Bulgarian Chemical Communications, Volume 47, Number 2 (pp. 613 – 618) 2015

Exploring the interactions of enkephalin and dalargin analogues with the μ-opioid receptor

F. I. Sapundzhi[1], T. A. Dzimbova*[2], N. S. Pencheva[1], P.B. Milanov[1,3]

[1]*South-West University "NeofitRilski", Bulgaria, 2700 Blagoevgrad*
[2]*Institute of Molecular Biology, Bulgarian Academy of Sciences, Bulgaria, 1113 Sofia*
[3]*Institute of Mathematics and Informatics, Bulgarian Academy of Sciences, Bulgaria, 1113 Sofia*

Received June 30, 2014, Accepted August 14, 2014

The μ-opioid receptor (MOR) is an important target in the search for novel analgesics. The recently published crystal structure of MOR gives the possibility of *in silico* investigations. The aim of the present work is to evaluate the method for finding the relationship between structure and activity of the selective ligands of MOR in order to develop a reliable approach for designing new potent analogues. We performed docking with enkephalin and dalargin selective analogues to MOR with GOLD 5.2 and we found a correlation between data obtained *in vitro* and the scoring function from the computational method. The docking procedure can help to explain *in vitro* results and could be successfully used in design of new agonists of the MOR receptor.

Keywords: Docking, scoring functions, μ-opioid receptor, correlation, GOLD.

INTRODUCTION

Opioid receptors are a family of G-protein-coupled receptors. This family consists of three principal receptor subtypes, termed μ-opioid receptor (MOR), δ-opioid receptor and κ-opioid receptor [1]. Opioid agonist drugs are potent analgesics that are used clinically for pain management [2]. Knockout mouse studies have shown that MOR is the opioid receptor subtype primarily responsible for mediating the analgesic and rewarding effects of opioid agonist drugs [3]. However, chronic use of opioid agonist drugs may cause tolerance and dependence, thus limiting their therapeutic efficacy [3]. Development of new opioid drugs that provide analgesia without producing dependence is important for pain treatment.

In the last decades computer-aided drug design has taken a more significant place in the field of natural sciences. Predicting the binding modes and affinities of compounds when they interact with a protein-binding site lies at the heart of structure-based drug design. Consequently, the number of algorithms available for protein–ligand docking is large. DOCK [4], FlexX [5], PRO_LEADS [6], and GOLD [7, 8] are examples of docking programs, but many more are reported in the literature (for an overview of docking strategies see Taylor *et al.* [9]). Most approaches consider the protein to be (mostly) rigid and allow the ligand to be flexible.

A characteristic of a good docking program is the ability of its scoring function to score and rank ligands according to their experimental binding affinities.

In this article, we describe the implementation of the ChemScore function as a scoring function for GOLD 5.2 and its usefulness to perform docking precisely, to predict the binding energies, and to realise the biological effects of investigated compounds.

MATERIALS AND METHODS

Objects

- *Receptor-MOR*

The crystal structure of MOR published in RCSB Protein Data Base (PDB id: 4dkl, www.rcsb.org) was used. It was obtained by X-ray diffraction with 2.8 Å resolution.

- *Ligands*

[Cys(O_2NH_2)^2-Leu^5]-enk, [Cys(O_2NH_2)^2-Met^5]-enk, dalargin, dalarginamide, dalarginethylamide, DAMGO ([D-Ala^2,N-Me-L-Phe^4,Gly-ol^5]-enkephalin), [D-Phe^4]- dalarginamide, [L-Ala^2]-dalargin, [Leu^5]-enkephalin, [Met^5]-dalargin, [Met^5]-enkephalin, N-Me-[D-Phe^4]-dalarginamide, and N-Me-[L-Phe^4]-dalarginamide.

Software:

- *Avogadro Version 1.1.0.*

Ligand preparation was done with Avogadro: an open-source molecular builder and visualization tool (Version 1.1.0, http://avogadro.openmolecules.net).

Avogadro is an advanced molecule editor and visualiser designed for use in computational

* To whom all correspondence should be sent:
E-mail: tania_dzimbova@abv.bg

chemistry, molecular modeling, bioinformatics, materials science, and related areas. It offers flexible high-quality rendering and powerful plugin architecture. The Molecular builder/editor is developed as a cross-platform for Windows, Linux, and Mac OS X. All source codes are available under the GNU GPL. Plugin architecture for developers includes: rendering, interactive tools, commands, and Python scripts. The Avogadro python API (Application-Programming Interface) resembles the [C++ API] as much as possible. This means that the C++ documentation also applies to Python. In addition to serving as a set of user-level tools, Open Babel offers a C++ library and interface in other languages (e.g., Perl and Python) for general chemical software development, both in-house and to encourage open source chemistry packages.

- *GOLD 5.2*

GOLD 5.2 has proven successful in virtual screening, lead optimisation, and identifying the correct binding mode of active molecules. GOLD 5.2 is highly configurable allowing full advantage to be taken of the knowledge of a protein-ligand system in order to maximise docking performance. GOLD 5.2 enables complete user control over speed *versus* accuracy settings, from efficient virtual screening of large compound libraries, to highly accurate exhaustive sampling for lead optimisation. With a wide range of available scoring functions and customisable docking protocols, GOLD 5.2 provides consistently high performance across a diverse range of receptor types. Most parts of the GOLD 5.2 program have been described by Jones et al. [7,8]. Like all other docking programs, GOLD 5.2 consists of three main parts.

The first part is a *scoring function* to rank different binding modes. The ChemScore scoring function [10] estimates the total free energy change that occurs on ligand binding:

$$(1)\ \Delta G_{binding} = \Delta G_0 + \Delta G_{hbond} S_{hbond} + \Delta G_{metal} S_{metal} + \Delta G_{lipo} S_{lipo} + \Delta G_{rot} H_{rot}$$

where S_{hbond} is score for hydrogen bonding, S_{metal} is score for acceptor-metal bonding, S_{lipo} is lipophilic interactions, H_{rot} –loss of conformational entropy of the ligand upon binding to the protein, and ΔG are coefficients derived from a multiple linear regression analysis. The expression for the ChemScore function [10] was adapted for docking by Baxter *et al*. [11], where they added the following three elements to the so called free energy of binding of a ligand to a protein ($\Delta G_{binding}$): a protein–ligand clash–energy term, (E_{clash}), a ligand–internal–energy term, (E_{int}) and a covalent energy term, (E_{cov}):

$$(2)\ \Delta G'_{binding} = \Delta G_{binding} + E_{clash} + E_{int} + E_{cov}$$

The second part is a *mechanism for placing the ligand* in the binding site. GOLD 5.2 uses a unique method to do this, which is based on fitting points. It adds fitting points to hydrogen-bonding groups on the protein and ligand, and maps acceptor points on the ligand on donor points in the protein and *vice versa*. Additionally, GOLD 5.2 generates hydrophobic fitting points in the protein cavity onto which the ligand CH groups are mapped.

The last part is a *search algorithm* to explore possible binding modes. GOLD 5.2 uses a genetic algorithm in which the following parameters are modified/optimised: - dihedrals of ligand rotatable bonds; - ligand ring geometries (by flipping ring corners); - dihedrals of protein OH groups and NH_3^+ groups; - the mappings of the fitting points (i.e., the position of the ligand in the binding site). Of course, at the start of a docking run, all these variables are randomised.

Molegro Molecular Viewer (MMV)

MMV is an application for studying and analysing how ligands interact with macromolecules. MMV can be used to: (1) inspect docking results consisting of high-scoring poses found by Molegro Virtual Docker (MVD) – the molecular docking software product offered by Molegro; (2) inspect and visualize molecular structures obtained from other sources, such as the Protein Data Bank.

The main focus of MVD and MMV is on studying protein-ligand interactions. MMV does not currently support DNA and RNA molecules.

The MolDock scoring function (MolDock Score) used by MVD [12] is derived from the PLP scoring functions originally proposed by Gehlhaar *et al*. [13, 14] and later extended by Yang *et al*. [15]. The MolDock scoring function further improves these scoring functions with a new hydrogen bonding term and new charge schemes. The docking scoring function, E_{score} is defined by the following energy terms:

$$(3)\ E_{score} = E_{inter} + E_{intra}$$

where E_{inter} is the ligand-protein interaction energy and E_{intra} is internal energy of the ligand.

- *GraphPad Prism®*

GraphPad Prism combines non-linear regression (curve fitting), basic biostatistics, and scientific graphing (www.graphpad.com). Prism uses the term

"analyze" more generally than many programs. The term includes data manipulation (i.e. mathematical transforms) as well as statistical analyses and regression. Prism quantifies correlations by calculating the Pearson correlation coefficient, *r*. In statistics, the Pearson product-moment correlation coefficient (sometimes referred to as the PPMCC or PCC, or Pearson's *r*) is a measure of the correlation (linear dependence) between two variables *X* and *Y*, giving a value between +1 and −1 inclusive. It is widely used in science as a measure of the strength of linear dependence between two variables. Pearson's correlation coefficient between two variables is defined as the covariance of the two variables divided by the product of their standard deviations. The form of the definition involves a "product moment" i.e. the mean (the first moment about the origin) of the product of the mean-adjusted random variables; hence the modifier *product-moment* in the name. Pearson's correlation coefficient when applied to a sample is commonly represented by the letter *r* and may be referred to as the *sample correlation coefficient* or the *sample Pearson correlation coefficient*. We can obtain a formula for *r* by substituting estimates of the covariance and variances based on a sample into the formula above. That formula for *r* is:

$$(4)\ r = \frac{\sum_{i=1}^{n}(X_i-\bar{X})(Y_i-\bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i-\bar{X})^2}\sqrt{\sum_{i=1}^{n}(Y_i-\bar{Y})^2}}$$

Concerning the choice of the criterion it has to be kept in mind that the Spearman correlations are based on ranks, not actual values, and so it could be assumed that in our investigation, the proper criterion would be that of Pearson.

- *Docking of ligands*

Thirteen peptides ([Cys(O_2NH_2)^2-Leu^5]-enk, [Cys(O_2NH_2)^2-Met^5]-enk, dalargin, dalarginamide, dalarginethylamide, DAMGO, [D-Phe^4]-dalarginamide, [L-Ala^2]-dalargin, [Leu^5]-enkephalin, [Met^5]-dalargin, [Met^5]-enkephalin, N-Me-[D-Phe^4]-dalarginamide, and N-Me-[L-Phe^4]-dalarginamide) were chosen for docking with the receptor. All of them were synthesised, *in vitro* biologically tested, and have already been published [16, 17]. Docking was carried out with GOLD 5.2 software, which uses a generic algorithm and considers full ligand conformational flexibility and partial protein flexibility. From the literature [18], the binding site for MOR was defined as residues within 10 Å radius of aspartic acid of the third TM domain, which is involved in the most crucial interaction. In the case of MOR this is Asp147. The ChemScore algorithm was used and scoring function was calculated for each ligand. The conformations of the ligands with best scoring functions were selected and parameters of the scoring functions were used in order to find correlations between them and the *in vitro* results (Table 1).

RESULTS AND DISCUSSION

Docking results

Docking was performed with MOR and all 13 ligands. The results of docking studies of ligands are described below and the best and the worst of them are presented in Fig. 1.

All of the ligands bind to the receptor by forming many H-bonds. A very important residue in the receptor sequence is Asp147, which forms a salt bridge with NH_3^+ of the ligand's molecule. Less potent MOR ligand N-Me-[L-Phe^4]-dalarginamide does not bind to Asp147. However the effect of the compounds is not connected to this interaction, because in the case of dalarginamide there is no such interaction, but it is still very potent. A key part of the ligand structure is the phenolic hydrogen group (Tyr residue). In all cases, except for dalargin and [D-Phe^4]-dalarginamide, it binds to different residues in the receptor structure.

The best poses obtained from docking for each ligand with MOR are described in Table 2 and the ligands with the best and the worst scoring functions are presented in Fig. 1.

According to this observation at least one of these interactions must be present in order to have some biological effect.

Number of interactions does not correlate with biological activity, only their strength is important. All the potent ligands bind to MOR electrostatically.

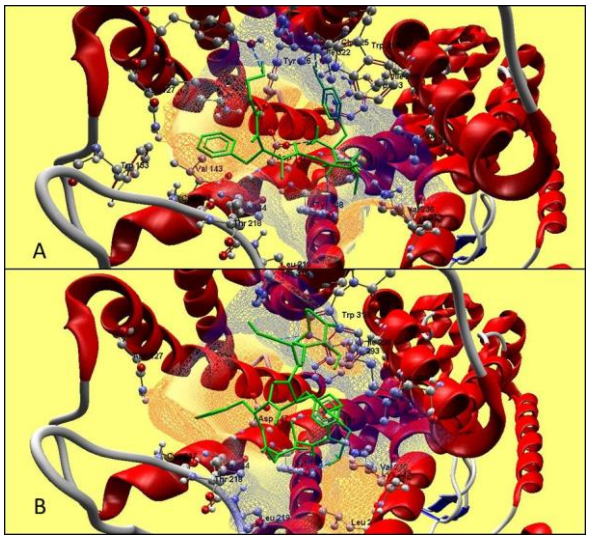

Fig. 1. Ligands with the best (A – DAMGO) and the worst (B–[D-Phe4]-dalarginamide) scoring functions.

Correlations

Correlations of docking data and *in vitro* experiments results were performed with GraphPad Prism 3.0. Scoring function was taken for all compounds and compared with the results of *in vitro* experiments. Good Pearson's correlation was obtained between scoring function from the GOLD5.2 docking procedure and IC_{50} value in the guinea-pig myenteric plexus.

Total energies of all compounds were calculated in MMV after docking (MolDoc algorithm). They were also compared with the results from *in vitro* experiments, but the correlation was not significant (Pearson r = 0.69742, P value = 0.008). (Fig. 2).

According to the correlations obtained, we can conclude that the ChemScore algorithm is more suitable for docking studies of MOR with this series of enkephalin analogues, as compared with MolDoc algorithm.

Table 1. The inhibitory effect (IC_{50}, nM) [17, 18] of: - dalargine, its analogues, endogenous ligands – [Leu^5]-enkephalin and [Met^5]-enkephalin and μ-selective ligand – DAMGO, on electrically evoked contractions of the myenteric plexus-longitudinal muscle of the guinea-pig (μ-selective tissue).

Ligands	IC_{50}, (nM)	Score	Total Energy
[Cys(O_2NH_2)^2-Leu^5]-enk	3960±740	20.44	-111.281
[Cys(O_2NH_2)^2-Met^5]-enk	1378±245	19.6	-107.904
Dalargin	12.3±1.7	22.05	-135.245
Dalarginamide	5.8±0.7	20.67	-148.221
Dalarginethylamide	6.0±0.7	28.75	-163.106
DAMGO	5.8±0.4	29.02	-93.278
[D-Phe^4]-Dalarginamide	5300±408	14.31	-115.856
[L-Ala^2]-Dalargin	234±46	21.28	-130.171
[Leu^5]-enkephalin	65.3±8.2	25.95	-148.483
[Met^5]-dalargin	11.9±1.7	23.27	-173.298
[Met^5]-enkephalin	28.6±8.4	25.11	-120.651
N-Me-[D-Phe^4]-Dalarginamide	3350±850	19.17	-115.122
N-Me-[L-Phe^4]-Dalarginamide	0.57±0.08	20.08	-107.216

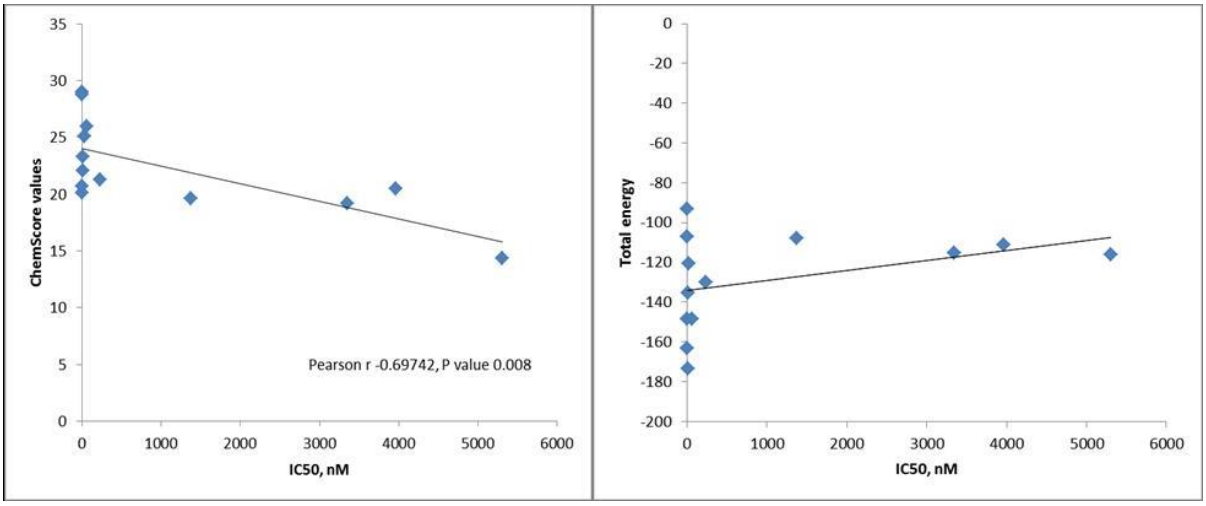

Fig. 2. Pearson's correlation between:
A- IC_{50} and ChemScore function; and **B** - IC_{50} and Total energy.

Table 2. Interactions of ligands with MOR.
Designations: the symbols in italic are the Asp residue in the binding site of the MOR, while the Tyr residue of the ligand molecule is shown in bold.

Ligand	Number of H-bonds	Residues and groups involved in interactions
[Cys(O_2NH_2)^2-Leu^5]-enk	5	Tyr128 – SO, Asp147 – COOH, Asp147 – **OH (Tyr)**, Trp318 – SO, Tyr326 – COOH
[Cys(O_2NH_2)^2-Met^5]-enk	7	Gln124 – COOH, Tyr128 – COOH, Tyr128 – CO, *Asp147 – NH_3^+*, Leu219 – SO, Lys233 – CO, His297 – **OH (Tyr)**

Dalargin	4	Tyr128 – NH, *Asp147 – NH_3^+*, Lys233 – COOH, Tyr326 – CO
Dalarginamide	8	Tyr128 – CO, Tyr148 – NH, Tyr148 – NH_3^+, Leu219 – CO, 2 H-bonds His297 – **OH (Tyr)**, Trp318 – CO, Tyr326 – CO
Dalarginethylamide	7	Tyr128 – CO, Tyr148 – NH_3^+, Lys233 – CO, 2 H – bonds His297 – **OH (Tyr)**, Gln314 – Gu, Trp318 –CO
DAMGO	5	Tyr128 – OH, *Asp147 – NH_3^+*, Tyr148 – CO, Ile322 – **OH (Tyr)**, Lys233 – CO
[D-Phe[4]]-Dalarginamide	4	Tyr148 – NH_3^+, Tyr148 – NH, Trp318 – CO, His319 – NH_2 (amide)
[L-Ala[2]]-Dalargin	7	Tyr128 – COOH, *Asp147 – NH_3^+*, Tyr148 – CO, Lys233 – CO, Trp318 – COOH, Cys321 – **OH (Tyr)**, Ile322 – **OH (Tyr)**
[Leu[5]]-enkephalin	5	Gln124 – COOH, Tyr128 – COOH, *Asp147 – NH_3^+*, His297 – **OH (Tyr)**
[Met[5]]-dalargin	8	Asn127 – COOH, *Asp147 – NH_3^+*, 2 H-bonds Asp147 – Gu-group, Tyr148 – CO, Cys217 – COOH, Ile322– **OH (Tyr)**, Tyr326 – **OH (Tyr)**
[Met[5]]-enkephalin	5	Asp147 – NH, *Asp147 – NH_3^+*, Cys217 – COOH, Leu219 – COOH, Ile322 – **OH (Tyr)**
N-Me-[D-Phe[4]]-Dalarginamide	5	*Asp147 – NH_3^+*, Tyr148 – CO, Lys233 – CO, Trp318 – CO, Ile322 – **OH (Tyr)**
N-Me-[L-Phe[4]]-Dalarginamide	6	Asp147 – NH_2 (amide), Tyr148 – NH, Lys233 – CO, Ile306 – OH (Tyr), 2 H-bonds Ile322 – Gu –group

CONCLUSIONS

Previously published results from *in vitro* experiments significantly correlate with docking data obtained with GOLD5.2, using the ChemScore algorithm. The docking procedure can help to explain *in vitro* results and could be successfully used for *in silico* design of new potent agonists of μ-opioid receptor, saving time, experimental animals and expense.

Acknowledgments: *This work is partially supported by the project of the Bulgarian National Science Fund, entitled: "Bioinformatics research: protein folding, docking and prediction of biological activity", code NSF I02/16, 12.12.14.*

REFERENCES

1. P.Y. Law, Y.H. Wong, H.H. Loh, *Ann.Rev.Pharmacol.Toxicol*., **40**, 389 (2000).
2. J.K. Zubieta, Y.R. Smith, J.A. Bueller, Y. Xu, M.R. Kilbourn, D.M. Jewett, C.R. Meyer,R.A. Koeppe, C.S. Stohler, *Science*, **293,** 311 (2001).
3. H.W.D. Matthes, R. Maldonado, F. Simonin, O. Valverde, S. Slowe, I. Kitchen, K. Befort, A. Dierich, M. Le Meur, P. Dollé, E. Tzavara, J. Hanoune, B.P. Roques, B.L. Kieffer. *Nature*, **383**, 819 (1996).
4. S. Makino, I. D. Kuntz, *J. Comput. Chem.*, **18**, 1812 (1997).
5. M. Rarey, B. Kramer, T. Lengauer, G. Klebe, *J. Mol. Biol.*, **261**, 470 (1996).
6. C.A. Baxter, C.W. Murray, D.E. Clark, D.R. Westhead, M.D. Eldridge, *Proteins*, **33**, 367 (1998).
7. G. Jones, P.Willett, R.C. Glen, A.R. Leach, R.Taylor, *J. Mol. Biol.*, **267**,727 (1997).
8. G. Jones, P.Willett, R.C. Glen, *J. Mol. Biol.*, **245**, 43 (1995).
9. R.D. Taylor, P.J. Jewsbury, J.W. Essex, *J. Comput.-Aided Mol. Des*., **16**, 151 (2002).
10. M.L. Verdonk, J. C. Cole, M.J. Hartshorn, C.W. Murray, R.D. Taylor, *Proteins*, **52**, 609 (2003).
11. C. A. Baxter, C. W. Murray, D. E. Clark, D. R. Westhead, M. D. Eldridge, *Proteins*, **33**, 367 (1998).
12. R.Thomsen, M. H. Christensen, *J. Med. Chem.*, **49**, 3315 (2006).
13. D. K. Gehlhaar, G. Verkhivker, P. A. Rejto, D. B. Fogel, L. J. Fogel,S. T. Freer, Proceedings of the Fourth International Conference on Evolutionary Programming, 1995, p. 615.
14. D. K.Gehlhaar, D. Bouzida, P. A. Rejto, Proceedings of the Seventh International Conference on Evolutionary Programming, 1998, p. 449
15. J-M. Yang, C-C. Chen, *Proteins*, **55**, 288 (2004).
16. N. Pencheva, J. Pospińek, L.Hauzerova, T. Barth, P. Milanov, *Br. J. Pharmacol*.,**128**, 569 (1999).
17. N. Pencheva, L.Vezenkov, E. Naydenova, P.Milanov, T. Pajpanova, S. Malamov, L. Bojkova, *Bul. Chem. Comm*., **33**, 47 (2001).
18. K. Befort, L. Tabbara, S. Bausch, C. Chavkin, C. Evans, B. Kieffer, *Mol.Pharmacol.*, **49**, 216 (1996).

ИЗСЛЕДВАНЕ НА ВЗАИМОДЕЙСТВИЯТА НА ЕНКЕФАЛИНОВИ И ДАЛАРГИНОВИ АНАЛОЗИ С μ-ОПИОИДЕН РЕЦЕПТОР

Ф. И. Сапунджи[1], Т. А. Дзимбова[2], Н. С. Пенчева[1], П. Б. Миланов[1, 3]

[1]Югозападен Университет „Неофит Рилски“, България, 2700, Благоевград
[2]Институт по молекулярна биология „Румен Цанев“, БАН, България, 1113, София
[3]Институт по математика и информатика, БАН, България, 1113, София

Постъпила на 30 юни, 2014 г., приета на 14 август 2014 г.

(Резюме)

Мюопиоидният рецептор (MOP) е особено важен в процеса на търсене на нови аналгетици. Наскоро публикуваната кристална структура на MOP дава възможност за *in silico* изследвания. Целта на представената работа е да се оцени метода за намиране на връзката структура-активност на селективните лиганди на MOP и да се разработи надежден подход за създаване на нови мощни аналози. Проведени са докинг изследвания на енкефалинови и даларгинови селективни MOP аналози с GOLD 5.2. и е установена корелация между данните получени от *in vitro* тестовете и оценъчната функция. Докинг процедурата може да помогне за обясняването на резултатите от *in vitro* тестовете и за успешно използване при дизайна на нови агонисти на MOP.