RESEARCH ARTICLE

Exploring the Binding Affinity and Molecular Interactions: A Comprehensive Study on the Molecular Docking of Benzimidazole Derivatives

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ABSTRACT

The aim of this research work was to study the comparative binding affinities of benzimidazole derivatives (2-methyl-1H-benzo[d]imidazole and 2-phenyl benzimidazole) against COX, LOX, and estrogen receptor. Benzimidazole stands as a vital heterocyclic aromatic organic molecule, playing a pivotal role in medicinal chemistry due to its essential pharmacophore and structural significance. The three-dimensional structures of COX, LOX, and the estrogen receptor were sourced from the PDB database. Concurrently, the structures of the benzimidazole derivatives, namely 2-methyl-1H-benzo[d]imidazole and 2-phenyl benzimidazole, were obtained from the PubChem database. Docking studies were conducted using the PyRx software. A total of nine modes for each receptor were generated, and 2E77 was selected as the best dock. The docking result shows that the interaction of 2-methyl-1H-benzo[d]imidazole with E77 has the highest binding energy. A total of nine modes for each receptor were generated, and 1CX2 was selected as the best dock. The docking result shows that the interaction of 2-phenyl benzimidazole with 1CX2 has the highest binding energy. The *in-silico* studies show that 2-phenyl benzimidazole has more binding energy with receptors as compared to 2-methyl-1H-benzo[d]imidazole.

Keywords: Benzimidazole derivatives, Molecular docking, Affinity, Receptor, COX, LOX, Estrogen.

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INTRODUCTION

Heterocyclic compounds hold considerable importance in the field of drug design and medicinal chemistry, owing to their diverse array of biological activities. Researchers are actively directing their attention towards heterocyclic compounds, driven by their potential for efficiency, accessibility, and reduced adverse effects. Among various heterocyclic compounds, the benzimidazole ring has gained substantial interest. Its ring is often deemed "privileged" owing to its extensive biological significance. The benzimidazole's structure and its ligands' chemistry stand as focal points of significant interest among researchers.[1]

Over the past few decades, extensive research in the field of heterocycles has taken place with the intention of developing novel therapeutic and pharmaceutical agents. Among these, benzimidazole derivatives have emerged prominently.[2] In medicinal chemistry, benzimidazole is a crucial heterocyclic aromatic organic compound with significant pharmacophoric elements and structural features.[3] It consists of a benzene ring fused with imidazole, which possesses many pharmacological properties.[4] The cyclic ring consists of two nitrogens in the form of a heteroatom; hence, it is considered a heterocyclic aromatic compound[5] (Figure 1). Benzimidazoles, characterized by a fused ring structure with benzene in the 4 and 5 positions, serve as valuable intermediates or subunits in the creation of small molecules with biological activity. The imidazole moiety, a five-membered ring, is a prevalent structural element in a myriad of natural products and pharmacologically active compounds.[6]

Benzimidazole derivatives find diverse applications, serving as analgesics,[7] anti-inflammatory agents,[8] antibacterials,[9] antifungals,[10] antivirals,[11] antihelminthics,[12] anticonvulsants,[13] anticancer agents,[14] antiulcer medications,[15] antihypertensives, antiprotozoals, antioxidants,[7] as well as playing roles in antimalarial and antiparasitic therapies. The medicinal value of benzimidazole and its analogs has gained significant attention.[5]

Benzimidazole's initial synthesis dates back to 1872, credited to Hoebrecker, who acquired 2,5-(or 2,6)-dimethylbenzimidazole by utilizing 2-nitro-4-methylacetanilide.[4] Notably, 2-substituted analogs of benzimidazole stand out as potent biologically active

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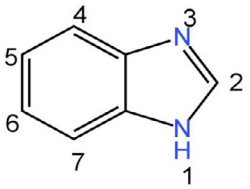

Figure 1: Structure of benzimidazole ring

compounds. Given the widespread utility of the benzimidazole moiety in medicinal chemistry, efforts have been dedicated to producing numerous 2-substituted benzimidazole derivatives with diverse functional groups at different positions. Recent findings highlight the noteworthy antioxidant activity exhibited by benzimidazole derivatives substituted at the 1 and 2 positions.[2]

Cyclooxygenase (COX), lipoxygenase (LOX), and Estrogen receptors are key components involved in various physiological processes within the human body. COX enzymes are responsible for catalyzing the synthesis of prostaglandins, which are lipid compounds involved in inflammation, pain signaling, and regulation of various physiological processes. LOX enzymes catalyze the oxygenation of polyunsaturated fatty acids, leading to the formation of leukotrienes. Leukotrienes play a role in inflammation and immune responses. Estrogen receptors (ERs) are proteins that mediate the biological effects of estrogen, a crucial hormone in the regulation of the female reproductive system and other tissues.[16,17] Understanding the functions and interactions of COX, LOX, and estrogen receptors is essential for developing therapeutic interventions targeting these pathways, particularly in inflammation, pain management, and hormonal regulation.

Benzimidazole and its derivatives showed affinity towards COX, LOX, and estrogen receptors, but very extensive research in this area is still required.[2] Identifying benzimidazole derivatives that interact with COX, LOX, and estrogen receptors may open avenues for the development of new therapeutic agents. These derivatives could be explored for their potential in managing conditions related to inflammation, pain, and hormonal regulation. Benzimidazole derivatives that exhibit affinity towards COX, LOX, and estrogen receptors have the potential to target multiple physiological pathways simultaneously. This multitarget approach could be advantageous in designing drugs with enhanced efficacy and a broader spectrum of therapeutic applications. The absence of reports regarding the affinity of 2-methyl-1H-benzo[d] imidazole and 2-phenyl benzimidazole derivatives towards COX, LOX, and estrogen receptors highlights a gap in current knowledge. Conducting research on these derivatives will help fill this gap, contributing valuable insights to the scientific community. In summary, the study of 2-methyl-1H-benzo[d] imidazole and 2-phenyl benzimidazole derivatives towards COX, LOX, and estrogen receptors is essential for advancing our understanding of their pharmacological potential, aiding in rational drug design and potentially leading to the development of novel therapeutic agents with diverse applications in medicine.

Molecular docking, a computational method, plays a crucial role in predicting how small molecules bind to their target macromolecules, offering valuable insights into drug discovery and development. Its applications extend to guiding the design and optimization of potential drugs, examining the structure-activity relationship of lead compounds, and minimizing the time and cost associated with experimental methods. Additionally, molecular docking aids in identifying new therapeutic targets and potential drug leads.[18] In the specific study referenced, molecular docking simulations were employed to rationally understand the observed biological outcomes related to novel 2-methyl-1H-benzo[d]imidazole and 2-phenyl benzimidazole derivatives. This information is instrumental in steering the design and development of more potent and selective benzimidazole derivatives.

MATERIALS AND METHODS

Derivatives

This study selected 2-methyl-1H-benzo[d]imidazole and 2-phenyl benzimidazole as two derivatives. The structures of these derivatives as 3D conformers in SDF format were retrieved from the PubChem Database, also called the Library of Drug Molecules.[19] The PubChem IDs of these molecules are presented in Table 1.

Protein Preparation

3D crystallographic structure of COX receptor[20] (PDB ID-1CX2, 5GMN, 7OCS), LOX receptor (2E77, 3FG1, 1YXJ), Estrogen receptor (4MOE, 2IOG, 5AAU, 2POG, 1XPC, 2QXS) were retrieved from Protein Data Bank[21] and used as protein target for our *in-silico* studies which is shown in Figure 2. In the process facilitated by Discovery Studio Visualizer, any bound water, hetero atoms, and undesired side chains were eliminated from the protein molecule. Subsequently, the protein's format was transformed to .pdb. pdbqt, wherein 'q' denotes the addition of Kollman charges, and 't' signifies the removal of hetero atoms.

The Pymol software was employed to visualize the protein structure, serving as the receptor for the investigated molecule. Utilizing this tool, cartoon structures of the protein were generated as shown in Figure 3.

Ligand Preparation

The structures of the chosen ligands were obtained from the PubChem Database (19) in.SDF format. Each ligand was then examined using Discovery Studio 2016 and subsequently saved in .pdb format for further processing. To facilitate a successful docking run, both components need to be in .pdb format before the commencement of the study. The 2D structures of both ligand molecules are illustrated in Figure 4. The details of ligands are presented in Table 2.

Table 1: Ligand and PubChem ID

S. No.	*Ligand*	*PubChem ID*
1.	2-methyl-1H-benzo[d]imidazole	11984
2.	2-phenyl benzimidazole	12855

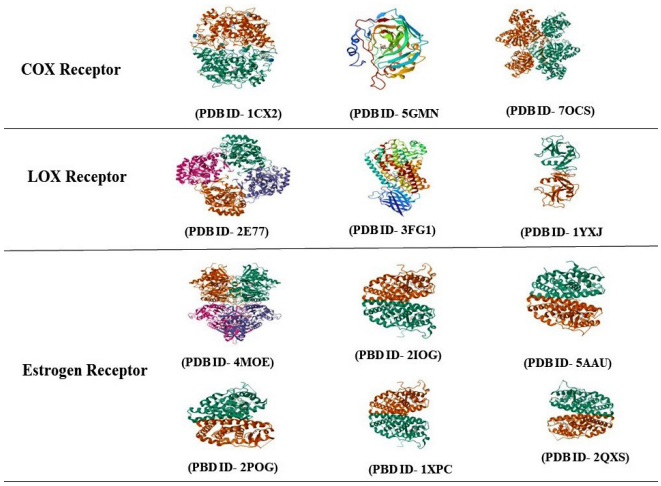

Figure 2: 3D structure of protein

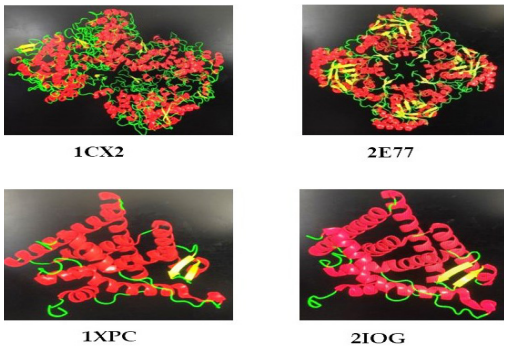

Figure 3: Pymol cartoon structure

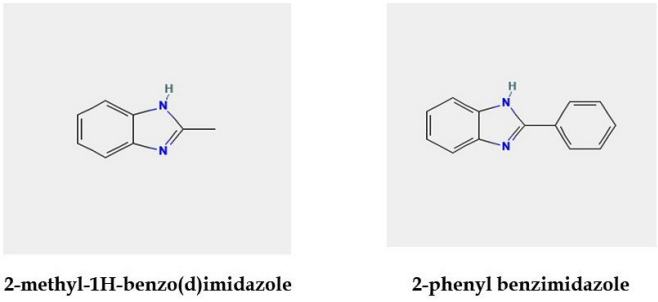

Figure 4: 2D structures of ligand molecule

Docking Procedure

Docking studies were conducted using the PyRx tool, an extensive open-source platform that integrates Autodock 4, Autodock Vina, and Auto Wizard into a unified environment. PyRx stands out for its ability to efficiently streamline the docking process for multiple ligands. Unlike Autodock Vina, PyRx eliminates the need for prompt command knowledge. Additionally, PyRx incorporates Open Babel, a valuable tool for enhancing. SDF files of ligand molecules, facilitating their conversion into the desired .pdb format compatible with the receptor molecule. Furthermore, PyRx offers the convenience of simultaneously performing energy minimization for all selected ligands, aiding in the identification of stable molecular conformations.[22-24] The grids of 2-methyl-1H-benzo[d] imidazole and 2-phenyl benzimidazole are presented in Figures 5 and 6.

RESULTS AND DISCUSSION

Both selected ligands such as 2-methyl-1H-benzo[d]imidazole and 2-phenyl benzimidazole, were subjected to docking studies. This study is conducted using PyRx software. After successful autodocking, result files are generated and that is saved in the form of an Excel sheet. The result can be viewed using an Excel sheet. A total of nine modes of both ligands were generated, and one of the best docks was selected for each ligand.

Binding interaction of 2-methyl-1H-benzo[d]imidazole with COX, LOX, and Estrogen Receptor

The details of the binding energy of 2-methyl-1H-benzo[d] imidazole are presented in Table 3 and it has been observed

Table 2: Ligand parameters

Ligand	*Formula*	*Molecular weight*	*No. of atoms*
2-methyl -1H-benzo(d) imidazole	$C_8H_8N_2$	132.16	18
2-phenyl benzimidazole	$C_{13}H_{10}N_2$	194.23	25

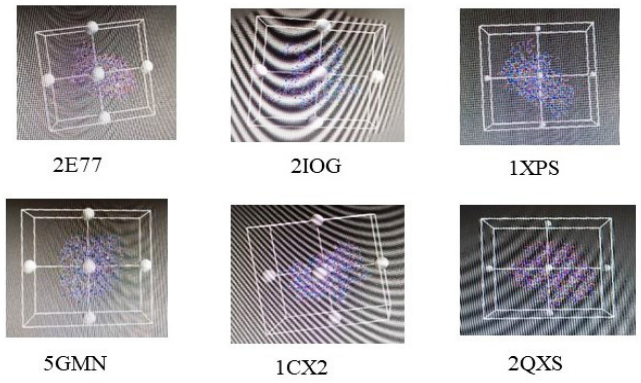

Figure 5: Grids of 2-methyl-1H-benzo[d]imidazole

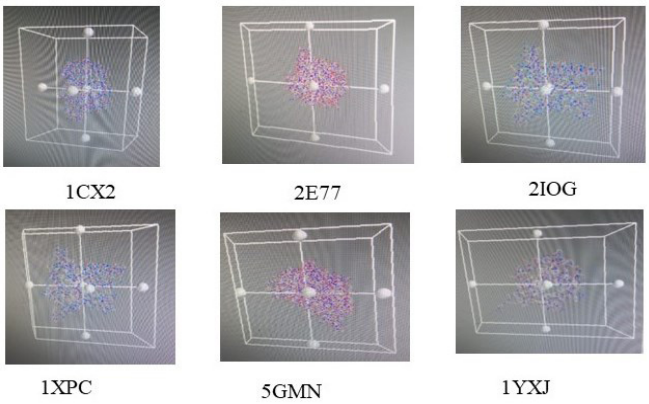

Figure 6: Grids of 2-phenyl benzimidazole

that the highest binding energy of -6.5 kcal/mol was observed with 2E77. Based on the highest binding energy 2E77 was selected as the best dock for 2-methyl-1H-benzo[d]imidazole. The binding of 2-methyl-1H-benzo[d]imidazole with 2E77 is presented in Figure 7.

Binding Interaction of 2-phenyl benzimidazole with COX, LOX, and Estrogen Receptor

The details of the binding energy of 2-phenyl benzimidazole are presented in Table 4 and it has been observed that the highest binding energy of -7.9 kcal/mol was observed with 1CX2. Based on the highest binding energy 1CX2 was selected as the best dock for 2-phenyl benzimidazole. The binding of 2-phenyl benzimidazole with 1CX2 is presented in Figure 8.

The comparative analysis revealed that 2-phenyl benzimidazole has a higher binding affinity (-7.9 kcal/mol) with COX, LOX, and estrogen receptor as compared to 2-methyl-1H-benzo[d]imidazole (-6.5 kcal/mol). In summary, the comparative analysis of binding affinities between 2-phenyl benzimidazole and 2-methyl-1H-benzo[d]imidazole provides crucial data for making informed decisions in drug development. It guides the selection of lead compounds, influences drug design strategies, and contributes to the overall success of pharmaceutical research and development efforts.

Table 3: Binding energy of 2-methyl-1H-benzo[d]imidazole

Receptor	*Binding energy of compound with receptor*								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1CX2	-5.5	-5.1	-5.1	-4.9	-4.9	-4.9	-4.9	-4.8	-4.8
5GMN	-5.7	-5.6	-5.2	-5.1	-4.8	-4.7	-4.7	-4.7	-4.5
7OCS	-4.7	-4.4	-4.3	-4.3	-4.3	-4.1	-4.1	-4	-4
2E77	-6.5	-6.5	-5.6	-5.5	-5.2	-5	-5	-5	-4.9
3FG1	-5.2	-4.5	-4.3	-4.1	-4	-4	-3.9	-3.8	-3.8
1YXJ	-4.9	-4.9	-4.9	-4.8	-4.7	-4.7	-4.6	-4.6	-4.5
4MOE	-4.6	-4.4	-4.3	-4.3	-4.2	-4.1	-4	-4	-3.9
2IOG	-6.3	-6.3	-6	-5.6	-5.2	-4.8	-4.8	-4.7	-4.7
5AAU	-4.8	-4.7	-4.6	-4.6	-4.5	-4.4	-4.4	-4.4	-4.4
2POG	-4.7	-4.6	-4.6	-4.6	-4.5	-4.4	-4.4	-4.3	-4.3
1XPC	-6	-5.6	-5.2	-5.1	-4.8	-4.6	-4.5	-4.4	-4.1
2QXS	-5.5	-4.7	-4.2	-4.1	-4.1	-4.1	-4	-4	-4

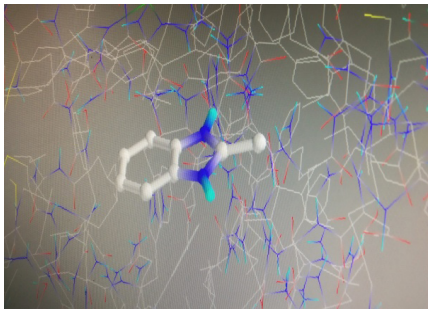

Figure 7: Binding of 2-methyl-1H-benzo[d]imidazole with 2E77

Table 4: Binding energy of 2-phenyl benzimidazole

Receptor	*Binding energy of compound with receptor*								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1CX2	-7.9	-7.7	-7.5	-7.2	-7.2	-7.1	-6.9	-6.6	-6.6
5GMN	-6.9	-6.6	-6.6	-6.5	-6.5	-6.5	-6.1	-6.1	-6
7OCS	-6	-5.8	-5.4	-5.3	-5.3	-5.2	-5.2	-5.3	-5
2E77	-7.6	-7.4	-7.3	-6.8	-6.6	-6.6	-6.5	-6.4	-6.4
3FG1	-5.4	-5.3	-5.2	-5.2	-5.1	-4.9	-4.8	-4.7	-4.7
1YXJ	-6.9	-6.7	-6.6	-6.6	-6.3	-6.2	-6.1	-5.9	-5.9
4MOE	-6	-6	-5.4	-5.3	-5.2	-5.2	-5.2	-5.2	-5.1
2IOG	-7.5	-7.1	-6.5	-6.3	-6.2	-6.1	-5.9	-5.6	-5.6
5AAU	-5.9	-5.9	-5.8	-5.8	-5.6	-5.5	-5.3	-5.3	-5.2
2POG	-5.9	-5.8	-5.7	-5.6	-5.5	-5.5	-5.5	-5.4	-5.4
1XPC	-7	-6.3	-6.2	-6.2	-6.1	-6	-6	-5.6	-5.6
2QXS	-5.9	-5.8	-5.8	-5.7	-5.7	-5.6	-5.6	-5.6	-5.3

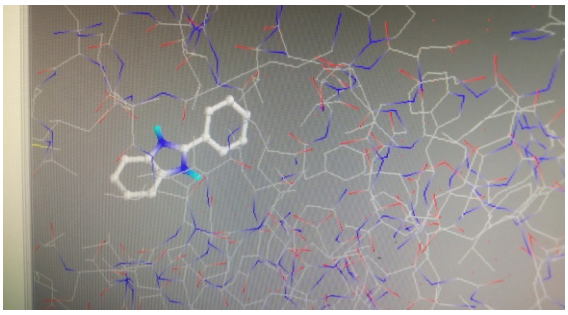

Figure 8: Binding of 2-phenyl benzimidazole with 1CX2

CONCLUSION

The 2-methyl-1H-benzo[d]imidazole and 2-phenyl benzimidazole showed better binding features with COX and estrogen receptors. Thus, these compounds can be effectively used for anti-inflammatory and antioxidant activity. This study concluded that 2-phenyl benzimidazole has a higher binding affinity with COX, LOX, and estrogen receptors than 2-methyl-1H-benzo[d]imidazole.

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