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RESEARCH ARTICLE

In-Silico Analysis of various Benzilate Derivatives towards Cyclooxygenase-2 Enzyme

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ABSTRACT:
Cyclooxygenases (COX-1 and COX-2) catalyze the rate-limiting step in the biosynthesis of prostaglandins, prostacyclins, and thromboxanes. Ibuprofen has classically fallen into the time-dependant class of COX inhibitors as it binds rapidly and reversibly to Cox and acts as a competitive inhibitor of arachidonic acid oxygenation.The discovery of new and novel anti-inflammatory drugs is an area of intense interest in both pharmaceutical industry and academic laboratories. Significant advances have been made in the treatment of inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, but most dramatically with new biologic agents. Perhaps due in part to the mixed experiences with COX-2 inhibitors, very few small molecule anti-inflammatory drugs with novel modes of action have made it to the market in the last decade.The various benzilic acid compounds synthesized have taken for in-silico analysis to study the structure activity relationship using crystal structure co-crystallized with 2-(4-isobtyl phenyl) propionic acid (PDB ID: 4PH9).The synthesized ligands were docked using three different docking strategies; High throughput virtual screening, Standard Precision and Extra Precision docking strategies. After three different analyses, the docking scores of the synthesized compounds were found to be in the range of -8.221 to –6.342 Kcal mol^{-1}. Finally the compounds are shortlisted based on the visual inspection of the amino acids interaction.

KEYWORDS: Cyclooxygenases, Docking, Benzilic acid and inflammatory diseases.

INTRODUCTION:
The discovery of new and novel anti-inflammatory drugs is an area of intense interest in both pharmaceutical industry and academic laboratories. Significant advances have been made in the treatment of inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, but most dramatically with new biologic agents. Perhaps due in part to the mixed experiences with COX-2 inhibitors [1], very few small molecule anti-inflammatory drugs with novel modes of action have made it to the market in the last decade.

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Therefore, there remains an enormous unmet medical need for new, effective and safe small molecules disease-modifying therapies to expand treatment options for these and other indications, including asthma and chronic obstructive pulmonary disease, allergic diseases, atherosclerosis, psoriasis, inflammatory bowel disease and pain.All synthesized compounds show moderate to extent antioxidant activity [2]. The synthesised molecule showed stacking interaction and the compound has also found to be surrounded by non-polar amino acids, which makes this molecule potent toward antibacterial drug discovery [3].

Cyclogenase-2 Enzyme Structure:
Human cyclogenase-2 enzymes are homodimer with 581 amino acids [1], Each subunit of the dimer consists of three domains, the epidermal growth factor domain the membrane binding domain and the catalytic domain

comprising the bulk of the protein, which contains the cyclooxygenase and peroxidase active sites on either side of the heme prosthetic group [4]. The structure of the active site helps to the promiscuous substrate specificity of the COX peroxidase, which reduces a wide range of primary and secondary organic hydroperoxides.
Active site of Cox-2

The cyclooxygenase active site lies on the opposite side of the heme from the peroxidase active site at the top of an L-shaped channel that originates in the membrane binding domain. The mouth of the channel consists of the lobby, a large volume that narrows to a constriction that must open before substrates or inhibitors can pass deeper into the channel. Above the constriction, the channel is surrounded by hydrophobic residues, which outline the nearly right angle bend and the narrow terminus. When an inhibitor or substrate binds in the cyclooxygenase active site, it lies with its carboxyl group at the constriction and its ω-methyl group at the narrow terminus of the channel [1].

Role of Cox-2 in Anti-inflammatory Drug Discovery:
Cyclooxygenases (COX-1 and COX-2) catalyze the rate-limiting step in the biosynthesis of prostaglandins, prostacyclins, and thromboxanes. These potent lipid-signaling molecules regulate "housekeeping" functions required for normal physiological activities [1]. COX-1 and COX-2 are the pharmacological targets of non-steroidal anti-inflammatory drugs and COX-2 selective inhibitors. COX inhibitors fall into four different categories based on their mechanism of inhibition. Time- independent inhibitors bind to COX in a rapidly reversible manner resulting in competitive inhibition [4] mixed inhibitors display an initial time-dependent decrease in enzyme activity without completely inhibiting the enzyme and covalent inhibitors chemically modify the cyclooxygenase active site. Ibuprofen has classically fallen into the time-dependant class of COX inhibitors as it binds rapidly and reversibly to Cox and acts as a competitive inhibitor of arachidonic acid oxygenation [4]

Crystal structure of Cox-2 Enzyme:
Crystal structures of COX-2 in complex with a myrid of inhibitors and substrates have been determined (PDB ID: 4PH9). The crystal structure of ibuprofen bound to COX-2 has been determined. The analgesic and anti-inflammatory effects of ibuprofen are thought to arise from the inhibition of COX-2. The binding mode of ibuprofen to COX-2 versus COX-1 and to reveal a possible mechanism of ibuprofen mediated substrate selective inhibition [1].

MATERIALS AND METHODS:

Computational Details:
All computations were carried out in an Intel Core 2 Duo E7400 2.80 GHz capacity processor with memory of 2GB RAM running with the RHEL 5.2 operating system. PHASE 3.3 implemented with Maestro 9.3 software package (Schrodinger, LLC) was used to generate pharmacophore models [5]. The virtual screening options for HTVS (High Throughput Virtual Screening), SP (Standard Precision) and Glide XP (Extra Precision) docking were all checked to be executed. Glide XP (extra precision) module of Schrodinger 9.3 (Glide, version 5.7, Schrodinger, LLC, New York, NY, 2016) was utilised for docking. Bond orders and formal charges were added to the hetero groups and hydrogen atoms were added to all atoms in the system [6].

Protein Preparation Using Protein Preparation Wizard:
Protein preparation using protein preparation wizard and impact energy minimization, the protein file was prepared. About 500 cycles of steepest descent (SD) and 5000 cycles of conjugate gradient (CG) methods with optimized potential for liquid simulations (OPLS) 2005 force field using Schrodinger suite version 9.3 were employed [5, 6]. The active site of the protein was located and grid files were generated using receptor grid generation panel. The "Write XP descriptor information" option was selected and "Compute RMSD" option was enabled and rest of the parameters were kept as default. The XP Glide scoring function was used to order the best ranked compounds and the important interactions like π-cation and π-π stacking were analysed using XP visualizer in Glide module. The input RMSD of the crystal ligand was also ascertained [7].

Molecular docking:
Virtual screening of the compound was carried out by using Glide module of Schrodinger, LLC, 2016 Primarily, by using Glide module (Grid based ligand docking with energetic), we examined for favorable interactions between screened ligand hits and the protein of interest in the flexible mode docking [8]. The Glide module with three modes of docking, high-throughput virtual screening (HTVS), standard precision (SP), and extra precision (XP) mode were employed sequentially. The XP mode was used for exhaustive sampling and advanced scoring, resulting in even higher enrichment [9]. Final short listing of hit molecules were performed based on visual inspection of important amino acid interactions in the active site cavity, docking scores and the hydrogen bonds involved in binding [10, 11].

RESULTS AND DISCUSSIONS:

The various benzilic acid and its derivatives synthesized [12]. have taken for in-silico analysis to study the structure activity relationship using crystal structure co-crystallized with 2-(4-isobtyl phenyl) propionic acid (PDB ID: 4PH9) for our study. Initially the active site was validated and the reference ligand is re-docked and the binding energy was found to be -7.567 kcalmol^{-1}. The original ligand interaction and the redocked ligand interactions revealed similar interactions and the superimposition were carried out and found that the RMSD was 1.23. Now after preliminary docking studies and active site validation was carried out by generating the grid with the size of 20 A°. Grid is the rectangular box generated using grid generation panel where the compounds will get docked into the particular space of the co-crystallized protein (Saxena et al., 2014). After validation, now the synthesized compounds are made into dock with the co-crystallized protein and their respective docking scores were identified.

The synthesized ligands were docked using three different docking strategies. High throughput virtual screening, Standard Precision and Extra Precision docking strategies. After three different analyses, the docking scores of the synthesized compounds were found to be in the range of -8.221 to –6.342 Kcalmol^{-1}. Finally the compounds are shortlisted based on the visual inspection of the amino acids interaction like Arg 121 and Tyr 356. The binding energy of the synthesized compounds and the aminoacid interactions were tabulated in the table 3.1.

The synthesized compound 2'-chloro-3-nitro-4-methoxy benzilic acid showed the docking score of -8.221 kcal mol-1 after three prominent docking studies. On closer analysis of this compound in the protein active site revealed hydrogen bonding interactions with the Phe519. The compound also showed hydrophobic interaction with Leu385, Trp388, Phe382, Tyr386 and Met523 amino acid residues. Some polar contacts were also observed such as Ser254, Ser531, Hie90, Thr94 and Gln193 (Fig. 3.2).Further in silico investigation into binding profile of the molecules in the Cox-2 domain revealed the importance of the hydrophobic interactions in increasing the specificity of the molecule towards the protein.

The synthesized compound Diisopropyl ammonium benzilate showed the docking score of -7.739 kcal mol-1 after three prominent docking studies. On closer analysis of the compound, it's seen that the compound is well fitted into the active site of the co-crystallized protein. The ligand interaction diagram shows that the compound is showing polar interaction with Arg521. Also the bulky phenyl group's are well bounded with the non-polar aminoacids Phe519, Leu353, Ile518, Trp388, Phe382, Tyr386, Thr349 and Val524. When compared to reference ligand this compound shows non-polar interaction and this makes the compound well fitted into cyclooxygenase-2 molecule.

The compound 4,4'-Dibromo benzilic acid was made to dock into the active site of the co-crystallized protein and the docking score of the compound was found to be -5.681 kcalmol^{-1}. The figure 4.1.2 shows the binding pattern and the ligand interaction diagram. When looking it to closer it reveals that, the carboxyl group shows positively charged aminoacids interaction with Arg171. Also the bulky bromo phenoxy groups are well associated with Leu353, Met523, Thyr386, Ile518, Phe382, Trp388, Leu385, Thr349, Val524 and Ala528. The three dimensional figure shows that the compound has flexible in binding to the active site. Based on this analysis, the docking score was comparable less due to bulky halogenic group present in the compound (sudha et al., 2017).

All synthesised compounds were well occupied in the hydrophobic pocket within the vicinity of Leu385, Phe382, Met523 and Ile518 and few polar amino acid residues Arg121 respectively. Though these two compounds were able to fit in to active site but failed to interact with active site residues through hydrogen bonding. This is the reason these molecules were showing low docking score. The binding analysis and ligand interaction diagram for both the compounds were shown in Figure 3.4 and Figure 3.5.

CONCLUSION:

In this in-silico analysis, we have studied the Molecular docking study for the various benzilic acid and its derivatives towards the active site of cyclooxygenase-2 co-crystallized enzyme. Among the five synthesized compounds two showed good binding energy towards the enzyme. This analysis is the starting point for further developing the synthetic leads for various anti-inflammatory diseases which are affecting the society.

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