Molecular docking study of the interactions of prostanoid EP4 receptor with potent ligands†

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Abstract: In this work, a previously reported homology model of prostanoid EP4 was used for docking studies of potent EP4 ligands, in order to provide information about protein - ligand interaction patterns. Glide software, from the Schrödinger package, with XP option, was used for docking simulations. Among the amino acids residues from the EP4 binding site that made interactions with the ligands taken in our study, the key residue Ser285 (highlighted, also, in mutagenesis studies) was noted. The observed interactions between ligands and amino acid residues consist in several hydrogen bonds (e.g. with Thr175, His181, Ser95, Ser103, Asp311) and hydrophobic interactions (e.g. with Ala314, Tyr186). The outcome resulted from the docking studies led to a better understanding of how the agonists and antagonists bind in situ and may lead to the discovery of new active compounds.

Keywords: prostaglandin EP4 receptor; agonists docking; antagonists docking

1. Introduction

It is known from the specialty literature that many biological processes, like: bone and vascular remodeling, carcinogenesis, renal function, cardiac hypertrophy, gastrointestinal homeostasis, and reproductive function, are closely related to EP4 signaling [1]. EP4 receptor has, also, a major role in pain and inflammation induced by prostaglandin E2 (PGE2) mediator [2]. Recent studies on EP4 receptor from rat shows that the administration of the selective agonist AE1-329 in subarachnoid hemorrhage considerable improve neurological dysfunction [3].

This protein is one of the four receptor subtypes identified for PGE2, namely: EP1, EP2, EP3 and EP4, which belong to the larger class of G-protein-coupled receptors. EP4 have been identified for the first time in the piglet saphenous vein [4], and it was observed to be insensitive to agonists of the other types of EP receptors (EP1, EP2, and EP3) [1]. The expression of EP4 receptor was found in a vast variety of tissues (e.g. cardiovascular, immune, gastrointestinal, skeletal, cancer tissue) [1, 5].

It was observed that both agonists and antagonists of EP4 receptor are responsible for the influence of various pathologic states [1]. In order to gain insight about the interaction patterns between EP4 receptor and its ligands (agonists and antagonists) the homology model of EP4 waspreviously built [6] and was used for docking experiments in this study.

2. Materials and Methods

2.1. Ligand Preparation

21 compounds with affinity for prostanoid EP4 receptor (Ki (μM)), which act as antagonists were selected from literature [7] and 32 compounds with affinity for EP4 lower than 10 nM (EC50 (nM)), which acts as agonists, were downloaded from CHEMBL database [8]. The 2D structure of the agonists and antagonists were generated using the Marvin Sketch software, version 17.18, from Chemaxon [http://www.chemaxon.com.], as isomeric smiles (see Table 1).

Table 1. ID, SMILES code for compounds of dataset

No	ID	Smiles Code
1	CHEMBL127204	OC(=O)CCc1ccccc1c2cccc(c2)c3ccc(OCc4ccccc4)cc3
2	CHEMBL127482	OC(=O)CCc1ccccc1c2cccc(c2)c3ccccc3COc4ccccc4
3	CHEMBL338388	OC(=O)CCc1ccccc1c2cccc(c2)c3ccccc3OCc4ccccc4
4	CHEMBL124199	OC(=O)c1ccc(CCCc2ccccc2OCc3ccccc3)cc1
5	CHEMBL125269	OC(=O)CCCc1ccccc1c2cccc(c2)c3ccccc3OCc4ccccc4
6	CHEMBL434247	CC(Cc1ccccc1c2cccc(c2)c3ccccc3OCc4ccccc4)C(=O)O
7	CHEMBL125087	OC(=O)CCc1ccccc1c2cccc(c2)c3ccccc3OCc4c(Cl)cccc4Cl
8	CHEMBL124738	OC(=O)CCc1ccccc1c2csc(c2)c3ccccc3OCc4ccccc4
9	CHEMBL434637	O=C(CCc1ccccc1c2cccc(c2)c3ccccc3OCc4ccccc4)NS(=O)(=O)c5cccs5
10	CHEMBL125588	OC(=O)C1CC1c2ccccc2c3csc(c3)c4ccccc4OCc5ccccc5
11	CHEMBL123855	OC(=O)CCc1ccccc1c2cc(cs2)c3ccccc3OCc4ccccc4
12	CHEMBL123844	OC(=O)Cc1ccccc1c2cccc(c2)c3ccccc3OCc4ccccc4
13	CHEMBL340501	OC(=O)\C=C\c1ccccc1c2cccc(c2)c3ccccc3OCc4ccccc4
14	CHEMBL332446	CC(CC(=O)O)c1ccccc1c2csc(c2)c3ccccc3OCc4ccccc4
15	CHEMBL124675	OC(=O)CNc1ccccc1c2csc(c2)c3ccccc3OCc4ccccc4
16	CHEMBL446098	CC(Cc1ccccc1c2csc(c2)c3ccccc3OCc4ccccc4)C(=O)O

17	CHEMBL126472	OC(=O)CCc1ccccc1c2cccc(c2)c3ccccc3
18	CHEMBL124574	OC(=O)\C=C\c1ccccc1c2ccc(Cl)c(Cl)c2
19	CHEMBL87371	Clc1ccc(cc1Cl)c2ccccc2\C=C\C(=O)NS(=O)(=O)c3cccs3
20	CHEMBL123794	Cc1cccc(\C=C\Cc2ccccc2\C=C\C(=O)O)c1OCc3ccccc3
21	CHEMBL125110	OC(=O)CCc1ccccc1c2ccc(s2)c3ccccc3OCc4ccccc4
22	CHEMBL251294	CCCC[C@H](O)\C=C\[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O
23	CHEMBL222715	CCCCC[C@H](O)\C=C\[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O
24	CHEMBL272276	O[C@@H](Cc1ccccc1)\C=C\[C@H]2CCC(=O)N2CCc3ccc(cc3)C(=O)O
25	CHEMBL258332	O[C@@H](Cc1cccc(Cl)c1)\C=C\[C@H]2CCC(=O)N2CCc3ccc(cc3)C(=O)O
26	CHEMBL222677	Cc1cc(Cl)ccc1c2cccc(c2)[C@H](O)CC[C@H]3CCC(=O)N3CCc4ccc(cc4)C(=O)O
27	CHEMBL251294	CCCC[C@H](O)\C=C\[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O
28	CHEMBL251505	CCCC\C(=C/C=C/[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O)\C
29	CHEMBL249953	O[C@H](CCN1CCC(=O)N1CCc2ccc(cc2)C(=O)O)Cc3cccc(Br)c3
30	CHEMBL222782	O[C@@H](CCC1CCC1)\C=C\[C@H]2CCC(=O)N2CCc3ccc(cc3)C(=O)O
31	CHEMBL249538	OC(CCN1CCC(=O)N1CCc2ccc(cc2)C(=O)O)Cc3cccc(Br)c3
32	CHEMBL249744	OC(CCN1CCC(=O)N1CCc2ccc(cc2)C(=O)O)Cc3cccc(Cl)c3
33	CHEMBL1645138	C[C@H](NC(=O)c1cccc2CCN(Cc3cccc(c3)C(F)(F)F)c12)c4ccc(cc4)C(=O)O
34	CHEMBL272277	CCCCC1(CCC1)[C@@H](O)\C=C\[C@H]2CCC(=O)N2CCc3ccc(cc3)C(=O)O
35	CHEMBL1645142	C[C@H](NC(=O)c1cccc2CCN(Cc3cc(Br)cc(Br)c3)c12)c4ccc(cc4)C(=O)O
36	CHEMBL248679	C\C(=C/C=C/[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O)\c3ccccc3
37	CHEMBL1933725	O[C@@H](Cc1ccccc1)\C=C\[C@H]2CCC(=O)N2CCSc3nc(cs3)C(=O)O
38	CHEMBL298026	Cc1cc(Cl)ccc1c2cccc(c2)C(O)\C=C\[C@H]3CCC(=O)N3CCCCCCC(=O)O

39	CHEMBL1645133	C[C@H](NC(=O)c1cccc2CCN(Cc3cccc(Cl)c3)c12)c4ccc(cc4)C(=O)O
40	CHEMBL251709	CCCC\C=C/C=C/[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O
41	CHEMBL275667	O[C@@H](Cc1ccccc1)\C=C\[C@H]2CCC(=O)N2CCCCCCc3nnn[nH]3
42	CHEMBL548	CCCCC[C@H](O)\C=C\[C@H]1[C@H](O)CC(=O)[C@@H]1C\C=C/CCCC(=O)O
43	CHEMBL3754586	CCCC[C@H](C)C[C@H](O)\C=C\[C@H]1CCC(=O)N1CCSc2nc(cs2)C(=O)O
44	CHEMBL251710	CC\C(=C/C=C/[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O)\C
45	CHEMBL548	CCCCC[C@H](O)\C=C\[C@H]1[C@H](O)CC(=O)[C@@H]1C\C=C/CCCC(=O)O
46	CHEMBL257658	CCCCC(C)(C)[C@H](O)\C=C\[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O
47	CHEMBL398947	CC(=C\C=C\[C@H]1CCC(=O)N1CCc2ccc(cc2)C(=O)O)C
48	CHEMBL3752377	CCCCC(C)(C)[C@H](O)\C=C\[C@H]1CCC(=O)N1CCSc2nc(cs2)C(=O)O
49	CHEMBL249136	CCCCCC(O)CCN1CCC(=O)N1CCc2ccc(cc2)C(=O)O
50	CHEMBL222834	COCc1cccc(C[C@H](O)\C=C\[C@H]2CCC(=O)N2CCc3ccc(cc3)C(=O)O)c1
51	CHEMBL413509	CCCCC[C@H](O)\C=C\[C@H]1[C@H](O)CC(=O)N1C\C=C\CCCC(=O)O
52	CHEMBL192743	Cc1cc(Cl)ccc1c2cccc(c2)[C@H](O)CC[C@H]3CCCC(=O)N3CCSCCCC(=O)O
53	CHEMBL548	CCCCC[C@H](O)\C=C\[C@H]1[C@H](O)CC(=O)[C@@H]1C\C=C/CCCC(=O)O

*Compounds 1 to 21 acts as antagonists and 22 to 53 acts as agonists on the prostanoid EP4 receptor

2.2. Docking

The 3D structure for EP4 prostanoid receptor used in this investigation was achieved previously by homology modelling [6]. The Maestro suite version 2016-3 [9] was used in all the preliminary stages for the docking process with Glide [10, 11]. Thus, the database comprising 21 antagonists and 32 agonists, was prepared for docking procedure by generating energetically minimized tautomers (with the force field OPLS_2005) and ionization states at physiological pH (7.2 ±0.2), using LigPrep software [12]. Glide software [10] with the extra precision (XP) option was engaged in the docking process. The Grid generated was centred on the Asp311 residue and the default settings were used during the docking. For the docking step, no further restrictions were applied to the default settings. The XP GScore scoring function was used to select the best poses for each ligand.

2.3. Pharmacophore modeling

For a better understanding of the features necessary for a ligand to be recognized by the target, a pharmacophore study was achieved with the aid of Phase software [13, 14]. The pharmacophore

model was developed based on the multiple ligands resulted from the docking poses and by using the prealigned ligands option. Other settings used were: hypothesis should match at least 40% of actives; number of features in the hypothesis from 3 to 5, and all the features found were taken in account.

3. Results and Discussion

The pattern of the antagonist binding mode at EP4 receptor is rendered in Figure 1. The observed interactions between antagonists and amino acid residues from EP4 binding site are: hydrogen bonds with: Ser285 (also highlighted by mutagenesis study [15]), Ser103, Leu100, Ser95, Thr175, Thr179, His181, Arg291, Ser307, Asp311, and $\pi-\pi$ *stacking* interactions with Tyr186.

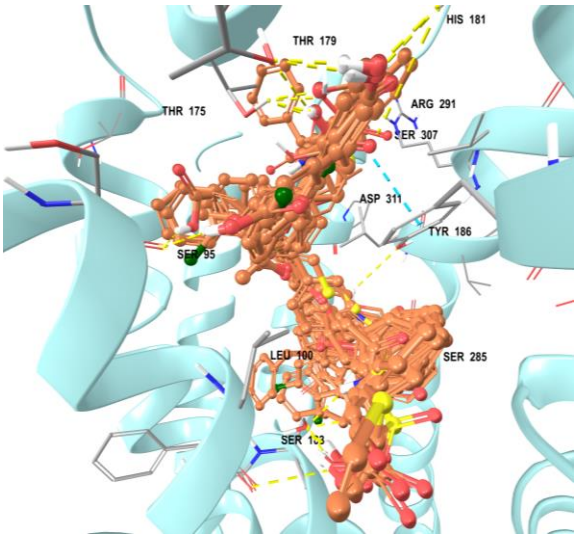

Figure 1. The superposition of compounds from Gallant's series [7] in the binding site of EP4 homology model [6] resulted from docking with the Glide XP software [10].

From the pharmacophore modeling process resulted that the common features for the aligned antagonists in the binding site (for 9 active compounds: **21**, **12**, **6**, **8**, **9**, **7**, **5**, **18**, **1**) with the reference ligand 21, were three aromatic rings: R5, R6 and R8 (see Figure 2).

The pharmacophore pattern of compound **21** (see Table 1) along with the interaction profile with residues from the EP4 receptor binding site is shown in the Figure 2. The most important interaction between compound **21** and the amino acid residues of the EP4 binding site is represented by the hydrogen bond with Asp311. All the possible pharmacophore features of this antagonist are: three hydrogen bond acceptors, one hydrogen bond donor, and four aromatic rings (Figure 2).

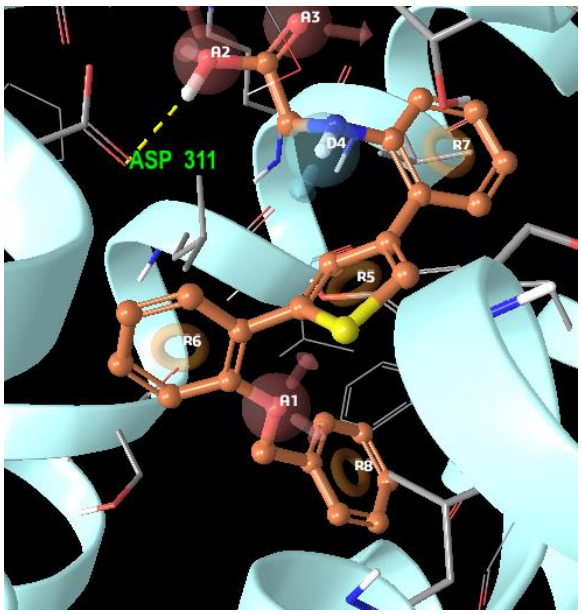

Figure 2. One of the most active antagonist, compound 21, of the Gallant's series [7] in the binding site of the EP4 homology model [6] resulted from docking with the Glide XP software [10].

The pattern of the agonists binding mode at the EP4 receptor is rendered in the Figure 3. The observed interactions between agonists and the amino acid residues of the EP4 binding site are: hydrogen bonds with: Arg304, Lys308, Arg291, Ser307, Asp311, His181, Thr175, Thr179; $\pi - \pi$ *stacking* interactions with Tyr 80 and Tyr 186, and halogen bonds with: Tyr 186 and Leu 99.

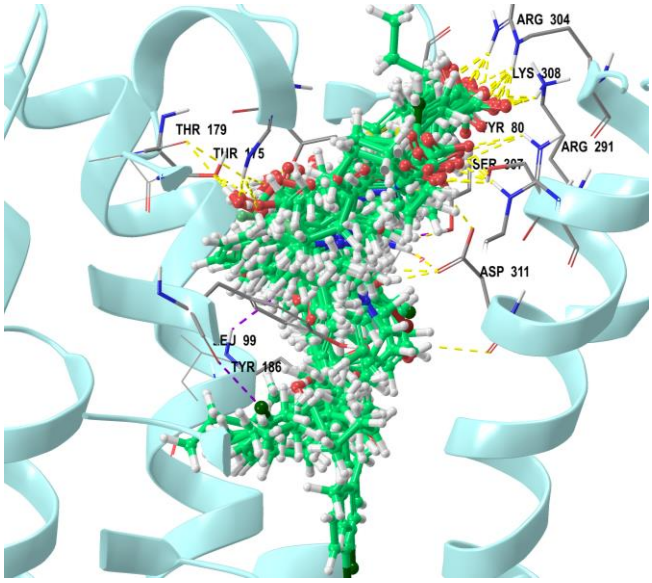

Figure 3. The superposition of dataset agonists in the binding site of the EP4 homology model [6] resulted from docking with the Glide XP software [10].

From the pharmacophore modeling process resulted that the common features for the aligned agonists in the binding site (for 13 active compounds: 38, 33, 51, 37, 49, 36, 52, 43, 39, 44, 47, 14) with the reference ligand no. 22 (Table 1), were: one negative charged site N7, one hydrophobic site H4 and one aromatic ring: R8 (see Figure 4).

The pharmacophore pattern of the most active agonist from the dataset (see Table 1), along with the interaction profile with residues from the EP4 receptor binding site is shown in the Figure 4. The most important interactions between the most active agonist from the dataset, (CHEMBL ID: CHEMBL251294) and amino acid residues from the EP4 binding site are represented by: hydrogen bond with Arg304 and Lys 308 and $\pi - \pi$ stacking interactions with Tyr 80. The possible pharmacophore features of this agonist are: two hydrogen bond acceptors, one hydrogen bond donor, one negative charged, three hydrophobic, and one aromatic ring.

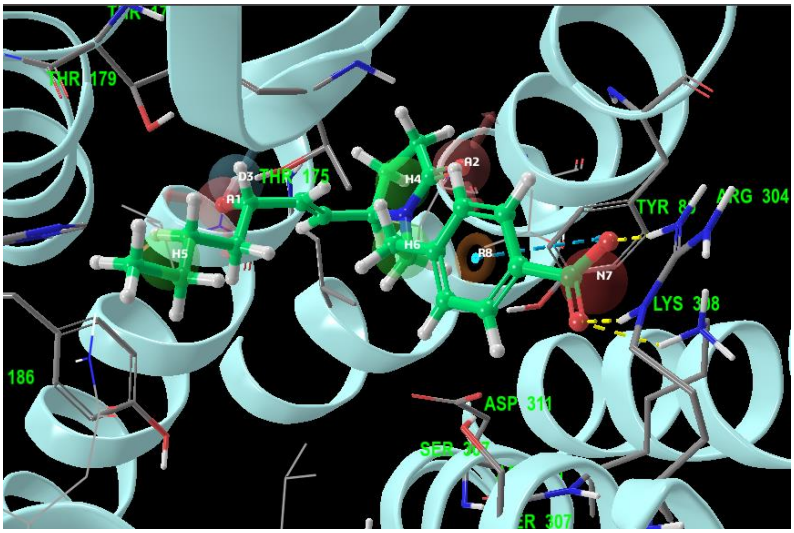

Figure 4. The most active agonist of the dataset (CHEMBL ID: CHEMBL251294) in the binding site of the EP4 homology model [6] resulted from docking with the Glide XP software [10].

Comparing the results obtained by docking the EP4 agonists versus antagonists we observed that beside the common interaction pattern with amino acids from the EP4 binding site, there are some distinct feature represented by: hydrogen bonds formed with Arg304, Lys308 for agonists and with Ser285, Ser103, Leu100, Ser95 for antagonists; agonists make additional $\pi - \pi$ stacking interactions with Tyr 80 and moreover two halogen bonds with: Tyr 186 and Leu 99.

4. Conclusions

In silico study for agonists and antagonists against the prostanoid EP4 receptor was undertaken using docking and paharmacophore modeling protocols. The docking results show all the interactions made by the ligands taken in our study and the amino acid residues from the homology model of the EP4 binding pocket, while the constructed pharmacophore models show the common features necessary for a ligand (agonist or antagonist) to interact with the target protein. Thus, the most important characteristics for agonists were found to be: one negative site, one hydrophobic feature and one aromatic ring, and for the antagonists: three aromatic rings. The findings of our study can be useful for a better understanding of the interaction patterns between the EP4 receptor and its ligands and can serve as a starting point for the rational drug design for this target.

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Conflicts of Interest: The authors declare no conflict of interest

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