THE USE OF THE DOCKING STUDIES WITH THE PURPOSE OF SEARCHING POTENTIAL CARDIOPROTECTORS

Article · September 2018

CITATIONS
0

READS
50

5 authors, including:

Lina Perekhoda
National University of Pharmacy
35 PUBLICATIONS **7** CITATIONS

SEE PROFILE

THE USE OF THE DOCKING STUDIES WITH THE PURPOSE OF SEARCHING POTENTIAL CARDIOPROTECTORS

I. Drapak[1], L. Perekhoda[2], O. Vynogradova[3], M. Protopopov[4], M. Suleiman[2], I. Sych[2], N. Kobzar[2], O. Kiz[2]

[1]Department of general, bioinorganic, physical and colloidal chemistry,
DanyloHalytskyLviv National Medical University,
Pekarska str. 69, Lviv, 79010, Ukraine
[2]Department of medicinal chemistry, National University of Pharmacy,
Pushkinska str. 53, Kharkiv, 61002, Ukraine
[3]Department of therapeutical stomatology,
DanyloHalytskyLviv National Medical University,
Pekarska Str. 69, Lviv, 79010, Ukraine
[4]Department of medicinal chemistry,
Institute of Molecular Biology and Genetics of the NAS of Ukraine,
AcademikaZabolotnoho Str. 150, Kyiv, 03143, Ukraine

Abstract. This work is one of the stages of searching for effective cardioprotectors of the metabolic action based on derivatives of 2-imino-1,3-thiazoline and 1,3,4-thiadiazole.Our study gives possibility to save laboratory animals and time, to carry out purposeful and effective research in future.

The receptor-based flexible docking of 154 compounds synthesized among 2-imino-1,3-thiazoline derivatives, which contain hydroxyethyl, methylpiperazine, morpholine, propylmorpholine, ethylmorpholine, ethyl fragments in the structure, and 2,5-disubstituted derivatives of 1,3,4-thiadiazoletothe active site of gamma-butyrobetaine hydroxylase (IUBMB Enzyme Nomenclature: 1.14.11.1) has been conducted with the purpose of directed search for inhibitors of this enzyme as potential cardio protectorsof the metabolic action.

According to the docking results, 15studiedderivatives of 2-imino-1,3-thiazoline, which contain in the structure the morpholine and propyl morpholine fragment, and 3 derivatives of2-R,5-R-amine-1,3,4-thiadiazole,have values of affinity to the enzyme, which are comparable with the values of the classical inhibitors L-carnitine and reference drug mildronate. It is the argument for the study of the metabolic action of the 18 compounds under research *in vivo*. It should also be noted that compounds ***8***, ***9*** and ***18*** are the most promising according to the results of *in silico* studies. Besides, 2-imino-1,3-thiazoline derivatives containing the morpholine and propyl morpholine fragment in the structure and derivativesof 2-R,5-R-amine-1,3,4-thiadiazole can be regarded as a promising scaffolds for creating combinatorial libraries of potential cardio protectors of the metabolic action.

Key words: enzyme gamma-butyrobetaine hydroxylase, cardio protective drug, derivatives of 2-imino-1,3-thiazoline and 1,3,4-thiadiazole, receptor-based flexible docking.

Introduction

According to the WHO data, pathologies of the cardiovascular system lead the world in their prevalence. During the past decades a lot of experimental and clinical studies that proved the effectiveness of the concept of the metabolic approach to the therapy of ischaemic heart disease were conducted. According to this concept a number of medicines with a positive effect on the metabolism of the cardiac muscle in conditions of hypoxia have been introduced into medical practice and proven their clinical efficacy. Currently, the therapy is leading in the treatment regimens for cardiovascular disease and is included in the international recommendations [1-3].

Currently *in silico* studies are widely used to decrease the numbers of laboratory animals, which are needed for scientific research. At present the methods of computer simulation accumulate all the latest achievements based on the application of advanced mathematical and statistical algorithms, which accuracy is more than 80%. The use of the molecular docking, i.e. three-dimensional attachment of the structure with the cavity of the target receptor, allows to determine affinity of compounds to a particular biological target to a large extend and select compounds for which a certain kind of biological activities predicted taking into account specificity of the interaction of a low-molecular chemical compound with the appropriate target[4-5].

Gamma-butyrobetaine hydroxylase enzyme (IUBMB Enzyme Nomenclature: 1.14.11.1) catalyzes the process of transformation of gamma-butyrobetaine into l-carnitine, which is involved in the generation of metabolic energy from long-chain fatty acids, and it, in turn, leads to relaxation of vascular smooth muscles, improvement of microcirculation and the endothelial function.All these processes are very important in the pathology of the cardiovascular system, especially at the early stages of the disease [6]. Gamma-butyrobetaine hydroxylase enzyme inhibitor 3-(1,1,1-trimethylhydrazin-1-ium-2-yl)propanoate (mildronate), which is an approved, clinically used cardioprotective drug, is a relatively poor gamma-butyrobetaine hydroxylase enzyme inhibitor and requires high daily doses.

Among heterocyclic derivatives of 2-imino-1,3-thiazoline and 2,5-disubstituted derivatives of1,3,4-thiadiazole a significant number of highly active compounds with diverse pharmacological actions were identified. This work is one of the stages of searching for effective cardio protectors of the metabolic action based on derivatives of 2-imino-1,3-thiazoline and 1,3,4-thiadiazole.Our study gives possibility to save laboratory animals and time, to carry out purposeful and effective research in future. The aim of our study is to conduct the receptor-based virtual screening of the sample of 154 compounds among derivatives of 2-imino-1,3-thiazoline (77compunds), and2,5-disubstituted derivatives of 1,3,4-thiadiazole(77compunds) to the active site of gamma-butyrobetaine hydroxylase for the directed search of inhibitors of this enzyme as potential cardio protectors.

Materials and Methods

For the receptor-based flexible docking the software packageAutodock4.2.6 was used [7]. Preparation of ligands was performed using such programs as Vega ZZ command line [8] andMGL Tools 1.5.6 [7].

For calculations inAutodock4.2.6 program the input data for the receptor and ligands were converted in a special format PDBQT. The PDBQT file creation, calculation of torsion angles and removal of hydrogen atoms in non-polar atoms for the ligands studied were performed using Vega ZZ program.

As a biological target for docking the active site of the macromolecule from Protein Data Bank of gamma-butyrobetaine hydroxylase enzyme PDB ID: 3O2G was used [9]. The choice of a biological target is stipulated by the literature data concerning the mechanism of action of such reference drugs as mildronate. The receptor maps were prepared in MGL Tools and AutoGrid programs. The docking studies were conducted by the method of the flexible

molecular docking as an approach to search molecules with affinity to a specific biological target. [9]. FromPDB file ID: 3O2G water molecules, ions and the ligand were removed. The Autodock 4.2.6 program setting was taken from the article [10]. The following parameters of docking were determined: the translational motion step was equal to 2 Å, the quaternion angle – 50°, the torsion angle – 50°. The torsion degree of freedom and the coefficient were 2 and 0.274, respectively; the cluster tolerance – 2 Å; the external lattice energy– 1000, the maximal initial energy – 0, the maximal number of attempts – 10 000; the number of structures in the population – 300, the maximal number of stages of energy evaluation – 850 000, the maximal number of generations – 27 000, the number of structures, which pass to the next generation – 1, the level of gene mutation – 0.02,the level of crossover – 0.8, the way of crossover – arithmetic. The α-parameter of Gaussian distribution was equal to 0, and β-parameter of Gaussian distribution – 1; the number of iterations of the Lamarckian genetic algorithm – 50 for each ligand.

Visual analysis of the complexes of compounds from the active site of gamma-butyrobetaine hydroxylase was performed using Discovery Studio Visualizer 4.0 program [11].

Results and Discussion

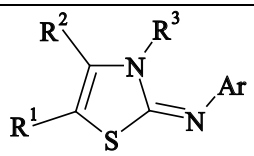
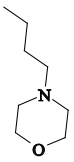
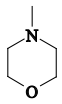
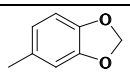
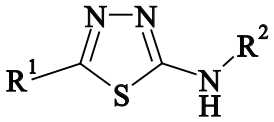
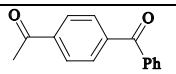
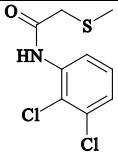
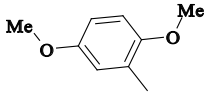
For the study 2,5-disubstituted derivatives of 1,3,4-thiadiazoleand 2-imino-1,3-thiazoline derivatives, which contain hydroxyethyl, methylpiperazine, morpholine, propylmorpholine, ethylmorpholine, ethyl fragments in the structure, in the amount of 154 compounds were selected.

The methodology of the studies conducted consisted in the following stages. To determine the main interactions the docking in the active site of gamma-butyrobetaine hydroxylase of reference drug mildronate was conducted. Then under the same conditions the docking of all 154 compounds in the sample studied was carried out. All results were ranked by the scoring function of Autodock 4.2.6 program and visually assessed by the presence of key interactions with the active site of gamma-butyrobetaine hydroxylase enzyme. According to the results of studying affinity of the substances to the active site of gamma-butyrobetaine hydroxylase15 promising derivatives of 2-imino-1,3-thiazoline which contain morpholine and propyl morpholine fragments in the structure and 3promising derivatives of2-R-amine-5R-1,3,4-thiadiazole were selected. Based on the data obtained it was found that both groups derivatives of 2-imino-1,3-thiazoline and derivatives of2-R-amine-5R-1,3,4-thiadiazole under research are characterized by a high level of affinity calculated for the target studied, and it is comparable with the value of the reference drug mildronate.

The greatest number of promising compoundsare in the groups of 2-imino-1,3-thiazoline derivatives containing the *morpholine* and *propyl morpholine* fragment in the structure, it gives grounds to consider it to be promising for further search of cardioprotectors. The results obtained were compared with the results of the control experiment, namely with the values of the scoring functions of the reference drug–mildronate and classical inhibitor L-carnitine: EDoc -5.68 kcal/molandEDoc -5.41 kcal/mol, respectively.

The structures and the values of the scoring functions of the molecular docking calculated for 18 promising compounds are given in Table 1.

Table 1. *The structures and the values of the scoring functions of the molecular docking calculated for 18 promising compounds.*

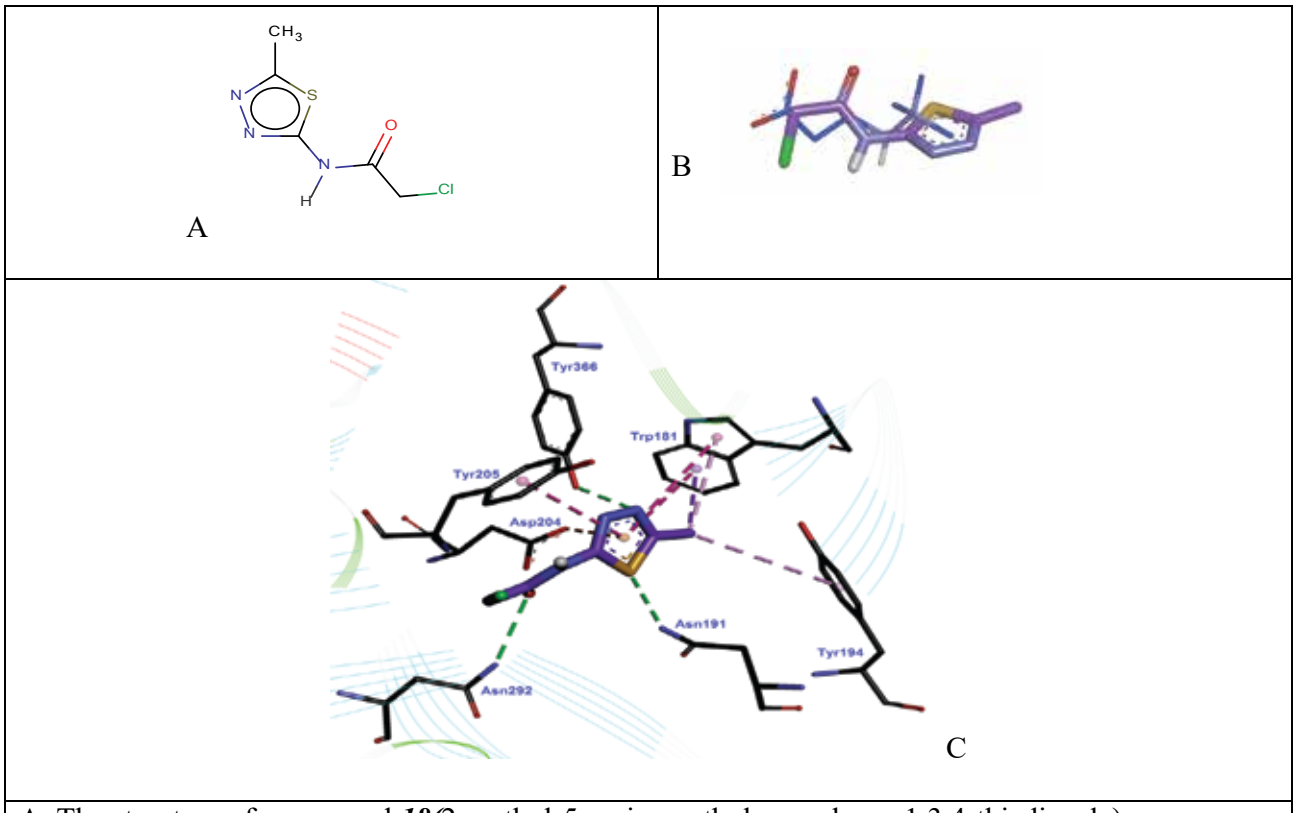
No. of the compound					EDoc kcal/mol
	R^1	R^2	R^3	Ar	
1	Ac	Me		3-MeO-C_6H_4	-9.89
2	Ac	Me		3-Cl-C_6H_4	-8.77
3	Ac	Me		2,5-diMe-C_6H_3	-9.44
4	Ac	Me		2,4-diMe-C_6H_3	-6.67
5	Ac	Me		2-Me-C_6H_4	-6.70
6	Ac	Me		4-MeO-C_6H_4	-6.89
7	Ac	Me		4-Me-C_6H_4	-4.89
8	H	3-NO_2-C_6H_4		Ph	-10.18
9	H	4-Me-C_6H_4		Ph	-10.14
10	H	3,4-diMeO-C_6H_4		Ph	-8.99
11	H	Me		2,5-diMe-C_6H_3	-8.82
12	H	Me		Ph	-9.28
13	H	4-Cl-C_6H_4		4-EtO-C_6H_4	-6.93
14	H			Ph	-7.89
15	H	4-MeO-C_6H_4	Allyl	4-Br-C_6H_4	-8.00
					
16	Ph		-	-	-8.6
17			-	-	-9.25
18	CH_3	$CO-CH_2Cl$	-	-	-10.20

Therefore, the results of the flexible molecular docking of 2-imino-1,3-thiazoline derivatives, which contain morpholine andpropylmorpholine fragments in the structure and 2-R-5-R-amine-1,3,4-thiadiazole to the active site of gamma-butyrobetaine hydroxylase indicate the thermodynamic probability and the energy usefulness of forming complexes between molecules of the substances under research and the enzyme specified.

It is the argument for the study of the metabolic action of the compounds under research *in vivo*. Compounds ***8***, ***9*** and ***18*** are the most promising according to the results of *in silico* studies.

Using Autodock 4.2.6 program the complexes of inhibitors with gamma-butyrobetaine hydroxylase were constructed PDBID: 3O2G.-Figure1shows the type of binding one of the most active inhibitor – 2-methyl-5-aminomethylenecarboxy-1,3,4-thiadiazole ***18*** with the acceptor site of gamma-butyrobetaine hydroxylase PDBID: 3O2G.

Figure1. *Compound 18 in the site of binding the fragment of gamma-butyrobetaine hydroxylase protein (the crystallographic model 3O2G).*



A. The structure of compound ***18(***2-methyl-5-aminomethylenecarboxy-1,3,4-thiadiazole**).**
B. Comparison of the arrangement of the inhibitor and the ligand ***18***of 3O2G crystal
C. The complex of the one of promising compound***18***with the active site of gamma-butyrobetaine hydroxylase PDB ID: 3O2G.

When visualizing the results of the docking the formation of hydrogen bonds with such amino acids as tyrosine Tyr205, asparagi-nAsn191 and asparagin Asn292 was considered to be the key interactions of binding between the ligand and the enzyme specified for all the 2-R-amine-5R-1,3,4-thiadiazole studied. As we can see from figure 1 a number of hydrophobic

contacts with amino acidresidues of tyrosine Tyr 366 and tryptophane Trp181 and cation-π interaction between thiadiazole ring and nearby aromatic groups, and a positive charge at one end of the ligand is crucial for binding facilitated stabilization of the complexes which is formed.

As shown by the control studies, such drug as L-carnitine and 4-(Trimethyl-azaniumyl) butanoate (a crystallized substrate) have the similar arrangement in the active site of gamma-butyrobetaine hydroxylase enzyme [12].The control experiments with L-carnitine confirmed the importance of these interactions. The results of the docking confirm that between the compounds of the test sample and the target it is possible to form stable complexes, in which the arrangement of ligands in the active site of the receptor and amino acid residues of side chains involved in the formation of non-covalent bonds is similar to the geometry and types of binding of levocarnitine. The insignificant differences in the arrangement of the ligand and the active site of the enzyme are explained, first, by the difference of the chemical structures of ligands themselves, and secondly, by the small size of a ligand compared to the volume of the box, and it is taken into consideration by the docking program. It is worth noting the difference in size of molecules of the test sample and the known data on inhibitors of gamma-butyrobetaine hydroxylase. According to CHEMBL data the ligands with the activity from 90 нМto 7 мкМ have the molecular weight from 143uto 274u. This tendency is also confirmed by the relatively compact site of interaction.

2-Imino-1,3-thiazoline derivatives and 2-R,5-R-amine-1,3,4-thiadiazole can be regarded as a promising scaffolds for creating combinatorial libraries of potential biologically active substances, including through the introduction of new pharmakophorecenters in 2nd position thiadiazole and 1 position2-imino-1,3-thiazoline, as evidenced by the results of virtual screening procedures.Our study gives possibility to save laboratory animals and time, to carry out purposeful and effective research in future.

Conclusions:

1. The docking studies have been conducted with the purpose of directed search for inhibitors of gamma-butyrobetaine hydroxylase (PDBID: 3O2G) as potential cardioprotectors of the metabolic action.

2. According to the results of the docking 2-imino-1,3-thiazoline derivatives and 2,5-disubstituted derivatives of 1,3,4-thiadiazolehave the calculated values of affinity to the enzyme, and they are comparable with the values of the classical inhibitor for 15 derivatives of 2-imino-1,3-thiazoline and 3derivatives of2-R,5-R-amine-1,3,4-thiadiazole.

3. The 18 substances from 154 have been chosen for the study of the metabolic action *in vivo*. According to the results of *in silico* studies leadcompounds ***8***, ***9*** and ***18*** are the most promising.The lead compounds may be proposed as a potential cardiotrophic agent for in-depth pharmacological studies.

References:

1. Kuryata AV, Geychenko VP, Muzhchil'L, Karavanskaya IL. Effektivnost' ispol'zovaniya preparata metabolicheskogo deystviya Mildronatv kompleksnoy terapii khronicheskoy serdechnoy nedostatochnostis sokhranennoys istolicheskoy funktsiyey v bol'nykhs ishemicheskoy bolezn'yu serdtsa I yeye vliyaniye na funktsional'noye sostoyaniye endoteliya sosudov, uroven' insulinaikortizola. Lekarstva Ukrainy 2011; 4: 107-112.

2. Dinicolantonio JJ, Lavie CJ, Fares H, Menezes AR, o'Keefe JH. L-Carnitine in the Secondary Prevention of Cardiovascular Disease: Systematic Review and Meta-analysis. Mayo Clinic Proceedings 2013; 88: 544–51.

3. Dambrova M, Makrecka-Kuka M, Vilskersts R, Makarova E, Kuka J, Liepinsh E. Pharmacological effects of meldonium: Biochemical mechanisms and biomarkers of cardiometabolic activity. Pharmacological research. 2016: http://dx.-doi.org/10.1016/j.phrs.2016.01.019

4. Schulz-Gasch T, Stahl M. Scoring-functionsforprotein–ligandinteractions:

acriticalperspective. DrugDiscov.Today-Tech2004; 1: 231-239.

5. Elektronnaya baza dannykh Protein Data Bank [Elektronnyyresurs] // Baza dannykh rasshifrovannykh trekh mernykh struktur belkovykh kompleksov – Rezhimdostupa: http://www.rcsb.org/pdb/[available:2013.12.15].

6. Johri AM, Heyland DK, Hétu MF. Crawford, JD, SpenceB. Carnitine Therapy for the Treatment of Metabolic Syndrome and Cardiovascular Disease: Evidence and Controversies. Nutr. Metab. Cardiovasc.2014: Dis. http://dx.doi.org /10.1016/j.[available:2014.03.07].

7. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ, Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry2009; 16: 2785–2791.

8. Pedretti A, Villa LV. VEGA - An open platform to develop chemo-bio-informatics applications, using plug-in architecture and scriptprogramming. J.C.A.M.D2004; 18: 167–173.

9. Krojer T, KochanG, McDonough-MA,VonDelftF, LeungIKH, HenryL, ClaridgeT-DW, PilkaE, UgochukwuE, MunizJ, FilippakopoulosP, BountraC, ArrowsmithCH, WeigeltJ, EdwardsA, KavanaghKL, SchofieldCJ, OppermannU.Crystal Structure of Human gamma-butyrobetaine,2-oxoglutarate dioxygenase 1 (BBOX1)2010:http://dx.doi.org/10.2210/pdb3o2g/pdb

10. Syniugin AR, Ostrynska OV, Chekanov MO, Volynets GP, Starosyla SA, Bdzhola VG, Yarmoluk SM,. Design, synthesis and evaluation of 3-quinoline carboxylic acids as new inhibitors of protein kinase CK2. J. Enzyme Inhib. Med. Chem. 2016:http://dx.-doi.org/10.1080/14756366.2016.1222584.

11. Discovery Studio Visualizerhttp://accelrys.com/.

12. Tars K, Leitans J, Kazaks A, Zelencova D, Liepinsh Edgars, Kuka J, Makrecka M, Lola D, Andrianovs V, Gustina D, Grinberga S, Liepinsh E, Kalvinsh I, Dambrova M, Loza E, Pugovics O. Targeting Carnitine Biosynthesis: Discovery of New Inhibitors against γ-Butyrobetaine Hydroxylase. J. Med. Chem. 2014;57: 2213–2236.