10.37021/ijper.v5i2.02

RESEARCH ARTICLE

Molecular Docking Studies of Benzimidazole Analogues as Novel Anti-Inflammatory Agents

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ABSTRACT

Introduction: The benzimidazole nucleus is an integral part of legion bioactive heterocyclic compounds that are of great interest due to their various biological and clinical properties. The primary goal of this research is to design (*in-silico*) novel benzimidazole chemical entities as anti-inflammatory and analgesic agents.

Material and Method: Molecular docking calculations were carried out to identify the interactions of designed benzimidazole analogues with COX-2 (PDB ID: 6COX). The docked complex conformations were studied in terms of binding energy, hydrogen bonding, and hydrophobic interactions. Molecular docking experiments were carried out at MGL Tools 1.5.7 software suite AutoDock 4.2.6.

Results And Discussion: The Bindings revealed that the four novel molecules had binding energy greater than Indomethacin (Standard molecule). 2-fluorophenyl derivative has a binding energy of -11.34 kcal/mol and binding interactions with amino acids; (vizALA:199, PHE:200, HIS:207, ASN:207, HIS:386, HIS:388, LEU:390, LEU391).It Identifies benzimidazole analogues as promising molecules for anti-inflammatory activities comparable with standard.

Key Words: Benzimidazole, *In-silico* docking, anti-inflammatory and analgesic, COX2, Auto Dock

How to cite this article: Chauhan H, Bansal SK, Agrawal A. Molecular Docking Studies of Benzimidazole Analogues as Novel Anti-Inflammatory Agents . Int. J. Pharm. Edu. Res. 2023;5(2):7-10.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Cyclooxygenase (COX) is a rate-limiting enzyme involved in the transformation of arachidonic acid into various inflammatory prostaglandins. There are two form of COX isoenzyme involved in prostaglandin biosynthesis: COX-1 and COX-2. COX-1 is a housekeeping enzyme that is constitutively expressed in all tissues, whereas COX-2 is exclusively present in the kidney, brain, and ovaries.[1,2] During inflammatory conditions, COX-2 increasingly release pro-inflammatory molecules such as IL-1, TNF-α, LPS and its expression is absent or low in healthy individuals. Although COX-2 inhibitors are widely prescribed as anti-inflammatory agents, several important side effects have been associated with the simultaneous inhibition of COX-1 activity. Therefore, the development of compounds that would inhibit COX-2 almost exclusively is an important target in order to reduce adverse side effect during non-steroidal anti-inflammatory treatment, thus improving therapeutic benefits.[3,4]

One of the oldest known nitrogen-containing, promising heterocyclic aromatic compounds is benzimidazole. The nucleus of benzimidazole molecule is formed by the fusion of benzene and imidazole ring. The benzimidazole ring has been used as a privileged scaffold (a pharmacophore with exclusive structural features and electron-rich environment) to synthesize a variety of drugs with medical applications, including antiulcer, antioxidant, HIV-RT inhibitor, anticancer, anthelmintic, antimicrobial, and antihistamine.[5-7]

Molecular docking studies are one of the in-silico tools to get an insight into ligand-receptor interactions. All molecular docking calculations were performed on the Auto Dock software. The Auto Dock Tools (ADT) graphical user interface was used to calculate Kollman charges for the protein and to add polar hydrogen. Docking is a computer approach used to anticipate the non-covalent interaction of macromolecules or, more commonly, a macromolecule (receptor) and a small molecule (ligand) efficiently, starting with their unbound structures, structures obtained from MD simulations, homology modelling, etc.[8]

Therefore, it was worthwhile to (i) design novel benzimidazole analogues; (ii) perform molecular docking studies against the COX-2 enzyme; and (iii) study the interaction of ligands with amino acids in the binding pocket.

MATERIAL AND METHODS

The ligands were prepared using Advanced Chemistry Development, Inc.'s ACD/ChemSketch 12.01; the proteins were prepared using UCSF Chimera, the binding energy was calculated using Auto dock tools 4.2.6 and MGL Tools 1.5.7, and the docking findings were visualized using Discovery studio 2019.

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Docking Procedure

Protein preparation

The X-ray crystallographic structure of the protein COX-2 (PDB ID: 6COX) at a resolution of 2.8 0Å was downloaded from the Protein Data Bank. Water molecules, ligands, and other hetero atoms were deleted with the help of chimera software. Chain A was chosen, with the deletion of all other chains.[9-11] , The Three dimensional structure of protein has been shown in Figure 1.

Ligand Preparation

The 2D Structured of compound was drawn with the help of ACD/ChemSketch, and the energy minimization was done with the help of Chem 3D Draw Ultra 8.0.3. The molecules were saved in PDB format, which is compatible with Auto Dock software.[12,13]

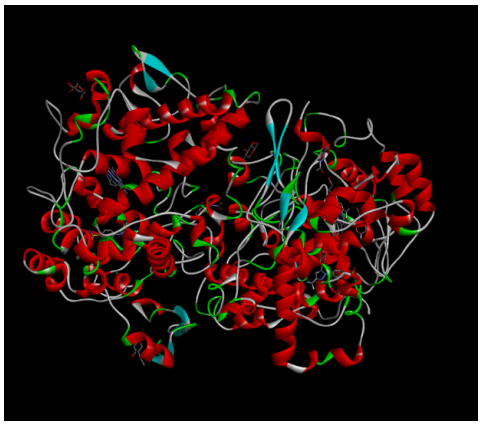


Figure 1: The 3D structure of Protein (PDB ID: 6Cox)

Molecular Docking

For docking simulations, AutoGrid and AutoDock (Version; 4.2.6) were used.[14,15] The Lamarckian genetic algorithm with local search was employed as the search engine throughout the course of 30 runs. The region of interest, used by AutoDock 4.2.6 for docking runs and by Auto Grid 4.2.6 for preparing affinity grid maps, was defined in such a way as to include the entire catalytic binding site using a grid of 60 x 56 x 68 points with a grid space of 0.514and centers of the grid box: x=25.190, y=25.805, and z=46.378.

Visualization and Analysis

Finally, the more energetically favourable cluster poses were evaluated using Discovery studio. The hydrogen bond, hydrophobic and pi-pi interactions were analyzed. Discovery studio produced high quality 2D &3D images of small molecules and macromolecules.[16]

RESULT AND DISCUSSION

A total of Seven molecules were conceptually designed and included in the present study. The docking interactions (the Binding Energy, Inhibition constant & Amino acid residue) results are tabulated in Table

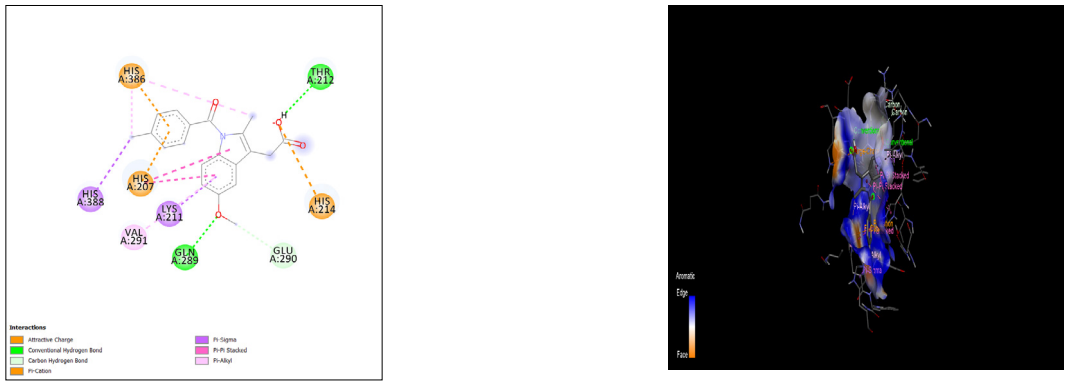


Figure 2: 2D and 3DInteractions of Indomethacin with COX-2

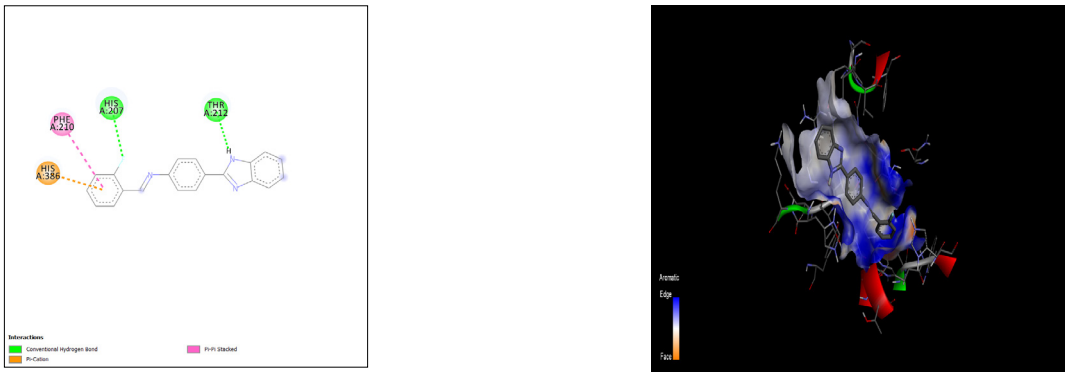
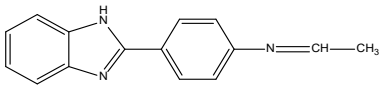
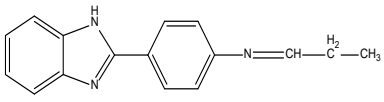
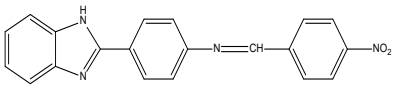
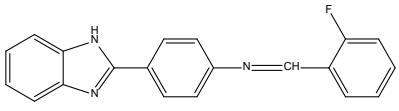
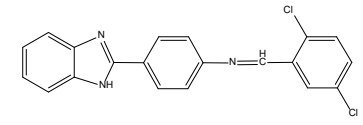
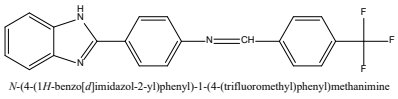
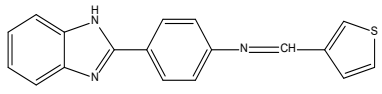
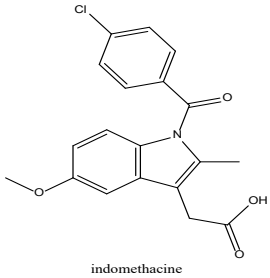


Figure 3: 2D and 3DInteractions of 2-fluorophenyl derivative analogue with COX-2

Table 1: Docking interaction and binding energy of designed benzimidazole molecule & Standard molecule (Indomethacin) has been tabulated in the table.1

Sr. No	*Compound*	*Binding Energy*	*KI (Inhibition Constant)*	*Amino acid residue*
1.	 *N*-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)ethanimine	-9.08	219.68nM	MET:196,ALA:199,PHE:200,HIS:207,TYR:385,HIS: 386,HIS:388,LEU:390,LEU:391,PRO:392
2.	 *N*-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)propan-1-imine	-9.56	97.85nM	ASP:125, ILE:124, THR:149, ALA:151, PHE:209, ARG376, ALA378, PHE381, PHE:529, LYS:532, LEU:534
3.	 *N*-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1-(4-nitrophenyl)methanimine	-10.91	10.14nM	HIS:207, LYS:211, THR:212, LYS:215, ARG:222, ASN:382, HIS:386
4.	 *N*-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1-(2-fluorophenyl)methanimine	-11.34	3.90nM	ALA:199, PHE:200, HIS: 207, ASN: 207, HIS: 386, HIS:388, LEU:390, LEU391
5.	 *N*-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1-(2,5-dichlorophenyl)methanimine	-10.33	52.98nM	ALA:516, ASP:515, LEU:352, PRO:514, SER:353, VAL:523
6.	 *N*-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1-(4-(trifluoromethyl)phenyl)methanimine	-9.38	133.75nM	ALA:202, GLN:203, HIS:207, HIS:214, TYR:385, TRP: 387, HIS:388, VAL: 447, ALA: 450, GLN: 454,
7.	 *N*-(4-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-1-(thiophen-3-yl)methanimine	-11.0	8.66nM	HIS:207, VAL:295, TYR:385, HIS:386, HIS:389, LEU:391, PHE:407, LEU:408
8.	Standard Molecule  indomethacine	-9.57	97.16nM	PHE:210, THR:212, TYR:385, HIS:207, HIS:386, HIS:388, LEU:294, VAL:291, VAL:295, LEU:298

1. Docking data demonstrated that four compounds had higher binding energies, as shown in Table 1, than the standard compound, Indomethacin. The binding energies of the proposed benzimidazole analogues are -9.08, -9.56, -10.91, -11.34, -10.33, and -11.0 kcal/mol, respectively. 2D and 3D interactions of indomethacin and its 2-fluorophenyl derivative are shown in Figure 2 and 3, respectively. The interaction of the 2-fluorophenyl derivative (the most promising molecule) are with ALA: 199, PHE: 200, HIS: 207, ASN: 207, HIS: 386, HIS 388, LEU: 390, LEU391. Figure 2 depicts the 2D & 3D interaction of Standard (indomethacin) and Protein. Figure 3 depict the 2D and 3D interaction of most Promising molecule (2-flurophenyl analogues) & Proteins.

CONCLUSION

In this study, molecular docking studies were carried out with conceptually designed benzimidazole analogue using Auto dock software to describe the binding mechanism of ligands to the target COX-2 enzyme. These docking results precisely indicated that the 2-fluorophenyl derivative has good interactions with ALA:199, PHE:200, HIS:207, ASN:207, HIS:386, HIS:388, LEU:390, LEU:391 as compared to other molecules. According to current

study, newly developed benzimidazole analogues have improved binding sites and better protein ligand interactions with Anti-inflammatory properties. These potent molecules can be further synthesized and subsequently validated for in-vivo biological activity as prospective hits.

ACKNOWLEDGEMENT

The authors are thankful to Ram-Eesh Institute of vocational & Technical Education, Greater Noida, UP. For providing Research facility.

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