Influence of *N*–1 substituent properties on binding affinities of arylpiperazines to the binding site of 5-HT$_{1A}$ receptor

MARIO V. ZLATOVIĆ[1*#], VLADIMIR V. ŠUKALOVIĆ[2#], SLAĐANA V. KOSTIĆ-RAJAČIĆ[2#], DEANA B. ANDRIĆ[1#] and GORAN M. ROGLIĆ[1#]

[1]Faculty of Chemistry, University of Belgrade, POB 158, 11000 Belgrade and [2]ICTM, Department of Chemistry, University of Belgrade, POB 473, 11000 Belgrade, Serbia (e-mail: mario@chem.bg.ac.yu)

Abstract: Serotonin receptors (5-HTRs), especially the 5-HT$_{1A}$ subtype, have been the subject of intensive research for the past decade, due to their function in human physiology. Several structurally different classes of ligands are known to bind to the 5-HT$_{1A}$ receptor, but arylpiperazine derivatives are among the most important ligands. In the work, docking analyses were used to explain the binding affinities of a series of ligands with different *N*-1 substituent. All ligands had in common the arylpiperazine structure, while the *N*-1 substituent was modified to investigate the influence of ligand structure on its binding affinity. The shape and size, as well as the rigidity of the substituents were altered to investigate the possible effects on the formation of the receptor – ligand complex.

Keywords: 5-HT$_{1A}$, binding site, hydrophobic pocket, arylpiperazine.

INTRODUCTION

G protein-coupled receptors (GPCRs) comprise not only the largest superfamily of proteins in the body, with more than 1000 different proteins described as GPCRs, but also the most interesting target for protein targeting pharmaceuticals. Serotonin receptors (5-HTRs) have been the subject of intensive research in the past decade because of their function in human physiology.[1] To date, seven classes, including 14 subtypes, of 5-HTR, have been found, and only one subtype, 5-HT$_3$, does not belong to GPCRs. Among these transmembrane serotonin receptors, the 5-HT$_{1A}$ subtype is the best studied. Several structurally different classes of ligands are known to bind to this receptor[2] and arylpiperazine derivatives are among the most important 5-HT$_{1A}$ ligands. The main obstacle to research in this field is the fact that, despite the intensive research, the 3D structure and mechanism of action of GPCRs are still not known.

* Corresponding author.

\# Serbian Chemical Society active member.

The great drawback for arylpiperazine derivatives as potential pharmacologically usable ligands for the 5-HT$_{1A}$ receptor is that they are not sufficiently selective. Many of them show a tendency to bind strongly to other 5-HTR subtypes as well as to other GPCRs (D2, adrenergic...). Knowledge about the details of the binding process of the ligand to the 5-HT$_{1A}$ binding site would be helpful for designing ligands with a satisfactory selectivity to fulfill the pharmacological demands.

The binding of small molecules with the arylpiperazine moiety to the active site of the 5-HT$_{1A}$ receptor was the object of various studies and investigations, resulting in several reasonable suggestions for the binding mechanism,[3,4] but no unified theory was postulated for arylpiperazine derivatives with a high affinity for 5-HT$_{1A}$. The main goal of this work was to shed additional light on the influence of the *N*-1 substituent of *N*-arylpiperazines on the binding process of these ligands. A series of ligands having in common the arylpiperazine structure but with different *N*-1 substituents ("head" part) varying in shape, size and rigidity was used in order to investigate their possible effects on the formation of the receptor – ligand complex.

EXPERIMENTAL

The model of the 5-HT$_{1A}$ receptor was built by comparative modeling using the MODELLER program,[5] which is a part of the InsightII software[6] and the binding site of that model determined as described in an earlier paper.[7] The amino acids forming the binding site of the 5-HT$_{1A}$ model are listed in Table I. The position of the key amino acids in this binding site can be seen in Fig. 1. The binding site designed in this way corresponds well to the rather conserved binding domain of rhodopsin-like receptors between helices III, V, VI and VII of Class A transmembrane receptors.[8] Some of the listed amino acids were earlier identified by point mutations to have key interactions with different types of ligands.[9,10] The proposed binding site includes well-defined, conserved amino acid residues and amino acid residues found by computer analysis of the 5-HT$_{1A}$ receptor model.

TABLE I. Amino acids in the proposed binding site of the 5-HT$_{1A}$ model

Amino acids in				
TM3	*e2*	TM5	TM6	TM7
Phe 112 (3.28)	Asp 185	Tyr 195 (5.38)	Cys 357 (6.47)	IIe 385 (7.38)
IIe 113 (3.29)	Ala 186	Thr 196 (5.39)	Trp 358 (6.48)	Asn 386 (7.39)
Ala 114 (3.30)	Cys 187	Ser 199 (5.42)	Leu 359 (6.49)	Trp 387 (7.40)
Asp 116 (3.32)	Thr 188	Thr 200 (5.43)	Pro 360 (6.50)	Leu 388 (7.41)
Val 117 (3.33)	IIe 189	Gly 202 (5.45)	Phe 361 (6.51)	Gly 389 (7.42)
Leu 118 (3.34)		Ala 203 (5.46)	Phe 362 (6.52)	Tyr 390 (7.43)
Cys 119 (3.35)		Phe 204 (5.47)		Ser 391 (7.44)
Cys 120 (3.36)				Asn 392 (7.45)
Thr 121 (3.37)				

Although several of envisaged 5-HT$_{1A}$ models exist today,[11,12] they are usually models without loops, because there is almost no information about the spatial arrangement of amino acids in the loops. However, one important fact is known, *i.e.,* that there is a conserved disulfide bond connecting Cys 109 (C 3.25)[13] on TM3 and Cys 187, amino acid from the *e2* loop.[14] In an earlier paper, the possible importance of the *e2* loop in modeling receptor – ligand interactions[7] was highlighted, especially

Fig. 1. The binding site of the 5-HT$_{1A}$ receptor model.

of Thr 188, situated near the conserved region in that loop (Cys 109 – Cys 187), which is capable of forming a hydrogen bond with a convenient substitutent on the arylpiperazine part of the ligand.

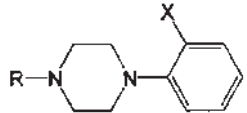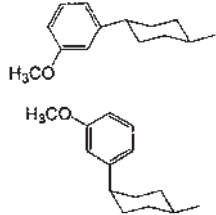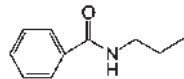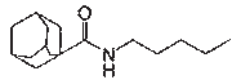TABLE II. Structures and activities of the investigated ligands
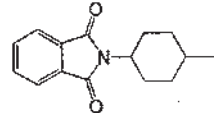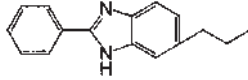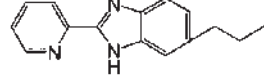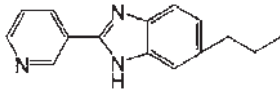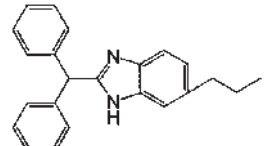
Ligand	X	R	K_i/nM
1	–OCH$_3$		0.02[19]
2	–OCH$_3$		79.6[19]
3	–OCH$_3$		1.3[20]
4	–OCH$_3$		0.4[21]
5	–OCH$_3$		8.0[22]
6	–H		10.1[23]
7	–H		2.8[23]

TABLE II. Continued

Ligand	X	R	K_i/nM
8	–H		70.7[23]
9	–H		4.2[23]

All ligands used in the docking analysis were modeled using the Accelrys InsightII program Build module. The initial geometry was optimized until energy minima were reached. The geometry obtained in this manner was the starting point for the docking analysis and the conformation of the arylpiperazine part corresponds to the conformations of the X-ray structures of the arylpiperazines deposited in the Cambridge Structural Database (CSD).[15] This conformation did not change during the calculations.

Ligand docking of the ligands in Table II was done by simulated annealing using the Affinity module from InsightII on SGI Octane2 workstation.[16] All ligands were docked as protonated, using the CFF91 force field. The charges of the amino acid residues were adjusted as required. The protein bind site was determined by combining the results from experimental data and the InsightII bind site analysis module. The initial position of the ligand in the bind site was arbitrary in respect of the arylpiperazine part facing TM6 and TM7, while the protonated nitrogen on the piperazine part was kept in close proximity to Asp 116. After the initial ligand placement, no further constrains were applied and a docking procedure based on the Monte Carlo methodology was carried out. Up to 100 structures were produced in every run and each was finally optimized in order to remove steric interaction with a gradient limit of 0.0042 kJ/mol or 4000 optimization steps.

The obtained docked structures were examined, and those with the lowest total energy were further filtered to obtain docking structures with the best ligand fit. The structures were selected based on the following criteria: lowest total energy of the complex, shortest salt bridge formed between Asp 116 and the proton on the piperazine ring nitrogen, conformation of the arylpiperazine ring analogous to the crystal structure[15] and the aryl part of the molecule positioned in the rear hydrophobic pocket of the ligand. After an initial criterion was satisfied, the second step was an examination of the different interactions that could be formed between the receptor and the front part of the ligand. In this way, the best possible docking structures were selected. The structures were rendered using PovRay raytracer v3.6.[17]

RESULTS AND DISCUSSION

At present, it is well known that for the binding of ligands with an arylpiperazine moiety the formation of a salt bridge between the protonated *N*-1 nitrogen of arylpiperazine and the negatively charged Asp 116 is required. This interaction guides the ligand towards its binding site, most probably by zipper-like mechanisms,[18] leading to interactions with key residues in the receptor binding site. For 1,4-disubstituted arylpiperazines, hydrophobic and/or H-bonding interactions of the 4-*N*-aryl substituent with amino acid residues from TM6, TM7 and *e2* also play an important role both in the binding affinity as well as in the correct positioning of the entire ligand in the binding site. After these interactions are established, the rest of the molecule is placed in the best possible position, accomplishing the position

and conformation that will provide the optimal energy stabilization and the least steric and torsional strain.

The results of the docking analysis show that all the investigated ligands bind in a similar manner. A short salt bridge between Asp 116 and a protonated nitrogen atom is present, which is a precondition for good binding affinity. All the ligands form an aromatic–aromatic interaction between the arylpiperazine part and Phe 361 (F 6.51) and Tyr 390 (Y 7.43). In addition, the ligands **1 – 5** can form a hydrogen bond between their methoxy group on the aryl ring and Thr 188 in the *e2* loop of the receptor, as described earlier.[7]

Binding of the head part of the ligand is influenced by its shape, size and chemical character (aromatic or aliphatic moiety, capability of forming hydrogen bonds, presence of heteroatom in the aromatic ring, *etc.*).

Fig. 2. Ligand **1** docked in the active site.

Fig. 3. Ligand **2** docked in the active site.

Docking analysis of ligand **1** (Fig. 2) shows all the features mentioned above. A short salt bridge between Asp 116 and a protonated nitrogen is present as well as two edge-to-face (ETF) interactions together with a hydrogen bond positioned beteeen the methoxy group located on the arylpiperazine part and Thr 188. The head section of the ligand, arylcyclohexyl part, forms a hydrogen bond connecting its methoxy group and Thr 200 (T 5.43), together with several CH – π interactions with Phe 204 (F 5.47) and Phe 362 (F 6.52). All those interactions, combined with the ligand shape and size (distance between arylpiperazine part and arylcyclohexyl part) and the rigidity of the ligand structure, lead to the highest activity toward the 5-HT$_{1A}$ receptor in the investigated group (Table II).

Ligand **2** (Fig. 3) is the *cis* isomer of the ligand **1**. Docking analysis shows that the *cis* isomer cannot form a hydrogen bond with Thr 200, nor CH – π interactions, because of a significant difference in shape compared with the *trans* isomer, ligand **1**. This isomer is positioned in a different part of the binding site, in the cavity near the top of the 5-HT$_{1A}$ binding site, faced toward the extracellular space. The only significant interaction this ligand can make is a hydrogen bond with Ser 200 (S

5.42), but this bonding would lead to a weakening of the hydrogen bond with Thr 188, losing the favorable position for ETF interactions of the aryl group and, possibly, even to an extension of the salt bridge between the ligand and Asp 116. One of such structures is shown in Fig. 3. This is not an effective position for binding and ligand **2** shows low affinity toward 5-HT$_{1A}$.

Analysis of the best docked structure of the ligand **3** (Fig. 4) shows that a somewhat shorter *N*-1 substituent of the ligand (*N*-ethylbenzamide) cannot form a hydrogen bond with Thr 200, but there is a possibility of a slightly weaker hydrogen bonding with Trp 358 (W 6.48). This interaction, together with the aromatic–aromatic interactions between the benzamide part of the ligand and Phe 204 and Phe 362, leads to a lower binding to 5-HT$_{1A}$ when compared with **1**.

Fig. 4. Ligand **3** docked in the active site

Fig. 5. Ligand **4** docked in the active site

Ligand **4** (Fig. 5) is characterized by a longer alkyl chain and the bulky adamantyl group, instead of an aromatic moiety present in the other ligands. Docking analysis shows that the longer alkyl chain can be coiled inside the binding site, pushing the adamantylamide part toward Phe 204, Phe 362 and Leu 359, thus hiding the lipophilic adamantyl group deep inside the hydrophobic pocket formed by these amino acid residues. An additional hydrogen bond is formed with Ser 199, leading to a high 5-HT$_{1A}$ affinity. Although the hydrogen bond with Thr 188 is longer (and weaker), the position of the aromatic hydrogens in this aryl group, unlike in ligand **2**, still enables the formation of good ETF interactions.

Ligand **5** (Fig. 6) represents a somewhat larger group in the head part; the 2-cyclohexylisoindoline-1,3-dione group cannot form hydrogen bonds with either Ser 199 or Thr 200, but it can form weak bonding with the hydrogen from the peptide bond between Phe 362 and Phe 361. Docking analysis shows that the isoindoline group can fit between Phe 204 and Phe 362, forming aromatic–aromatic interactions, thus stabilizing the receptor–ligand complex. This ligand exhibits moderate binding affinity.

Fig. 6. Ligand **5** docked in the
active site.

Fig. 7. Ligand **6** docked in the
active site.

Ligands **6** to **9** cannot form an additional bond in the arylpiperazine part, being without the 2-methoxy substituent. They also share a common 6-ethyl-1*H*-be - nzo[*d*]imidazole moiety, linked to the arylpiperazine part.

Docking analysis of ligand **6** (Fig. 7) shows the formation of the following inter- actions: a short salt bridge between Asp 116 and the protonated nitrogen atom of the piperazine part of the ligand, ETF interactions between the arylpiperazine part and Phe 361 and Tyr 390. The head section of the ligand can form additional aro- matic–aromatic and hydrophobic interactions, *via* the 2-phenyl substituent of the benzo[*d*]imidazole part and Phe 204, Phe 362 and Leu 359. Docking analysis shows that aromatic–aromatic interactions in this part can be π-stacking interactions due to the distance and orientation of the aromatic rings. This ligand cannot form hydrogen bonds with the receptor, therefore its binding affinity is weak or moderate.

Ligands **7** and **8** (Figs. 8 and 9) are similar in structure, with a pyridinyl substi- tuent in the benzo[*d*]imidazole part. Docking analysis of ligand **7** shows the presence of an intramolecular hydrogen bond between the nitrogen atom in the pyridine and the proton on the nitrogen atom in the benzimidazole ring. Thus the structure of

Fig. 8. Ligand **7** docked in the
active site.

Fig. 9. Ligand **8** docked in the
active site.

Fig. 10. Ligand **9** docked in the active site.

ligand **7** is far more rigid than those of ligands **6** and **8**. Ligand **7** is stabilized by aromatic–aromatic and hydrophobic interactions with Phe 204, Phe 362 and Leu 359. Due to its inflexibility and the position of the nitrogen atom in the pyridine ring in position 2 with respect to the rest of the molecule, the affinity toward the 5-HT$_{1A}$ receptor is moderate but, nevertheless, slightly higher than for ligand **6**.

Ligand **8** has a nitrogen atom in position 3 to the benzo[*d*]imidazole moiety. Experimental data show a low binding affinity toward the 5-HT$_{1A}$ receptor. A first glance at the docking structures shows that there are no major differences in the general position of ligands **7** and **8** in the binding site, but there is a significant decrease in affinity. This can be explained if different orientations of pyridine ring are considered. This part of the ligand is responsible for stabilizing aromatic–aromatic and hydrophobic interactions with Phe 204, Phe 362 and Leu 359, but the position and electrostatic properties of the nitrogen atom, together with, to some extent, a less restricted rotation of this part of the molecule than in ligand **7**, lead to an unfavorable orientation and position for π-stacking bonding interactions.

The benzo[*d*]imidazole part of ligand **9** (Fig. 10) has a bulky diphenylmethyl substituent. Although it is a large and rotationally restricted group, docking analysis shows that it can still be docked inside the 5-HT$_{1A}$ receptor. One phenyl ring in the diphenylmethyl group can easily fit between Phe 204 and Phe 362, while the other is located in a binding cavity facing the extracellular space, similar to the space occupied by ligand **2**. Stabilization of the receptor–ligand complex is achieved by aromatic–aromatic and hydrophobic interactions. Since rigid structures are more favorable than flexible ones, the binding affinity is moderate and comparable to ligands **6** and **7**.

CONCLUSION

Docking analysis showed that all the investigated ligands bind in a similar manner. The first step in the ligand binding is the formation of a short salt bridge

between the negatively charged oxygen atoms on Asp 116 and a positively charged nitrogen atom in the piperazine part of the ligand. This is followed by the formation of an aromatic–aromatic interaction (edge-to-face type) between the arylpiperazine part of the ligand and the rear hydrophobic pocket of the receptor, formed by Phe 361 and Tyr 390 on the TM6 and TM7 helices, respectively. An additional hydrogen bond can be formed with Thr 188 located in the *e2* loop, closing the pocket on the extracellular side. For binding ligands with an arylpiperazine moiety, these interactions are crucial.[7,24]

Careful comparison of the docking analysis results shows the existence of another hydrophobic pocket, located between TM5 and TM6, in the front part of the 5-HT$_{1A}$ receptor, formed by Phe 204 on TM5, as well as Phe 362 and Leu 359 on TM6. Ligands **1** and **3– 9**, in their binding to the 5-HT$_{1A}$ receptor, form as least one aromatic–aromatic interaction with these amino acid residues.

In case of ligands **1, 3** and **4**, the binding affinity is influenced by the formation of hydrogen bonds with Thr 200, Trp 358 or Ser 199, respectively, while ligands **5 – 9** cannot form these hydrogen bonds and their affinity depends only on the number and strength of aromatic–aromatic and hydrophobic interactions.

The importance of the formation of hydrogen bonds between a ligand and Ser 199 and/or Thr 200 was investigated earlier,[25] but since many ligands with a moderate to high affinity cannot form this type of interactions, it can be concluded that they are not a decisive factor in the formation of the receptor–ligand complex.

The results of docking analysis show that the front hydrophobic pocket can accommodate both aromatic and aliphatic substituents, ranging from the methoxy group (ligand **1**) up to the adamantyl group (ligand **4**). This finding shows that the parts of TM5 and TM6 near the extracellular side of the protein are to some extent flexible and can adjust their conformation during ligand binding. In support of this, the experimental results show that ligands with rigid structures (**7** and **9**) possess higher affinity than similar flexible ligands (**6** and **8**).

In case of aromatic substituents, docking analysis shows that the preferable orientation of the Phe 204 and Phe 362 residues and the aromatic part of the ligand is a stacked one (sandwich) but exact orientation (parallel, displaced or parallel-displaced) cannot be asserted by docking analysis alone. The introduction of a heteroatom in the aromatic part of the ligand (as in ligands **7** and **8**) shows mixed influence on the binding affinity. While a nitrogen in position 2 of the aromatic ring leads to an increase in the binding affinity, a nitrogen in position 3 causes a large decrease compared to ligand **6**. A possible explanation lies both in the rigidity of the system (ligand **7** is more rigid than **6** and **8**), as well as a charge distribution which can affect the formation of aromatic–aromatic interactions.

Comparing the ligand afinity, it can be concluded that ligands **1, 3** and **4**, capable of forming hydrogen bonds, show a somewhat higher affinity than the other investigated ligands. A rigid structure is preferable to a flexible one, because it facili-

tates the formation of a hydrogen bond, by placing corresponding functional groups in close proximity. Ligands which form only aromatic–aromatic interactions with the receptor bind with a moderate affinity. The binding affinity of the ligands **5**–**9** is determined by the strength of the interactions formed between the ligand and amino acid residues in the front hydrophobic pocket. In these ligands, a rigid structure is also preferable and results in higher ligand affinity.

To shed some more light on ligand binding to the 5-HT_{1A} receptor, regarding the postulated front hydrophobic pocket, a large training set, comprised of a number of different ligands sharing a common arylpiperazine moiety and an aromatic or aliphatic head segment, with or without a heteroatom, is currently being built. In this way, it is hoped a clearer picture regarding interactions leading to the stabilization of the receptor–ligand complex can be obtained.

ИЗВОД

УТИЦАЈ ОСОБИНА *N*-1 СУПСТИТУЕНТА НА АФИНИТЕТ АРИЛПИПЕРАЗИНА ПРЕМА ВЕЗИВНОМ МЕСТУ 5-HT_{1A} РЕЦЕПТОРА

МАРИО В. ЗЛАТОВИЋ[1], ВЛАДИМИР В. ШУЋАЛОВИЋ[2], СЛАЂАНА В. КОСТИЋ-РАЈАЧИЋ[2], ДЕАНА Б. АНДРИЋ[1] и ГОРАН М. РОГЛИЋ[1]

[1]*Хемијски факулшеш, Универзишеш у Беоīраду, ū. ūр 158, 11000 Беоīрад и* [2]*ИХТМ Ценшар за хемију, Универзишеш у Беоīраду, ū. ūр. 473, 11000 Беоīрад*

Серотонински рецептори су, а нарочито 5-HT_{1A} подтип, због значајне улоге у физиологији људског организма, предмет интензивног изучавања током протекле деценије. Познато је да се за 5-HT_{1A} рецептор везује неколико структурно различитих класа лиганада, али су арилпиперазински деривати међу најзначајнијим. Да би објаснили везивање серије лиганада са различитим *N*-1 супституентима за рецептор користили смо анализу везивања (docking анализу). Сви лиганди су имали заједничку арилпиперазинску структуру док су им *N-1* супституенти модификовани тако што је мењан облик, величина као и крутост супституента да би се истражио њихов евентуални утицај на формирање комплекса рецептор – лиганд.

(Примљено 19. маја, ревидирано 24. јула 2006)

REFERENCES

1. I. Lucki *Biol. Psychiatry* **44** (1998) 151
2. B. Olivier, W. Soudijn, I. van Wijngaarden, *Progress in Drug Res.* **52** (1999) 103
3. M. F. Hibert, M. W. Gittos, D. N. Middlemiss, A. K. Mir, J. R. Fozard, *J. Med. Chem.* **31** (1988) 1087
4. M. F. Hibert, I. McDermott, D. N. Middemiss, A. K. Mir, J. R. Fozard, *Eur. J. Med. Chem.* **24** (1989) 31
5. A. Sali, T. L. Blundell, *J. Mol. Biol.* **234** (1993) 779
6. InsightII, Binding Site Analysis, Affinity, Discover, WebLab Viewer are molecular modeling software, corresponding modules and visualization software from Accelrys, Inc., San Diego, CA, USA (http://www.accelrys.com).

7. M. V. Zlatović, V. V. Šukalović, C. Schneider, G. M. Roglić, *Bioorg. Med. Chem.* **14** (2006) 2994
8. K. Bondensgaard, M. Ankersen, H. Thøgersen, B. S. Hansen, B. S. Wulff, R. P. Bywater, *J. Med. Chem.* **47** (2004) 888
9. M. L. López-Rodríguez, M. J. Morcillo, E. Fernández, B. Benhamú, I. Tejada, D. Ayala, A. Viso, M. Olivella, L. Pardo, M. Delgado, J. Manzanares, J. A. Fuentes, *Bioorg. Med. Chem. Lett.* **13** (2003) 1429
10. L. Shi, J. A. Javitch, *Annu. Rev. Pharmacol. Toxicol.* **42** (2002) 437
11. M. L. López-Rodríguez, B. Vicente, X. Deupi, S. Barrondo, M. Olivella, M. J. Morcillo, B. Benhamú, J. A. Ballesteros, J. Salles, L. Pardo, *Mol. Pharmacol.* **62** (2002) 15
12. I. Sylte, A. Bronowska, S. G. Dahl, *Eur. J. Pharmacol.* **416** (2001) 33
13. J. A. Ballesteros, H. Weinstein, *Methods Neurosci.* **25** (1995) 366
14. L. Oliveira, T. Hulsen, D. Lutje Hulsik, A. C. M. Paiva, G. Vriend, *FEBS Lett.* **564** (2004) 269
15. F. H. Allen, *Acta Crystallogr.* **B58** (2002) 380
16. SGI Octane2 is a trademark of Silicon Graphics, Inc, CA, USA (http://www.sgi.com/)
17. PovRay Persistence of Vision Raytrace is a trademark of the PovRay team (http://www.povray.org/)
18. A. S. Burgen, G. C. Roberts, J. Feeney, *Nature* **253** (1975) 733
19. R. Perrone, F. Berardi, N. A. Colabufo, M. Leopoldo, E. Lacivita, V. Tortorella, *J. Med. Chem.* **44** (2001) 4431
20. W. Kuipers, C. G. Kruse, I. van Wijngaarden, P. J. Standaar, M. T. M. Tulp, N. Veldman, A. L. Spek, A. P. Ijzerman, *J. Med. Chem.* **40** (1997) 300
21. R. K. Raghupathi, L. Rydelek-Fitzgerald, M. Teitler, R. A. Glennon, *J. Med. Chem.* **34** (1991) 2633
22. M. H. Paluchowska, M. J. Mokrosz, A. Bojarski, A. Wesolowska, J. Borycz, S. Charakchieva-Minol, E. Chojnacka-Wojcik, *J. Med. Chem.* **42** (1999) 4952
23. V. Šoškić, J. Joksimović, *Curr. Med. Chem.* **5** (1998) 493
24. F. Fiorino, E. Perissutti, B. Severino, V. Santagada, D. Cirillo, S. Terraciano, P. Massarelli, G. Bruni, E. Collavoli, C. Renner, G. Caliendo, *J. Med. Chem.* **48** (2005) 5495
25. M. L. López-Rodríguez, D. Ayala, B. Benhamú, M. J. Morcillo, A. Viso, *Curr. Med. Chem.* **9** (2002) 443.