



COMPUTATIONAL STUDIES AND SUPERIMPOSITION OF CELECOXIB OVER CRYSTAL LIGAND USING SCORING FUNCTION

AJAY P. BABU^{*a}, P. SATISH^a, BHAGVATI SARMA^a, BHANU KIRAN^a,
SAI LAXMI and SRINIVAS LODAGALA^b

^aProGene biosciences, 103, Bharat Towers, Dwaraka Nagar,
VISAKHAPATNAM – 530016 (A.P.) INDIA

E-mail: ajay@progenebio.com

^bDepartment of Pharmaceutical Sciences, Andhra University,
VISAKHAPATNAM – 530003 (A.P.) INDIA

ABSTRACT

Computational studies were initiated on one of the selective COX–2 inhibitors, Celecoxib to identify the orientation and geometry for superimposition within active site space. Superimposition of Celecoxib onto X–ray crystal ligand, SC–58 carried out in 3 stages, by superimposing onto (i) SO₂–NH₂ group (ii) 5 membered ring and (iii) 6 membered group with respect to the identified 4 dihedral angles. Potential Energy maps generated on each superimposed molecule against each dihedral angle and the orientations were subjected to X–score, scoring function program against COX–2 enzyme, 1CX2, taken from PDB (Protein Data Bank). Each dihedral oriented molecule is scored and ranked based on interaction with active site residues. Therefore, we attempted to identify the orientation and binding energy of 4 dihedral angles of Celecoxib superimposed over 3 different groups on X–ray crystal ligand. Of the data generated, it was identified that the 5 membered ring superimposition resulted in better orientation than the other 2 groups.

Key words : Protein Data Bank (PDB), X–Score, Non–steroidal anti–inflammatory drugs (NSAIDs), Cyclooxygenase (COX), van der Waals.

INTRODUCTION

The non–steroidal anti–inflammatory drugs (NSAIDs) have been among the most widely used drugs for the treatment of pain and inflammation. NSAIDs develop their mode of action by blocking the Cyclooxygenase (COX) enzyme and thus the biosynthesis of PGs (Prostaglandins).¹ Two isoforms of the COX enzyme have been characterized: cyclooxygenase–1 (COX–1) and cyclooxygenase–2 (COX–2)²

* Corresponding author

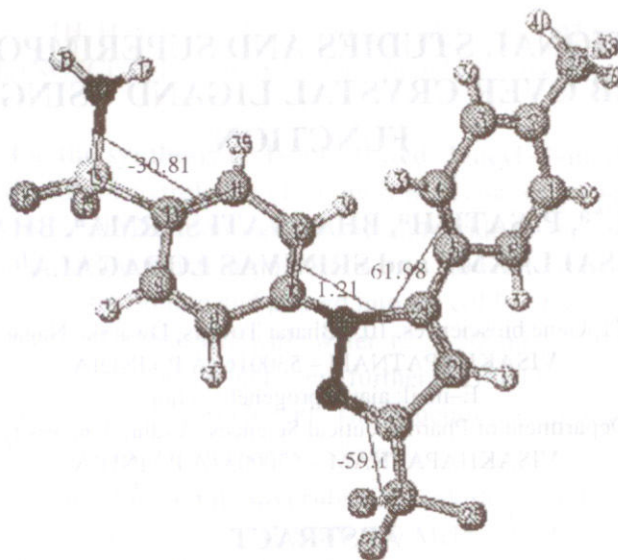


Fig. 1. The ball and stick representation of Celecoxib showing 4 dihedral angles

COX prostaglandin H₂ synthase is a key enzyme in the biosynthesis of PGs mediating inflammation and other important physiological processes. COX-1, a housekeeping enzyme, is constitutively expressed in nearly all tissues, and mediates physiological responses (e.g., cytoprotection of the stomach, platelet aggregation, and regulation of renal blood flow). On the other hand, COX-2 expressed by cells that mediate inflammation (e.g., macrophages, monocytes, synoviocytes) has been recognized as the isoform that is primarily responsible for the synthesis of the prostanoids involved in pathological processes, particularly those related to acute and chronic inflammatory states.³

Based on selectivity for COX-1 and COX-2, COX inhibitors are classified into, selective COX-1 inhibitors, non-selective COX inhibitors, moderately selective COX-2 inhibitors, and highly selective COX-2 inhibitors⁴. Celecoxib, the first highly selective COX-2 inhibitor approved by US FDA is indicated against osteoarthritis and rheumatoid arthritis⁵. The anti-inflammatory activity of Celecoxib has been studied in various animal models. In vitro studies on human enzymes revealed that Celecoxib is 375 times more selective for COX-2 than for COX-1.⁶ Various classes of selective COX-2 inhibitors have been identified, where tricyclic molecules bearing a methylsulfonyl or sulfamoyl group (coxibs) have been extensively studied. Celecoxib is a 3-substituted 1,5-diaryl pyrazole^{7,8}.

Evidence has suggested that selective COX-2 inhibitors may also provide an opportunity for both cancer prevention and therapy.⁹

EXPERIMENTAL

X-ray crystallographic 3D structure of COX-2 protein, 1CX2¹⁰ selected for study was downloaded from protein data bank, PDB.¹¹ 1CX2 is a mouse cyclooxygenase-2 (prostaglandin synthase-2) enzyme with EC No.1. 14.99.1, having 3.0 Å resolution and with 0.216 R-value, respectively. The crystal ligand SC58 isolated from 1CX2 and selective COX-2 inhibitor, Celecoxib were chosen for investigating superimposition groups.

Chemical drawings software ISIS Draw¹² and chemical analysis software, CaChe Workspace Pro 6.1.1¹³ were chosen for analysis. 2-Dimensional celecoxib structure was drawn using ISIS Draw and the chemical properties were identified based on Lipinski's rule¹⁴ of 5 evaluated from logp.com.¹⁵ A three dimensional molecule, Celecoxib was drawn using CaChe 6.1.1 workspace. The structure of Celecoxib with 4 dihedral angles and atom number are shown in Figure 1. All bond lengths and bond angles were set as per the standards. The final structure was adjusted and refined with respect to valence, hybridization, rings and geometry of the molecule. Geometry optimization carried out on Celecoxib by employing Conjugate Gradient and MM2/MM3 Augmented Force Field method with a convergence value 0.001 kcal/mol. The remaining work was carried under CaChe workspace.

Table 1. Four dihedral angles of Celecoxib with initial angles (geometry optimized structure) given in parenthesis. ^aDihedral angles calculated using Cache 6.1.1 software

Dihedral Angles	Atoms numbers (initial angles) ^a
dihedral angle 1	N14 – S13 – C10 – C11 (– 30.81)
dihedral angle 2	C12 – C7 – N19 – C20 (1.21)
dihedral angle 3	N19 – C20 – C5 – C4 (61.98)
dihedral angle 4	F26 – C24 – C22 – N23 (–59.18)

SC-58 x-ray crystal ligand extracted from 1CX2.pdb. Celecoxib structure copied onto SC-58 and the coordinates (x, y, z) of both the SO₂-NH₂ groups were matched and superimposed by selecting C8, C10 and C12 carbon atoms of benzene ring attached to SO₂-NH₂ group. Dihedral angles representing the number of rotatable bonds with their respective atom numbers on Celecoxib are displayed in Table 1. A total of four dihedral angles were identified on the molecule. Dihedral search carried out for single, ionic and coordinate bonds between – 180° and + 180° in 2 steps along with a double bond search between – 180° and 180° in 1 step. The search performed by including terminal groups. Each dihedral angle varied for 150° rotation.

A potential energy map generated by selecting a 'Standard procedure' method using 'conformations of long chain' property. The optimization method was set to Block-Diagonal Newton Raphson with CaChe MM3 Augmented force field. The convergence criterion is set at

about 0.001 kcal/mol with maximum updates of up to 300. van der Waals cut-off distance is 9.0 Å while electrostatic interactions were defined using MM2/MM3 bond dipoles. The resulted P.E. map against selected molecules with lowest energy was displayed and all the conformers were subjected to X-score scoring function¹⁶ program under Linux. An example of the Potential Energy map with the lowest possible energy dihedral for SO₂-NH₂ superimposed groups is given in Figure 2.

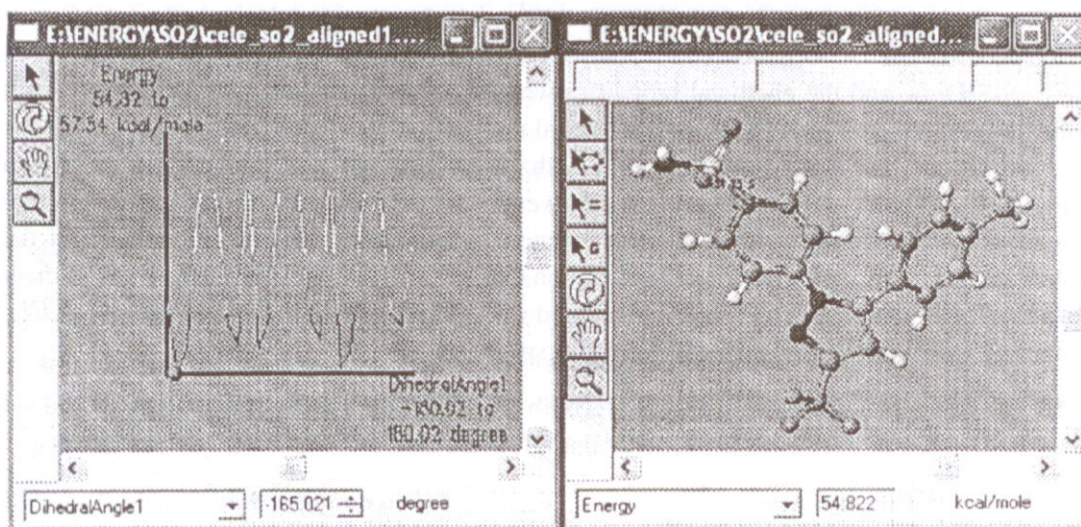


Fig. 2. The potential Energy map for SO₂-NH₂ superimposed group showing the lowest possible energy (54.822 kcal/mole) with dihedral angle - 165.021 degrees

X-Score computes a binding score for a given protein-ligand complex structure based on the three empirical scoring functions viz. HPScore, (Hydrophobic Pair) HMScore (Hydrophobic Match) and HSScore, (Hydrophobic Surface), respectively. The interaction energy between protein and ligand is computed as the binding affinities and by steric and electrostatic interactions, however, the energy computed is only an approximation to the enthalpy change in the binding process.

The overall binding energy term in X-Score accounts for van der Waals interaction, H-bonding, deformation effect and hydrophobic effect between ligand and the protein¹⁶. The program calculated binding energy that corresponds to the non-bonded interactions of the ligand with respective active site amino residues. Apart from generating scores, the program resulted in generating ranks based on highest score and highest binding energy. The best three ranking hits for SO₂-NH₂ superimposed groups on Celecoxib (for 4 dihedral angles) are given in Table 2.

Table 2. SO₂-NH₂ superimposed results for dihedrals 1, 2, 3 and 4 with the first three ranked dihedrals displayed the average X-score and binding energy

Rank	HP ^a Score	HM ^b Score	HS ^c Score	Average Score	Dihedral molecule	Dihedral angle	Binding Energy
SO₂-NH₂ superimposed dihedral 1 (N14 – S13 – C10 – C11)							
1	5.31	5.45	4.99	5.25	SO2_dihedral_11.csf	-30.01	-7.16
2	5.30	5.46	4.99	5.25	SO2_dihedral_17.csf	59.93	-7.16
3	5.30	5.46	4.99	5.25	SO2_dihedral_10.csf	-44.94	-7.16
SO₂-NH₂ superimposed dihedral 2 (C12 – C7 – N19 – C20)							
1	5.32	5.70	5.00	5.34	SO2_dihedral2_25.csf	177.53	-7.31
2	5.28	5.67	4.97	5.31	SO2_dihedral2_24.csf	165.49	-7.27
3	5.22	5.61	4.90	5.24	SO2_dihedral2_23.csf	150.33	-7.19
SO₂-NH₂ superimposed dihedral 3 (N19 – C20 – C5 – C4)							
1	5.31	5.75	5.01	5.35	SO2_dihedral3_22.csf	134.96	-7.30
2	5.28	5.73	4.98	5.33	SO2_dihedral3_23.csf	149.79	-7.27
3	5.28	5.71	4.98	5.32	SO2_dihedral3_9.csf	-60.02	-7.26
SO₂-NH₂ superimposed dihedral 4 (F26 – C24 – C22 – N23)							
1	5.35	5.63	5.03	5.34	SO2_dihedral4_22.csf	135.07	-7.30
2	5.36	5.51	5.04	5.31	SO2_dihedral4_6.csf	-104.99	-7.26
3	5.32	5.59	5.00	5.30	SO2_dihedral4_23.csf	150.10	-7.26

a, b and c represent hydrophobic pair, hydrophobic match and hydrophobic surface. The molecular formula, molecular weight and log P for all examples are C₁₇H₁₄N₃O₂SF₃, 381.3 and 4.40, respectively

Similar procedure was carried out by superimposing 5 membered ring and 6 membered ring on the crystal SC-58 ligand and Celecoxib. The resulted best three scores and ranks with respect to different dihedrals are listed in Tables 3 and 4, respectively.

Table 3. 5meb superimposed results for dihedrals 1, 2, 3 and 4 with the first three ranked dihedrals displayed the average X-score and binding energy

Rank	HP ^a Score	HM ^b Score	HS ^c Score	Average Score	Dihedral molecule	Dihedral angle	Binding Energy
5meb superimposed dihedral 1 (N14 – S13 – C10 – C11)							
1	3.38	4.90	3.24	3.84	5meb_dihedral1_1.csf	-172.85	-5.24
2	3.38	4.90	3.24	3.84	5meb_dihedral1_2.csf	-164.55	-5.24
3	5.56	5.06	4.83	5.15	5meb_dihedral1_3.csf	-150.02	-5.24
5meb superimposed dihedral 2 (C12 – C7 – N19 – C20)							
1	5.59	5.11	4.86	5.19	5meb_dihedral2_21.csf	120.18	-7.08
2	5.56	5.07	4.84	5.16	5meb_dihedral2_23.csf	150.33	-7.03
3	5.56	5.06	4.83	5.15	5meb_dihedral2_22.csf	135.25	-7.02
5meb superimposed dihedral 3 (N19 – C20 – C5 – C4)							
1	5.49	5.06	4.83	5.13	5meb_dihedral3_4.csf	-134.98	-7.00
2	5.49	5.02	4.78	5.10	5meb_dihedral3_3.csf	-150.47	-6.96
3	5.45	5.04	4.80	5.10	5meb_dihedral3_5.csf	-120.01	-6.95
5meb superimposed dihedral 4 (F26 – C24 – C22 – N23)							
1	5.50	5.05	4.84	5.13	5meb_dihedral4_23.csf	150.10	-7.00
2	5.50	5.05	4.84	5.13	5meb_dihedral4_24.csf	165.19	-7.00
3	5.50	5.05	4.84	5.13	5meb_dihedral4_8.csf	-75.00	-7.00

a, b and c represent hydrophobic pair, hydrophobic match and hydrophobic surface. The molecular formula, molecular weight and log P for all examples are C₁₇H₁₄N₃O₂SF₃, 381.3 and 4.40, respectively

Table 4. 6meb superimposed results for dihedrals 1, 2, 3 and 4 with the first three ranked dihedrals displayed the average X-score and binding energy

Rank	HP ^a Score	HM ^b Score	HS ^c Score	Average Score	Dihedral molecule	Dihedral angle	Binding Energy
6meb superimposed dihedral 1 (N14 – S13 – C10 – C11)							
1	3.89	4.63	3.92	4.15	6meb_dihedral1_11.csf	-30.01	-6.45
2	3.87	4.59	3.89	4.12	6meb_dihedral1_10.csf	-44.94	-6.43
3	3.86	4.57	3.88	4.10	6meb_dihedral1_18.csf	75.04	-6.43
6meb superimposed dihedral 2 (C12 – C7 – N19 – C20)							
1	3.85	4.58	3.87	4.10	6meb_dihedral2_25.csf	177.53	-6.55
2	3.84	4.59	3.86	4.10	6meb_dihedral2_12.csf	-14.70	-6.46
3	3.83	4.59	3.86	4.10	6meb_dihedral2_13.csf	-2.63 [°]	-6.49
6meb superimposed dihedral 3 (N19 – C20 – C5 – C4)							
1	3.89	4.74	3.93	4.19	6meb_dihedral3_8.csf	-75.01	-6.70
2	3.89	4.74	3.92	4.18	6meb_dihedral3_7.csf	-90.01	-6.68
3	3.89	4.74	3.92	4.18	6meb_dihedral3_20.csf	105.02	-6.67
6meb superimposed dihedral 4 (F26 – C24 – C22 – N23)							
1	3.88	4.48	3.91	4.09	6meb_dihedral4_14.csf	15.34	-6.46
2	3.88	4.48	3.91	4.09	6meb_dihedral4_16.csf	45.02	-6.45
3	3.88	4.48	3.91	4.09	6meb_dihedral4_17.csf	60.01	-6.45

a, b and c represent hydrophobic pair, hydrophobic match and hydrophobics surface. The molecular formula, molecular weight and logP for all examples are C₁₇H₁₄N₃O₂SF₃, 381.3 and 4.40, respectively

RESULTS AND DISCUSSION

Dihedral angles selected as the criteria to evaluate the binding energy of Celecoxib pharmacophore with active site residues. Interactions between ligand and protein play an important role in defining the activity of the protein.

For the four dihedral angles identified, the first run was carried out by considering dihedral angle 1 (atoms N14 – S13 – C10 – C11) and subjected to potential energy map generation. Similar procedure employed for the remaining dihedral angle 2 (atoms C12 – C7 – N19 – C20), dihedral angle 3 (atoms N19 – C20 – C5 – C4) and dihedral angle 4 (atoms F26 – C24 – C22 – N23), respectively. The potential energy map for the dihedral angle search carried out by defining geometry label search between -180° to $+180^{\circ}$ angles with 15° rotations resulted in 25 molecules for each dihedral angle (1, 2, 3 and 4) selected. All molecules were converted from .csf format of CaChe to SYBYL Mo12 format using Mol2Mol format conversion program¹⁷. The molecules are then selected and screened by employing X-Score program under Linux.

It is evident from Table 2, the first best hit having good interaction with the receptor amino acid residues has an average HP, HM, HS Score (for SO₂-NH₂ superimposed dihedral1) of about 5.25 represented by a dihedral angle of -30.01° with a binding energy of -7.16 kcal/mol. Similarly, for dihedral 2, 3 and 4, the average scores for the first best hits were, 5.34, 5.35 and 5.34, respectively, represented by dihedral angles of 177.53° , 134.96° and 135.07° with a predicted binding energy of -7.31 , -7.30 and -7.30 kcal/mol, respectively.

When compared with the statistics from Tables 3 and 4, the average HP, HM, HS Scores for 5membered and 6membered groups are not much significant when compared to the scores generated for different dihedrals of SO₂-NH₂ runs. This is because of the interactions of SO₂-NH₂ group with surrounding residues. The binding energy for 5-membered and 6membered superimposed groups ranged from -5.24 to -7.08 kcal/mol and -6.31 to -6.70 kcal/mol respectively for all the 4 dihedral angles under study, whereas the binding energy for SO₂-NH₂ superimposed groups ranged from -6.87 to -7.31 kcal/mol, which suggest that the SO₂-NH₂ group is an important pharmacophore to elicit biological interactions with active site residues.

However, superimposition of all the dihedrals for SO₂-NH₂ groups, 5-membered and 6membered groups onto crystal ligand have revealed that the 5-membered ring superimposition is most favored than others. This is due to the orientation of 5-membered ring dihedrals which exhibited same orientation and conformation as crystal ligand. Therefore, it can be sought that the superimposition of 5-membered ring leads to better orientation but leads to poor scoring. The difference in scoring pattern with SO₂-NH₂ groups for 5-membered ring is due to the conformational flexibility of ligand interactions with the active site residues.

As the score being generated using X-score is based on interactions of pharmacophore groups on ligand, the SO₂-NH₂ group superimposition exhibited better results, whereas the 5membered group resulted in poor interaction and a low score than the former group. When the orientation of ligand is considered, it is the 5membered ring that had shown good orientation within the active site space than the SO₂-NH₂ group. Therefore, changing the conformers of better oriented 5membered superimposed molecule would lead to better results and proper

interactions. As orientation of ligand within active site region is more important in invoking biological, structural and functional activity of the protein, the better oriented 5membered ring superimposition suggested that in order to study any analogs of either SC-58 crystal ligand or Celecoxib, it is necessary to look into the 5-membered ring orientation and SO₂-NH₂ group interaction with the ligand.

The property of ligand, its orientation in 3 dimensional space and geometry of the molecule are an important characteristic feature for better interactions with amino acid residues in the active site space. This feature is evident from our studies, which represent a method to study interactions of any ligand with reference to the 4 dihedral angles selected.

Not much difference observed in the predicted binding energy between 5membered and 6membered group superimposition, whereas the binding energy for SO₂-NH₂ group differed to a little extent, which shows that the orientation of Celecoxib within the active site should be done over the 5membered ring and the ligand – residue interactions to be calculated with the SO₂-NH₂ group. In this case, as evidenced by X-score scoring function, the orientation of ligand and interactions with residue for 5-membered ring superimposition leads to better results.

REFERENCES

1. J. R. Vane, Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature – New Biology*. **231**, 232 (1971).
2. M. K. O'Banion, H. B. Sadowski, V. Winn and D. A. Young, A serum- and glucocorticoid regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein, *J. Biol. Chem.*, **266**, 23261 (1991).
3. Chun Kyung-Soo and Surh Young-Joon, Signal transduction pathways regulating cyclooxygenase-2 expression: potential molecular targets for chemoprevention, *Biochem. Pharmacol.*, **68**, 1089 (2004).
4. D. O. Stichtenoth, Classification of COX-1 and COX-2 inhibitors. *Eur. J. Clin. Pharmacol.* **52**, A110 (1997).
5. B. Horatio, Fung, Harold L. Kirschenbaum, Selective Cyclooxygenase-2 Inhibitors for the Treatment of Arthritis. *Clinical Therapeutics*, **21**, 7 (1999).
6. T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, L. F. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. D. Anderson, E. G. Burton, J. N. Cogburn, S. A. Gregory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang and P. C. Isakson, Synthesis and biological evaluation of the 1,5-diarylpirazole class of cyclooxygenase 2 inhibitors: Identification of 4-[methylphenyl]-3- (trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulfonamide (SC-58635, Celecoxib) *J. Med. Chem.*; **40**, 1347 (1997).

7. H. Hashimoto, K. Imamura, J. Haruta and K. Wakitani, 4-(4-Cycloalkyl/aryl-oxazol-5-yl) benzenesulfonamides as selective cyclooxygenase-2 inhibitors: Enhancement of the selectivity by introduction of a fluorine atom identification of a potent, highly selective, and orally active COX-2 inhibitor JTE-522, *J. Med. Chem.*, **45**, 1511 (2002).
8. R. W. McMurray and K. J. Hardy, Cox-2 inhibitors: today and tomorrow, *Am. J. Med. Sci.* **323**, 181 (2002).
9. W. F. Anderson, A. Umar and E. T. Hawk, *Expert Opin. Pharmacother.* **4**, 2193 (2003).
10. R. G. Kurumbail, A. M. Stevens, J. K. Gierse, J. J. McDonald, R. A. Stegeman, J. Y. Pak, D. Gildehaus, J. M. Miyashiro, T. D. Penning, K. Seibert, P. C. Isakson and W. C. Stallings, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, **384**, 644 (1996).
11. <http://www.rcsb.org/pdb>
12. <http://www.mdli.com/>
13. <http://www.cachesoftware.com/>
14. Christopher A. Lipinski, Franco Lombardo, Beryl W. Dominy and Paul J. Feeney "Experiment and computational approaches to estimate solubility and permeability in drug discovery and development settings", *Adv. Drug Delivery Rev.*, **23**, 3 (1997).
15. <http://www.logp.com>
16. R. Wang, L. Lai and S. Wang, Further development and validation of empirical scoring functions for structure-based binding affinity prediction. *J. Comput.-Aided Mol. Des.* **16**, 11 (2002).
17. <http://web.interware.hu/frenzy/mol2mol/index.html>

Accepted: 23.1.2005