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Supercomputer Simulations of Dopamine-Derived Ligands Complexed with Cyclooxygenases

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An *in silico* approach was adopted to identify potential cyclooxygenase inhibitors through molecular docking studies. Four potentially active molecules were generated by fusion of dopamine with ibuprofen or ketorolac derivatives. The binding mode of the considered ligands to cyclooxygenase-1 and cyclooxygenase-2 isoforms was described using Autodock Vina. Preliminary docking to full cyclooxygenase isoforms structures was used to determine possible binding sites for the described dopamine-derived ligands. The following more accurate docking iteration to the described binding sites was used to achieve better conformational sampling. Among the studied molecules, IBU-GABA-DA showed preferable binding to cyclooxygenase active site of cyclooxygenase-1, while IBU-DA bound to peroxidase site of cyclooxygenase-1, making these ibuprofen-comprising ligands a base for further research and design of selective cyclooxygenase-1 inhibitors. Keterolac-derived ligands KET-DA and KET-GABA-DA demonstrated binding to both cyclooxygenase isoforms at a side pocket, which does not relate to any known functional site of cyclooxygenases and needs to be further investigated.

Keywords: molecular docking, non-steroidal anti-inflammatory drugs, ibuprofen, dopamine, cyclooxygenase.

Introduction

Cyclooxygenases (COX), or prostaglandin-endoperoxide synthases, are a family of membrane-bound isozymes located on the lumenal surfaces of the endoplasmic reticulum and on the inner and outer membranes of the nuclear envelope. There are two human COX isoforms, COX-1 and COX-2, which mediate basic housekeeping functions in various tissues and play key roles in inflammation process. Non-steroidal anti-inflammatory drugs (NSAIDs) are COX inhibitors that exhibit analgesic, antipyretic, and anti-inflammatory actions [1]. Most NSAIDs are known to inhibit COX enzymes by binding at the cyclooxygenase active site, but several NSAIDs have alternative binding locations on COX surface [3]. Development of selective COX-1 inhibitors might be highly relevant for diseases, such as neuro-inflammation, atherosclerosis and gastrointestinal toxicity, while COX-2 selective NSAIDs are needed for treatment of rheumatoid arthritis and as a preventative agent for colon cancer [1].

Dopamine, one of the major neurotransmitters in the central nervous system, is involved in regulation of the immune system and host defense. Dopamine-derived drugs are a largely unexplored but promising class of mediators involved in the regulation of neuroinflammation, exhibiting reduction of prostaglandin E2 level in primary microglial cells without alteration of COX-2 gene expression [9].

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An established technique of developing new COX inhibitors is modification of existing non selective inhibitors, such as ibuprofen. Ibuprofen and other NSAIDs esterified from their carboxyl group showed higher binding affinity and good selectivity for COX-2 in both *in silico* and *in vitro* studies [2]. In this study we investigate the binding mode to COX isozymes for four potential dopamine-derived COX inhibitors designed by ibuprofen and ketorolac esterficaton (Fig. 1). The preferable binding sites were determined by the estimated binding affinities using molecular docking approach. IBU-GABA-DA is selected as a lead molecule for further design of COX-1 selective inhibitors.

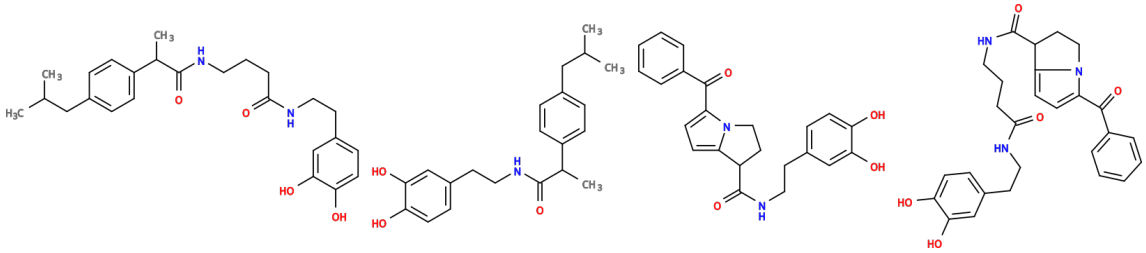


Figure 1. Chemical structures of chosen dopamine-derived COX inhibitors on base of ibuprofen From left to right: IBU-DA, IBU-GABA-DA, KET-DA, KET-GABA-DA

1. Methods

3D structures for four dopamine-derived ligangs (DDLs), the cyclooxygenase substrate arachidonic acid (AA) and NSAID ibuprofen (IBU) were generated from SMILES strings using Open Babel 2.3.2 [5]. The AutoDock Tools 1.5.6 software was used to assign atomic partial charges [4].

Three crystal structures of COX-1 and COX-2 bound to AA (PDB ID 1DIY for COX-1, 3HS5 for COX-2), ibuprofen (1EQG for COX-1, 4PH9 for COX-2) and other inhibitors (flurbiprofen - 2AYL for COX-1, naproxen - 3NT1 for COX-2) were used to sample ligand binding to different COX conformations. The macromolecules were treated to be rigid.

We first performed 140 docking iterations per COX structure of AA to box containing the whole protein structure (95.21Å x 96.96Å x 121.05Å) with `exhaustiveness`=8, using Autodock Vina [8]. AA:COX complexes were clustered with Affinity Propagation method implemented in Affbio python package [6]. The centroids of the resulting clusters were used as new centers of docking boxes (20Å x 20Å x 20Å). 140 docking iterations with `exhaustiveness`=256 per docking box per ligand per protein structure were performed.

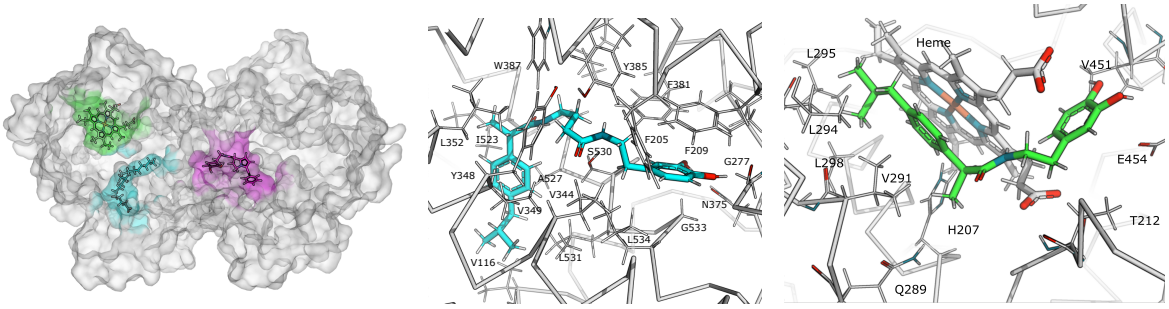
2. Results

As NSAIDs are known to target various binding sites, we performed docking of AA to search space covering the whole COX isoform structure. Despite the fact that AA poses bound to active sites in both COX isoforms had highest binding scores (-7.9 kcal/mol in COX-1, -8.2 kcal/mol in COX-2), they represent only 4.8% and 6.7% of poses for COX-1 and COX-2. Thus, we performed docking of DDLs to smaller boxes formed around AA-located sites in order to achieve better pose sampling.

As a control study, we compared poses for AA and IBU with crystal structures by docking to all the selected boxes. The best binding energy was observed at active sites. The root mean square

deviation (RMSD) to the crystal structures were 0.98Å and 0.92Å in COX-1 and COX-2 for AA, 0.56Å and 1.27Å for IBU, indicating the obtained poses correspond to actual conformations.

All four DDLs were then docked to all the selected docking boxes. For COX-1, IBU-GABA-DA demonstrated the best binding energy (-9.4 kcal/mol) at the subunit A cyclooxygenase active site (Fig. 2 (a)). Most interactions in this binding had hydrophobic character, except for hydrogen bonds with ASN-375 (see Fig. 2 (b)). For COX-2, IBU-GABA-DA had the best binding energy (-10.1 kcal/mol) at the side pocket on the subunit B lumenal surface. The pose of IBU-DA with the best binding energy (-9.3 kcal/mol) binds closely to peroxidase (POX) site of subunit A in COX-1 (Fig. 2 (a, c)). In COX-2, IBU-DA best binding site (-9.6 kcal/mol) was located on the subunit B membrane surface. In COX-2 both KET-DA and KET-GABA-DA bound better (-10.6 kcal/mol and -11.6 kcal/mol, for KET-DA and KET-GABA-DA) at the same side pocket on the subunit B lumenal surface as IBU-GABA-DA, and in COX-1 they demonstrated the highest binding score (-10.6 kcal/mol and -10.4 kcal/mol) at the corresponding site.



(a) Possible binding sites on COX-1 surface, green - POX site of subunit A, cyan - active site of subunit A, magenta - a pocket on the subunit B lumenal surface

(b) IBU-GABA-DA bound at active site of COX-1

(c) IBU-DA bound at the POX site of COX-1

Figure 2. Possible binding sites of DDLs on COX-1 surface

3. Discussion

Molecular docking is a powerful instrument for prediction of binding conformations for enzyme inhibitors. Autodock Vina is one of the most widely used tools for this task. Note that while the software provides a valuable insight into the geometry of intermolecular interactions, its binding affinity estimates should be treated with care [8].

Among the DDLs considered in this study, only IBU-GABA-DA shows preferable binding to one of the cyclooxygenase active sites of COX-1. The preferable pose interacts with the same set of COX-1 aminoacids as AA in the crystal structure, yet the estimated binding energy for IBU-GABA-DA is lower, -9.4 kcal/mol versus -7.9 kcal/mol for AA. As it does not demonstrate preferable binding to the active site of COX-2, we may suggest that IBU-GABA-DA can serve as a base for further design of COX-1 selective inhibitors.

The predicted IBU-DA pose interacts with Heme molecule bound to the POX site of COX-1. Such interaction may affect the activity of COX-1, as several other NSAIDs, such as resveratrol,

bind at the POX site [3]. The binding energies for the POX site, active site and a pocket on the lumenal surface of COX-1 have a difference of 0.2 kcal/mol, indicating that IBU-DA may have different modes of action. The predicted binding mode of this ligand to COX-2 may not be functional, as the top scoring pose interacts with the membrane part of COX-2.

KET-DA and KET-GABA-DA bind to both COX isoforms at a side pocket, which does not relate to any known functional site. Despite the fact that the binding energy of these potential inhibitors is lower than the other DDLs' considered in this study, we can not state whether this binding would affect the activity of any COX isoform.

Conclusion

In this study we have described the binding mode of four dopamine-derived potential COX inhibitors using molecular docking. Among the candidates, IBU-GABA-DA is predicted to bind selectively at the active site of COX-1, making it a possible target for further drug development. IBU-DA is predicted to have a set of equivalent target sites on COX-1. A putative functional site was located with KET-DA and KET-GABA-DA docking experiments, however further research is needed to prove its significance.

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