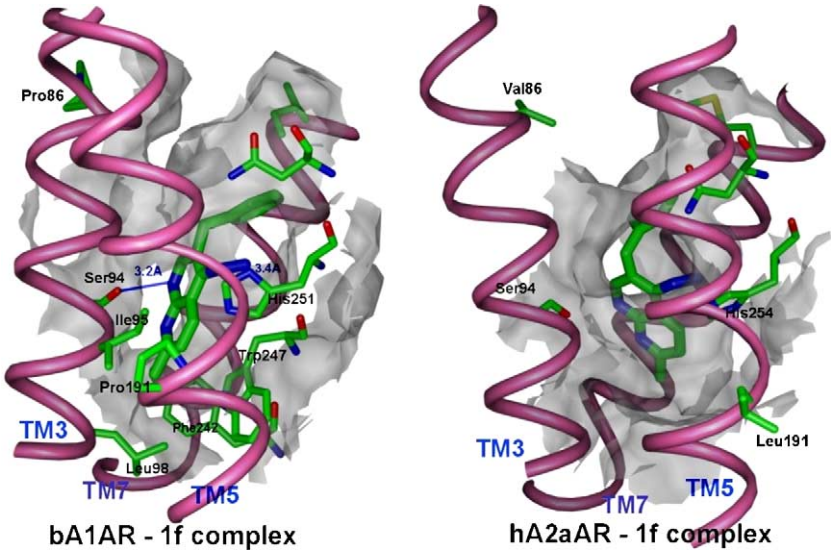


Figure 2. Compound **1f** docked into the bA_1AR (left) and $hA_{2A}AR$ (right) binding site. Interatomic distances in Angstroms between H-bonded atoms are reported in yellow. The surfaces of the receptors cavities are shown in grey, and TM3 and TM5 backbones are represented through magenta ribbons.

relative positions of TM3 and TM5 are different and the cavity is larger, consequently **1f** is not parallel to TM3, and the naphthyridine nucleus shifts toward TM7, without any interaction with Ser94 (see Fig. 2).

The difference in interaction energy between the bA_1AR–**1f** and the $hA_{2A}AR$–**1f** complexes is −3.8 kcal, in agreement with the higher stability of the A_1 complex. On the contrary, compound **1b**, without any polar substituent on the naphthyridine is able to form hydrogen bond, has no activity on the bA_1AR or the $hA_{2A}AR$, and has a similar docking in the two subtypes, without any strong interactions with the binding site residues.

Compounds of types **2** and **3** possess a longer amidic chain which seems slightly to favor the affinity for the $A_{2A}AR$. The docking studies on these compounds show that the larger $hA_{2A}AR$ binding cavity allows different orientations of ligands in this receptor, whereas in the bA_1AR they are generally arranged in the same manner between TM3 and TM6, shifted towards TM7 and without any interaction with TM5. Among the possible orientations of compounds **1b**,**f**, **2c**,**d**,**h**, **3e**,**f**,**h**, and **3l** in the $hA_{2A}AR$, only **2d**, **3f**, and **3l**, fit into in the crevice, inserting the naphthyridine nucleus turned towards TM5, into the lipophilic pocket formed by Trp246 (TM6), Pro189 (TM5), and Ile92 (TM3) and interact with the residue Asn253 through the amidic group.

Figure 3 shows, as an example, the binding mode of **2d** with the $hA_{2A}AR$: the presence of the methyl (or *N*-dimethyl substituent) on the naphthyridine core raises the benzylic chain and allows an effective interaction between Asn253 and the amidic group of the ligand, in

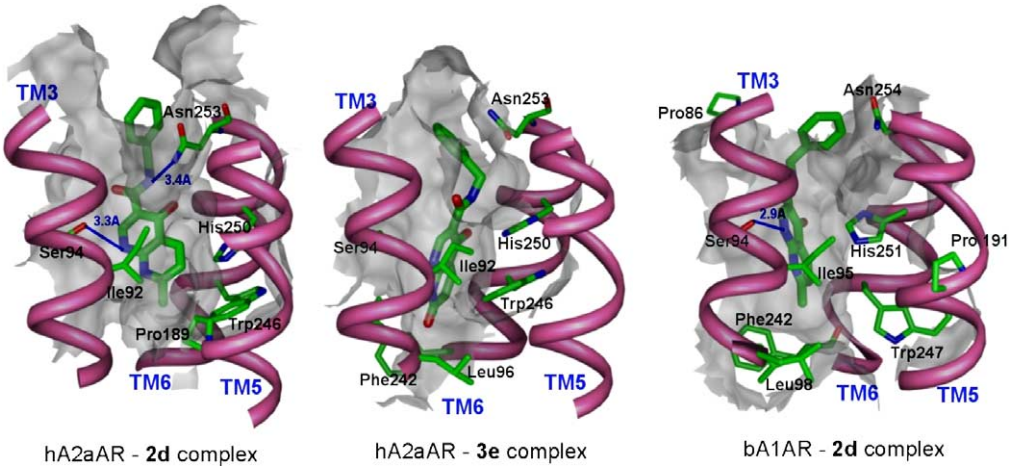


Figure 3. Comparison between the arrangement of the $hA_{2A}AR$ and bA_1AR. Compounds **2d** and **3e** occupy different lipophilic pockets of the $hA_{2A}AR$, due to the variation of the substituent in position 7. In the smaller cavity of the bA_1AR, also the compound **2d** stretches parallel to TM3 without interactions with TM6.

addition to the hydrogen bond between Ser94 and naphthyridine N1, and a strong lipophilic stabilization thanks to His250.

Compounds such as **3e** or **3h**, which have smaller substituents in position 7, do not interact with Asn253, and shift into a lower lateral lipophilic pocket formed by Trp246 and Phe242 of TM6, Ile92, Leu96 of TM3; this is shown in Figure 3 for compound **3e**.

In the bA_1AR, **2d**, like the other compounds **1b**,**f**, **2c**,**h**, **3e**,**f**,**h**, and **3l**, can interact only with Ser94 (see Fig. 3) and its interaction energy is 3.1 kcal higher than the $A_{2A}AR$–**2d** complex.

We may thus conclude that the insertion of an amidic group into the benzylic chain of the naphthyridine favors the interaction of the ligands with the larger cavity of the $hA_{2A}AR$, because of the possibility of a interaction with Asn253. This interaction is favoured if the substituent in position 7 is quite small and slightly lipophilic, such as methyl (**2d**) or *N*-dimethyl (**3l**) with the result that the antagonist can occupy the crevice delimited below by Trp246 and above by Asn253.

Acknowledgment

Financial support from Italian MURST is gratefully acknowledged.

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