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Original Research Article

Pharmaceutical Chemistry for better Medicinal drug

Design, Molecular Docking, Synthesis And Biological Evaluation Of Novel 2,5-Substituted Imidazole Derivatives As Anti-Inflammatory Agents

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Abstract: Imidazoles have occupied a unique position in heterocyclic chemistry, and its derivatives Imidazoles are heterocyclic compounds which possesses biological and pharmaceutical importance owing to its diversified activities like antibacterial, anti-tumor, antiviral, antiretroviral etc. In the current study 2,5-substituted imidazole were synthesized by using 2-acetyl thiophene as the starting material and 12 derivatives of 2,5- substituted imidazoles were subjected to *insilico* docking studies (PDB ID:1N26) using AUTODOCK vina software. The compounds that showed the best results were synthesized. The newly synthesized derivatives were characterized using IR, 1H NMR and Mass spectra. All the synthesized derivatives were evaluated for *invitro* anti-inflammatory activity. The compounds IV c showed good activity whereas compounds IV a ,IV b, IV d, and IV e showed moderate activity which was comparable to that of the standard Diclofenac drug.

Keywords: Molinspiration, Osiris, Molecular docking, Imidazoles, anti-inflammatory activity.

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1. INTRODUCTION

The name (Fig:1) "imidazole" was coined in 1887 by the German chemist Arthur Rudolf Hantzsch (1857–1935). It is classified as diazole having nonadjacent nitrogen atom. Imidazole can serve as a weak acid and weak base. It is a planer five-member heterocyclic ring with 3C and 2N atom and in ring N is present in 1st and 3rd positions having molecular formula of $C_3N_2H_4$ (Fig 1). The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. Being a polar and ionisable aromatic compound, it improves pharmacokinetic characteristics of lead molecules and thus used as a remedy to optimize solubility and bioavailability parameters of proposed poorly soluble lead molecules. It has m.p of 89-91°C and having a high B.P. 256°C resonance value of 14.2 Kcal/ mol.[1]

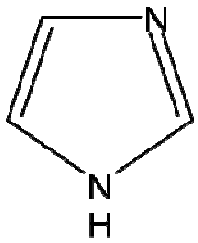


Fig 1. Imidazole

Thiophene (Fig:2) belongs to a class of heterocyclic compounds containing a five membered ring made up of one sulphur as heteroatom with the formula C_4H_4S. Thiophene and its derivatives exist in petroleum or coal. In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications. It is insoluble in water but soluble in most organic solvents including alcohol and ether. Melting Point of thiophene is -38°C while boiling point is 84°C. [2]The heat of combustion indicates resonance stabilization to the extent of 22-28 K.cal./mol.[3]

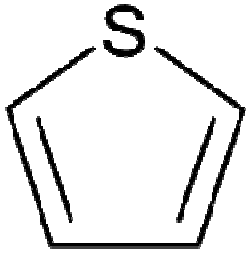


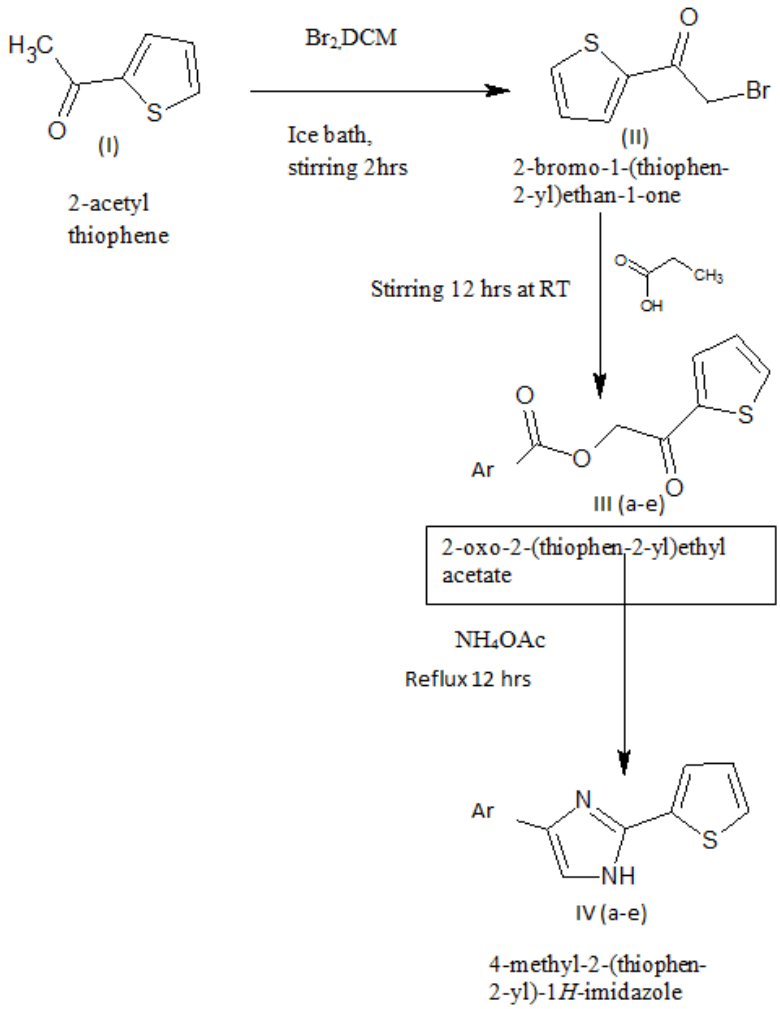
Fig 2. Thiophene

The Objectives for the present study are:

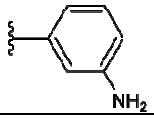
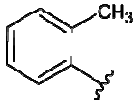
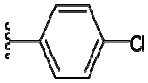
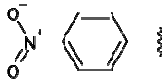
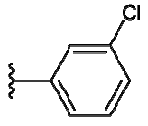
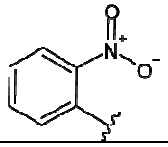
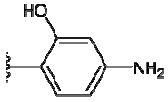
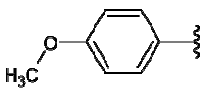
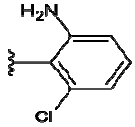
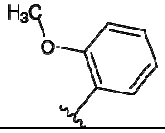
- To perform *insilico* studies (docking studies) for 2,5-substituted imidazole derivatives.
- To synthesize potent and safer anti-inflammatory agents.
- To characterize the synthesized compounds by melting point, TLC, IR, ^{1}HNMR, and MASS spectra.
- To screen the synthesized compounds for their *in vitro* anti-inflammatory activity by Albumin denaturation method.

2. MATERIALS AND METHODS

Many drugs like Indomethacin and Nimesulide acts as the Imidazole group which is essential for activity but each drug has its own side effects. Most of the available drugs may not be safe as they have serious side effects. Potency and safety is of utmost importance in the use of these drugs. Hence, still, there is a need to synthesize such new compounds that will be potent and safer than the existing agents.



Scheme I

Compound	Ar	IV f	
IV a		IV g	
IV b		IV h	
IV c		IV i	
IV d		IV j	
IV e		IV k	

2.1 Experimental procedures

2.1.1 Synthesis of 2-Bromo-1-(Thiophene-2-yl) ethanone

A solution of Bromine (1.98 ml, 0.039 mmol) in dichloromethane (20ml) was added dropwise to (Fig:3) 2–acetyl thiophene (5g, 0.039 mmol) in dichloromethane (30ml) over 20 min. The reaction mixture was stirred at 25°C for 2hrs and neutralized with a saturated solution of $NaHCO_3$. The progress of the reaction was monitored by TLC using Toluene: Dichloromethane (5:5) as a solvent system. The organic layer was washed with water followed by brine and dried over Na_2SO_4 and filtered. The removal of the solvent afforded a residue the yield obtained was yellow oil and the Boiling point -213°C and Percentage yield - of the obtained product was found to be 81.6%.

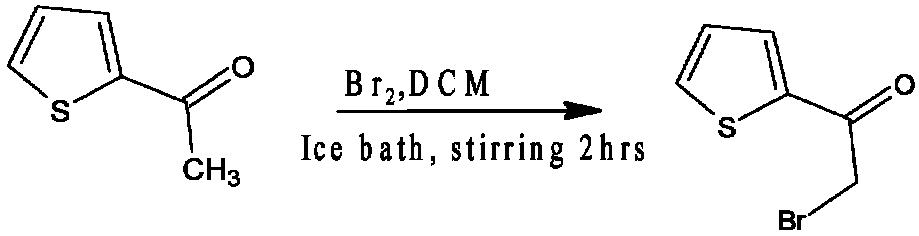


Fig 3. 2-Acetyl thiophene 2-Bromo-1-(Thiophene-2-yl) ethanone

2.1.2 Synthesis of 2-oxo-2-(thiophen-2-yl)ethyl acetate

A mixture of para aminobenzoic acid (1.0 mol) (11grams) and triethylamine (3.0mol) (15ml) in dichloromethane (15ml) was cooled in an ice bath to below 25°C. To this (Fig:4) acyl bromide (1.2 mol), was added dropwise and the solution was stirred for 12hrs at room temperature. The completion of the reaction was monitored by TLC using Petroleum ether: ethyl acetate (2:8). The reaction mixture was diluted with chloroform and successively washed with water and brine solution, and dried over Na_2SO_4, after the removal of the solvent under the reduced pressure and the residue is obtained.

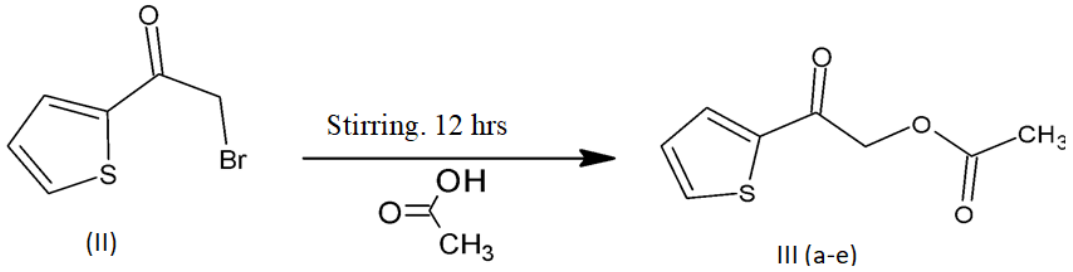


Fig 4. 2-Bromo-1-(Thiophene-2-yl) ethanone 2-oxo-2-(thiophen-2-yl)ethyl acetate

2.1.3 General procedure for the synthesis of compounds IV (a-e)

Mechanism: It is a cyclisation reaction where the ester converted into imidazole ring in the presence of NH4OAc. To the mixture of (Fig: 5) 4-amino-3-{[2-oxo-2-(thiophen-2-yl)ethoxy]carbonyl}benzoic acid was dissolved in toluene (10ml) and treated with ammonium acetate (5.0 mol) (11 grams). The reaction mixture was refluxed for 12hrs and was monitored by TLC. The reaction mixture was quenched with sodium bicarbonate. The product was extracted with ethyl acetate (2 times), and successively washed with water and brine solution, and dried over Na_2SO_4. After the removal of solvent under reduced pressure, the crude product was obtained

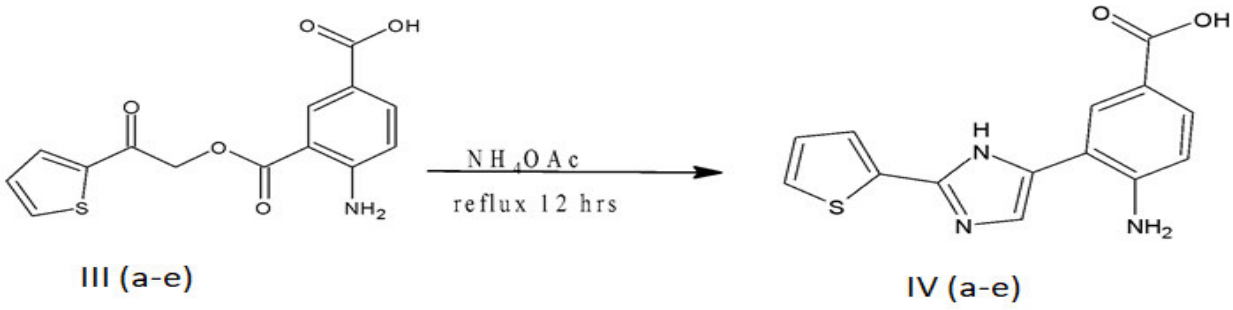
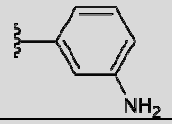
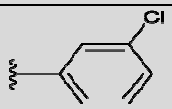
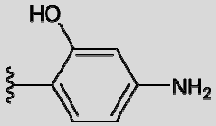
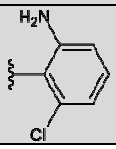


Fig 5. 2-oxo-2-(thiophen-2-yl)ethyl acetate 4-amino-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid

Table 1. List of Aryl substituents

Compound	Ar
IV a	
IV b	
IV c	
IV d	
IV e	

Docking studies and molecular toxicity prediction are studied for the compounds a to k But the derivatives with the high docking scores and the availability of the chemicals we have synthesized the derivatives from a to e

2.1.4 Biological activity

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury form due to stress. The Inflammation of tissue is due to the response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function occurs depends on the size and extent of the injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion [4]. Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the management of inflammatory conditions such as rheumatoid arthritis and other infectious diseases. They reportedly bind to plasma albumin, preventing or inhibiting the thermal denaturation of albumin.

2.2 Procedure for biological activity

2.2.1 Method used

Bovine serum albumin assay

2.2.2 Preparation of Protein solution

Bovine Serum Albumin Solution (BSA) solution (0.2%, w/v) was prepared in Tris Buffered Saline. The pH was adjusted to 6.8 with glacial acetic acid.

2.2.3 Preparation of Test solution

Stock solutions of the synthesized compound were prepared in methanol at a concentration of 1000 mg/mL or 0.005%, w/v. From the stock solution three different concentrations of 10, 30, 50 µg/ml was prepared by using methanol as solvent.

2.2.4 Protein denaturation

The test solutions was transferred to Eppendorf tubes using 1ml micro pipette. 5ml of 0.2% W/V BSA was added to all the above eppendorf tubes. The standard consists of 10 µg/ml of Diclofenac sodium in methanol with 5ml 0.2% W/V BSA solution[5]. The solutions were incubated in BOD incubator at 37°C for 15 min then heated in a water bath at 72 °C for 5 minutes. The turbidity of the solutions (level of protein precipitation) was measured at 660 nm in a UV Spectrophotometer. The percentage inhibition of precipitation (protein denaturation) was determined on a percentage basis, relative to the negative control using the following equation:

$$\% \text{ Anti denaturation activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} X\,100$$

The % anti-denaturation was calculated and was related to the anti-inflammatory activity of the compounds tested.

3. RESULTS AND DISCUSSION

3.1 Molinspiration and OSIRIS

Molecular properties were calculated using molinspiration online tool[6] . This tool enables us to predict the molecular properties of the drug molecule. Molinspiration uses the Lipinski rule of five to evaluate the molecular properties. OSIRIS Property Explorer [7] is a very good webserver to predict molecular properties and other drug-associated factors. It was used to estimate the risks of side effects, such as mutagenic, tumorigenic, irritant and reproductive effects,

as well as drug-relevant properties including Toxicity prediction, Partition coefficient, Solubility prediction, Molecular Weight, drug-likeness and overall drug-score.

Table 2. Physical properties of the title compounds using Molinspiration

S.no	Compound	Log P	TPSA	N atoms	Molecular weight	No.of rotatable bonds
IV a	4-amino-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	2.93	54.71	17	241.32	2
IV b	2-chloro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	2.58	54.71	17	241.32	2
IV c	4-chloro-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.13	28.68	17	260.75	2
IV d	5-amino-2-hydroxy-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.18	28.68	17	260.75	2
IV e	2-amino-6-chloro-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	3.41	74.51	19	271.30	3
IV f	4-methyl-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	3.46	74.51	19	271.30	3
IV g	2-methyl-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	3.90	28.68	17	240.33	2
IV h	4-nitro-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	3.95	28.68	17	240.33	2
IV i	2-nitro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	3.51	37.92	18	256.33	3
IV j	4-methoxy-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	3.51	37.92	18	256.33	3
IV k	2-methoxy-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	3.48	36.14	17	243.0	2

The log P value or the partition coefficient for a drug molecule affects its absorption and solubility. If a compound has a higher logP value it is considered as a hydrophobic molecule and if the logP value is less it is considered as a hydrophilic molecule. An ideal drug molecule should contain moderate logP value. All the compounds that were analyzed have moderate logP values. TPSA or Topological Polar Surface Area is the total surface area of a drug molecule that allows the compound to permeate into the cells. The TPSA should be less than 140 sq. angstroms to enter into the cells and the TPSA should be less than 90 sq. angstroms to cross the blood-brain barrier. All the title compounds molecules were found to have TPSA of less than 90 sq. angstroms.[8-9] According to Lipinski's rule of five, a drug molecule must have a molecular weight less than 500 daltons so that the drug molecule can be easily transported through the membranes by passive transport. All the title compounds show less than 500 daltons of molecular weight. They should have less than or equal to 10 Hydrogen bond Acceptors (nON) so that the molecule can interact with the active site of the target protein. All the proposed compounds have shown the number of hydrogen bonds to be less than 10. The number of rotational bonds in a molecule gives an impression about the stability of the molecule. The number of rotational bonds should be less than or equal 8 in order to be highly stable. The title compounds have rotational bonds between 5-8 and thus are concluded to be stable molecules.

Table 3. Drug likeliness and toxicity calculation of title compounds using OSIRIS property calculator

S.no	IUPAC name	Log S	Drug likeness	Drug score	Mutagenicity
IV a	4-amino-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	5.01	3.15	0.62	None
IV b	2-chloro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	5.01	3.61	0.63	None
IV c	4-chloro-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.35	0.354	0.354	None
IV d	5-amino-2-hydroxy-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.35	2.87	0.757	None
IV e	2-amino-6-chloro-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.618	2.474	0.676	None
IV f	4-methyl-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.618	2.474	0.676	None
IV g	2-methyl-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.734	4.554	0.643	None
IV h	4-nitro-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.734	7.577	0.373	None
IV i	2-nitro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.292	2.565	0.737	None
IV j	4-methoxy-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.292	2.891	0.737	None
IV k	2-methoxy-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	4.588	1.35	0.646	None

Log S corresponds to the aqueous solubility of a compound. Prevailingly, most of the compounds that are present have a logS value above -4 and below +4. If the drug solubility is less, the.re are minimum chances for the drug to be absorbed into the tissues. Therefore it is preferred to have a moderate solubility for the drug molecules. All the compounds showed

logS value lesser than[4] The Drug score of a molecule is based on the fragments of the molecules that have already present and valuated. These fragment scores must not have any negative value, thus corresponding to the drug molecules that are already present. The compound scores for compounds IV a to IV e were positive. Drug like properties of a compound is based on the fragment comparison of the drug molecule with the pre-knowledge of the standard compounds. The drug likeliness of the compound can give values between 1.0 (no risk), 0.8 (moderate risk) and 0.6 (high risk) that reveal the toxicity and risk that the compound may cause.[8] Compounds IV a to IV e have shown drug likeness score between 1 to 0.8. Mutagenicity was also calculated by the OSIRIS property calculator. The compounds IV a through to IV e have shown no mutagenic properties and hence were further evaluated by Ligand Flexible Docking.

3.2 Molecular docking

Over the decade, research and development of drug have become an uncomplicated journey. All this is owing to the dramatic increase in the usage of *in silico* drug designing and optimization. *In silico* studies enables the researchers to develop a molecule within less time by assessing the entire affirmative and the unaffirmative characteristics[10]. Computational prediction of protein−ligand binding poses has become an important tool in drug discovery[11-12]. A number of docking programs have been developed[13-15] and applied to discover agonists or antagonists of therapeutic targets[16-18].Although protein receptors often undergo conformational changes upon ligand binding, docking methods that do not consider protein flexibility are still used extensively due to their simplicity and efficiency. However, consideration of receptor flexibility is crucial for docking success, as has been emphasized in previous studies[19-23]. After thorough screening of all the 12 molecules, 5 molecules were docked by using AUTODOCK vina software. The protein used was Biotin Ligase (PDB ID: 1N26) . The docking site or active site was selected and a grid box was laid out to specify the region. The Amino acids present in the active site of the protein were found to be ARG122, GLU 286, ARG125, PHE123, THR285, ASP320, ASN 287, ILE 317, ARG 227 and HIS 126. Hydrogen bonds, as well as non-bonded interactions, were found between the various compounds and the active site of Biotin ligase protein. Diclofenac was taken as the standard drug and docking was performed at the same active site. 9 conformations were identified and docked on the protein. The highest docking score was -6.5Kcal/mol and various conformations of the streptomycin molecule has shown interactions with TYR 55, ALA 58, ARG 65, ARG 68, LEU 64, LEU 63, LEU 68, LEU 162 and TRP 57 All the bonds were identified to be hydrogen bonds. ARG 65, ARG 68, LEU 63 and LEU 162 made hydrophobic bonds while ALA 58, TRP 57, TYR 55, LEU 68 and LEU 64 made hydrophilic interactions with the docked compounds. Docking interactions for the compound IV a were hydrophobic interactions with ARG 65 and hydrophilic interactions with TYR 55, LEU 162 (Fig 6). The best docking score for this compound was found to be -6.3. Compound IV b showed hydrophobic interaction with ARG 65 and LEU 63, while the interactions with LEU 64 and TRP 57 were hydrophilic interaction (Fig 7). -6.4 is the highest docking score for this molecule. Docking interactions for compound IV c showed hydrophobic and hydrophilic interactions with ALA 58 , ARG 68, LEU 64, LEU 63 and TRP 57(2 interactions) (Fig 8). The best docking score was -6.5 amongst the conformations of IV c. Compound IV d interacted with LEU 123, SER 112, SER 72, LEU 69 and VAL 91 by hydrophilic interactions and hydrophobic interactions (Fig 9) as hydrogen bonds. The best docking score for the molecule was -6.3. The compound IV e has shown interactions as hydrophobic interactions with ARG 65 and hydrophilic interactions with LEU 64, LEU 68, TRP 58. (Fig 10). The best docking score for the compound was -5.9. These are the five synthesized compounds and showed good hydrophobic and hydrophilic interactions. And from the fig 11 to fig 17 those are the docking pictures which were not synthesized so I have not mentioned the results.

Table 4. Molecular docking scores of the compounds IV a – IV e

S.no	Sample	K cal/mol	Rmsdl.b	Rmsdu.b
IV a	4-amino-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-6.3	15.616	17.171
IV b	2-chloro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-6.4	17.482	19.914
IV c	4-chloro-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-6.5	28.962	30.383
IV d	5-amino-2-hydroxy-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-6.3	24.327	27.634
IV e	2-amino-6-chloro-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-5.9	17.878	19.176
IV f	4-nitro-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-5.8	15.025	24.220
IV g	2-nitro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-5.8	15.027	24.220
IV h	4-methyl-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-6.0	4.443	6.750
IV i	2-methyl-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-6.1	4.842	7..176
IV j	4-methoxy-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-5.9	17.667	19.981
IV k	2-methoxy-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid	-6.0	15.678	16.220
Standard	Diclofenac	-6.9	32.981	35.129

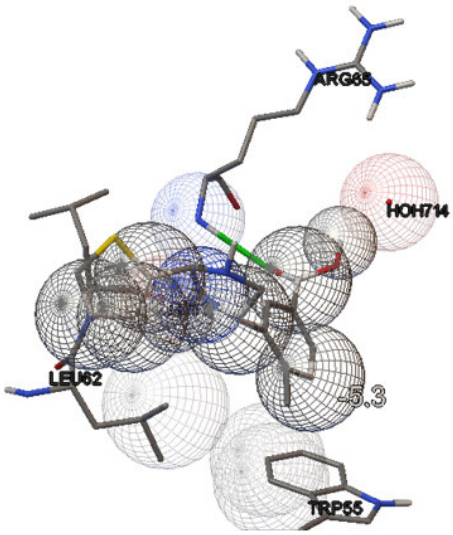


Fig 6. Hydrophobic interactions of compound IV a with the active site of 1N26

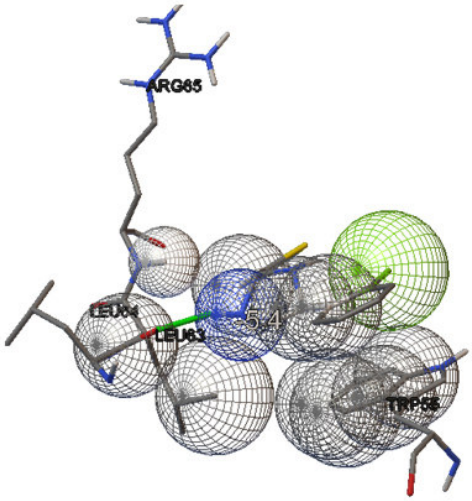


Fig 7. Hydrophobic interactions of compound IV b with the active site of 1N26

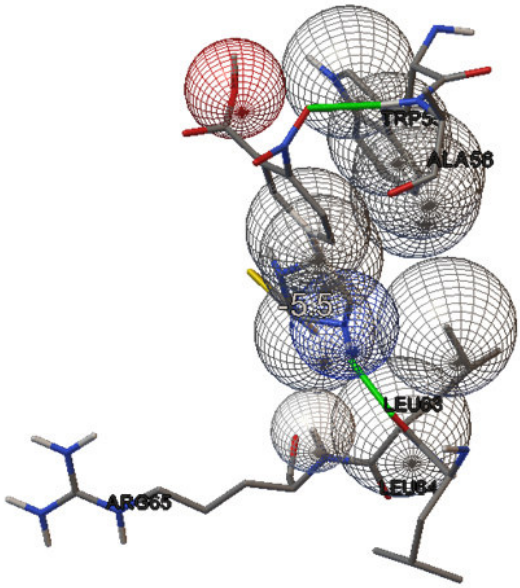


Fig 8. Hydrophobic interactions of compound IV c with the active site of 1N26

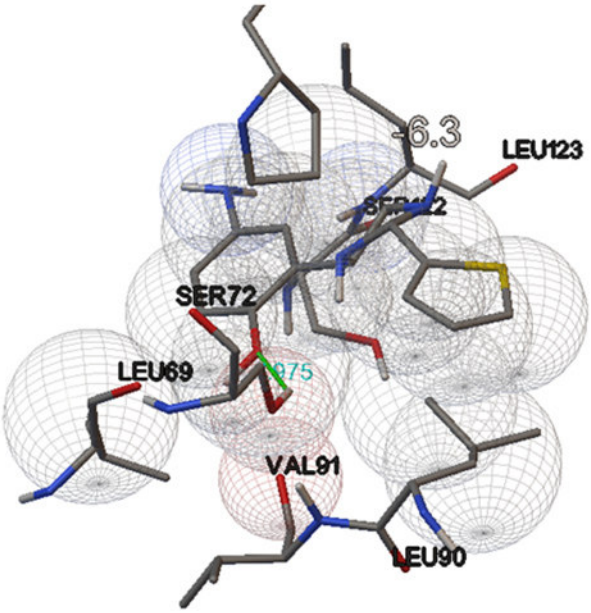


Fig 9. Hydrophobic interactions of compound IV d with the active site of 1N26

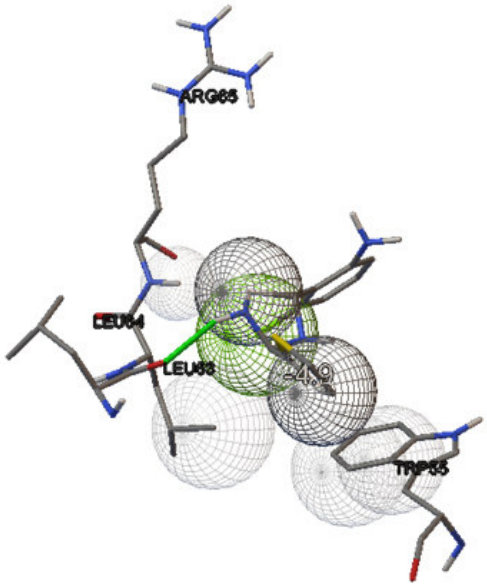


Fig 10. Hydrophobic interactions of compound IV e with the active site of 1N26

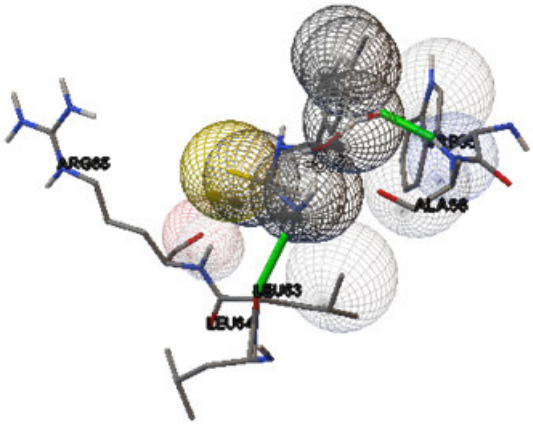


Fig 11. Hydrophobic interactions of compound IV f with the active site of 1N26

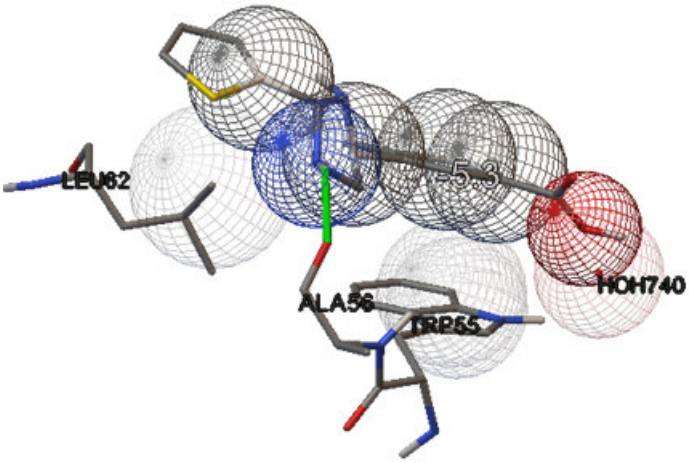


Fig 12. Hydrophobic interactions of compound IV g with the active site of 1N26

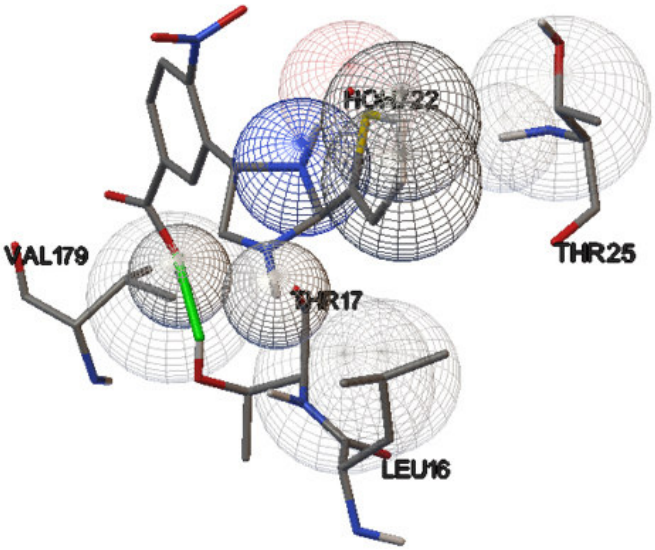


Fig 13. Hydrophobic interactions of compound IV h with the active site of 1N26

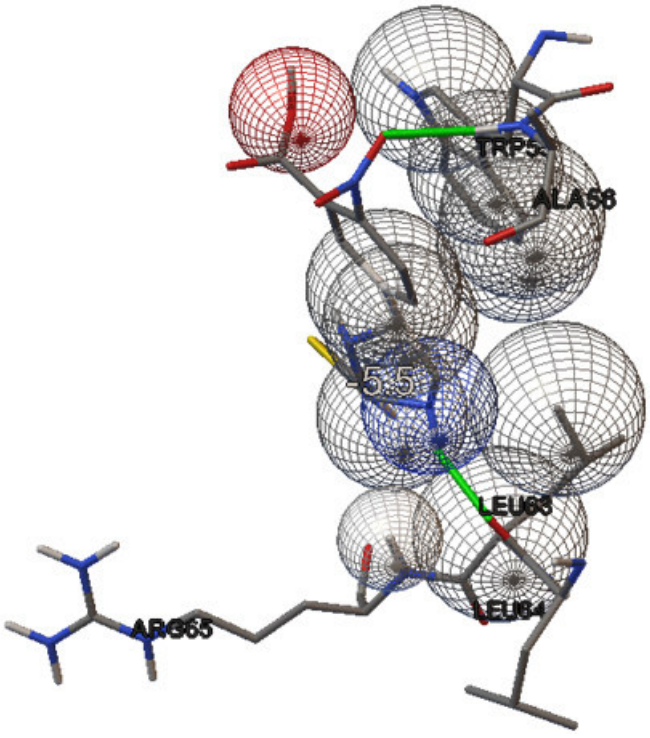


Fig 14. Hydrophobic interactions of compound IV i with the active site of 1N26

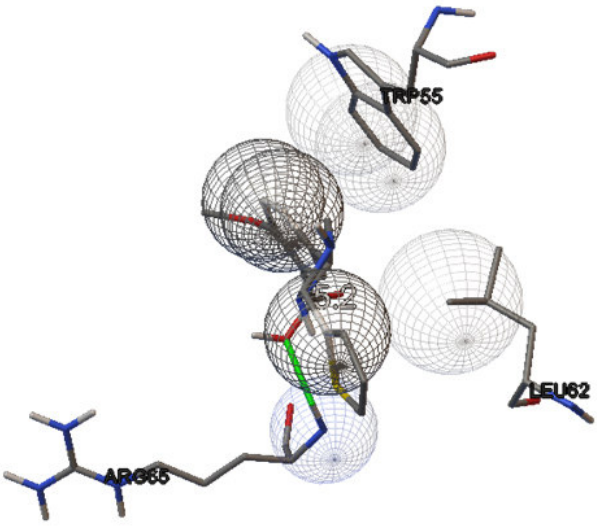


Fig 15. Hydrophobic interactions of compound IV j with the active site of 1N26

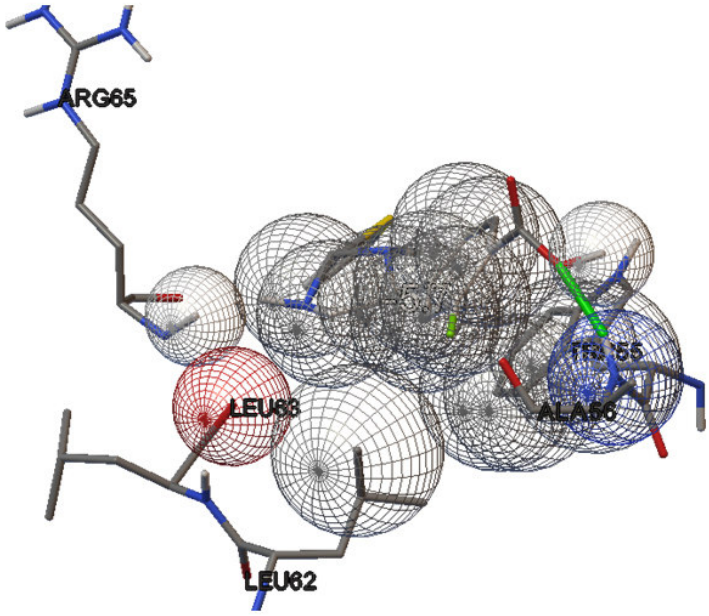


Fig 16. Hydrophobic interactions of compound IV k with the active site of 1N26

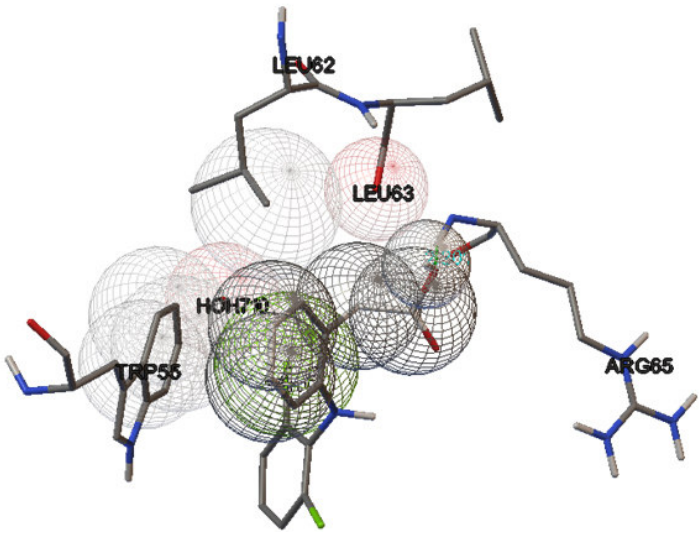
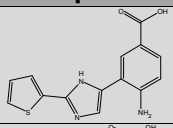
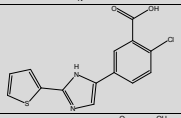
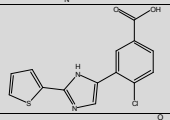
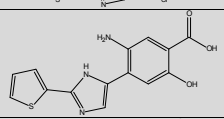
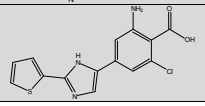


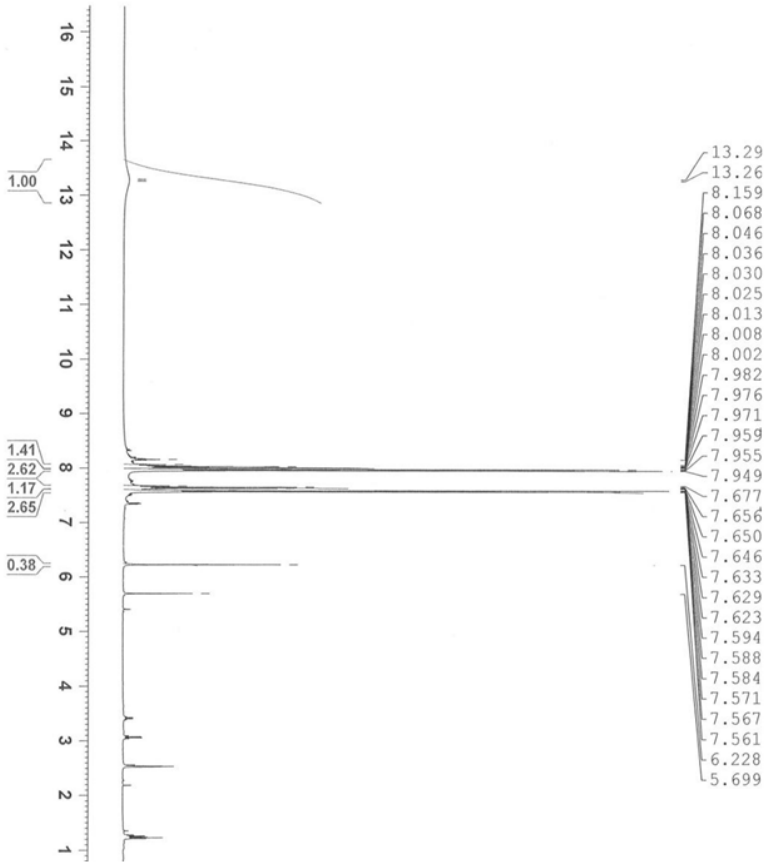
Fig 17. Hydrophilic and hydrophobic interactions of Diclofenac (std) with the active site of 1N26

3.3 Chemistry

After performing an evaluation of the molecular properties and molecular docking studies, Table 5 five derivatives of 2,5 substituted imidazoles have been synthesized. The completion of the reaction was confirmed by TLC. All the compounds were purified by recrystallization. The compounds were identified by using physical data and were characterized by using .NMR and MASS spectra.(Fig 18 and Fig 19)

Table 5. Physical data of synthesized derivatives

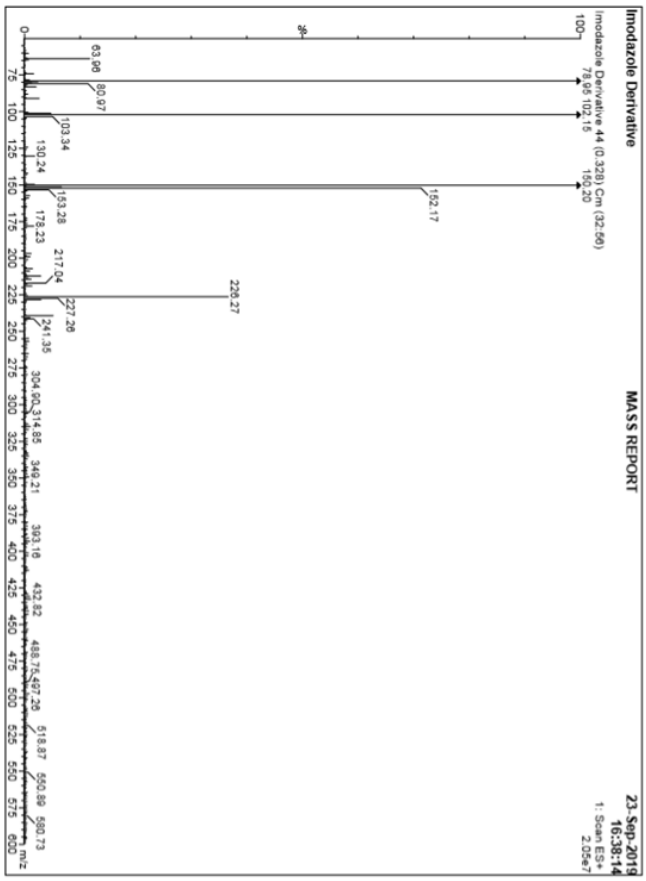
S.No	Name of the Derivative	Synthesized compound	Molecular formula	Molecular weight	Colour	Melting point	*Rf* values
IV a	4-amino-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid		$C_{13}H_{11}NO_3S$	261.29	Cream	160 °C	0.92
IV b	2-chloro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid		$C_{14}H_{11}N_3O_2S$	285.32	White	140 °C	0.95
IV c	4-chloro-5-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid		$C_{14}H_9ClN_2O_2S$	304.75	White	140 °C	0.98
IV d	5-amino-2-hydroxy-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid		$C_{13}H_{11}N_3OS$	257.31	Brown	190 °C	0.87
IV e	2-amino-6-chloro-4-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid		$C_{13}H_{10}ClN_3S$	275.75	Dark Brown	290 °C	0.84



^{1}H NMR: δ 6.228 (1H, s), 7.5840 (1H, dd), 7.594 (1H, dd), 7.588 (1H, dd), 7.65-7.8 (2H, 7.677, dd., 7.659 dd), 8.068 (1H, dd).

Fig 18. Proton NMR of 4-amino-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid

ES+ scan

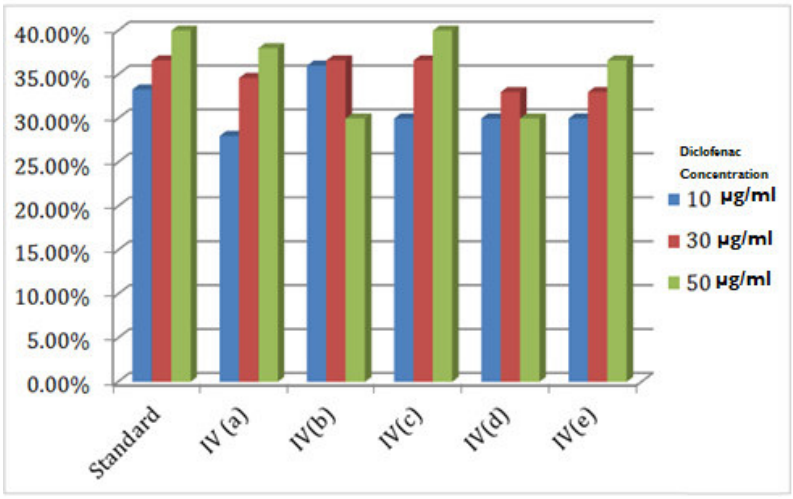


[M – CO_2] peak was absorbed at 241.35
[M- CO_2 and NH_2] peak was absorbed at 226.27

Fig 19. Mass spectrum of 4-amino-3-[2-(thiophen-2-yl)-1*H*-imidazol-5-yl]benzoic acid

Table 6. Absorbance and Percentage inhibition of synthesized compounds

Concentration	Absorbance 10 µg/ml	Percentage Inhibition /10 µg/ml /ml	30 µ Absorbance g/30 µg/ml	Percentage Inhibition In30 µg/ml	50 µg/ Absorbance m50 µg/ml	Percentage Inhibition n50 µg/ml
Control	0.030	-	0.030	-	0.030	-
Standard	0.020	33.3%	0.019	36.6%	0.018	40%
IV(a)	0.023	28%	0.022	34.6%	0.024	38%
IV(b)	0.022	36%	0.022	36.6%	0.021	30%
IV(c)	0.019	30%	0.019	36.6%	0.018	40%
IV(d)	0.019	30.%	0.020	33%	0.021	30.%
IV(e)	0.021	30%	0.020	33.%	0.019	36.6%



Graph 1. Percentage inhibition of compounds IV a – IV e

After a thorough screening of all the 5 molecules were docked by using AUTODOCK vina software. The protein used was Biotin Ligase (PDB ID: 1N26). The docking site or active site was selected and a grid box was laid out to specify the region. The Amino acids present in the active site of the protein were found to be Arginine122, Glutamic acid 286, Arginine125, Phenylalanine123, Threonine 285, Aspartic acid 320, Aspargine 287, Isoleucine 317, Arginine 227 and Histamine 126. Hydrogen bonds, as well as non-bonded interactions, were found between the various compounds and the active site of Biotin ligase protein. The compound IV a – IV e were tested for anti-inflammatory activity by using Protein denaturation assay (Bovine serum albumin assay) the compound IV c has the highest docking score and the same results are reflected in the *invitro* biological activity. Graph 1 The percentage inhibition was found to be 40% which was comparable to the standard Diclofenac concentrations (10, 30, 50 µg /ml). The activity was found to be comparable to that of the standard Diclofenac and the highest amount of denaturation was seen at 50 µg /ml of concentration. The compounds IVa, IVb,IVd, IVe, have moderate anti-inflammatory activity.

4. CONCLUSION

Imidazoles and Thiophenes are therapeutically important class of heterocyclic building blocks. The method used in the present study is one of the best method for synthesizing 2,5 substituted compounds. In the present work we designed a series of synthesized derivatives of imidazoles and were subjected to docking studies by using PDB ID:1N26. The compounds with good docking scores were synthesized. The docking results revealed that most of the novel compounds synthesized showed good interaction with protein as compared with Diclofenac which were taken as reference so we proceeded for biological activity. The anti-inflammatory activity of the synthesized imidazole derivatives was studied by using *in vitro* method.

5. ACKNOWLEDGEMENT

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6. AUTHORS CONTRIBUTION STATEMENT

Monalika A gathered the data with regard to this work. Dr.M.Vijayabhargavi analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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