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The influence of dispersive interactions on the binding affinities of ligands with an arylpiperazine moiety to the dopamine D2 receptor

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Abstract: Several isosteric 1,3-dihydro-5-[2-(4-aryl-1-piperazinyl)ethyl]-2*H*--benzimidazole-2-thiones were used to investigate the interactions of different ligands with the binding site of the D2 receptor. Due to limitations of the simulation methods, docking analysis failed to show precisely the interactions that influence the binding affinity of the ligands. It is presumed that dispersive forces or more precisely edge-to-face interactions play an important role in the binding process, especially for the lipophilic part of the ligands. In order to confirm this hypothesis, *ab initio* calculations were applied on a model system in order to find the stabilization energies of potential edge-to-face interactions and then to correlate them with the ligand affinity. The obtained results indicate that there is a significant correlation between the strength of dispersive interactions and ligand affinity. It was shown that for the calculation of stabilization energies of modeled receptor–ligand complexes the Becke "half-and-half" hybrid DFT method can be used, thus speeding up the usually long calculation time and reducing the required computer strength.

Keywords: dispersive interactions; hybrid DFT; ligand affinity; correlation; D2.

INTRODUCTION

Dopaminergic systems have been the focus of much research over the past 30 years, mainly because several pathological conditions, such as Parkinson's disease, schizophrenia, Tourette's syndrome and hyperprolactinemia, have been linked to a dysregulation of dopaminergic transmission. Dopamine (DA) receptor antagonists have been developed to block hallucinations and delusions that occur in schizophrenic patients, whereas DA receptor agonists are effective in alleviating the hypokinesia of Parkinson's disease. However, blockage of DA receptors

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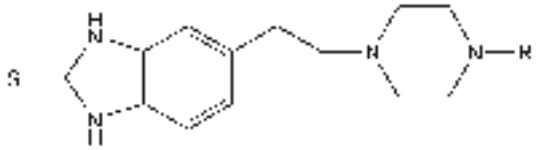
can induce extrapyramidal effects similar to those resulting from DA depletion and high doses of DA agonists can cause psychoses. The therapies of disorders resulting from DA imbalances are thus associated with severe side effects.[1]

The development of new, selective dopaminergic ligands devoid of adverse effects has become a challenging field of research.

The main feature of many substances that exhibit significant dopamine D2 receptor affinity is the presence of the arylpiperazine moiety.[1] The focus of our research was on the lipophilic part of 1,3-dihydro-5-[2-(4-aryl-1-piperazinyl)--ethyl]-*2H*-benzimidazole-2-thiones ligands, changing it in order to study the effects of different substituents on the activity of the ligand (Table I). Seven ligands were synthesized and their dopamine D2 receptor affinity tested. Only two ligands (**3** and **4**) showed a substantial increase in biological activity, while the others demonstrated no or only a small increase in activity or were inactive when compared to ligand **1**.

TABLE I. Structure of the synthesized ligands and their activities[2] towards the dopamine D2 receptor



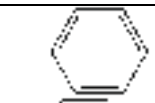
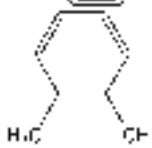
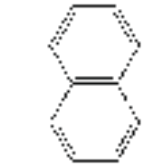
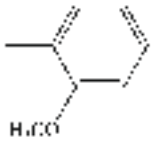
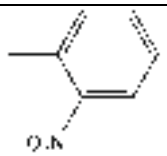
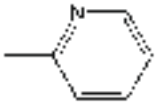
No.	R	D_2 K_i±*SEM* / nM
1		15.7±2.0
2		15.8±2.3
3		3.4±0.4
4		1.7±0.4
5		20.7±2.2

TABLE I. Continued

No.	R	D_2 K_i±*SEM* / nM
6		11.79±0.9
7		128

Earlier studies of the D2 receptor set up the criteria that a salt bridge to the carbonyl group of aspartate on TM3 and two hydrogen bonds to the serines (Ser 122, 141) on TM5 are essential for D2 activation.[3] However, these criteria alone were incapable of explaining differences in affinity between ligands possessing all these interactions. It was later concluded that more complex decisive factors for ligand binding must exist, including additional interactions.[4]

To investigate the interactions that are responsible for receptor–ligand complex formation, methods of computational chemistry, namely docking analysis and molecular properties calculations for ligands (ClogP and electrostatic isopotential) were employed.[5] Docking results showed that a short salt bridge to Asp 86 (TM3) together with multiple hydrogen bond formation to the serines on TM5 were present in all structures. Since the docked ligands showed different affinity towards the D2 receptor, it was obvious that there must be one or more additional interactions that determine ligand affinity. Based on docking structures and on ligand properties, a hypothesis was postulated that variations in affinity may originate from different distributions of edge-to-face (ETF) interactions in the docked structures. These interactions control the crystal structure of aromatic molecules, the stability of biological systems and molecular recognition processes.[6] In ETF interactions, a partially positive hydrogen atom on one aromatic ring interacts with a partially negatively charged region on the second ring, hence the name edge-to--face (Fig. 1).[7] The orientation of the amino acid residues of the ligands and re-

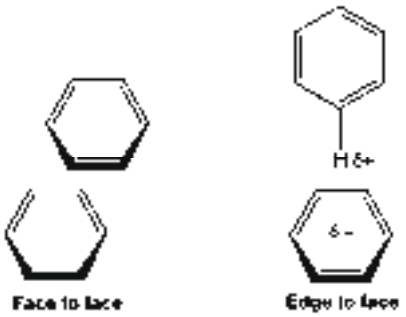


Fig. 1. Possible orientations for benzene aromatic rings. π-π stacking interaction parallel, (face-to-face) and T-shaped (edge-to-face).[6]

ceptor in results of docking simulations and calculated electrostatic isopotential maps strongly support the theory concerning ETF interactions as additional interactions and a decisive factor determining differences in ligand affinity.[8]

Observation of all interactions contributing to receptor–ligand complex formation is important when computer based methods are employed to model or explain such assemblies. Neglecting one type of interaction, such as, for instance, edge-to-face interactions, may lead to a series of wrong conclusions. However, research of structures possessing a large number of atoms, such as a dopamine D2 receptor–ligand complex, is usually limited to the methods of molecular mechanics, because only those kinds of calculations can be performed employing computer equipment with a reasonable processing power in a reasonable time. However, molecular mechanics force fields (even advanced class II ones, such as CFF)[9] cannot guarantee that all of the influencing forces are taken into account. Dispersive forces, such as edge-to-face interactions fall well beyond the computing capabilities of MM force fields. Due to this, aromatic–aromatic interactions (namely ETF interactions) cannot be investigated without employing more precise, *ab initio* methods. Unfortunately, the use of these methods is limited to smaller molecules because of high demands for computational resources. Hence, for the application of *ab initio* calculations in research of large molecules, smaller model systems need to be postulated.

According to docking analysis, the ETF interaction between the N-aryl part of a ligand and the three aromatic amino acids (Tyr 216, Trp 182 and Phe 178) plays a significant role in receptor-ligand complex formation (Fig. 2). This conclusion is based on indirect evidence, namely on the values of the angles, distances and on mutual orientation of the corresponding amino acids and ligands, as well as on the calculated electrostatic potential.

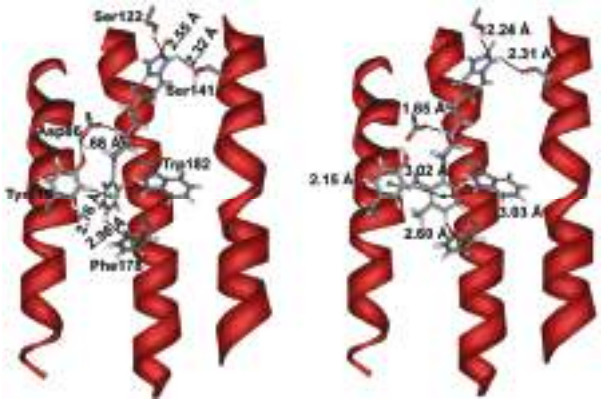


Fig. 2. Structures of benzimidazole-2-thione ligands **1** (left) and **2** (right) docked at the D2 receptor model. The orientation and distances between ligand and the lipophilic part of the binding site suggest that ETF interactions may be responsible for ligand affinity.

In order to present further evidence that will uphold the hypothesis concerning ETF interactions, a simplified binding site model system of dopamine D2 receptor–ligand ETF interactions was constructed and subjected to *ab initio* calculations in order to determine if the complex stabilization energy could be correlated to ligand affinity.

To utilize *ab initio* methods in the research of receptor–ligand interactions, several approximations had to be made, taking into account given the computer resources and the available (or reasonable) time. Thus the system had to be made smaller and the calculations more efficient. To simplify the system, our effort was concentrated on key amino acid residues and the part of the ligand that was held responsible for edge-to-face interactions. Ethylbenzene was used instead of phenylalanine, 3-ethyl-1*H*-indole instead of tryptophan and 4-ethylphenol was used to replace tyrosine. This kind of simplifications did not influence the properties of the aromatic moieties and gave a smaller and more compact model system to work on. In a similar way, the ligand molecule was shortened to arylpiperazine (Fig. 3).

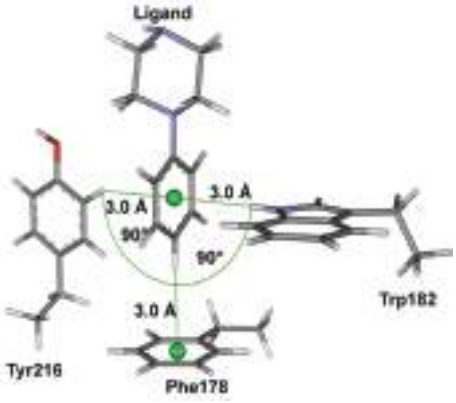


Fig. 3. Model system used in the present calculations mimics the hydrophobic pocket in the D2 receptor formed by 3-ethyl-*H*-indole (representative of Trp 182), ethylbenzene (representative of Phe 178) and 4-ethylphenol (representative of Tyr 216).

The initial receptor ligand position in this model system was taken directly from the results of docking analysis. These results positioned the arylpiperazine ligand moiety at distances of up to 3.00 Å to the receptor amino acid residues Tyr 216, Trp 182 and Phe 178 (Fig. 2). Based on these results, a model was constructed in which the ligand moiety was positioned perpendicular at 3.00 Å from each amino acid residue (Fig. 3). The same tetramer model was constructed for each ligand and subjected to *ab initio* DFT BH&H (Becke half & half) and MP2 calculations to determine the stabilization energy as described further. It is known that density function theory (DFT) calculations can not take into the account the existence and strength of dispersive forces such as ETF interactions but the BH&H hybrid DFT method was shown earlier to give rather correct results when applied on similar systems.[10,11] The obtained results were correlated to ligand affinity but failed to show any significant correlation.

It was obvious that the docking analysis had failed to predict the exact distances between the ligand and the amino acid residues, mainly due to the fact that the employed molecular mechanics force field could not take into account the existing ETF interactions. To establish the optimal amino acid residue–ligand distance, a more complex approach was required.

First, the tetramer model system had to be further disassembled. As shown earlier, the energy of a complex system can be obtained by summing the energy of the system parts calculated separately.[12] Bearing this in mind, the system was treated as three separate dimers, each formed by ligand and one of the amino acid residues responsible for ETF interactions. The results calculated in this manner did not show significant differences, since the difference in the stabilization energy for ligand **1** calculated using the BH&H method for the tetramer and as a sum of each dimer separately was 0.59 kJ/mol (less than 1.5 % difference).

In order to swiftly determine the stabilization energy of the complex, the BH&H DFT *ab initio* method was used together with the 6-31g+* basis set. In this way, the calculation time was significantly reduced with no major loss in the quality of the result, compared to that obtained by the MP2 method.

Each ligand–amino acid residue pair was subjected to further *ab initio* calculations in which the distance was varied between 1.75 and 3.50 Å in order to establish the optimal value. The results are shown in Table II and Fig. 4. The optimal distance varies between 2.25 and 2.50 Å, depending on both the ligand and the amino acid residue. This result supports the docking analysis results, placing the ligand at an average distance of 3.00 Å from the amino acid residues. If Fig. 4 is taken into account, it can be seen that the difference in stabilization energy between 2.25 or 2.50 and 3.00 Å are noteworthy and that this is the main reason why docking analysis alone cannot be used to explain the fine differences between the investigated ligands. Finally, all the stabilization energies at the optimal distances were summed to obtain the stabilization energy of the system. These results were again correlated with the ligand affinity and the results are shown in Table III.

TABLE II. Calculated optimal distances to the corresponding amino acid residues

Ligand	Calculated optimal distance (Å) from		
	Phe178	Trp182	Tyr216
1	2.25	2.25	2.50
2	2.50	2.25	2.50
3	2.50	2.25	2.50
4	2.25	2.25	2.50
5	2.25	2.25	2.50
6	2.25	2.25	2.50
7	2.25	2.25	2.50

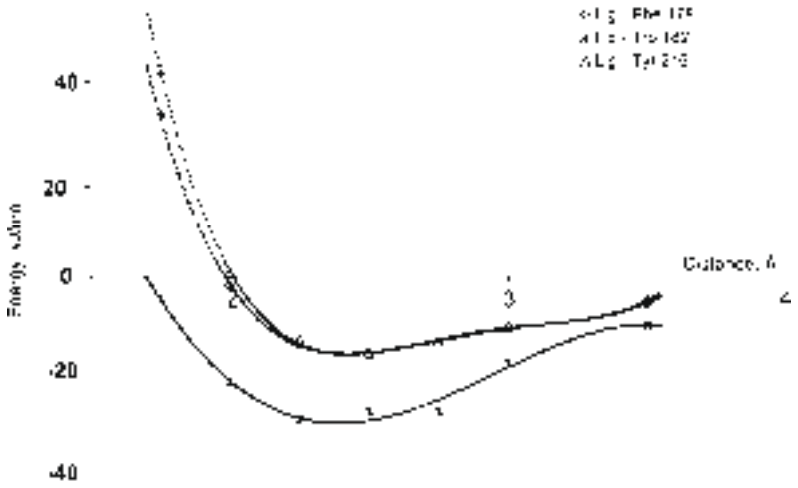


Figure 4. Calculated optimal distances between receptor amino acid residues and ligand **4**.

In order to verify these results, one *ab initio* MP2 calculation was performed using the same basis set and the obtained results were similar to those obtained with BH&H DFT calculations (Table III).

TABLE III. Stabilization energies for various ligands calculated using the MP2 and BH&H method

Ligand	Stabilization energy, kJ/mol	
	MP2	BH&H
1	–38.07	–56.48
2	–38.49	–56.90
3	–42.68	–60.67
4	–30.12	–53.97
5	–30.12	–51.88
6	–38.49	–56.07
7	–26.36	–45.19

EXPERIMENTAL

Docking analysis

Ligand docking of the ligands in Table I was performed by simulated annealing using the Affinity module from InsightII in the SGI Octane2 workstation.[13] All ligands were docked in the protonated form (protonated N-1 nitrogen on the piperazine part), using the CFF91 force field. Docking was performed using the dopamine D2 model and the binding site published earlier.[8] During the docking procedure, the amino acid residues within the binding site were flexible, while the other amino acid residues of the protein were constrained. The initial position of the ligand in the binding site was arbitrary, with respect to the arylpiperazine part facing TM6 and TM7 (*i.e.*, Tyr 216, Try 182 and Phe 178), while the protonated nitrogen on the piperazine part was kept in close proximity to Asp86. After the initial placement of the ligand,

no further constraints were applied. A docking procedure based on the Monte Carlo methodology was performed and the obtained structures were finally optimized in order to remove steric strain, with a gradient limit of 0.0042 kJ/mol or 4000 optimization steps.[14]

The results were visualized using DS Visualizer, version 1.7, and the obtained images were rendered using PovRay Raytracer, version 3.6.[15] Structures and affinities of the investigated ligands are given in Table I and the docking results are shown in Fig. 2.

Ab initio calculations

Gaussian 03W[16] was used to carry out the calculations of the energy contribution of the chosen ligand–receptor assembly. All structures were subjected to geometry optimization using the HF method and the 6-31g+* basis set until the energy minima were attained. The mutual orientation of the interacting groups were taken from the results of docking analysis and later adjusted as required. The stabilization energies of the paired structures were calculated as the difference between the dimer and the separate molecular entities using the BH&H DFT and MP2 method, with the 6-31g+* basis set. All the reported energies were corrected for the basis set superposition error using the counterpoise method of Boys and Bernardi.[17]

RESULTS AND DISCUSSION

At present, it is well known that for the binding of ligands with the arylpiperazine moiety, the formation of a salt bridge between the protonated N-1 nitrogen of arylpiperazine and the negatively charged Asp 86 is required.[4] This interaction guides the ligand towards its binding site, most probably by a zipper-like mechanism,[17] leading to interactions with key residues in the receptor binding site. For substituted arylpiperazines, both hydrophobic and/or H-bonding interactions of the substituent with amino acid residues from TM6 and TM7 also play an important role in the binding.

Docking results confirmed that all the investigated ligands, can form a short salt bridge with Asp 86, within range of 1.68 to 1.80 Å,[2,7] and fit inside the proposed hydrophobic pocket formed by amino acid residues on TM6 and TM7. Since all the investigated ligands form a short salt bridge with Asp 86, and have the same benzimidazole part of the ligand, it can be concluded that the differences in ligand affinity must arise from the number of proposed ETF interactions that the ligand can form with Tyr 216, Trp 182 and Phe 178 (Fig. 1).

BH&H Calculation of the stabilization energy for the receptor–ligand model system, designed to take into account only these ETF interactions, produced the following results:

– Although ligand **7** can fit into the hydrophobic pocket of the receptor, formed by Tyr 216, Trp 182 and Phe 178, the reason for its low affinity lies the fact that the introduction of a N atom into the aromatic part of the molecule decreases the ETF interactions, due to the withdrawal of electrons from the center of the aromatic ring. The calculated stabilization energy for this ligand showed destabilization by 11.72 kJ/mol compared to ligand **1**.

– Ligands **2**, **5** and **6** have a similar affinity or show a minimal increase in affinity compared to ligand **1**. They can form the same type and number of ETF

interactions as ligand **1**. The calculated energies for these ligands varied in range from –0.50 to 0.50 kJ/mol compared to ligand **1**, with the exception of ligand **5**, which showed destabilization by 4.60 kJ/mol. Such an amount of destabilization energy would usually lead to a significant loss of affinity, but ligand **5** showed a relatively small difference in affinity compared to ligand **1**. The reason for this unusual behavior could be that ETF interactions in ligand **5**, containing a halogen atom, could involve interactions with halogen atom itself instead of hydrogen. Although the measurements and crystal data analyzed so far confirm, without doubt, increased dispersion forces with the halogen atom present as a vicinal substituent in the aromatic ring, the real nature of the C–X···π interactions are still not completely resolved and this question is still open for discussion.[19] If this is the case, the geometry of the studied model system should be altered in order to take account of this possibility.

– Although they can form the same number of multiple ETF interactions as ligands **2**, **5** and **6**, ligands **3** and **4** show increased affinity compared with ligand **1**. Increased affinity of ligand **3** is a consequence of the enlarged aromatic surface with a stronger and larger electrostatic potential when compared to **1**. This leads, as expected, to stronger dispersive ETF interactions with Tyr 216 and Trp 182. Hence, as expected, the calculated stabilization energy for this ligand was larger by –4.19 kJ/mol than that of ligand **1**. On the other hand, ligand **4** displayed a decrease in stabilization energy calculated by these methods (2.51 kJ/mol less than **1**), but this is followed by a strong increase in affinity. It is obvious that, in this case, something else in addition to dispersive ETF interactions is responsible for the affinity increase, which was not taken into account. From the structure of the ligand and the binding site of the protein, it can be seen that it is very likely that the methoxy group acts as a hydrogen acceptor for one of the possible hydrogen donors in the vicinity of Tyr 216 or Trp 182. This hydrogen bond is an interaction much stronger than the dispersive ETF interactions considered herein and is a key interaction for the binding of this ligand into this part of the binding site.

CONCLUSIONS

From the results presented herein, it is obvious that there is a significant correlation between the strength of the calculated stabilization energies (and thus with ETF interactions) and affinity of the ligands. This finding supports the hypothesis about the importance of ETF interactions for the affinity of arylpiperazine-type ligands. Ligand **4** was excluded from the present calculations as there are reasons for believing that most important interaction responsible for its affinity is not a dispersive ETF interaction, calculated in this study, but a hydrogen bond. In this case, it can easily be seen that the correlation coefficient of affinities and stabilization energies calculated using BH&H is 0.81, and 0.91 for MP2, and r^2 is 65.5 and 82.0 %, respectively. Such high values identify a strong correlation

between the strength (and number) of edge-to-face interactions and the affinity of a ligand in this case.

Moreover, it can be seen that both the employed methods, although very different in their demands for computer time and resources, gave results with acceptable accuracy. This indicates that the BH&H method may be used in addressing the calculation of ETF stabilization energy in similar systems. The use of the BH&H method is recommended due to the fact that this method is, on average, 100 % faster than the MP2 method (2 h *vs.* 58 min for the pair ligand **4**–Phe 178 on a reference PC system*).

Although several different interactions are responsible for the binding of the investigated ligands to the dopamine D2 receptor, it is obvious that neglecting weaker interactions can lead one astray. Investigation of large systems can sometimes proceed with difficulties due to their size and limitations in time, space and instrumental strength. Is such cases, it is always wise to study ligand–receptor interactions separately and then to combine these results to give the final picture. In view of the fact that aromatic interactions are responsible for many important interactions and phenomena in chemistry and biology, a better understanding of these forces could lead to advancements in medicinal chemistry research.

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ИЗВОД

УТИЦАЈ ДИСПЕРЗИВНИХ ИНТЕРАКЦИЈА НА АФИНИТЕТ ВЕЗИВАЊА ЛИГАНАДА СА АРИЛПИПЕРАЗИНСКОМ ФУНКЦИЈОМ ЗА ДОПАМИНСКИ D2 РЕЦЕПТОР

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Неколико изостерних 1,3-дихидро-5-[2-(4-арил-1-пиперазинил)етил]-*2H*-бензимидазол--2-тиона је коришћено да би се испитале интеракције различитих лиганада са везивним местом допаминског D2 рецептора. Због ограничења метода коришћених за симулацију везивања, анализа ових резултата није могла да покаже прецизније које интеракције утичу на афинитет везивања лиганада. Претпостављено је да дисперзивне силе, или прецизније, тзв. *edge-to-face* интеракције, играју значајну улогу у процесу везивања, нарочито у липофилном делу лиганда. Да би се потврдила ова хипотеза применом *ab initio* израчунавања на модел систем, покушано је израчунавање стабилизационе енергије ових интеракција и њено довођење у везу са афинитетом лиганада. Добијени резултати указују да постоји значајна корелација између јачине дисперзивних интеракција и афинитета лиганда. Показано је да се при израчунавању стабилизационих енергија моделованих комплекса лиганд–рецептор може употребити Becke-ова „*half-and-half*" хибридна DFT метода, што значајно смањује време потребно за израчунавања и потребне рачунарске ресурсе.

(Примљено 17. фебруара, ревидирано 21 априла 2009)

* Intel® Pentium® Core™2Quad CPU processor 2.4 GHz, 4 GB RAM memory.

REFERENCES

1. C. Missale, S. R. Nash, S. W. Robinson, M. Jaber, M. G. Caron, *Physiol. Rev.* **78** (1998) 189
2. V. Šukalović, M. Zlatović, D. Andrić, G. Roglić, S. Kostić-Rajačić, V. Šoškić, *Arch. Pharm. Pharm. Med. Chem.* **337** (2004) 502
3. L. Shi, J. Javitch, *Annu. Rev. Pharmacol. Toxicol.* **42** (2002) 437
4. M. Y. S. Kalani, N. Vaidehi, S. E. Hall, R. J. Trabanino, P. L. Freddolino, M. A. Kalani, W. B. Floriano, V. W. T. Kam, W. A. Goddard, *Proc. Natl. Acad. USA* **101** (2004) 3815
5. M. V. Zlatović, V. V. Šukalović, C. Schneider, G. M. Roglić, *Bioorg. Med. Chem.* **14** (2006) 2994
6. A. K. Tewari, R. Dubey, *Bioorg. Med. Chem.* **16** (2008) 126
7. A. V. Muchldorf, D. V. Engen, J. C. Warner, A. D. Hamilton, *J. Am. Chem. Soc.* **110** (1988) 6561
8. V. Šukalović, M. Zlatović, D. Andrić, G. Roglić, S. Kostić-Rajačić, V. Šoškić, *Arzneim. Forsch./Drug Res.* **55** (2005) 145
9. *Insight II, Binding Site Analysis, Affinity, Discover, and WebLab Viewer are molecular modeling software*, corresponding modules and visualization software from Accelrys, Inc., San Diego, CA, http://www.accelrys.com (accessed 15 April, 2009)
10. M. P. Waller, A. Robertazzi, J. A. Platts, D. E. Hibbs, P. A. Williams, *J. Comput. Chem.* **27** (2006) 491
11. V. V. Šukalović, M. V. Zlatović, G. M. Roglić, S. V. Kostić-Rajačić, D. B. Andrić, *Acta Chim. Slov.* **56** (2009) 270
12. J. Vondrášek, L. Bendová, V. Klusák, P. Hobza, *J. Am. Chem. Soc.* **127** (2005) 2615
13. Silicon Graphics, Inc., CA, http://www.sgi.com/ (accessed 15 April, 2009)
14. M. V. Zlatović, V. V. Šukalović, S. V. Kostić-Rajačić, D. B. Andrić G. M. Roglić, *J. Serb. Chem. Soc.* **71** (2006) 1125
15. *PovRay Persistence of Vision Raytrace*, PovRay team, http://www.povray.org (accessed 15 April, 2009)
16. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, Hratchian, H. P.; J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03, Revision E.01*, Gaussian, Inc., Wallingford, CT, 2004.
17. S. F. Boys, F. Bernardi, *Mol. Phys.* **19** (1970) 553
18. A. S. Burgen, G. C. Roberts, J. Feeney, *Nature* **253** (1975) 753
19. C. D. Tatko, M. L. Waters, *Org. Lett.* **6** (2004) 3969.