



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 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 and new active compounds.



MATERIALS AND METHODS

2.1. Ligand Preparation

- ❑ 21 compounds with affinity for prostanoid receptors (K_i (μM)) which acts as antagonists were selected from literature [1] and 32 compounds with affinity on EP4 lower than 10 nM (EC_{50} (nM)) which acts as agonists were downloaded from ChEMBL database [9].
- ❑ The 2D structure of the agonists and antagonists were generated using the Marvin Sketch software version 17.18 from Chemaxon [[http://www.chemaxon.com.](http://www.chemaxon.com)] (Fig.1 and Fig2)



MATERIALS AND METHODS

2.2. Docking

- ❑ The 3D structure for EP4 prostanoid receptor used in this investigation was achieved previously by homology modelling [3].
- ❑ The Maestro suite version 2016-3 [4] was used in all the preliminary stages for the docking process with Glide [5, 6].
- ❑ Thus, the database comprising of 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[7].
- ❑ Glide software [5] 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.



MATERIALS AND METHODS

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 [8].
- ❑ 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.

MATERIALS AND METHODS

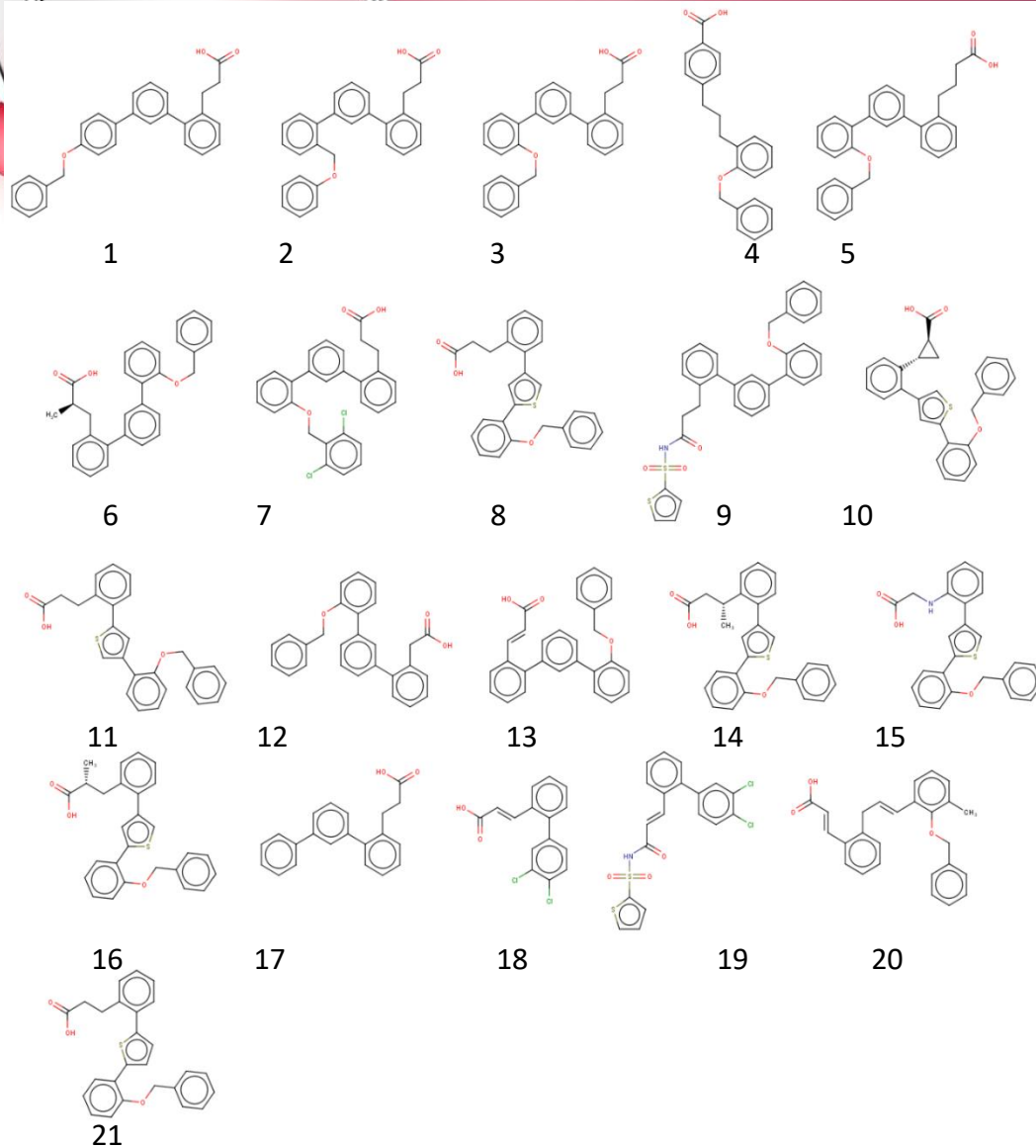


Fig. 1 The 2D structure of the antagonists from dataset [1]

MATERIALS AND METHODS

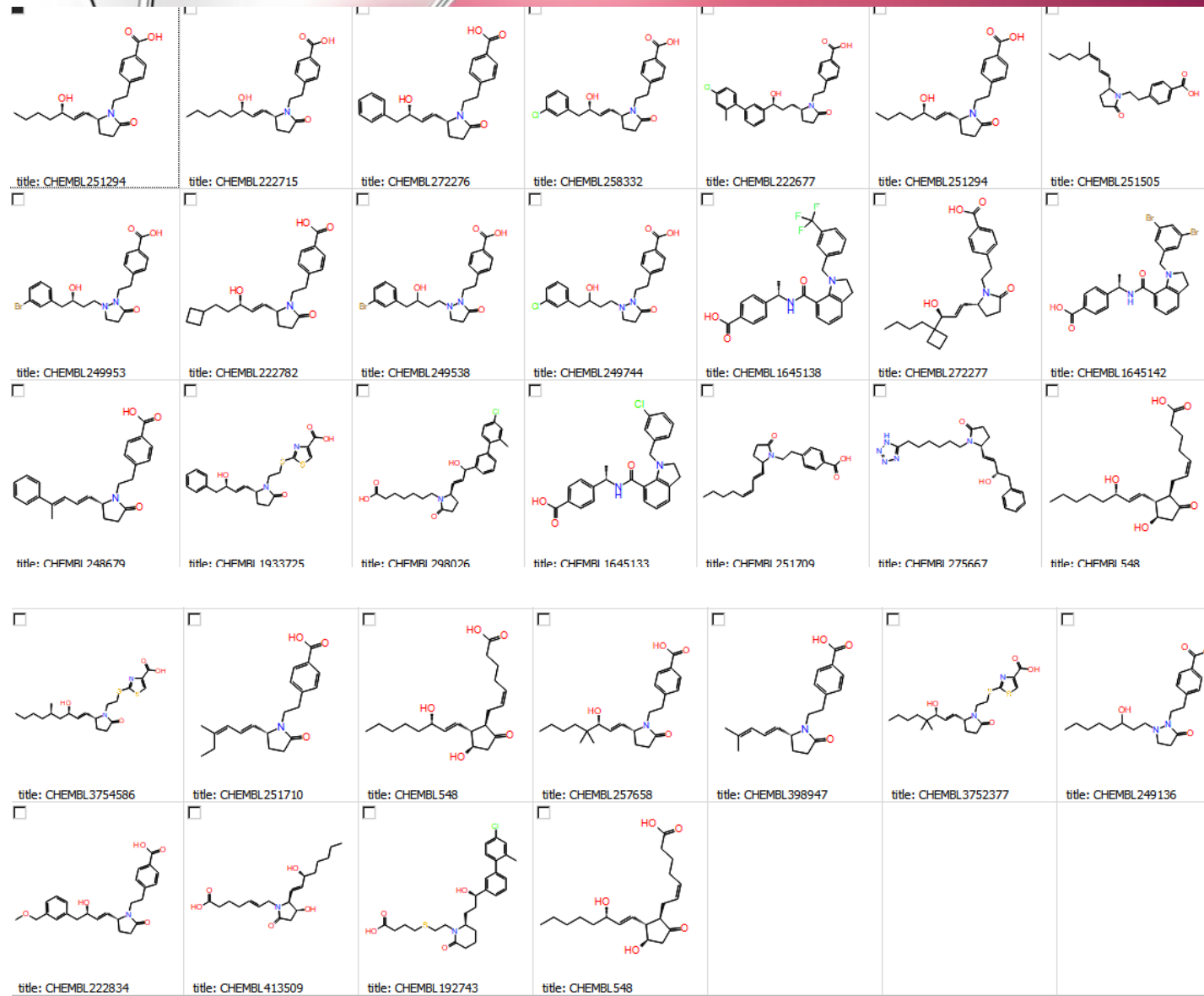
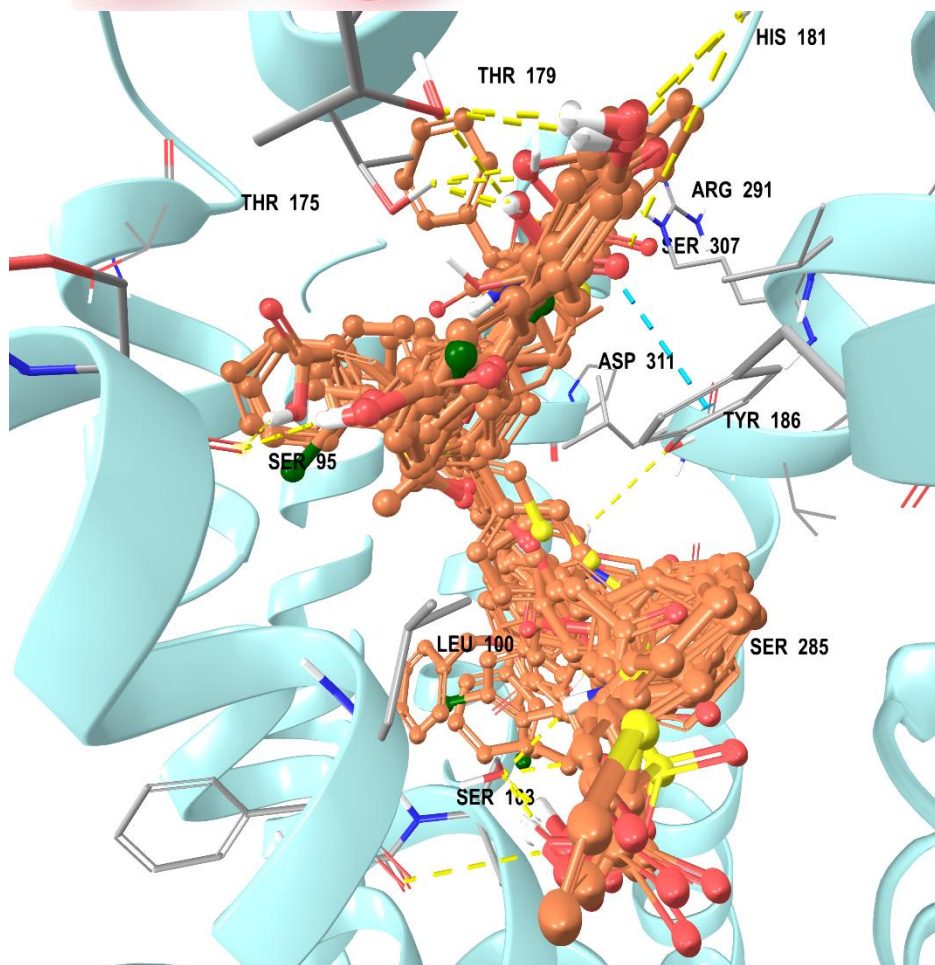


Fig. 2 The 2D structure of the agonists from dataset [2]

RESULTS AND DISCUSSION

The pattern of the antagonists binding mode at EP4 receptor is rendered in Fig. 3.



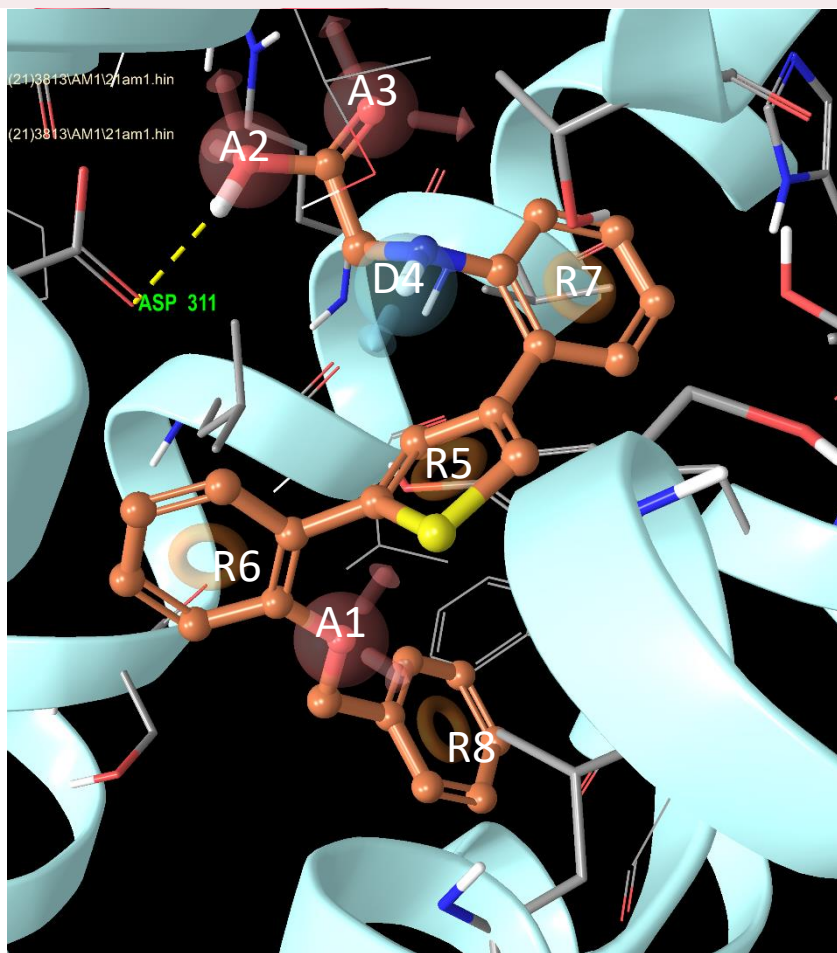
The observed interactions between antagonists and amino acid residues from EP4 binding site are:

- hydrogen bonds with: Ser285, Ser103, Leu100, Ser95, Thr175, Thr179, His181, Arg291, Ser307, Asp311
- $\pi - \pi$ *stacking* interactions with Tyr186.

Fig.3. The superposition of compounds from Gallant's series [1] in the binding site of EP4 homology model [3] resulted from docking with the Glide XP software[5].

RESULTS AND DISCUSSION

The pharmacophore pattern of compound **21** (see Fig.1) along with the interaction profile with residues from the EP4 receptor binding site is shown in the Fig. 4.



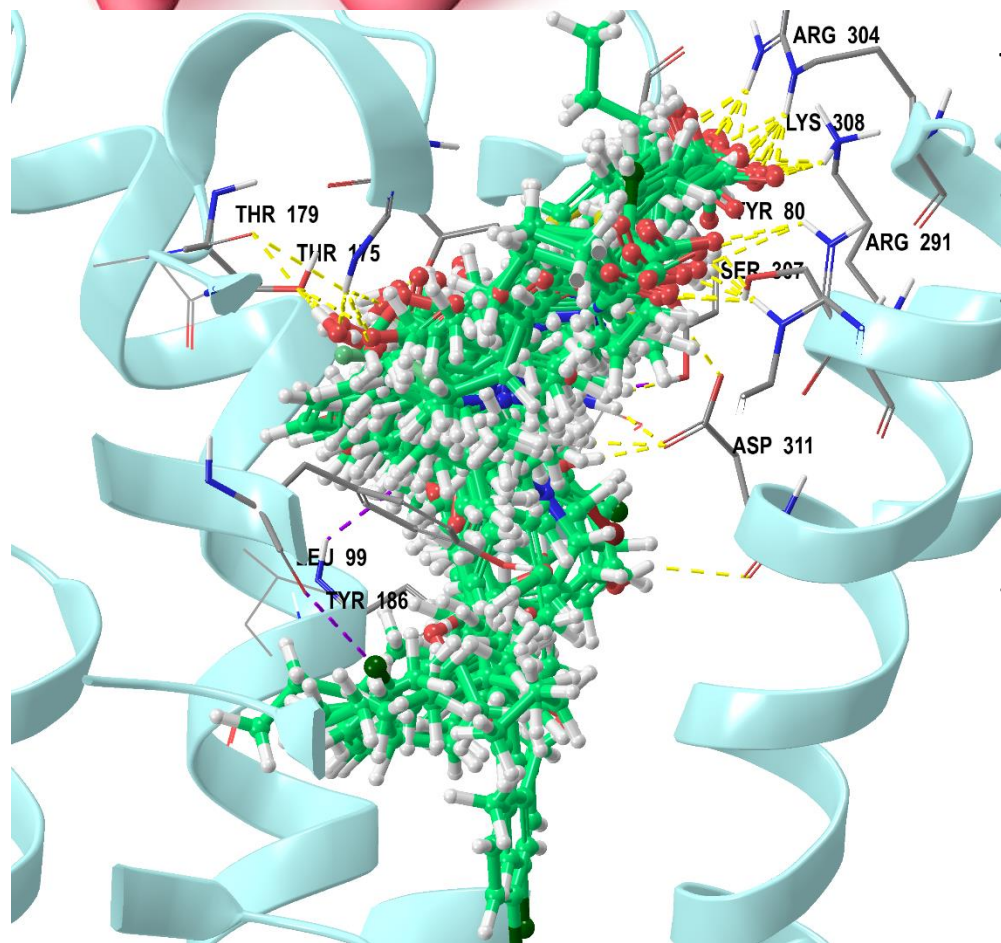
The most important interaction between compound **21** and the amino acid residues of the EP4 binding site is represented by the hydrogen bond with Asp311.

The possible pharmacophore features of this antagonist are: three Hydrogen bond acceptors, one Hydrogen bond donor, and four aromatic rings.

Fig.4. One of the most active antagonist, compound **21**, of the Gallant's series [1] in the binding site of EP4 homology model [3] resulted from docking with the Glide XP software[5].

RESULTS AND DISCUSSION

The pattern of the agonists binding mode at EP4 receptor is rendered in the Fig. 5.



The observed interactions between agonists and amino acid residues from EP4 binding site are:

- hydrogen bonds with: Arg304, Lys308, Arg291, Ser307, Asp311, His181, Thr175, Thr179
- π – π *stacking* interactions with Tyr 80 and Tyr 186
- halogen bonds with: Tyr 186 and Leu 99

Fig.5. The superposition of dataset agonists (Figure 2) in the binding site of the EP4 homology model [3] resulted from docking with the Glide XP software[5].

RESULTS AND DISCUSSION

The pharmacophore pattern of the most active agonist from dataset (see Fig.2) along with the interaction profile with residues from EP4 receptor binding site is shown in the Fig.6.

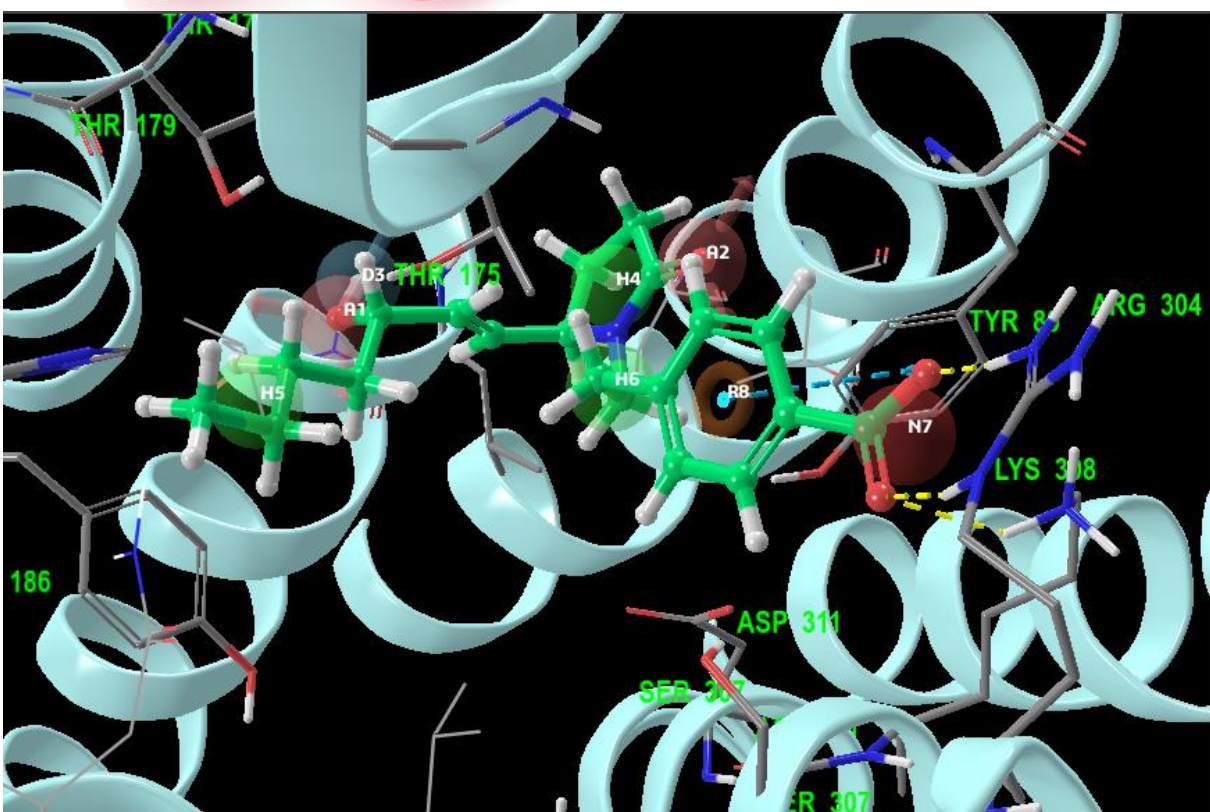


Fig.6. The most active agonist of the dataset (CHEMBL ID: **CHEMBL251294**) in the binding site of EP4 homology model [3] resulted from docking with Glide XP software[5].

The most important interactions between the most active agonist from dataset, with CHEMBL ID: **CHEMBL251294** (Fig. 2) and amino acid residues from 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 site, three hydrophobic sites, and one aromatic ring.



CONCLUSIONS

- ✓ In silico study for agonists and antagonists against the prostanoid EP4 receptor was undertaken using docking and pharmacophore 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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