Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(11):118-124
(http://derpharmachemica.com/archive.html)

Comparative evaluation of four scoring functions with three models of delta opioid receptor using molecular docking

Fatima Sapundzhi[1*], Tatyana Dzimbova[2], Nevena Pencheva[3] and Peter Milanov[1,4]

[1]*Faculty of Mathematics and Natural Sciences, South-West University "Neofit Rilski", Blagoevgrad, Bulgaria*
[2]*Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Faculty of Public Health and Sports, South-West University "Neofit Rilski", Blagoevgrad, Bulgaria*
[4]*Faculty of Mathematics and Natural Sciences, South-West University "Neofit Rilski", Blagoevgrad, Bulgaria*

ABSTRACT

Delta opioid receptor (DOR) is an attractive target for the treatment of brain disorders and design of selective and effective ligands is very important. Computer-assisted design of compounds could help much in this field if there is a good receptor model and appropriate algorithm for the corresponding receptor-ligand system. The aim of the present study was to find the most appropriate scoring functions and the model for docking of endogenous enkephalins and their analogues with delta-opioid receptor (DOR) that correlated well with preliminary data from in vitro bioassay such as: IC_{50} - potency, K_A - affinity, e_{rel} - efficacy. The capabilities of the four scoring functions, available in GOLD5.2 were explored with the following three different models of DOR: a) a theoretical model (ePDB id: 1ozc); b) a model obtained with homology modeling (Model B); and c) a crystal structure of human DOR (PDB id: 4ej4). Enkephalin analogues were consistently docked with each of the receptor models with each of the four scoring function. The analysis of the obtained results shows that after the docking with Model B the values of the scoring functions correlate negatively with the data from in vitro tests at the highest degree. Furthermore, the use of the ASP scoring function enable more precise docking of the test ligands as correlation coefficients were: ASP score/IC_{50} = - 0.86, and obtained correlation has a biological sense. Much higher value of fitness function is, the lower the value of concentration is, and i.e. its potency is greater.

Keywords: Scoring functions, Docking, GOLD Delta Opioid Receptor, Enkephalin analogues.

Highlights:

- Docking was performed with delta-selective enkephalin analogues and related compounds.
- Three models of DOR were studied.
- Four scoring functions with three models of DOR were applied.
- The best combination scoring function – model of DOR was found.
- The best results are obtained with our model of DOR.

Abbreviations: *ASP - Astex Statistical Potential; DOR – Delta Opioid Receptor; DPDPE – (4S,7S,13S)-7-Benzyl-3,3,14,14-tetramethyl-6,9,12-trioxo-13-(L-tyrosylamino)-1,2-dithia-5,8,11-triazacyclo tetradecane-4-carboxylic acid; GOLD - Genetic Optimisation for Ligand Docking; MMD – Molegro Molecular Dicking; PDB – Protein Data Bank; RDF – Radial Distribution Function; PLP – Picewise Liner Potential; RMSD – Root-Mean-Square Distance.*

INTRODUCTION

Delta opioid receptor (DOR) is an attractive target for the treatment of brain disorders has strengthened in recent years. It is broadly expressed in the brain, binds endogenous opioid peptides, and shows as functional profile highly

distinct from those of μ- and k-opioid receptors [1]. Design of selective and effective ligands of this receptor is still challenging task. Different analogues of enkephalins were synthesized and biologically tested [2]. Preliminary *in vitro* tests show that selective ligands could be obtained with modification in the primary structure of enkephalin molecule.

In the last years, in support of scientific work have appeared new approaches to computer-assisted design or so called *in silico* studies of new compounds. These methods help to shorten the time and reduce costs of research. In order to perform *in silico* studies important condition is to have the correct structure of the studied objects and suitable software for the experiment. First attempts in this field were made with published theoretical model (ePDB id: 1ozc), as docking was performed using Molegro Molecular Docker 1.1.1 (MMD) [3].This study did not lead to desired result, namely finding a correlation between the biological activity and data from docking most probably due to incorrect model of the receptor or inappropriate.

Further efforts in this direction [4], has led to the creation of new theoretical model, by using the homology modeling. Several models were obtained as the best of them gave the significant correlation with the results of docking with GOLD 5.2 (GoldScore scoring function) and *in vitro* tests. For some work along these lines, see [5-10].

After the publication of the crystal structure of the DOR (PDB id: 4ej4) [11] docking was conducted again and the results were very contradictory.

Therefore, in this study we tried to combine everything related to docking of delta-selective enkephalin analogues with DOR. The aim was to find a suitable model and the most exact scoring function describing ligand-receptor interactions. This investigation was carried out by docking of each of the three models of DOR with GOLD 5.2. The binding site was defined at a distance about 10Å from Asp128 [12]. Four scoring functions provided with GOLD 5.2 were used consistently for each model. The purpose was to find if there is a correlation between obtained docking results and the data from the *in vitro* bioassay. The following generally accepted are used: *A* – opioid agonist; IC_{50} (potency) – concentration of an agonist *A* (ligand), which produce 50% of the maximal response of the tissue; K_A (affinity) – dissociation constant with units [*A*] of the ligand; e_{rel} (efficacy) – relative efficacy of the agonist, which is unitless [2], [13-15]. For the function which gave the best correlation with *in vitro* results, docking was carried out 10 times to check the repeatability of the results, and hence the reliability of the corresponding functions of software.

MATERIALS AND METHODS

1.1. Delta-selective enkephalin analogues and related compounds.

Ligands used in this study and their values of IC_{50}, K_A, e_{rel} from *in vitro* test are presented in Table 1, [2].
For the receptor (DOR) three structures were used: the first two are theoretical models (Model B and 1ozc), a the third is the crystal structure (PDB id: 4ej4).

Table 1. Preliminary *in vitro* bioassay data of Cys[2]-containing and related analogues of enkephalins on their inhibitory effects of the *mouse vas deferens* tissue

Primary structure	Mouse vas deferens Ligand	IC_{50} (nM)	K_A (nM)	e_{rel}
Tyr-D-Pen-Gly-Phe-D-Pen	DPDPE	6.18±1.17	180±35	30.2±10.0
Tyr-Gly-Gly-Phe-Leu	[Leu[5]]-enk	11.45±2.06	54.9±13.1	5.8±1.0
Tyr-Gly-Gly-Phe-Met	[Met[5]]-enk	18.91±2.15	48.4±7.5	3.6±0.3
Tyr-Cys(Bzl)-Gly-Phe-Leu	[Cys(Bzl)[2], Leu[5]]-enk	8.30±1.40	68.5±29.7	9.3±3.2
Tyr-Cys(Bzl)-Gly-Phe-Met	[Cys(Bzl)[2], Met[5]]-enk	9.53±1.20	23.8±3.0	3.5±0.3
Tyr-Cys(O2NH2)-Gly-Phe-Leu	[Cys(O_2NH_2)[2], Leu[5]]-enk	1.29±0.31	36.4±16.4	29.2±9.5
Tyr-Cys(O2NH2)-Gly-Phe-Met	[Cys(O_2NH_2)[2], Met[5]]-enk	2.22±0.45	14.1±5.4	7.3±2.0
Tyr-D-Cys(O2NH2)-Gly-Phe-Leu	[DCys(O_2NH_2)[2], Leu[5]]-enk	11.40±2.01	73.4±12.7	7.4±1.9
Tyr-D-Cys(O2NH2)-Gly-Phe-Met	[DCys(O_2NH_2)[2], Met[5]]-enk	75.96±11.67	463±161	7.1±1.8
Tyr-HCys(O2NH2)-Gly-Phe-Leu	[HCys(O_2NH_2)[2], Leu[5]]-enk	31.92±5.10	76.4±7.1	3.4±0.2
Tyr-HCys(O2NH2)-Gly-Phe-Met	[HCys(O_2NH_2)[2], Met[5]]-enk	16.09±1.90	55.7±6.1	4.5±0.3

1.2. Docking and scoring functions

In the molecular modelling, docking is a method which predicts the preferred orientation of one molecule to other when bound to each other to form a stable complex. If we know the preferred orientation of the molecule, we may be to predict the strength of association or binding affinity between two molecules using, for example, scoring functions. In the molecular modelling, scoring functions are fast approximate mathematical methods used to predict the binding affinity between two molecules after they have been docked.

Four scoring functions provided with GOLD 5.2 were used in our investigation: Fitness results are immense, but in any case the size of the resulting value gives an indication of how good pose is. The higher is value the scoring function; the better is the docking results [16].

ChemPLP

ChemPLP (Piecewise Linear Potential) is empirical scoring function optimised for pose prediction [17]. It used the terms hydrogen bonds and numerous potentials for modelling van der Waals interactions and potentials of repulsion. The Piecewise Linear Potential is used to model the steric complementarity between protein and ligand. The function is for covalent docking, considering flexible sidechains and explicit water molecules.

GoldScore

The GoldScore scoring function is a molecular mechanics–like function optimized for prediction the binding sites of the ligand taking into account factors such as the energy of hydrogen bonds, van der Waals energy, metal interactions and torsion deformations [18].

ChemScore

Unlike GoldScore, the ChemScore function was trained by regression against measured affinity data. ChemScore [19,20] estimates the total free energy change that occurs on ligand binding.

Astex Statistical Potential (ASP)

Some scoring functions for molecular docking are based on force fields or on regression. ASP scoring function uses information about the frequency of interaction between ligand and protein atoms. It is gathered by analysing existing ligand-protein structures in the PDB (http://www.rcsb.org/pdb/home/home.do) and this information is used to generate statistical potentials. Depending on the data base where the atom-atom potentials are taken from, the scoring function created can be targeted to certain proteins [16].

From the literature binding site of the receptor is known [12]. It is the residues within 10 Å around an aspartic acid residue, Asp128. All four scoring functions were used, which makes it possible to verify binding ability of the appropriate ligand with the receptor. For each ligand, the program conducted 10 independent experiments for its binding to the active site of the receptor, and for each one of them the corresponding scoring functions are calculated. At the end of the experiment results are sorted automatically by the software in descending order of the values of the scoring function.

1.3. Correlations

In order to find relationship between sets of data derived from *in vitro* assay and docking results, we tried to predict it with the help of Pearson's correlation, using GraphPad Prism 3.0 (http://www.graphpad.com).

RESULTS AND DISCUSSION

The root mean square deviation (RMSD) is the most frequently used measure for comparing two protein three-dimensional (3-D) structures. The RMSD is 0 for identical structures, and its value increases as the two structures become more different. RMSD between three models used in the present study was defined. It is respectively Model B with 1ozc RMSD = 1.960, Model B with 4ej4 – 1.660, 1ozc with 4ej4 – 1.874. Average RMSD between three models is 1.835. From the values it can be seen that they are very high. Consequently, it cannot be obtained unambiguous results when using any model.

Docking

Analysing the resulting poses of ligands obtained from docking with different models of DOR characteristic interaction of α-amino group of the ligand with the carboxyl group of the side chain of Asp128 of the receptor

sequence was observed. An example is shown in Figure 1. This result indicates that the software used (GOLD 5.2) predicts the correct binding mode of the ligand to the receptor. The correct choice of function optimizes the structure of the ligand in the binding site, as the most accurate (in our case ASP scoring function) provides precise information about the nature and strength of interactions in the binding site.

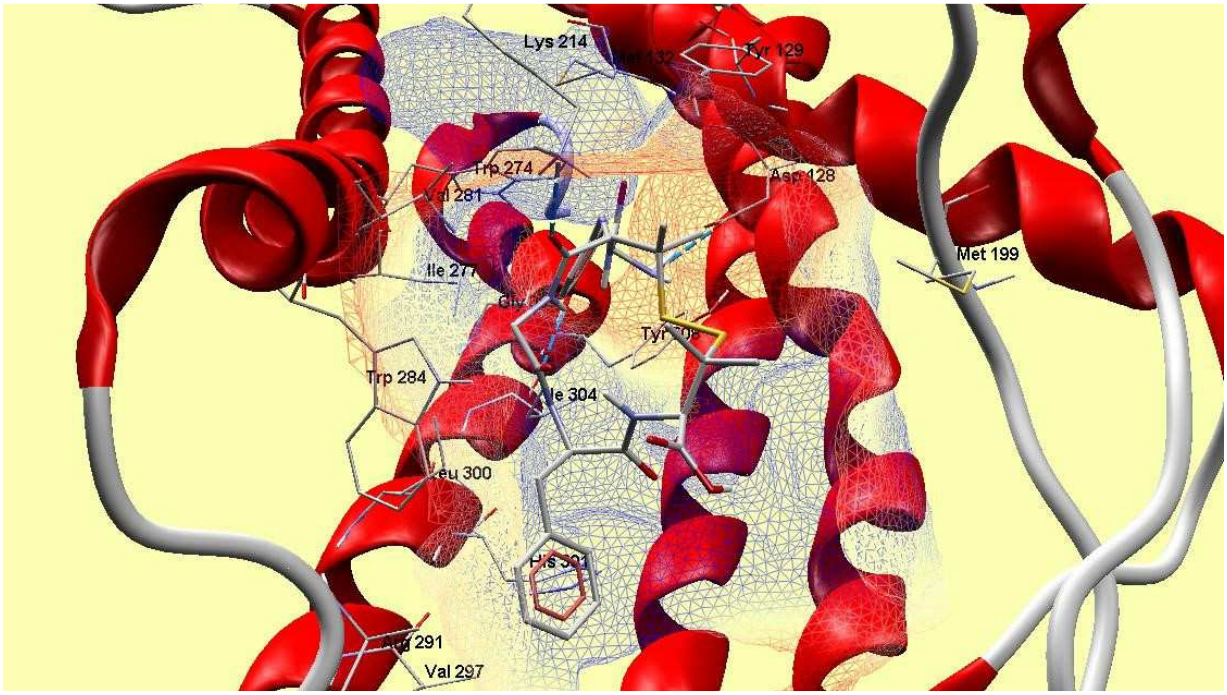

Figure 1. Protein-ligand docking of Model B of DOR and DPDPE (picture was generated by Molegro Molecular Viewer)

From the predefined information is necessary to carry out thorough study of models and functions in order to find an appropriate model that allows determining the relationship structure-activity. Following the docking of the three models and the four functions in Table 2 are presented the values of Pearson's correlation coefficient of all tested values.

Table 2. The values of Pearson's correlation coefficient between *in vitro* parameters (IC_{50} , K_A, e_{rel}) and the four scoring functions of GOLD 5.2 for different models of DOR

	4ej4			1ozc			Model B		
Functions	IC_{50}	K_A	e_{rel}	IC_{50}	K_A	e_{rel}	IC_{50}	K_A	e_{rel}
ASP	0.09075	-0.03759	-0.6366	-0.09349	-0.2418	-0.2518	-0.8600	-0.9381	-0.03214
ChemPLP	-0.07770	-0.1747	-0.6742	-0.05332	-0.3422	-0.4721	-0.2536	-0.07388	0.3506
ChemScore	-0.05391	0.09302	-0.01962	-0.2801	-0.2678	-0.08067	-0.4676	-0.3802	0.1219
GoldScore	0.1238	-0.09102	-0.4393	0.2069	-0.09418	-0.7209	0.2210	-0.1786	-0.6586

As can be seen from the table the values of correlations are higher in six cases: 4ej4 – ASP Score/e_{rel} = -0.6366, ChemPLP/e_{rel} = -0.6742; 1ozc – GoldScore/e_{rel} = -0.7209; Model B – ASP Score/IC_{50} = -0.86, ASP Score/K_A = -0.9381, and GoldScore/e_{rel} = -0.6589 (Figure 2).

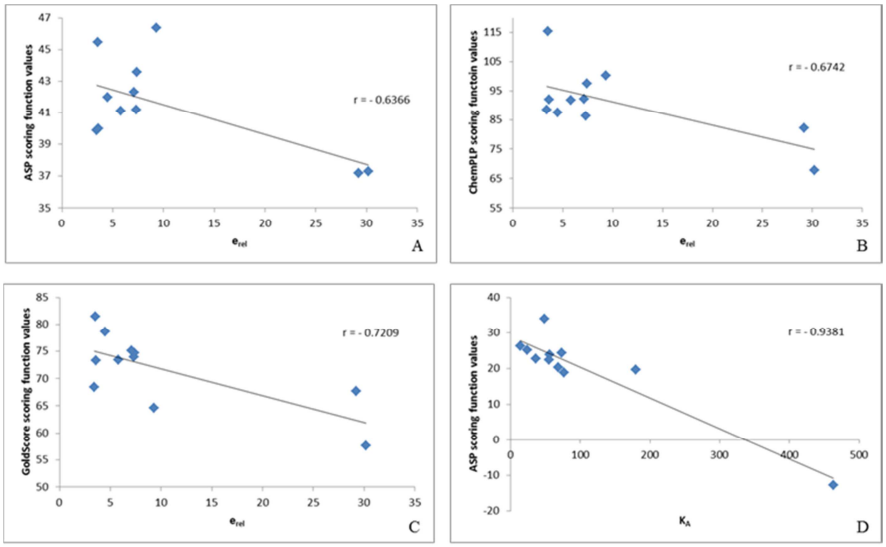

Figure 2. Pearson's correlation coefficient for: (A) ASP scoring function values and e_{rel} for model 4ej4; (B) ChemPLP scoring function values and e_{rel} for model 4ej4; (C) GoldScore scoring function values and e_{rel} for model 1ozc; and (D) ASP scoring function values and K_A

In all cases, the relationship is negative, i.e., as the value of the scoring function is greater, the smaller is the value of *in vitro* tests (IC_{50}, K_A, e_{rel}). However, it is known that the K_A has a higher value as the compound is more active with respect to the corresponding receptor, but the higher is its e_{rel}-value, the analogue is more efficient. In these two cases, the correlation does not have biological sense for explaining the effects of agonists. However, if test compounds act as antagonists which block the action of receptor by binding to its active center dependence might explain their effects. In order to analyse how agonists, antagonists and inverse agonists bind to the receptor docking was performed with ICI174864, which is inverse agonist for the DOR [21], and naltrexone - DOR antagonist [22]. Data for this docking with GOLD 5.2 of Model B and ASP scoring function is presented in Table 3.

Table 3. The values of ASP scoring function obtained for enkephalin analogues, agonist DPDPE, antagonist naltrexone, and inverse agonist ICI174864

Ligand	ASP scoring function values
DPDPE	23.207
[Leu^5]-enk	24.684
[Met^5]-enk	25.096
[$Cys(Bzl)^2$, Leu^5]-enk	24.386
[$Cys(Bzl)^2$, Met^5]-enk	20.534
[$Cys(O_2NH_2)^2$, Leu^5]-enk	19.759
[$Cys(O_2NH_2)^2$, Met^5]-enk	21.484
[$DCys(O_2NH_2)^2$, Leu^5]-enk	17.851
[$DCys(O_2NH_2)^2$, Met^5]-enk	11.973
[$HCys(O_2NH_2)^2$, Leu^5]-enk	20.133
[$HCys(O_2NH_2)^2$, Met^5]-enk	21.174
ICI174864	2.68
Naltrexone	30.96

From the docking is seen that the fitness function of the agonist DPDPE is 23.207, the inverse agonist ICI174864 is 2.68 and the antagonist naltrexone is 30.96. It follows that the best with the receptor binds antagonist follows an agonist and the least is the binding of the receptor with the inverse agonist. The values of scoring functions are within the range 2.68 – 25.096, and even the highest values are close to the value of the scoring function of the agonist DPDPE. This means that the compounds act as agonists, because their scoring function is a considerably smaller value than that of the antagonist naltrexone. Dependence that best describes the biological effects using the docking is between ASP scoring function and IC_{50}. Much higher value of fitness function is, the lower the value of concentration is (Figure 3).

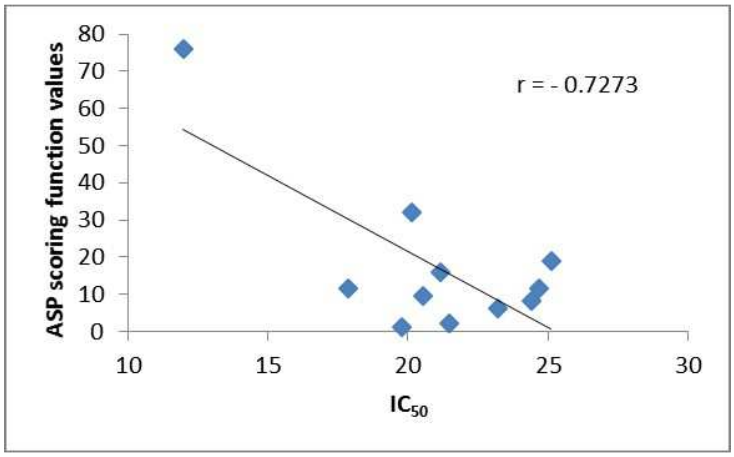

Figure 3. Pearson's correlation coefficient between average values of the ASP scoring functions of enkephalin analogues and IC_{50}

In order to verify the reliability of the docking program for determining the interaction between the ligands and receptors, the docking is performed a further nine times in a similar way: select the same receptor binding site is defined in the same way at each docking is used, the same ligands and scoring function. From the final results for each ligand the average scoring function was obtained. The average values of the functions again correlated well with the data from the in vitro test, Pearson's correlation coefficient -0.7273. This indicates that the use of GOLD 5.2 gives reliable results in the docking of ligands selective for DOR.

CONCLUSION

As a result of the presented work docking of enkephalin analogues with three models of DOR and four scoring functions was carried out. Docking is used to determine how delta selective enkephalin analogues interact with DOR as four fitness functions used in turn make possible to optimize this binding. It was established that the most appropriate combination for analysis of delta selective enkephalin analogues and DOR is that between the model of DOR suggested in our previous study the so-called Model B and the ASP scoring function (available in GOLD 5.2). This combination will enable us to create and test virtually a large number of potential DOR agonists, analogues of enkephalins. The presented work opens wide space for the design of novel compounds with a desired biological effect.

Acknowledgements
This work was supported by NFSR of Bulgaria project I02/16 - 12.12.2014.

REFERENCES

[1] C. Chung, BL. Kieffer, *Pharmacol. Ther.* **2013**, 140: 112-120.
[2] N. Pencheva, P. Milanov, L. Vezenkov, T. Pajpanova, E. Naydenova, *Eur. J. Pharmacol.* **2004**, 498: 249-256
[3] R. Thomsen, M. Christensen, *J. Med. Chem.* **2006**, 49: 3315-3321.
[4] T. Dzimbova, F. Sapundzhi, N. Pencheva, P. Milanov, *Int. J. Bioautomation.* **2013**, 17: 5-16
[5] F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Jurnal of Bulgarian Chemical Communications.* **2015**, 47: 613-618.
[6] F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Journal of Computational Methods in Molecular Design.* **2015**, 5: 98–108.
[7] F. Sapundzhi. *Proceedings of the Fifth International Scientific Conference - FMNS 2013*, 12– 16 June. **2013**, Blagoevgrad, Bulgaria, 193,
[8] F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Journal of Peptide Science*. **2014**, 20: 294–295.
[9] F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Proceedings of the Sixth International Scientific Conference - FMNS 2015*, 10 – 14 June. **2015**, Blagoevgrad, Bulgaria, 104-112.
[10] F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Biomath Communications*. **2014**, 84.
[11] S. Granier, A. Manglik, A. Kruse, T. Kobilka, F. Thian, W. Weis, *Nature.* **2012,** 485: 400-404.

[12] K. Befort, L. Tabbara, S. Bausch, C. Chavkin, C. Evans, B. Kieffer, *J. Mol Pharmacol.* **1996**, 49: 216-223.
[13] P. Milanov, N. Pencheva, *Serdica Journal of Computing.* **2011**, 5, 333-358.
[14] F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Proceedings of 7th Bulgarian Peptide Symposium, Emilia Naydenova, Tamara Pajpanova, Dancho Danalev (Eds).* 10-12 June. **2016**, Blagoevgrad, Bulgaria, p.89.
[15] P. Milanov, N. Pencheva, F.Sapundzhi. *Biomath Communications.* **2016**, 3, 47.
[16] http://www.ccdc.cam.ac.uk/Lists/DocumentationList/gold.pdf.
[17] O. Korb, T. Stutzle, T. Exner, *J. Chem. Inf. Model.* **2009**, 49: 84–96.
[18] M. Verdonk, J. Cole, M. Hartshorn, C. Murray, R. Taylor, *Proteins.* **2003**, 52: 609-623.
[19] M. Eldridge, C. Murray, T. Auton, G. Paolini, R. Mee, *J. Comput. Aided Mol. Des.* **1997**, 11: 425-445.
[20] C. Braxte, C. Murray, D. Clark, D. Westhead, M. Eldridge, *Proteins.* **1998**, 33: 367-382.
[21] T. Chiu, L. Yung, Y. Wong, *J. Mol Pharmacol.* **1996**, 50: 1651-1657
[22] B. Crabtree, *Clin Pharm*. **1984**, 3: 273-280