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IN SILICO DOCKING, SYNTHESIS OF 1,2-SUBSTITUTED BENZIMIDAZOLES FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
A potential class of biologically active molecules with a wide range of biological and pharmacological actions, such as anti-inflammatory, analgesic, and anti-bacterial properties, are derivatives of benzimidazoles, which are flexible heterocyclic compounds containing nitrogen. The series parent compound is approved known as imidazole, and its numbering corresponds to the usual pattern for heterocyclic compounds. In iminazole or iminazoline, an azapyrrole, one carbon atom separates the nitrogen atom. When this material was first made in 1958 from glyoxal and ammonia, it was referred to as glyoxalin. This research was conducted on various novel derivatives of benzimidazoles for their anti-inflammatory properties. Different derivatives of 1,2-substituted benzimidazoles were synthesized using different carboxylic acid derivatives by reacting with *o*-Phenylenediamine which has been used as the core ingredient for the synthesis. The Products obtained were confirmed through FT-IR spectroscopy.The obtained benzimidazoles then are docked for their analysis for the anti-inflammatory property and compared with the standard drug diclofenac sodium and found to have sufficient anti-inflammatory property.

KEYWORDS: Molecular docking, ligands, anti-inflammatory, PDB, benzimidazoles.

INTRODUCTION
A widespread medical, social, and economic burden are represented by chronic inflammatory illnesses and persistent pain of many origins, for which there is still no conclusive medication. Therefore, the need to create novel mechanisms of action for anti-inflammatory and analgesic drugs is considerable and urgent, yet the process is extremely difficult[1]. Similarly, another aspect of the body's defences against damaging outside stimuli is pain. The detection of potentially harmful pressures or substances is known as physiological or nociceptive pain, and preventing real tissue damage requires that the pain feeling be unpleasant. The nerve ends of primary sensory neurons with training to detect unpleasant thermal, mechanical, and chemical stimuli make up the nociceptors, which comprise the pain pathway. These neurons' central terminals travel into the spinal cord's dorsal horn, where they meet up with second-order neurons whose axons form the ascending pain-transmitting neural

tracts. Numerous brainstem nuclei and local interneurons modulate synaptic transmission in the dorsal horn in a facilitatory or inhibitory manner[ii]. Inflammation is a multifaceted reaction that arises from both endogenous and external stimuli in vascularized connective tissue. The host's natural defence mechanism, inflammation is a defensive reaction to tissue damage brought on by physical trauma, harmful substances, or microbiological pathogens. The initial step in tissue restoration is to try and eradicate alien organisms and remove irritants. The healing process normally causes the inflammatory process to lessen, but occasionally, inflammation can become severe and worsen the illness. In rare circumstances, it can even be fatal. Several phenomena, including the start, chemoattraction, and activation of inflammatory cells to release inflammatory mediators, are hallmarks of the inflammatory process[iii]. Inflammation is to eradicate pathogens and restore structural and functional integrity. It is the body's defensive reaction to either internal (tissue damage, infection) or external stimuli. The immune system can be divided into two main categories: innate immunity, which is a first line of defence against injury that is relatively nonspecific and has evolved through evolution; and adaptive immunity, which is more effective and specific and responds more slowly by producing specific antibodies. While both systems collaborate to respond to acute inflammation, malfunctions in the adaptive immune response are the main cause of persistent inflammation and autoimmunity[iv]

Types of inflammation: Acute inflammation and chronic inflammation are the two main categories into which inflammation can be classified. Different pathways mediate inflammatory reactions, which happen in phases as[v] inflammation is the host defence mechanism, which involves cell-cell, cell-mediator and tissue interaction[vi]. The mechanism of NSAID's is by inhibiting COX-2 selectively which are required for production of prostaglandins[vii].

ANALGESIC & ANTI-INFLAMMATORY ACTIVITY

Considered to be the fused ring of the aromatic chemicals benzene and imidazole, benzoimidazole is an aromatic heterocyclic compound. It is a white solid that crystallizes into a tubular form. Ladenberg and Wundt synthesized it in 1878, after Hoebrecker had done it in 1872. Benzomidazole is increasingly the drug of choice for therapeutic chemistry and drug creation because of its potential biological action. Many beneficial applications, such as anti-inflammatory, antibacterial, antifungal, antioxidant, anti-malarial, anticancer, and anti-parasitic properties, are known for the majority of scaffold types. A common ring system in heterocyclic pharmacophores is benzimidazole. These substructures' diverse recurrence in bioactive chemicals has earned them the moniker "privileged." Furthermore, benzimidazole, ligand, and its structural chemistry compounds are highly recognized; their biological activities are the main emphasis[viii]. (As Mentioned in figure – 1)

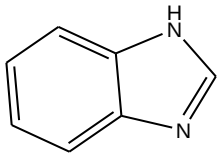


Fig 1: Benzimidazole

APPLICATIONS

Benzimidazole has a wide range of beneficial use

1. Benzimidazoles exhibit pharmacological activities like (As mentioned in Figure-2)

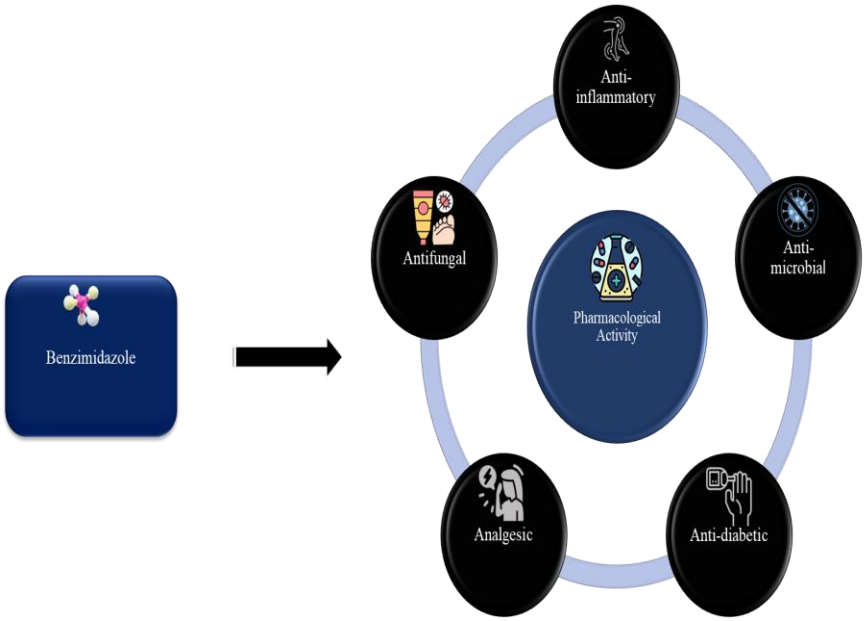


Fig 2: Pharmacological applications of Benzimidazole

2. General Applications of Benzimidazoles (As mentioned in figure- 3)

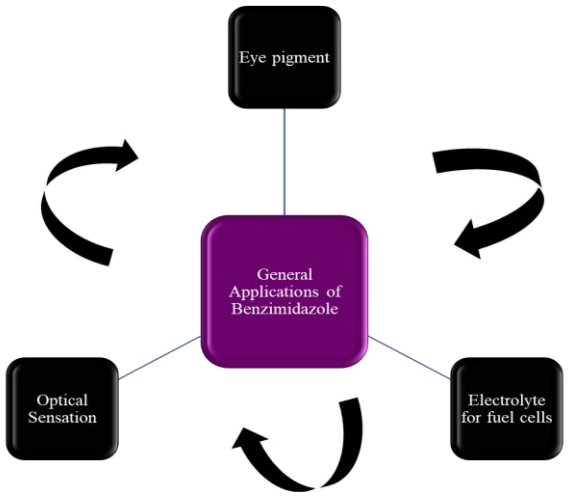


Fig 3: General Applications of Benzimidazoles

The benzimidazole scaffold has become the preferred pharmacophore among the many NSAID classes for creating analgesic and anti-inflammatory drugs that are active on various clinically recognized targets. In the early 1970s, a chemical based on benzimidazole was launched as an oral narcotic analgesic for the treatment of severe and chronic pain, its pharmacokinetic profile for both the parent molecule and despropionylbezitramide, the result of its hydrolysis, as well as its therapeutic efficacy against pain caused in experiments. A piperazine-linked benzimidazole derivative was described as a TRPV-1 antagonist with analgesic potential in a recent patent application from Amge. Investigating the viability of

developing novel analgesic medications was the aim of the current investigation, which focused on the analgesic activity displayed by the synthesized compounds[ix]

Molecular Docking

Molecular docking is a method that anticipates a molecule's preferred route when it jumps to another to form a stable complex. To predict the degree of participation or binding affinity connecting two molecules with one another, information about the rotation orientation selected can be worn. In order to minimize the free energy of the overall procedure, molecular docking aims to achieve an optimal conformation of both, protein and ligand as well as a fundamental direction between the two. In order to facilitate basic bimolecular processes including drug-protein, drug-nucleic acid, and enzyme substrate interactions, molecular recognition is essential[x].

Application of docking: In the fields of molecular biology and drug development, docking is the name given to a computer method that predicts which way molecules will bind together to create a stable complex. By mimicking the interaction between a tiny chemical (ligand) and a target protein (receptor), this technique is essential to the discovery of new drugs. Researchers can anticipate the strength of a possible drug candidate's binding and, consequently, its potential usefulness as a therapeutic agent by computationally docking it into the binding site of a target protein. Docking speeds up the process of finding new drugs by making it easier to screen through large libraries of chemicals and find good candidates for additional experimental validation. Furthermore, docking can provide details about the binding processes[xi]

MATERIALS AND METHODS:

Materials :

Beaker, Petridis, Condenser, Vacuum pump, Measuring cylinder, Glass rod, Water bath, Spatula

Pipette, Heating mantle, round bottom flask are the apparatus used in this research and *o*-phenylenediamine, cinnamic acid, nicotinic acid, *p*-hydroxy benzoic acid, chloroacetic acid, hydrochloric acid, ammonium solution, ethanol, distilled water are the regents.

Methods :

General Procedure for the Synthesis of 2-(Chloromethyl)- 1H-benzimidazole (Compound 1)

A 45-minute reflux heating period was used to combine o-phenylenediamine (5.4g, 0.05 mol), chloroacetic acid (7.1 g, 0.08 mol), and 4N hydrochloric acid (17.17 mL). After letting the combination sit for a full night, it was filtered, diluted with 100 milliliters of distilled water, chilled, and cautiously neutralized using a solution of 6N ammonium hydroxide. To stop gums from forming, the solution was kept cold throughout neutralization and rapidly agitated. Product formed was filtered, washed well with cold water and then was placed in vacuum desiccators until dry. Recrystallization was done using ethyl alcohol[xii].

General Procedure for the Synthesis of 2-[(E)-2-phenylethenyl]- 1H-benzimidazole (Compound 2)

A solution of 4N hydrochloric acid (17.17 mL), cinnamic acid (11.84 g, 0.08 mol), and o-phenylenediamine (5.4 g, 0.05 mol) was cooked at reflux for two hours. After letting the combination sit for a full night, it was filtered, diluted with 100 milliliters of distilled water, chilled, and cautiously neutralized using a solution of 6N ammonium hydroxide. To stop gums from forming, the solution was kept cold throughout neutralization and rapidly agitated. The resulting product was filtered, thoroughly cleaned with cold water, and then dried in vacuum desiccators. Ethyl alcohol was used in the recrystallization process.

General Procedure for the Synthesis of 2-(pyridine-3-yl)- 1H-benzimidazole (Compound 3)

Mixture of o-phenylenediamine (5.4g, 0.05 mol),nicotinic acid(9.84g, 0.08 mol), and 4N hydrochloric acid (17.17mL) was heated at reflux for 2hours.(scheme 1). The mixture was allowed to stand overnight, filtered, diluted with 100mL of distilled water, cooled, and carefully neutralized with 6N ammonium hydroxide solution. The solution was kept cold during the neutralization and stirred vigorously to prevent the formation of gums. Product formed was filtered, washed well with cold water and then was placed in vacuum desiccators until dry . Recrystallization was done using ethyl alcohol[xiii].

General Procedure for the Synthesis of 1-(1H-benzimidazol -2-yl)-2-phenylethan-1-amine (Compound 4)

Mixture of o-phenylenediamine (5.4g, 0.05 mol), phenylalanine (13.2g, 0.08 mol), and 4N hydrochloric acid (17.17mL) was heated at reflux for 2hours. (scheme 1). The mixture was allowed to stand overnight, filtered, diluted with 100mL of distilled water, cooled, and carefully neutralized with 6N ammonium hydroxide solution. The solution was kept cold during the neutralization and stirred vigorously to prevent the formation of gums. Product formed was filtered, washed well with cold water and then was placed in vacuum desiccators until dry. Recrystallization was done using ethyl alcohol[xiv].

General Procedure for the Synthesis of 4-(1H-benzimidazol)-2-yl) phenol (Compound 5) Mixture of o-phenylenediamine (5.4g, 0.05 mol), *p*–Hydroxy benzoic acid(10.5g, 0.08 mol), and 4N hydrochloric acid (17.17mL) was heated at reflux for 2hours.(scheme 1). The mixture was allowed to stand overnight, filtered, diluted with 100mL of distilled water, cooled, and carefully neutralized with 6N ammonium hydroxide solution. The solution was kept cold during the neutralization and stirred vigorously to prevent the formation of gums. Product formed was filtered, washed well with cold water and then was placed in vacuum desiccators until dry. Recrystallization was done using ethyl alcohol[xv].

Methodology

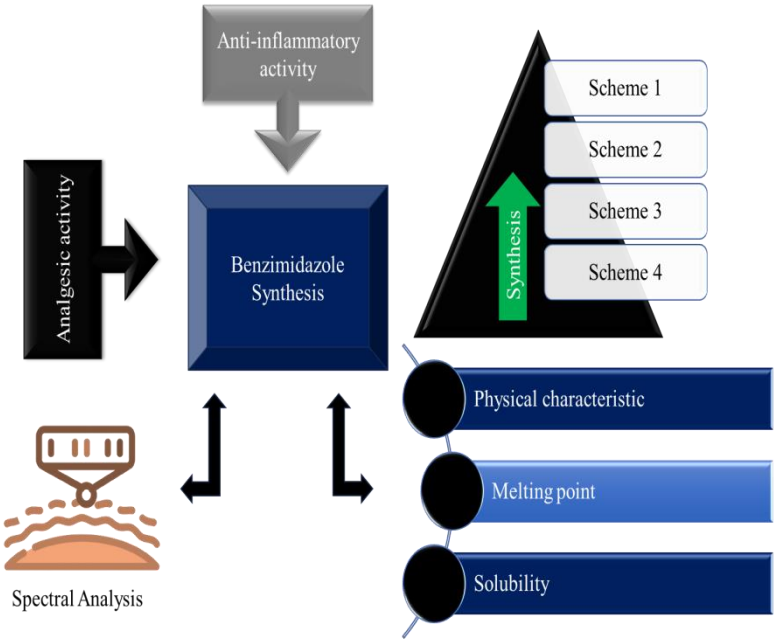


Fig 4 : Methodology

Molecular Docking

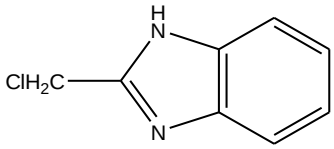
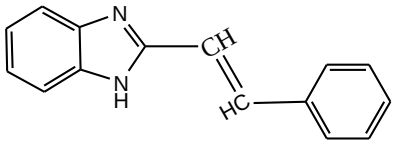
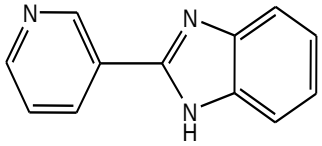
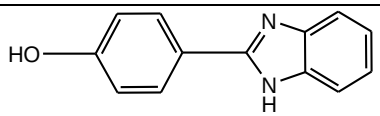
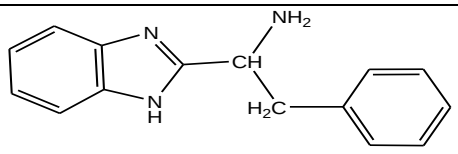
These days, a typical method in a computational drug discovery pipeline is molecular docking[xvi]. Medicinal chemists might possibly expedite structure-based drug creation by

using novel concepts for lead optimization that arise from a solid approximation of a ligand's crystal posture and combining a search method to sample the area in which a ligand conforms within a binding site with a scoring function to objectively assess the correctness of these poses is known as a docking technique. While the conformational search used by docking techniques is frequently successful in generating the ideal ligand pose, scoring algorithms frequently fall short of ranking them[xvii]. Since the scoring function selection has a major influence on the results, performance comparisons of many protocols are often conducted early in docking studies to rationalize protocol selection[xviii]. Autodock4[xix], was used and the results were discussed below.

RESULT AND DISCUSSION

PHYSICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

Table 1: Physical properties of synthesized Benzimidazoles.

Sl. NO	Compound	Colour	Solubility	Melting Point(°C)	Yield (%)
1		Reddish-Pink	DMSO	150-160	35
2		Yellowish-Brown	DMSO	152-156	40
3		Light Pink	DMSO	160-162	38
4		White	DMSO	150-158	30
5		Yellow-Light Pink	DMSO	160-165	33

IR Interpretation of Compound 1

2^{o} NH – 3348 cm^{-1}, *o* -disubstituted benzene- 746 cm^{-1},-C=C- of Imidazole-3042 cm^{-1}, Aromatic C=C benzene-1523-1625cm^{-1}, C-N Stretching-1303cm^{-1}.

IR Interpretation of Compound 2

2^{o}NH – 3024cm^{-1} ,-C=C of Imidazole- 2700 cm^{-1} ,-C=C Aromatic benzene-1447-1672cm^{-1}, -C-H Stretching-2950cm^{-1}, -C-N Stretching-1310cm^{-1}, Aliphatic-C=C- 1415cm^{-1}, - *o*-disubstituted benzene-870cm^{-1}, -monosubstituted benzene-763 cm^{-1}.

IR Interpretation of Compound 3

3- monosubstituted pyridine – 743cm^{-1}, -C=C- of Imidazole- 2359cm^{-1}, -C=C- of Aromatic benzene-1413-1693cm^{-1}, -*o*-disubstituted benzene -808cm^{-1}, -C-N Stretching-1292cm^{-1}.

I

R Interpretation of Compound 4

2^{o}NH- 3445cm^{-1}, -OH-3445 cm^{-1},-C=C-of Imidazole- 2813 cm^{-1}, -C=C- of Aromatic benzene- 1422-1651cm^{-1}-*o*- disubstituted benzene- 767cm^{-1}, -disubustituted benzene- 846 cm^{-1}.

IR Interpretation of Compound 5

2^{o}NH & 1^{o} NH2 --3187cm^{-1} , -C=C- of Imidazole -3028cm^{-1}, -CH Strecthing- 2900cm^{-1}, -*o*-disubstituted benzene-745cm^{-1}, monosubstituted benzene- 720cm^{-1}, - C-N Stretching-1336cm^{-1}, -C=C- Aromatic benzene- 1462-1669cm^{-1}.

MOLECULAR DOCKING ON DIFFERENT LIGANDS

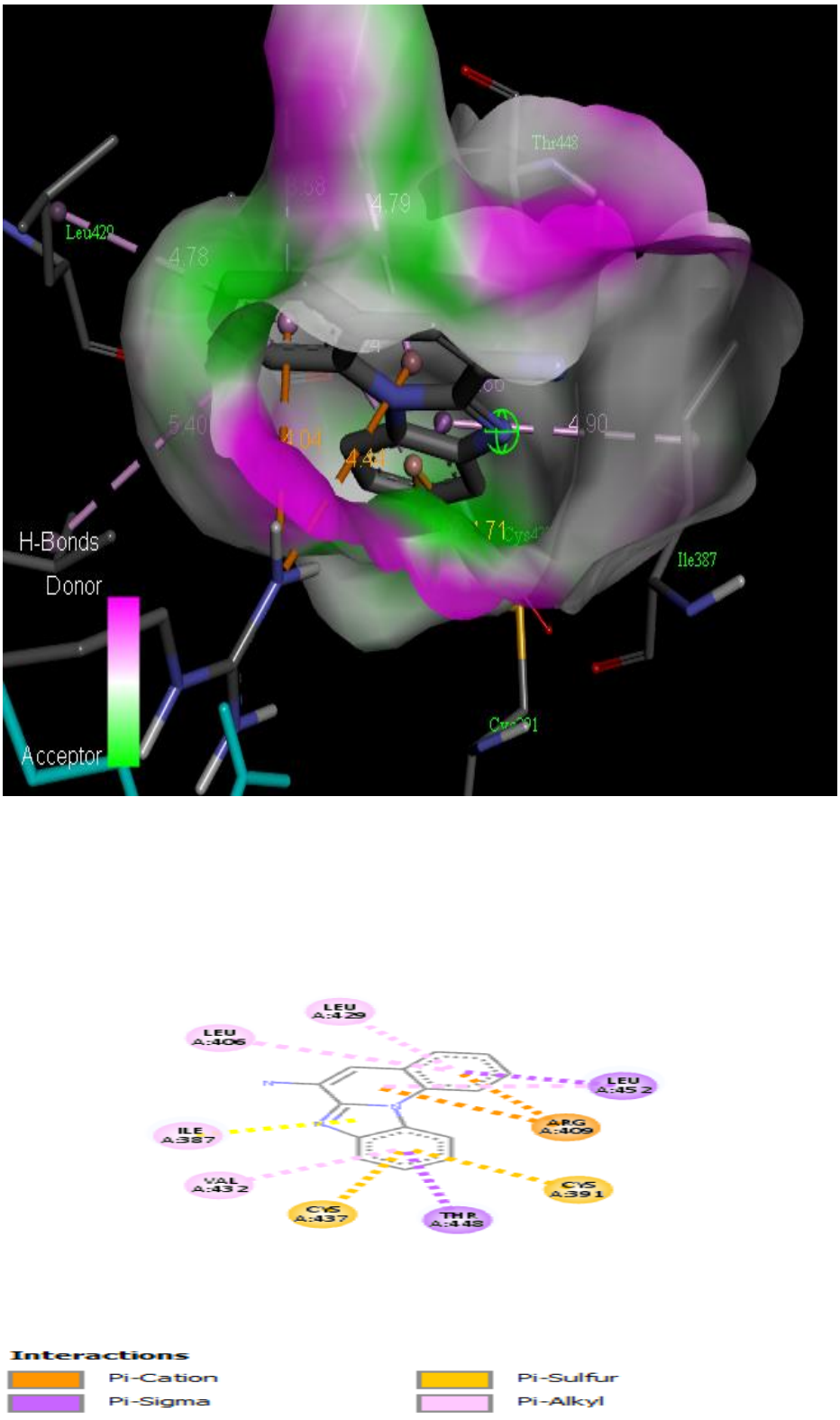


Fig. 5: Interaction of ligands on 6hn1 Compound 1

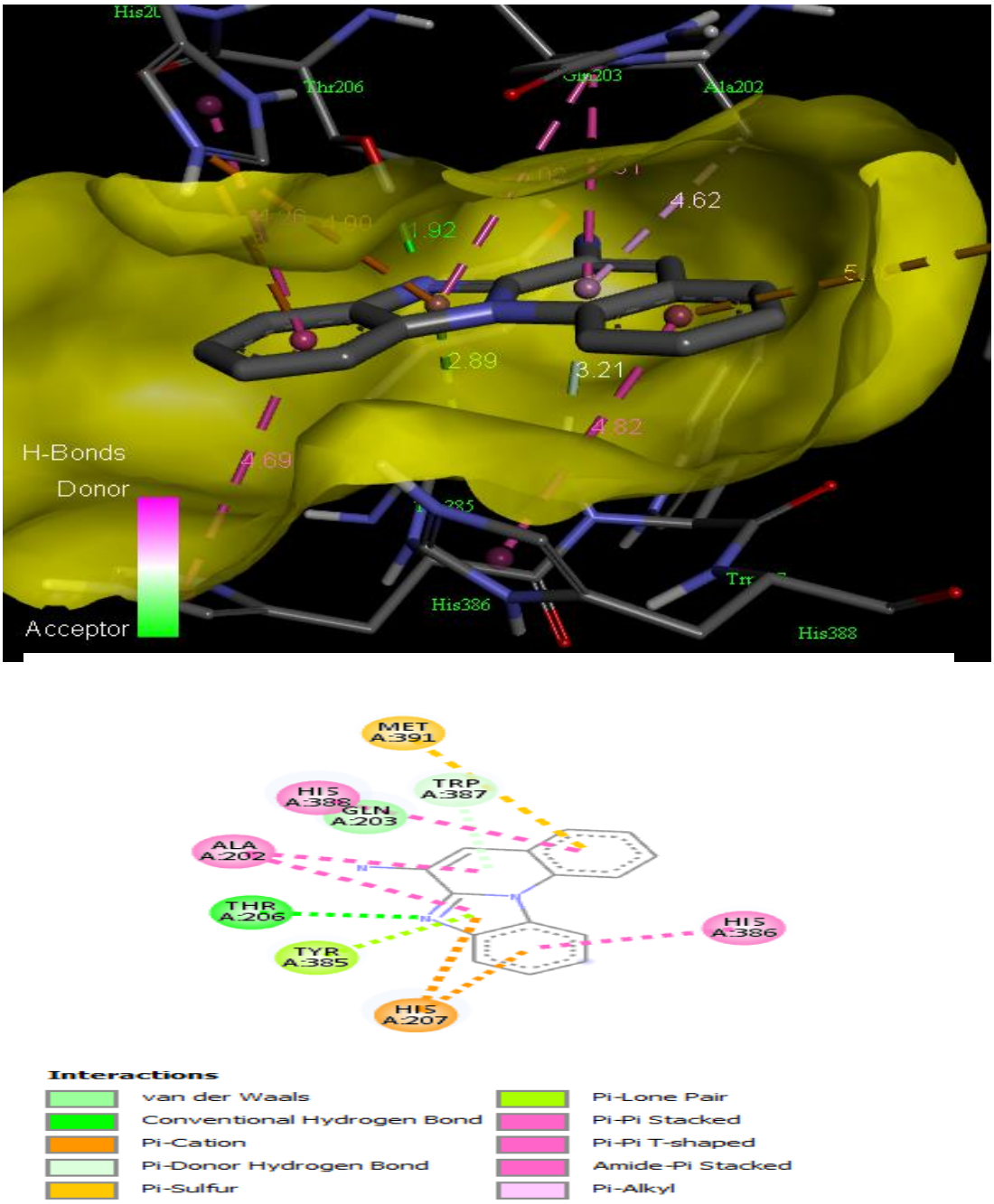
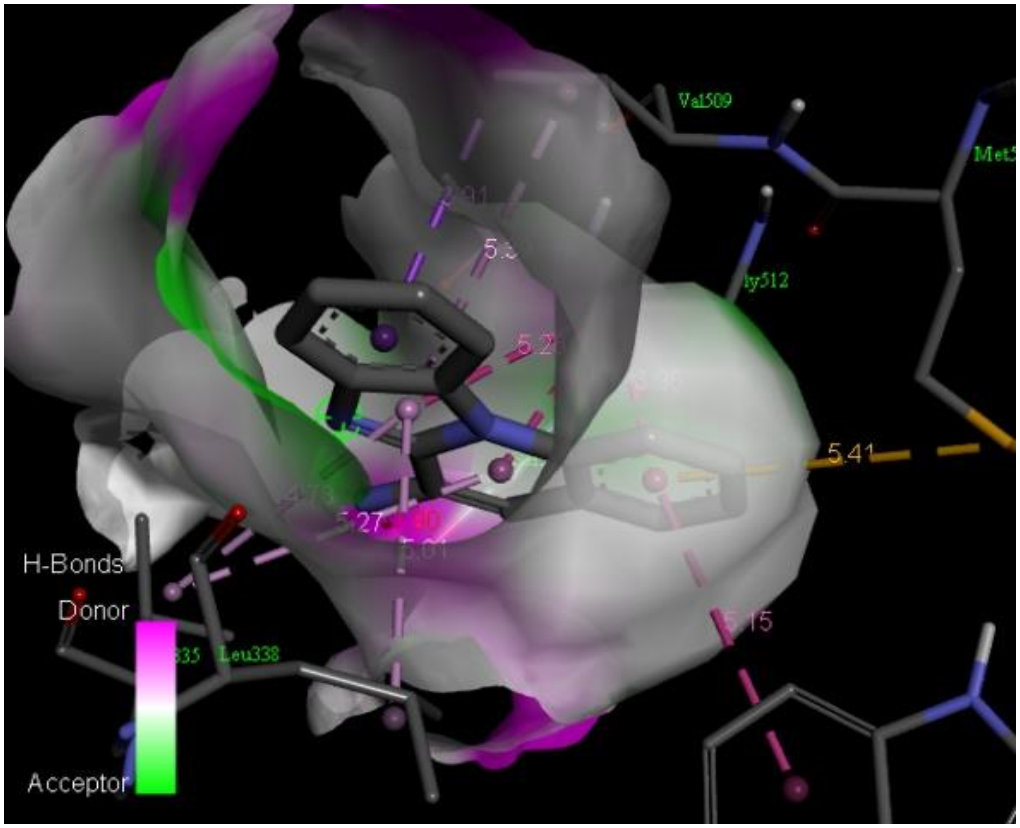


Fig 6: Interaction of ligands on 1ht8 Compound 2



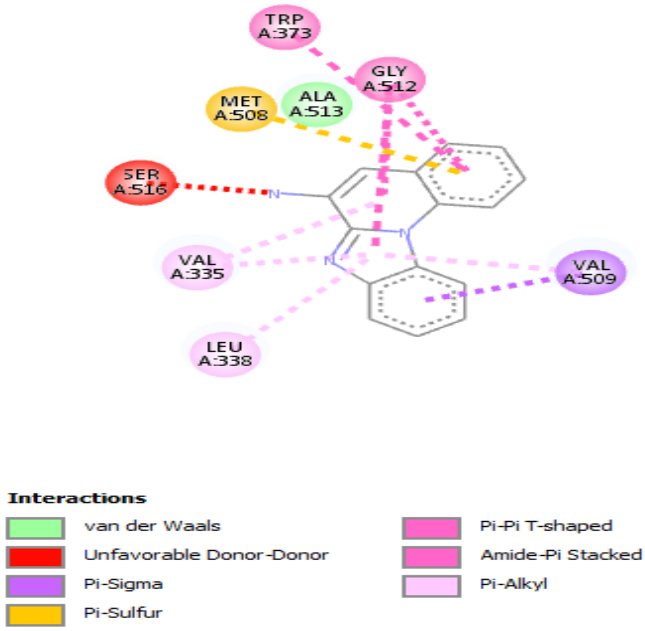
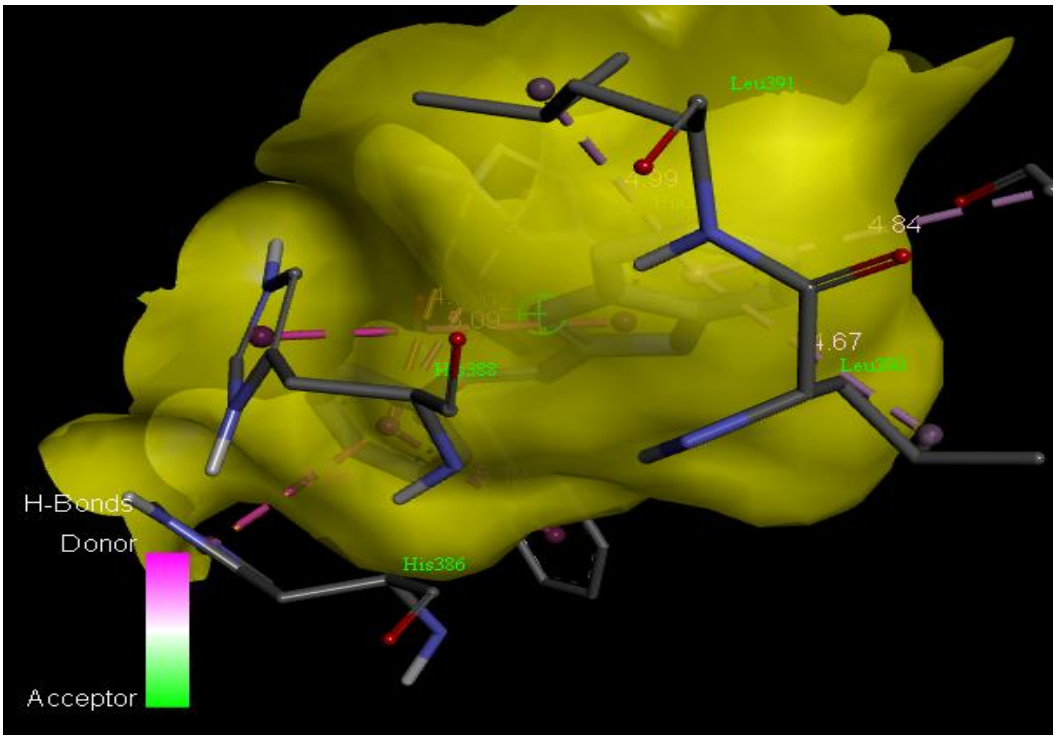


Fig.7: Interactions of ligand on 3ln1 Compound 3



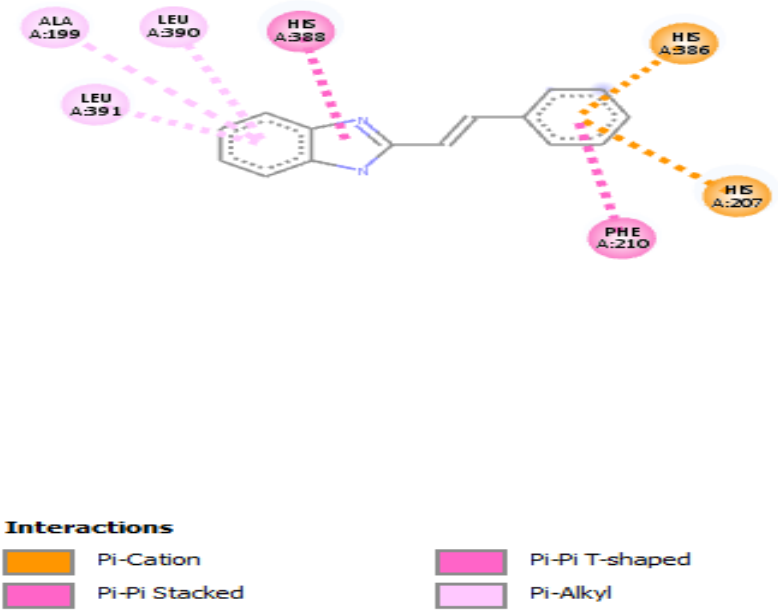


Fig.8: Interaction of ligand on 1pxx Compound 4

Table 2 : Docking Score of Synthesized Compounds on different proteins with Standard Diclofenac

Sl.No	Compounds	Rank	Sub-Rank	Protein	Run	Binding energy	Cluster RMSD	Reference RMSD
1	**1-(1H-benzimidazol-2-yl)-2-phenylethan-1-amine**	1	1	1ht8	2	-8.73	0.00	187.70
2	1-(1H-benzimidazol-2-yl)-2-phenylethan-1-amine	1	1	6hn1	1	-7.24	0.00	93.74
3	1-(1H-benzimidazol-2-yl)-2-phenylethan-1-amine	1	1	3ln1	5	-7.09	0.00	22.45
4	2-[(E)-2-phenylethenyl]-1H-benzimidazole	1	1	1pxx	6	-7.22	0.00	41.16
5	2-[2-(2,6-dichloroanilino)phenyl]acetic acid	1	1	1ht8	2	-7.12	0.00	213.05

DISCUSSION

The above synthesized compounds (1,2,3,4,) in table (2) were evaluated for anti-inflammatory and analgesic activities through molecular docking by using AutoDock Vina (O. Trott, The Scripps Research Institute, La Jolla, CA, USA). In the test, the conventional medication was diclofenac sodium. In table 9, the compounds (1,2,3,4) showed the lowest binding energies compared with the standard drug on binding to proteins (6hn1, 1ht8, 3ln1, 1pxx). FT-IR was done for all the compounds synthesized and found to fulfill the criteria for the same. Thus, reaction of Ortho-Phenylendiamine with different carboxylic acid derivatives found to obtain different derivatives of benzimidazoles for their Anti-inflammatory activity on 1ht8.

CONCLUSION

The present work has clearly demonstrated that the treatment of carboxylic acid derivatives with orthophenylenediamine (OPD) and certain amino acids may be used to efficiently synthesise pharmaceutical derivatives, including derivatives of benzimidazole. Furthermore, benzimidazole derivative synthesis is a one-step procedure. After the synthesis of five 1,2-substituted benzimidazole derivatives, compound 1 was shown to have the greatest analgesic and anti-inflammatory effects, the lowest binding energy, and the highest yield using molecular docking analysis. Strong 1,2-substituted benzimidazole derivatives are therefore being developed with the goal of using them as drug-like scaffolds in the future.

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