 doi:10.20944/preprints201803.0003.v1

Article

The docking studies of new derivatives of N-phenylanthranilic acids as inhibitors of microsomal prostaglandin E synthase-1 (mPGES-1)

Marharyta M. Suleiman, Diana A. Alferova, Valentina V. Druhovina, Olena M. Sergienko, Natalya P. Kobzar, Olga V. Kiz

National University of Pharmacy, 53, Pushkinska str., Kharkiv, Ukraine
suleiman.nfau@outlook.com

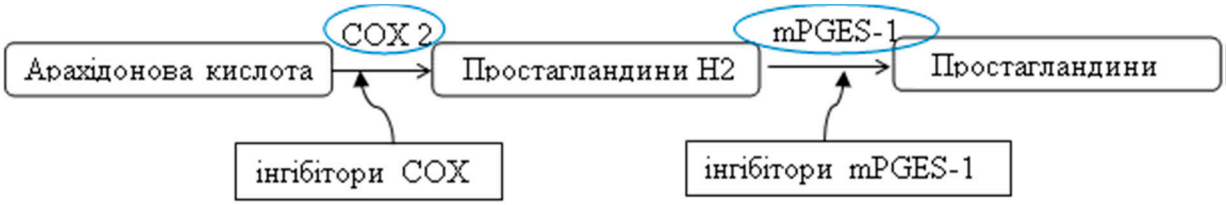
ABSTRACT

The aim of the study was to determine the possibility of suppression of the prostaglandin synthesis by new derivatives of N-phenylanthranilic acids; they inhibit the activity of the microsomal prostaglandin E synthase-1 (mPGES-1) enzyme using the method of a flexible molecular docking. For the docking studies the crystallographic structural models with high resolution from Protein Data Bank were used: mPGES-1 in the complex with glutathione (pdb code 4AL0). A flexible molecular docking was carried out using the Molecular Operating Environment (MOE) software package. According to the results of the docking studies four scoring functions were calculated (Affinity dG Scoring, Alpha HB Scoring, London dG Scoring, GBVI/WSA dG Scoring). The values of the scoring functions calculated indicate the thermodynamic probability and energy favorability of forming complexes between molecules of the substances under research and the specified receptor, in which arrangement of ligands in the active site of the receptor and residues of amino acids of side chains are of similar geometry and types of binding of the known inhibitors of mPGES-1 determined on the basis of the crystallographic studies.

Key words: derivatives of N-phenylanthranilic acids; anti-inflammatory activity; flexible molecular docking; microsomal prostaglandin E synthase-1.

INTRODUCTION

Microsomal prostaglandin E synthase-1 (mPGES-1) [1] – the final enzyme in the cascade of arachidonic acid transformations – is one of the new promising targets for searching biologically active substances exhibiting the anti-inflammatory action.



It belongs to the group of proteins involved in the metabolism of eicosanoids and glutathione (MAPEG proteins family), and is a membrane-associated protein, as well as a key enzyme in the catalytic isomerization of H2 (PGH2) prostaglandins to E2 (PGE2) prostaglandins. Glutathione is a cofactor required to provide the catalytic activity of mPGES-1. The possible mechanisms of action of mPGES-1 inhibitors are in their competitive interaction with the active sites of the cofactor or the substrate [2]. The scientific literature of recent years describes the results of studying phenanthrene imidazole derivatives as effective inhibitors of mPGES-1 [3,4]. Shan He et al. [5] report on the results of the docking studies of mPGES-1 inhibitors – derivatives of indole, pyrimidine and pyrazoline that

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

are able to compete with both the cofactor and the substrate for the active site in the protein structure. Fig. 1 shows the structures of the currently known inhibitors of mPGES-1.

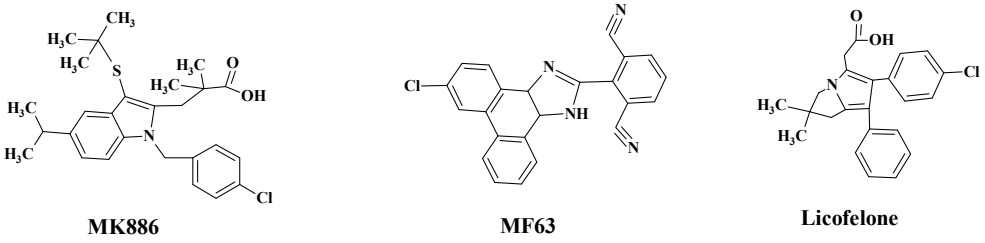


Fig. 1. The structures of the known inhibitors of mPGES-1

The previous *in silico* studies of new derivatives of N-phenylanthranilic acids as inhibitors of COX-1, COX-2 showed positive results [6, 7]. Therefore, it is expedient to continue the study in relation to the mPGES-1 target.

MATERIALS AND METHODS

For the docking studies the crystallographic structural models with high resolution from Protein Data Bank were used: mPGES-1 in the complex with glutathione (pdb code 4AL0) [8]. A flexible molecular docking was carried out using the Molecular Operating Environment (MOE) software package [9].

Before beginning the procedure of docking for 33 compounds synthesized – 3-oxamoyl-substituted and 3-succinoyl-substituted N-phenylanthranilic acids and their methyl esters – the base of conformers was created with the help of systematic conformation search using MOE. Minimization of the energy of all conformers obtained was carried out using MMFF94x force field and stopped when the value of the root mean square gradient (RMS gradient) was less than 0.01 with the number of iterations of not more than 200. Conformers, which energy values exceeded more than 7 kcal/mol of the minimum energy value found for this compound, were excluded from the database as energy unfavorable. In addition, for each compound the maximum number of generated conformers was set at the level of 200. Thus, the base of 2545 conformers for 33 compounds studied was formed. The preliminary optimization of the structure of receptors included the calculation of partial charges on atoms and the procedure of 3*D* protonation at pH=7.4, its purpose was to identify and correct the ionization state of acidic and basic functional groups of the residues of certain amino acids, as well as the position of hydrogen atoms in the structure of the peptide macromolecule. After that the final gradient minimization of the energy with the superposition of AMBER99 force field was performed till reaching the value of 0.01 by RMS gradient. In the active site of the receptor "dummy atoms" were created, and the residues of amino acids (alpha centers) within the radius of 4.5 Å from them were selected. The possible positions of ligands in the active site of the receptor was determined using the iterative procedure, in which a randomly selected conformer was placed in the binding sites in the way that the superposition of three arbitrary atoms of the ligand and three alpha centers of the receptor occurred [7].

RESULTS AND DISCUSSION

According to the results of the docking studies four scoring functions were calculated. The Affinity dG Scoring function determines the enthalpic contribution to the value of free energy of binding. The Alpha HB Scoring function is calculated as a linear combination of two values: the first of them determines the geometric location of the ligand in the acceptor active site, while the second one is the effects of formation of hydrogen bonds between them. The London dG Scoring function

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

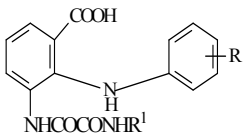
determines the free energy of binding for a particular conformation position of the ligand. The GBVI/WSA dG Scoring function determines the free energy of binding for a particular conformation position of the ligand and is calculated using force fields of MMFF94x and AMBER99.

The values of the scoring functions calculated as a result of the molecular docking of 3-substituted N-phenylanthranilic acids to mPGES-1 also indicate the thermodynamic probability and energy favorability of forming complexes between molecules of the substances under research and the specified receptor. Since there were no crystallographic models of the inhibitors of mPGES-1 co-crystallized with this protein, and the activity of the known inhibitors was found based on *in vitro* studies, the procedure of molecular docking was also carried out for MK886, MF63 and Licofelone; the scoring functions obtained for them were used as the standard functions.

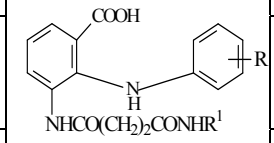
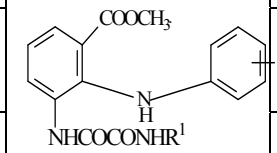
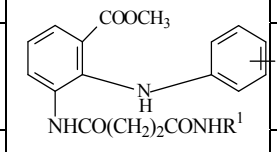
The analysis of the results of the docking studies (Tab. 1) shows that the values of Alpha HB and Affinity dG scoring functions for complexes of the compounds synthesized with the receptor of mPGES-1 are comparable with the values of these functions for the known mPGES-1 inhibitors, and for some compounds the values of these functions are even higher by absolute values. The absolute values of London dG are also comparable with the values of this function in standard substances, and only for some substances (compounds **15**, **32**) they are considerably lower by absolute values compared to the standards. It should be noted that for complexes of a significant part of the compounds synthesized with mPGES-1 the absolute values of GBVI/WSA dG scoring function are higher than for the known inhibitors.

In the analysis of types of binding and the amino acid residues of the active site of mPGES-1 forming bonds with ligands a special attention was paid to Arg126 and Ser127 probably involved in the mechanism of catalytic isomerization of PGH2 to PGE2. In general, in the complexes formed by molecules of the substances synthesized with mPGES-1 an increase in the number of interactions of various types is observed.

Table 1: The results of a flexible molecular docking of the compounds synthesized to the receptor of mPGES-1 (pdb code 4AL0)

Compound	Formula	R	R^1	GBVI/WSA dG	London dG	Alpha HB	Affinity dG
1		H	H	-4.2138	-7.7778	-41.1597	-2.9369
2		2'-CH_3	CH_3	-3.8088	-6.2663	-30.2639	-1.9620
3		3'-CH_3	$(CH_2)_2OH$	-3.8409	-7.9189	-39.9362	-2.1648
4		4'-CH_3	C_3H_7-i	-3.9510	-7.2311	-27.8405	-2.0386
5		3',4'-$(CH_3)_2$	C_4H_9-н	-3.7462	-7.9539	-34.5409	-1.9488
6		4'-CH_3	CH_3	-3.9581	-6.1561	-38.7788	-1.3565
7		4'-OC_2H_5	CH_3	-3.6711	-7.7513	-29.8789	-2.5746
8		2'-Cl	CH_3	-3.3521	-7.1179	-30.5325	-1.0587
9		4'-Cl	CH_3	-4.3030	-5.6423	-47.1469	-2.4576
10		2′-CH_3	CH_3	-4.4855	-7.4586	-40.9886	-2.1172

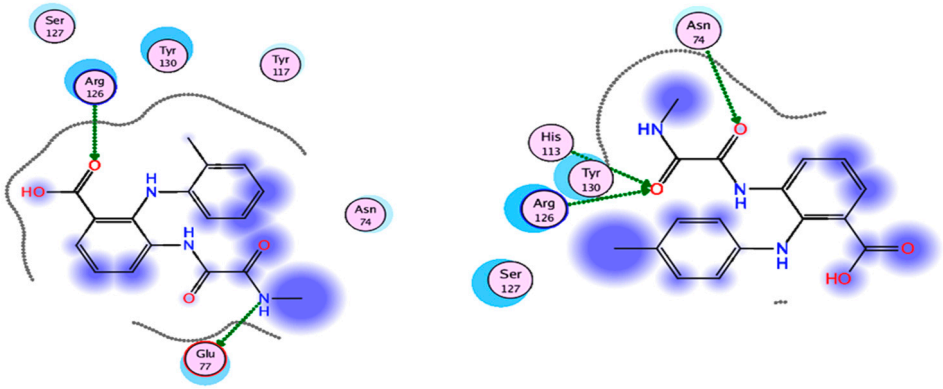
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

11		2′-CH_3	$(CH_2)_2OH$	-4.7620	-7.1531	-35.1506	-2.3335
12		4′-CH_3	CH_3	-4.5947	-5.8644	-28.7955	-1.2651
13		4′-CH_3	$(CH_2)_2OH$	-4.3816	-5.2325	-34.6931	-2.3243
14		3',4'-$(CH_3)_2$	CH_3	-4.1329	-7.4254	-38.7918	-2.1411
15		3',4'-$(CH_3)_2$	$(CH_2)_2OH$	-4.1109	-3.3687	-53.6302	-2.3066
16		4'-OC_2H_5	CH_3	-3.8837	-6.3415	-34.0936	-2.1403
17		4'-Cl	CH_3	-4.0017	-8.5599	-49.8055	-2.3292
18		H	H	-3.7772	-6.5356	-35.6719	-2.9369
19		2′-CH_3	CH_3	-4.3125	-6.5875	-46.1146	-1.5265
20		4′-CH_3	CH_3	-4.1226	-6.2504	-45.6104	-1.8903
21		I3',4'-$(CH_3)_2$	C_4H_{9}-н	-4.3845	-7.7308	-41.7009	-1.7571
22		4′-CH_3	C_3H_7-i	-3.9159	-8.2471	-32.8899	-1.6527
23		4'-OC_2H_5	CH_3	-4.2000	-8.2200	-37.3668	-1.0680
24		2'-Cl	CH_3	-4.4725	-6.2139	-22.1734	-1.9467
25		4'-Cl	CH_3	-4.3177	-7.0286	-17.6797	-1.9136
26		2′-CH_3	CH_3	-4.0853	-6.4538	-48.8882	-2.3934
27		2′-CH_3	$(CH_2)_2OH$	-4.2831	-6.6828	-40.7625	-1.2397
28		4′-CH_3	CH_3	-3.8333	-5.3968	-16.9914	-1.8399
29		4′-CH_3	$(CH_2)_2OH$	-4.2557	-5.8715	-40.3249	-1.5893
30		3',4'-$(CH_3)_2$	CH_3	-3.9983	-6.1287	-32.5156	-0.7423
31		3',4'-$(CH_3)_2$	$(CH_2)_2OH$	-4.4095	-6.0729	-36.6100	-1.7777
32		4'-OC_2H_5	CH_3	-4.1640	-3.1695	-21.8559	-1.5607
33		4'-Cl	CH_3	-4.7829	-6.1310	-24.6627	-1.4939

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

Analgin	-4.4452	-5.3778	-5.3732	-2.1121
MK886	-4.040	-7.472	-34.163	-2.326
MF63	-4.068	-4.599	-36.495	-1.919
Licofelon	-3.996	-7.811	-33.234	-2.741

Molecules of 3-substituted N-phenylanthranilic acids can bind in the complexes with mPGES-1 by forming one or more hydrogen bonds between the carbonyl oxygen atom of the carboxyl group of anthranilic acid and Arg126 (compounds **1**, **2**, **5**, **7** and **8**). In the complexes of molecules of compounds **1** and **7** with mPGES-1 there is another bond formed between the same oxygen atom and Ser127. Furthermore, molecules of compounds **1** and **2** form one more hydrogen bond between one of the nitrogen atoms of oxamoyl and Glu77 (Fig. 2), and the complexes of compounds **1** and **9** are also stabilized due to the π-π interaction between the phenolic substituent of anthranilic acid and Tyr130. In the complexes of compounds **3**, **4**, **6** and **8** the hydrogen bonds are formed between one or both carbonyl oxygen atoms of the oxalic acid residue and Arg126 (Fig. 2). Compounds **6** and **8** also form bonds between the same oxygen atoms and the amino acid residues of His113 and Asn74.



a) b)

Fig. 2. The diagrams of interactions of ligands in the complexes with mPGES-1 for compounds **2** (a) and **6** (b)

The superposition of glutathione and compound **8** in the active site of mPGES-1 is presented in Fig. 3. In the complex of compound **4** with mPGES-1 the carbonyl oxygen atom of the carboxyl group of anthranilic acid forms an additional bond with Arg77, while in the complex of compound **3** it is between the hydroxyl oxygen atom of 2-hydroxy-ethylaminooxalyl and Glu77.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

The complex formed with mPGES-1 and the molecule of compound **8** is additionally stabilized due to the H-π interaction of the phenyl ring of phenylanthranilic acid and Tyr130.

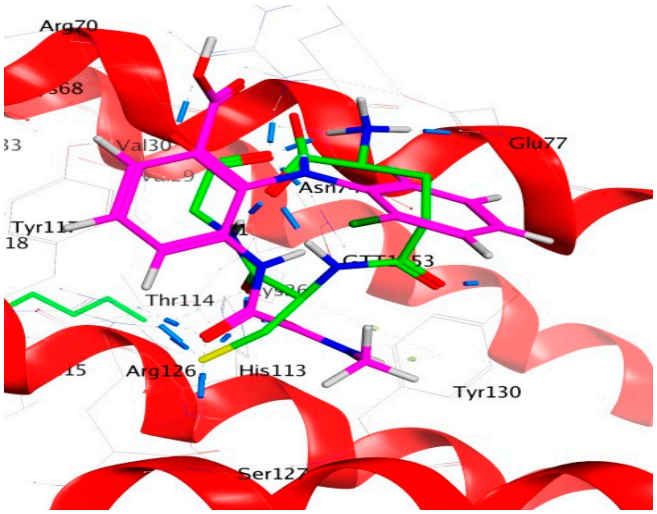
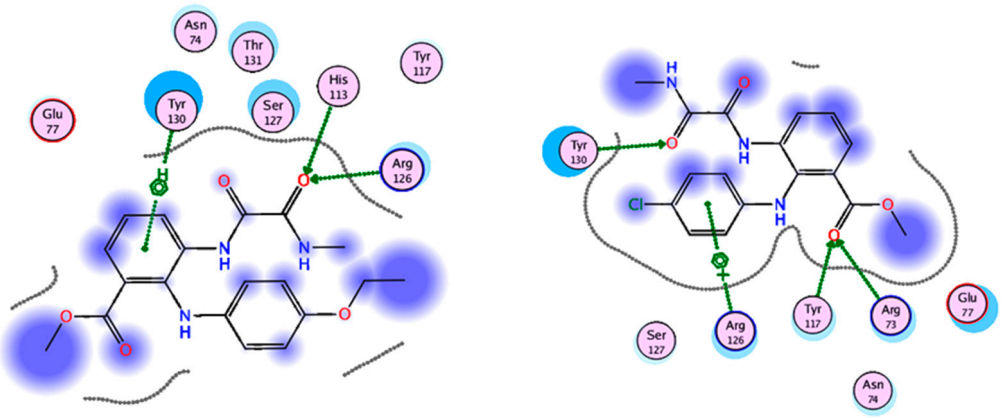


Fig. 3. The superposition of glutathione (green) and compound **8** (lilac) in the active site of mPGES-1

Molecules of all methyl esters of 3-oxamoyl-substituted N-phenylanthranilic acids studied form complexes with mPGES-1 due to the hydrogen bonds between one or both carbonyl oxygen atoms of the oxalic acid residue and Arg126, except of compound **24**, which molecule forms two bonds between carbonyl oxygen atoms of the aminooxalyl substituent with Arg73 and Tyr117 receptor, and compound **25**, in which the hydrogen bonds are formed by carbonyl oxygen atoms of anthranilic acid with Arg73 and Tyr117 in the molecule complex with mPGES-1. Furthermore, in the complexes the additional hydrogen bonds are formed between the abovementioned carbonyl oxygen atoms and the amino acid residues of Ser127, His113, Asn74 receptor. The complexes of compounds **23** and **25** are additionally stabilized due to the π-H and π-cationic interaction of the phenyl ring of phenylanthranilic acid with Tyr130 and Arg126, respectively (Fig. 4.).

Molecules of 3-succinoyl-substituted N-phenylanthranilic acids can bind with mPGES-1 by forming one or two hydrogen bonds between the carbonyl oxygen atom of the carboxyl group of anthranilic acid and Arg126 (compounds **10**, **11**, **13**, **16** and **17**) (Fig. 5.).



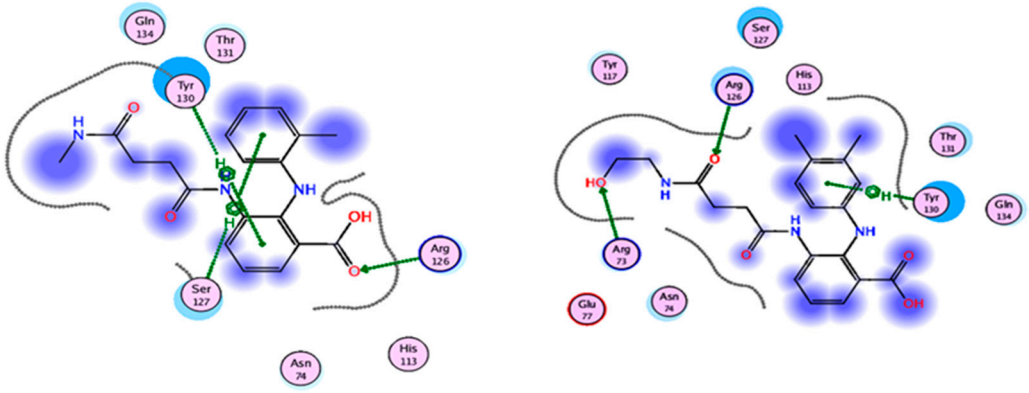
a) a)

b)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

Fig. 4. The diagrams of interactions of ligands in the complexes with mPGES-1 for compounds **23** (a) and **25** (b)

Moreover, additional stabilization of the complexes is possible due to the π-H interaction of the phenyl ring of phenylanthranilic acid with Ser127 and Tyr130 (compounds **10**, **11**, **13**) or the interaction of the phenyl ring of phenylanthranilic acid with Tyr130 (compound **17**). Molecules of compounds **12**, **14** and **15** form the complexes with mPGES-1 by forming the hydrogen bonds between Arg126 and carbonyl oxygen atoms of the propionyl amino group in position 3 of N-phenylanthranilic acid. The same oxygen atom of compound **14** forms also the bond with the amino acid residue of His113. The superposition of compounds **8** and **14** in the active site of mPGES-1 is given in Fig. 6.



a) b)

Fig. 5. The diagrams of interactions of ligands in complexes with mPGES-1 for compounds **10** (a) and **15** (b)

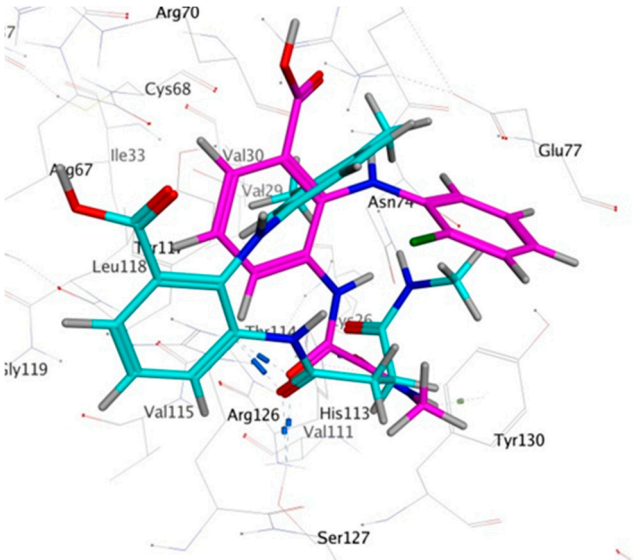


Fig. 6. The superposition of molecules of compounds **8** (lilac) and **14** (blue) in the active site of mPGES-1

In the complex of compound **15** with the receptor, except that the hydroxyl oxygen atom of the aminocarbonyl substituent forms the hydrogen bond with Arg73, there is also the π-H interaction of the phenyl ring of phenylanthranilic acid with Ser127 (Fig. 5).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

For methyl esters of 3-succinoyl-substituted N-phenylanthranilic acids formation of the complexes with mPGES-1 is typical due to the hydrogen bonds between carbonyl oxygen atoms of aminocarbonyl or propionyl aminocarbonyl groups and the amino acid residues of Arg126, Arg73, Tyr117, His113 receptor. Moreover, additional stabilization of the complexes is possible due to π-H and π-π interactions of the phenyl ring of phenylanthranilic acid with Tyr130 for molecules of compounds **28** and **33**, respectively. The superposition of molecules of compounds **22** and **29** in the active site of mPGES-1 is given in Fig. 7.

Tab. 2 shows the types, energy and localization of the interactions of molecules of 3-oxamoyl-substituted and 3-succinoyl-substituted N-phenylanthranilic acids and their methyl esters that exhibit the highest anti-inflammatory and analgesic activity, in the complexes with mPGES-1.

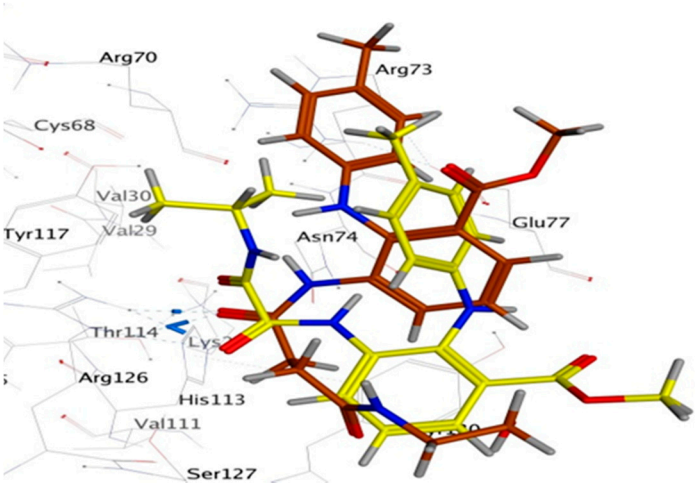


Fig. 7. The superposition of molecules of compounds **22** (yellow) and **29** (brown) in the active site of mPGES-1

Table 2. Residues of amino acids of the active site of the receptor, types and energy of interactions in the of 3-oxamoyl-substituted and 3-succinoyl-substituted of N-phenylanthranilic acids and their methyl esters with mPGES-1

The code of the compound	Atoms of the compounds studied that interact with the receptor	Residues of amino acids and types of bonds	The bond length, Å	The interaction energy, kcal/mol
1	**2**	**3**	**4**	**5**
3	Carbonyl oxygen atom of oxalyl *Ph*-NH-CO-	Arg126 hydrogen	3.54	-1.0
	Carbonyl oxygen atom of aminooxalyl *Ph*-NH-CO-CO-	Arg126 hydrogen	3.01	-1.3
	Hydroxyl oxygen atom of 2-hydroxy-ethylaminooxalyl	Glu77 hydrogen	3.27	-1.3
4	Carbonyl oxygen atom of oxalyl *Ph*-NH-CO-	Arg126 hydrogen	3.11	-2.9
	Carbonyl oxygen atom of the carboxyl group of anthranilic acid -COOH	Arg73 hydrogen	2.89	-5.1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0003.v1

8	Carbonyl oxygen atom of oxalyl *Ph*-NH-CO-	Arg126 hydrogen	2.97	-1.9
	Carbonyl oxygen atom of oxalyl *Ph*-NH-CO-	Ser127 hydrogen	3.19	-0.7
	Carbonyl oxygen atom of aminooxalyl *Ph*-NH-CO-CO-	His113 hydrogen	3.66	-0.6
	Phenyl ring of phenylanthranilic acid	Tyr130 H-π	3.63	-0.6
14	Carbonyl oxygen atom of aminooxalyl *Ph*-NH-CO-CO-	Arg126 hydrogen	3.19	-2.8
	Carbonyl oxygen atom of aminooxalyl *Ph*-NH-CO-CO-	His113 hydrogen	3.44	-1.4
15	Carbonyl oxygen atom of carbamoyl R^1NH-C(O)-	Arg126 hydrogen	3.15	-3.9
	Carbonyl oxygen atom of carbamoyl R^1NH-C(O)-	Arg126 hydrogen	3.14	-2.2
	Hydroxyl oxygen atom of the aminocarbonyl substituent -NH-$(CH_2)_2$-OH	Arg73 hydrogen	2.95	-2.3
	Hydroxyl oxygen atom of the aminocarbonyl substituent -NH-$(CH_2)_2$-OH	Arg73 hydrogen	3.25	-1.0
	Phenyl ring of phenylanthranilic acid	Tyr130 H-π	4.17	-0.6
22	Carbonyl oxygen atom of propionyl amino group *Ph*-NH-CO-	Arg126 hydrogen	3.38	-2.1
29	Carbonyl oxygen atom of propionyl amino group *Ph*-NH-CO-	Arg126 hydrogen	3.06	-3.3
	Carbonyl oxygen atom of propionyl amino group *Ph*-NH-CO-	Arg126 hydrogen	3.02	-1.4
30	Carbonyl oxygen atom of propionyl amino group *Ph*-NH-CO-	Arg126 hydrogen	2.94	-2.5
	Carbonyl oxygen atom of propionyl amino group *Ph*-NH-CO-	His113 hydrogen	3.51	-1.0

Thus, the results of a flexible molecular docking of 3-oxamoyl-substituted and 3-succinoyl-substituted N-phenylanthranilic acids and their methyl esters to mPGES-1 indicate the possibility of forming stable complexes between them, in which for all compounds studied binding between the ligand and the receptor occurs with participation of oxygen atoms of the carboxyl group of anthranilic

acid or oxygen carbonyl atoms in the residues of dicarboxylic acids in the form of hydrogen, as well as π-H or π-π interactions involving the phenyl ring of N-phenylanthranilic acid.

CONCLUSION

1- The docking studies conducted have found that the pharmacological activity is associated with suppression of the prostaglandin synthesis by new derivatives of N-phenylanthranilic acids; they inhibit the activity of the mPGES-1 enzyme.

2- The results obtained indicate the possibility of forming stable complexes of molecules of the substances studied with mPGES-1 with participation of oxygen atoms of the carboxyl group of anthranilic acid or oxygen carbonyl atoms in the residues of dicarboxylic acids in the form of hydrogen, as well as π-H or π-π interactions involving the phenyl ring of phenylanthranilic acid.

REFERENCES

1. Jegerschold, C.; Pawelzik, S.C.; Purhonen, P.; Bhakat, P.; Gheorghe, L.R.; Gyobu, N.; Mitsuoka, K.; Morgenstern, R.; Jakobsson, P.J.; Hebert, H. Structural basis for induced formation of the inflammatory mediator prostaglandin E2. *PNAS*. /. **2008**, *105*, 1110–11115.
2. Hamza, A.; Zhao, X.; Tong, M.; Tai, H.H.; Zhan, C.G. Novel human mPGES-1 inhibitors identified through structure-based virtual screening. *Bioorg. Med. Chem*. /. **2011**, *19*, 6077-6086.
3. AbdulHameed, A.; Hamza, J.; Liu, X.; Huang, C.G.; Zhan, M.D.M. Human Microsomal Prostaglandin E Synthase-1 (mPGES-1) Binding with Inhibitors and the Quantitative Structure-Activity Correlation. *J. Chem. Inf. Model*. /. **2008**, *48*, 179-185.
4. Dallaporta, M.; Pecchi, E.; Thirion, S.; Jean, A.; Troadec, J.D. Towards the management of inflammation: Recent developments of PGES-1 inhibitors. *Recent patents on cns drug discovery*. /. **2010**, *5*, 70-80.
5. He, S.; Lai, L. Molecular Docking and Competitive Binding Study Discovered Different Binding Modes of Microsomal Prostaglandin E Synthase-1 Inhibitors. *J. Chem. Inf. Model*. /. **2011**, *51*, 3254–3261.
6. Suleiman, M. M.; Isaev, S. G.; Klenina, O. V.; Ogurtsov, V. V. Synthesis, biological activity evaluation and QSAR studies of novel 3-(aminooxalyl-amino)and 3-(carbomoyl-propionylamino)-2-phenylamino-bCenzoic acid derivatives. *Journal of Chemical and Pharmaceutical Research*. /. **2014**, *6*, 1219-1235.
7. Suleiman, M.M.; Gritsenko, I.S.; Alferova, D.A.; Druhovina, V.V.; Sergienko, O.M. Docking studies of the derivatives of 3-oxamoyl(succinoyl)amides of N-phenylanthranilic acids as inhibitors of enzymes COX-1 and COX-2. *Der Pharma Chemica*. /. **2017**, *9*, 14-23.
8. Sjögrena, T.; Nordb, J.; Eka, M.; Johanssona, P.; Liub, G.; Geschwindnera, S. Crystal structure of microsomal prostaglandin E2 synthase provides insight into diversity in the MAPEG superfamily. *Proc.Natl.Acad.Sci. USA*. /. **2013**, *110*, 3806–3811.
9. Molecular Operating Environment (MOE), 2012.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2012.