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A model of the human M_2 muscarinic acetylcholine receptor

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Summary

The M_2 muscarinic acetylcholine receptor belongs to the family of rhodopsin like G-Protein Coupled Receptors. This subtype of muscarinic receptors is of special interest because it bears, aside from an orthosteric binding site, also an allosteric binding site. Based on the X-ray structure of bovine rhodopsin a complete homology model of the human M_2 receptor was developed. For the orthosteric binding site point mutations and binding studies with different agonists and antagonists are available. This knowledge was utilized for an initial verification of the M_2 model. Allosteric modulation of activity is mediated by structurally different ligands such as gallamine, caracurine V salts or W84 (a hexamethonium-derivative). Caracurine V derivatives with different affinities to M_2 were docked using GRID-fields. Subsequent molecular dynamics simulations yielded different binding energies based on diverse electrostatic and lipophilic interactions. The calculated affinities are in good agreement to experimentally determined affinities.

Introduction

Muscarinic cholinergic receptors belong to the large family of membrane receptors, which mediate signal transduction via coupling to heterotrimeric G-Proteins (GPCRs). For the muscarinic acetylcholine receptors five subtypes (M_1-M_5), sharing a large number of homologous amino acids, have been cloned. $M_2$and M_4 subtypes preferentially inhibit adenyl cyclase activity, whereas M_1, M_3 and M_5 preferentially stimulate phospholipase C activity.

After publication of the X-ray structure of bovine rhodopsin [1], a mammalian class A GPCR, the design of much more reliable GPCR models became possible. The model of the human M_2 muscarinic receptor (M_2 mAChR), reported here, gives insight into the binding of orthosteric agonists and antagonists.

Construction of the M_2 mAChR model

The model is based on the X-Ray structure of bovine rhodopsin (PDB 1F88 / 1HZX); the sequence of the human M_2 receptor was extracted from SwissProt (P08172) [2, 3]. Transmembrane regions of the M_2 receptor were predicted employing PsiPred [4, 5, 6] and SSPro2 [7], which performed best in predicting the lengths of the helices in the template structure. Another important information for the determination of the transmembrane parts (TMs) of the receptor is the so-called pin-points (Figure 1), detected by J.M. Baldwin *et al.* [8].

The extracellular and intracellular loops were created with a combination of different methods: secondary structure prediction [9, 10] as well as the application of a loop search routine based on homology aspects (implemented in the HOMOLOGY module of Insight II [11]).

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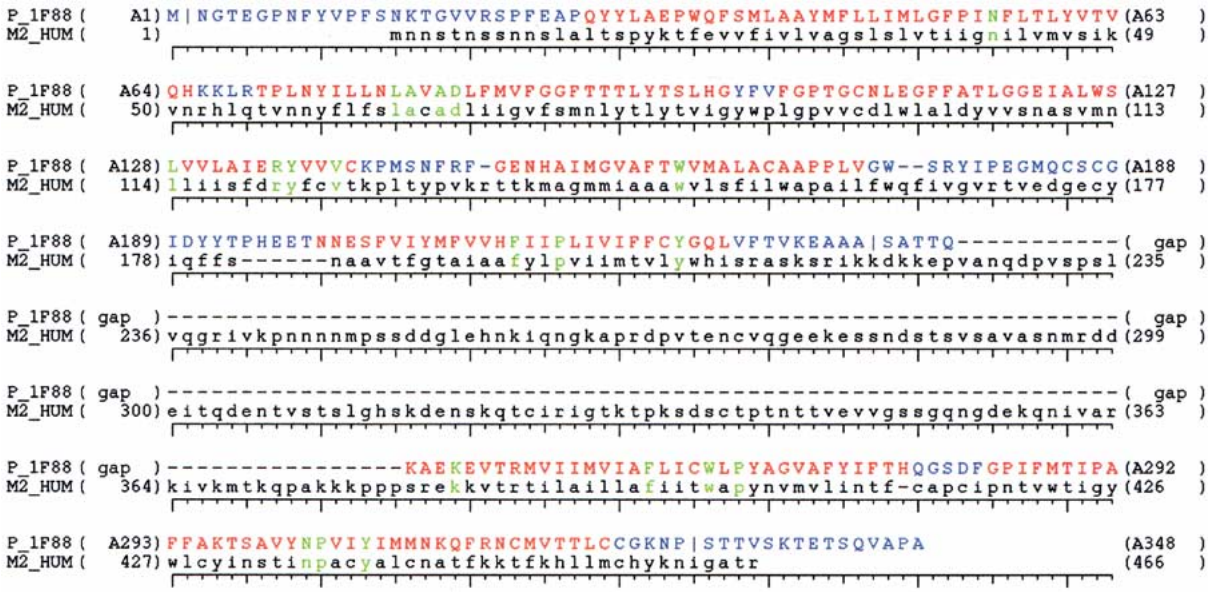


Figure 1. Alignment of the human M_2 sequence to bovine rhodopsin 1F88 blue, helices red, hM_2AchR black, pin-points green.

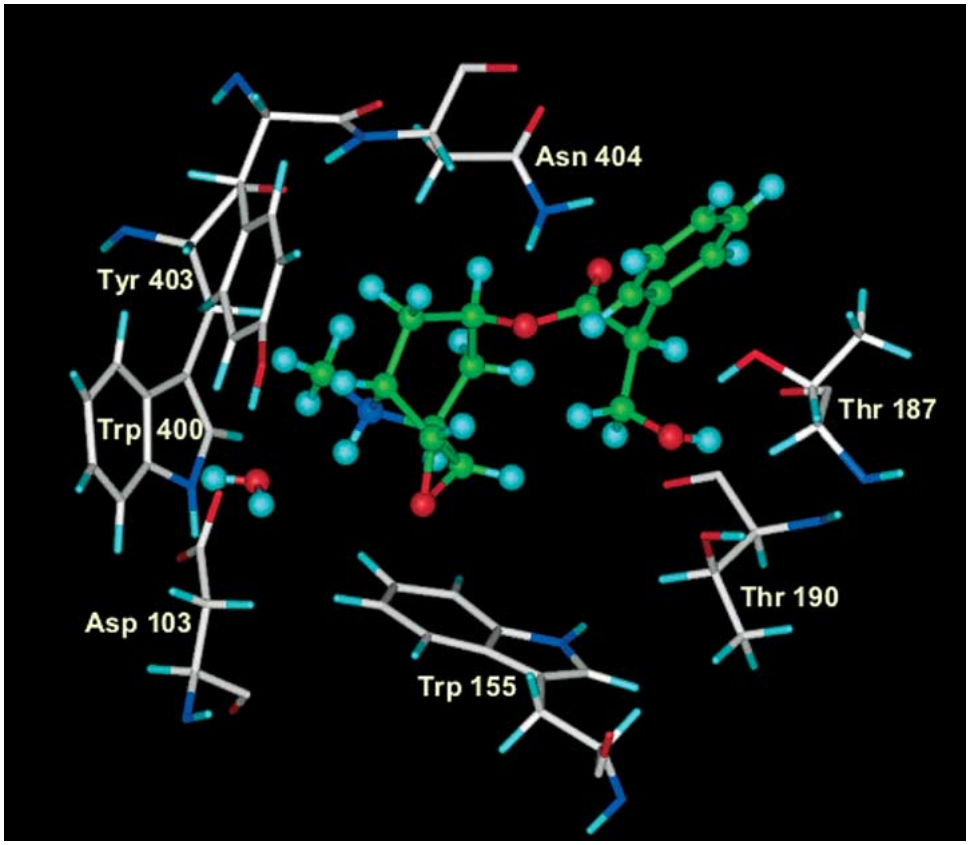


Figure 2. Orthosteric binding site of the M_2 model (white) with NMS (green).

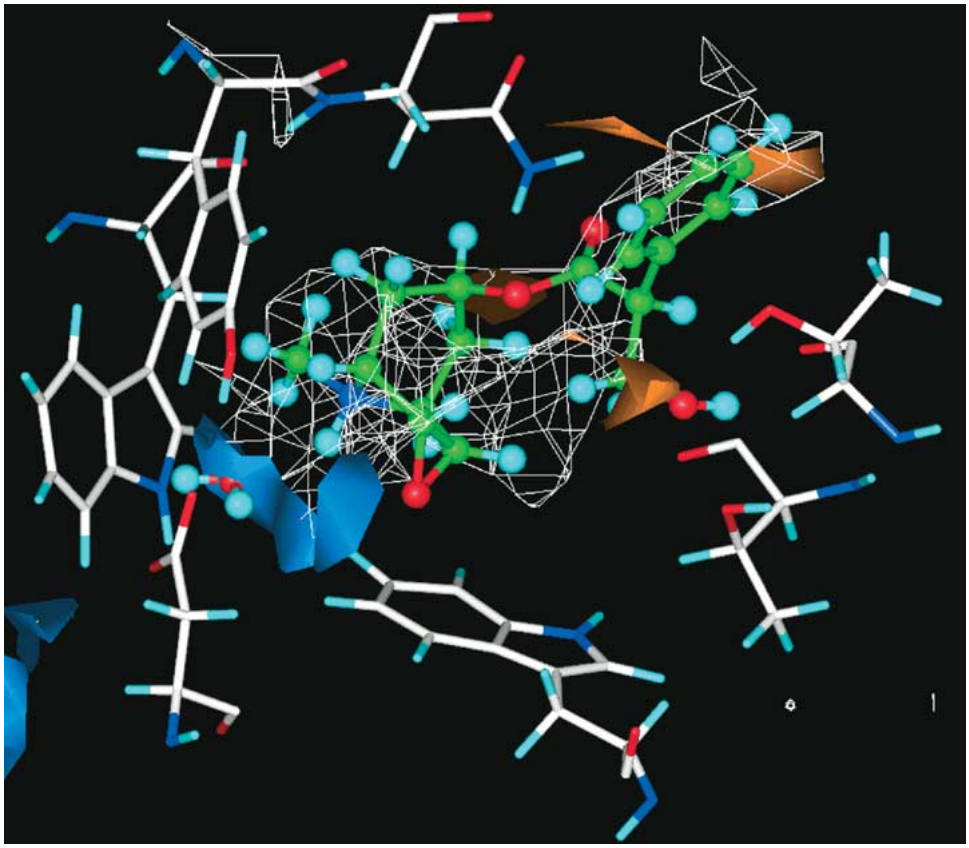


Figure 3. GRID interaction fields calculated for the uncomplexed hM_2AChR: Favourable regions of interaction between orthosteric site and ester probe, contoured at an energy level of −2.3 kcal/mol, orange; positively charged nitrogen probe, contoured at an energy level of −13.3 kcal/mol, blue; vinyl-aromatic probe, contoured at an energy level of −2,5 kcal/mol, white cage. NMS is displayed for comparison.

Extracellular loops

Length and predicted secondary structure of the first extracellular loop (E1) are very similar to the corresponding loop in bovine rhodopsin, so these coordinates were used. For the third loop (E3) a good template was found by homology searching. Acceptable coordinates for the second loop (E2) were found by taking a slightly different route. The coordinates of the conserved disulfide bridge between Cys96 (TM3) and Cys176 (E2) (including the directly neighboured amino acids) were adopted from the rhodopsin crystal structure. For the remaining segments on both sides of the disulfide bridge, loop searches were performed. According to secondary structure prediction, like in rhodopsin, a beta turn motif can be expected. However, in contrast to rhodopsin the turn probably is of β-I′-type in E2 compared to β-II′-type in rhodopsin. The loop search results were analysed and evaluated with regard to this proposition.

Intracellular loops

The first intracellular loop (I1) is, like its extracellular counter part, very similar in length and predicted secondary structure to the according structure in bovine rhodopsin. So again, the coordinates of bovine rhodopsin were accepted for the M_2 model. The second loop (I2) was still short enough for a loop search (< 20 amino acids) so coordinates could be generated along this line. However, the third intracellular loop is by far too long for a reliable loop search or even a loop generation, so it had to be replaced by 20 alanines.

N-terminus/C-terminus

Coordinates for the N-terminus were generated by secondary structure prediction [12]. The predicted secondary structure [7] for the C-terminal amino acids contains an extra helical part, similar to the structure found in bovine rhodopsin. Therefore, it seemed to

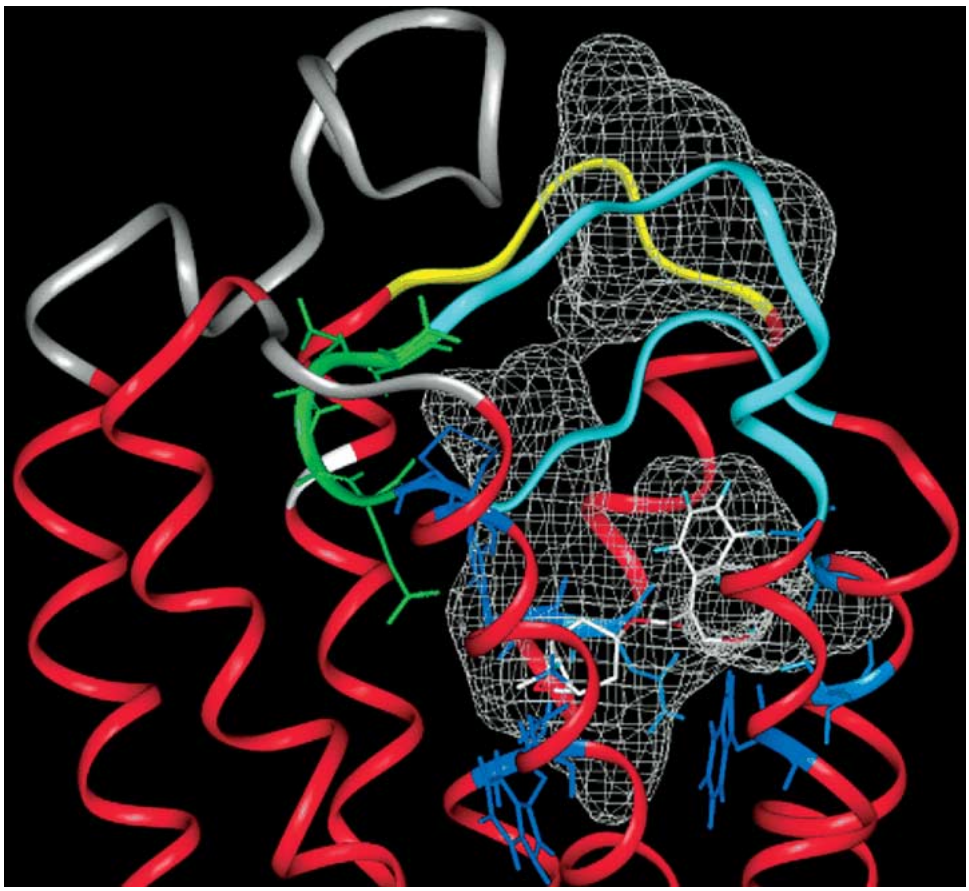


Figure 4. Position of the orthosteric and the common allosteric binding site in the M_2 receptor model. Helices red, E1 grey, E2 cyan /green, E3 yellow, orthosteric binding site blue, disulfide bridge magenta, EDGE green, accessible volumes white, NMS atom-type coded.

Figure 5. Pharmacophore of the caracurine V derivatives: Numbers according to Table 1: 1 yellow, 2 white, 3 orange, 4 green, 5 light blue, nitrogens dark blue, oxygens red.

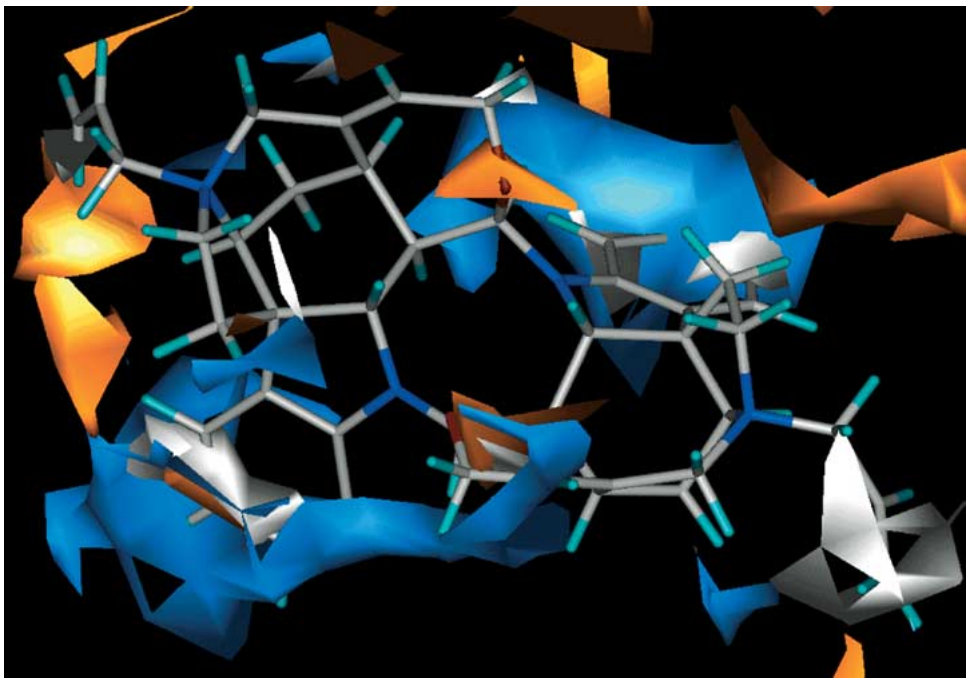


Figure 6. GRID interaction fields calculated for the uncomplexed hm2AChR projected onto diallylcaracurine V: Favourable regions of interaction between the allosteric site and ether probe, contoured at an energy level of −3.0 kcal/mol, orange; positively charged nitrogen probe, contoured at an energy level of −13.3 kcal/mol, blue; vinyl-aromatic probe, contoured at an energy level of −2,5 kcal/mol, white.

be reasonably to accept the coordinates of the X-ray structure also for this region of the model.

Molecular dynamics simulation

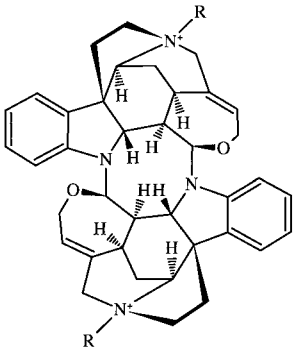
After completion, the model was minimized using steepest descent and conjugate gradient to obtain a stable low energy protein structure. To check whether the protocol for the molecular dynamics simulation (MDS) was suitable, identical simulations for the X-ray structure of bovine rhodopsin as well as the M_2 receptor model were performed. During the MDS, the geometry, which was monitored with PROCHECK [13, 14], was stable for both proteins. This pointed out that a backbone tether of 200 kcal/Å^2, a distance dependent dielectric constant of 2·r and the use of the physiological pH of 7.4 gave acceptable results when using the consistent valence force field (CVFF) [15].

Analysis of the orthosteric binding site

The orthosteric binding site is the classical binding site where the endogenous agonist acetylcholine or the competitive antagonist *N*-methylscopolamine (NMS) are bound. It is localized between helices three, four, five and six. Important amino acids for the binding of NMS are Asp103, Trp155, Trp400, and Tyr403. They interact with the protonated nitrogen of NMS via ion-ion and π-ion interactions. In addition, Asn404 is very important for donating a hydrogen bond to the ester oxygen of *N*-methylscopolamine. Less important for NMS binding are Thr187 and Thr190. A mutation from threonine to alanine reduces binding affinity to a third [16]. Considering these facts *N*-methylscopolamine was manually docked into the M_2 receptor model. For the sake of allowing a hydrogen bond between NMS's protonated nitrogen and Asp103, a bridging water molecule inside the binding site is postulated (Figure 2).

The position of *N*-methylscopolamine was stable in a MDS for 500 ps under above-mentioned conditions. After an initial equilibration, NMS showed the expected H-bonds to Asn404, Thr187, Thr190, and via the water molecule to Asp103. The location of NMS in the orthosteric binding site was further confirmed by applying the program GRID [17], which calculates non-covalent interaction potentials. For this purpose the positively charged nitrogen (N1+), the hydroxyl (O1), the ester (OES), the water (OH2) and the vinyl-aromatic probe (C1=) were used. As shown in

Table 1.



Compd	R	EC 50 [nM]
1	H	1187
2	CH_3	8
3		30
4		3
5		4

Figure 3 the generated interaction fields are in good agreement to the positions of the functional groups in NMS.

Analysis of the common allosteric binding site

The common allosteric binding site is located between E2, E3 and the upper part of TM 7, in direct contact to the orthosteric binding site. Although a common allosteric site is proposed, different epitopes of the protein are critical for ligand binding. In the case of gallamine, Asn419 in E3 and the acidic sequence Glu-Asp-Gly-Glu (EDGE) in E2 are important for muscarinic subtype selectivity [18]. Caracurine V derivatives are sensitive to a mutation of Thr423 in TM7 and a substitution of the E2 sequence with the corresponding sequence of M_5 [19].

As starting point for the detection of protein-ligand-binding modes, a set of five relatively rigid caracurine V derivatives with different binding affinities (Table 1) was used [20].

The free volume inside the receptor, calculated using the program SURFNET [21], was found to be large enough to accommodate the rigid ligands (Figure 4). Inside this cavity, GRID-fields [17] were computed to find clues for the ligand positioning. Subsequently the five ligand-receptor-complexes were minimized using conjugate gradients and a MDS was carried out (conditions as mentioned above). The resulting complexes were analysed considering the potential energy, the interaction energies as well as the complex geometries at both binding sites. When comparing the positions of the caracurine V derivatives after the MDS, it was found that the positions of the aromatic rings, the quaternary nitrogens and the ether oxygens of all five ligands are almost identical (Figure 5).

In order to confirm this finding from MDS, again GRID [17] was employed. In this case, the ether-probe (OC2), the positively charged nitrogen-probe (N1+), and the vinyl-aromatic probe (C1=) were taken (Figure 6). Especially the fields of the ether probe are in excellent agreement with the position of the caracurine V derivatives.

Conclusions

The described model of the human M_2 muscarinic acetylcholine receptor is in good agreement with the experimental knowledge and can be used to explain the experimental data at both binding sites. After further work on other allosteric modulators, it might be a useful tool for the design of new receptor mutants and hopefully might serve for the elucidation of the allosteric mechanism.

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References

1. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Le Trong, I., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M. and Miyano, M., Science, 289 (2000), 739.
2. Bairoch A. and Apweiler R., Nucleic Acids Res., 28 (2000), 45.
3. Gasteiger E., Jung E. and Bairoch A., Curr. Issues Mol. Biol., 3 (2001), 47.
4. McGuffin, L.J., Bryson K. and Jones, D.T., Bioinformatics, 16 (2000), 404.
5. Jones, D.T., J. Mol. Biol., 292 (1999), 195.
6. Jones, D.T., Taylor, W.R. and Thornton, J.M. Biochem., 33 (1994), 3038.
7. Baldi, P., Brunak, S., Frasconi, P., Pollastri, G. and Soda, G., Bioinformatics, 15 (1999), 937.
8. Baldwin, J.M., Schertler, G.F.X. and Unger, V.M., J. Mol. Biol., 272 (1997), 144.
9. Cuff, J.A. and Barton G.J., Proteins: Structure, Function and Genetics, 40 (1999), 502.
10. LOOPP: http://ser-loopp.tc.cornell.edu/loopp.html.
11. Insight II 2000, Accelrys Inc., San Diego, U.S.A.

12. Juretic D., Zoranic, L. and Zucic, D., J. Chem. Inf. Comp. Sci., accepted for publication.
13. Laskowski, R.A. MacArthur, M.W. Moss, D.S. and Thornton, J.M., J. Appl. Cryst., 26 (1993), 283.
14. Morris, A.L., MacArthur, M.W., Hutchinson, E.G. and Thornton, J.M., Proteins, 12 (1992), 345.
15. FDiscover, Accelrys, San Diego, U.S.A.
16. Heitz, F., Trumpp-Kallmeyer, S. and Hibert, M.F., Eur. J. Pharmacol., 380 (1999), 183.
17. Goodford P.J., J. Med. Chem., 28 (1985), 849.
18. Gnagey, A., Seidenberg, M. and Ellis, J., Mol. Pharmacol., 56 (1999), 1245.
19. Buller, S., Zlotos, D.P., Mohr, K. and Ellis, J., Mol. Pharmacol., 61 (2002), 160.
20. Zlotos, D.P., Buller, S., Tränkle, C. and Mohr K., Bioorg. Med. Chem. Lett., 10 (2000), 2529.
21. Laskowski, R.A., J. Mol. Graph., 13 (1995), 323.