Research Article

Design, Synthesis and Pharmacological Evaluation of Benzimidazole-Methylamine Bridged Phenyl-1,3,4-Thiadiazolamine Derivatives

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ABSTRACT

Aim/Background: Design, Synthesis and Pharmacological evaluation of Benzimidazole-Methylamine Bridged Phenyl- 1,3,4-Thiadiazolamine Derivatives based on the existing compounds having benzimidazole as a basic nucleus, because of advancement in the field of medicinal chemistry, several modifications are possible by molecular modelling. Benzimidazole derivatives are design with molecular docking studies gives an insight view of potent molecule interacting in a putative binding site. The ligands are studied for binding affinity of protein 3NT1 which is COX-2 inhibitors (Oxidoreductase/ Oxidoreductase inhibitor). The ligand 2C possess high affinity towards the active binding site and it shows good docking score against the protein 3NT1.

Materials and methods: In this scheme, a series of Phenyl-1,3,4-Thiadiazolamine containing Benzimidazole moieties were designed and well scored compounds get synthesized. Benzimidazole-Methylamine Bridged Phenyl- 1,3,4-Thiadiazolamine derivatives are synthesized in three steps. In step 1, Synthesis of 2-(Chloromethyl)-1H-Benzimidazole from O-phenylenediamine was carried out. Step 2 involves the preparation of substituted 5-phenyl-1,3,4-thiadiazol-2-amine from substituted aromatic aldehyde and thiosemicarbazide. Step 3 involves the preparation of Substituted N-[(1H-benzimidazol-2-yl) methyl]-5-phenyl-1,3,4-thiadiazol-2 amine from 2-chloromethyl benzimidazole and substituted 5-phenyl-1,3,4-thiadiazol-2-amine. The synthesized compounds were characterized and confirm the formation of the product by TLC, UV, FT-IR, 1H-NMR and Mass spectrometry.

Results and discussion: Synthesized compound 2C, which is having high energy value and docking score is selected for anti-inflammatory activity at three different doses. The test drug 2C at the dose of 100, 200, 400 mg/kg b.w p. o showed significant reduction in paw edema ($P<0.001$) after carrageenan administration. It was observed that 2C at the dose of 400 mg/kg b.w p. o produced 39.5% inhibition of paw edema at the 3rd hr of the drug administration, whereas, 45.5% was produced by Indomethacin.

Key words: Benzimidazole, Phenyl-1,3,4-Thiadiazolamine derivatives, molecular docking and anti-inflammatory activity.

1. INTRODUCTION

Inflammation is the local response of living mammalian tissues to injury due to any agent. It is body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent dead cells and tissues. It occurs when tissues are injured by bacteria, trauma, toxins, heat etc. The damaged cells release chemicals including histamine, bradykinin and prostaglandins, these chemicals cause blood vessels to leak fluid into the tissues causing swelling[1].

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All the chemicals and reagents used for present work are analytical and laboratory grade were not purified further, purchased from Loba chemicals, S.D. Fine Chem. Ltd., FINAR, Spectro chem, Yarrow chem products. Mumbai. Characterization of intermediates and final compounds was carried out utilizing several physicochemical and spectral techniques. Melting points of the compounds were determined by using Theil's tube and digital melting point instruments. UV spectra were captured by using UV-1700 pharma spec, Shimadzu, UV-Visible spectrophotometer. FT-IR spectra were obtained on Thermo Nicolate IR-700 spectrophotometer by using KBR pellets. NMR spectra of the synthesized compounds were recorded on ECZ 400 HIGH RESOLUTION, MULTINUCLEAR FT-NMR SPECTROMETER. Instrument: 400MHzNMR, Make: JEOL, Model: JNM-ECZ400/L1. Mass spectra of synthesized compounds were recorded by using Waters; Synapt G2 High detection Mass spectrometry. The physicochemical, analytical and spectral characterization data for the final compounds (1C-10C) were provided in Table.3 and Table.5 respectively.

2.2. Methodology:

In Silico Studies are carried out by using various software's like MGL Tools, AutoDockTools-1.5.6, Discovery Studio 2020 Client, ACD/3D Viewer ChemSketch with the protein having PDB ID: 3NT1 (Oxidoreductase/ Oxidoreductase inhibitor)[2].

2.2.1. Steps involved in docking studies:

2.2.1.1. Selection of target and protein cleaning:

The present study was focused on anti-inflammatory activity. From the literature review and the current research, we have selected Oxidoreductase / oxidoreductase inhibitor (3NT1) as the target for the present study. The pdb structure of protein was Oxidoreductase / oxidoreductase inhibitor (3NT1) downloaded from the RCSB protein data bank. Oxidoreductase / oxidoreductase inhibitor (3NT1) protein was downloaded from RCSB Protein Data Bank (PDB) in PDB format. Open discovery studio for cleaning protein selects the water molecule and small ligands attached then delete both, save as "control", so that this ligand molecule can be used as control for comparing with our ligand, then delete these ligands in working folder, save the cleaned protein[3].

2.2.1.2. Preparation of protein:

Protein preparation is done by using Auto dock select the address of working folder and copy, go to file -set preferences and paste, Click read molecule from file select cleaned protein and open then click on edit and add hydrogens, select polar only. hydrogens are added as white colour dots. Click on Edit and add charges select kollman charge, Save the protein in pdbqt format, Click on Grid then select macromolecule and choose.

Preparation of Ligand:

The ligands which are desired are drawn in ChemSketch software. Go to Tools, click Generate, Click SMILES notation (Simplified Molecular Input Line Entry System, which is a file format). save the SMILES in a word document, save the ligand in pdb format by using discovery studio, Open Auto dock, click on file then read molecule and select the ligand. Click on ligand, then click on input choose, select the ligand and click on select molecule for auto dock. Click on edit and add hydrogens, select polar only. Then merge nonpolar hydrogens. Click on edit and add charges, select compute gasteiger. Open ligand, select torsion tree, detect root, set no of torsions. Number of rotatable bonds is chosen. Open ligand, press output and select save as PDBQT (ligand. Pdbqt).

2.2.1.3. Generation of grid parameter file:

Open Grid and choose Grid box, select control as the ligand bring the grid box to the center of control, set the proper Grid Dimensions, adjust the spacing, select the File then click Close Saving Current, Open Grid and press on output and choose save GPF.

2.2.1.4. Generation of docking parameter file:

Open Docking, choose Macromolecule and set rigid file name, choose the 3nt1.fix document and open, open Docking press ligand, click on choose, select ligand, open Docking, search parameters, select genetic algorithm, set minimum number of run as 100 and click on accept. Open Docking choose output Lamarckian GA save docking parameter file 'Dpf'.

2.2.1.5. Running Auto grid:

Click on run choose auto grid**, s**elect Autogrid from folder as program path name, select Gpf file as parameter file, name and launch, Grid files will be generated as Mapfiles.glg.

2.2.1.6. Running Auto dock:

Click on run, choose Autodock, select Autodock from folder as program path name, select Dpf file as parameter file name and launch.

2.2.1.7. Analyzing and interpreting result:

Open the final folder and find the dlg file, find the histogram and lowest binding energy for the conformation and find the corresponding run number. Go to autodock select analyze open the dlg file. Go to analyze select confirmations play. type the run number with least binding energy. Press the & symbol write complex save in pdbqt format. give name 3nt1.1a.run.pdbqt. Open discovery studio, scripts ligand interaction opens the saved file, structure label adds choose amino acid. Press show 2D diagram and find the interactions Van der Waals, Conventional Hydrogen bond, Pi-Alkyl bond, P1-P2 T shaped, PI Sulphur, P2- Donor, H bond, PI Sigma bond, Carbon hydrogen bond, Alkyl bond[4].

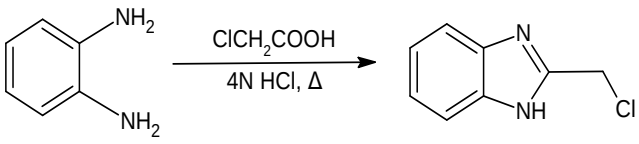
2.2.2. Determining the drug likeness property:

Open Swiss ADME, Copy the smiley notations produced in chemskech of the given ligands. Paste on Swiss ADME and the structure will be produced on left, click on 'run'. Determine the ADME properties as per Lipinski rule of five[5].

2.2.3. Scheme

2.2.3.1. Synthesis of 2-(Chloromethyl)-1H-Benzimidazole from O-phenylenediamine

A mixture of o-phenylenediamine (0.1 mol) and monochloroacetic acid (0.1 mol) was refluxed for 3 h in 4 N hydrochloric acid (50 mL) on a water bath. The reaction mixture was cooled and basified with ammonium hydroxide solution. The precipitate thus obtained was dried and recrystallized from methanol with activated charcoal treatment[6].

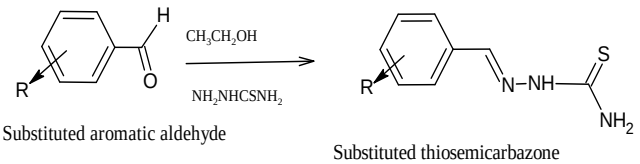


O-Phenylenediamine 2-(Chloromethyl)-1H-Benzimidazole

2.2.3.2. Preparation of substituted 5-phenyl-1,3,4-thiadiazol-2-amine from substituted aromatic aldehyde and thiosemicarbazide

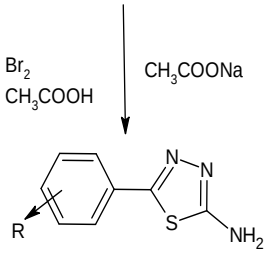
Substituted aromatic aldehyde (0.2 mole) in warm alcohol (300 ml) and thiosemicarbazide (0.2 mole) in warm water (300 ml) were mixed slowly with continuous stirring. The

product separated immediately on cooling which was filtered with suction, dried and recrystallized from ethanol to yield substituted thiosemicarbazone. Substituted thiosemicarbazone (0.01M) and Sodium acetate (0.02M) were dissolved in 30-40 ml of glacial acetic acid taken in a round-bottom flask equipped with a separating funnel for the addition of bromine. Bromine (0.7 ml in 5 ml glacial acetic acid) was added slowly to it, while stirring magnetically. After half an hour stirring, the solution was poured on crushed ice. The resulting solid was separated, dried and recrystallized from ethanol[7].



Substituted aromatic aldehyde

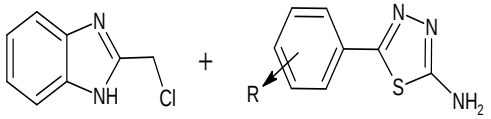
Substituted thiosemicarbazone



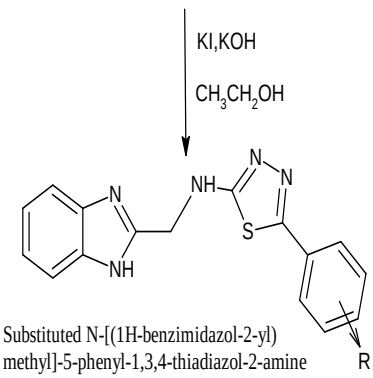
Substituted
5-phenyl-1,3,4-thiadiazol-2-amine

2.2.3.3. Preparation of Substituted N-[(1H-benzimidazol-2-yl) methyl]-5-phenyl-1,3,4-thiadiazol-2 amine

A mixture of 2-chloromethyl benzimidazole (0.01 mol), substituted 5-phenyl-1,3,4-thiadiazol-2-amine (0.01 mol) and KI (0.01 mol) in 50 mL of ethanol was heated under reflux for 6 hrs, KOH (0.01 mol in 5 mL of water) was added with continuous stirring for 2 hrs. Finally, the reaction mixture was left aside at room temperature and then poured into crushed ice. The solid product that precipitated was filtered off, recrystallized from ethanol[8].



2-(Chloromethyl)-1H-Benzimidazole Substituted 5-phenyl-1,3,4-thiadiazol-2-amine



Substituted N-[(1H-benzimidazol-2-yl) methyl]-5-phenyl-1,3,4-thiadiazol-2-amine

R= H, -2OH, 4-NO2, 3-NO2, 3-OH, 4-OCH3, 3,4,5-trimethoxy, 4-hydroxy and 3-methoxy, 4-N, N-dimethylamine, 4-Cl.

2.3. Pharmacological activity

The synthesized compound was screened for anti-inflammatory activity.

2.3.1. In-vivo anti-inflammatory method: carrageenan induced paw edema method[9]

2.3.1.1. Animals Approval

The study was conducted after obtaining permission from the Ethical Committee for the Purpose of Control and Supervision Experiments on Animals (CPCSEA) and Institutional animal ethics committee (IAEC), approval number **SACCP-IAEC/2021-02/48**.

2.3.1.2. Animals

Wistar albino rats of either male or female weighing about 140-200gms were used for the study. The animals were obtained from the animal house, Sri Adichunchanagiri College of Pharmacy B. G. Nagara, Karnataka, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 27±2ºC and relative humidity of 55±1%. A 12: 12 light: day cycle was followed. All animals were allowed free access to water and bed with standard commercial pelleted chow[10].

2.3.1.3. Method

Carrageenan-induced rat hind paw edema method.

2.3.1.4. Induction of Inflammation

A solution of 0.1 ml of 1% carrageenan in normal saline is injected subcutaneously in right hind paw of the rats and induces an acute swelling that becomes maximal 3–5 hours after the injection and subsides by 24 whereas a subcutaneous injection of complete Freund's adjuvant in saline induces a more prolonged swelling that becomes maximal at 24 hrs and persists for at least 7 days. The inflammation produced in these models can be used to assess the production of inflammatory mediators at sites of inflammation[11].

2.3.1.5. Procedure

Albino rats of either male or female were divided into five groups of six rats each.

Group I: Diseased Control (1% Carrageenan solution 0.1ml/kg b.w)

Group II: Standard (Carrageenan + Indomethacin - 10 mg/kg b.w)

Group III: Carrageenan + Benzimidazole (100 mg/kg b.w)

Group IV: Carrageenan + Benzimidazole (200 mg/kg b.w)

Group V: Carrageenan + Benzimidazole (400 mg/kg b.w)

The test is performed on 30 Wistar rats that are equally distributed into 5 groups (n=6). The diseased control group I is administered 1% carrageenan solution, the standard group II was administered 10 mg/kg indomethacin; and the 3 test groups were administered 100, 200, 400 mg/kg

Benzimidazole. All test and standard drugs were administered orally. The paw edema is estimated by using a plethysmometer, is the most efficient method for estimating the anti-inflammatory effect of a drug. The swelling was estimated as mL of edema at different time interval, that is, the paw volume before carrageenan administration (time=0 baseline) and paw volume after carrageenan administration from the 1st to 4th hour using Plethysmometer was noted. Changes in the paw volume estimated earlier, as well as the succeeding administration of phlogistic agent points toward the significance of edema. Lastly, percent reduction of paw swelling was estimated in terms of % Inhibition. % Inhibition of Paw Edema is calculated by using below formula. Data were analyzed by using software Graph pad-prism 8.0 version (Two-way ANOVA)[12].

$$\% \text{ Inhibition of Paw Edema} = \frac{Vc - Vt \times 100}{Vc}$$

Vc = Paw edema of control animals

Vt = Paw edema of drug treated animals

3. RESULTS AND DISCUSSION

3.1. Molecular Docking

The below mentioned Table.1 indicate the molecular drug binding affinity to protein of Oxidoreductase/ Oxidoreductase inhibitor (PDB ID: 3NT1). Molecular docking studies gives an insight view of potent molecule interacting in a putative binding site. Thus, with this knowledge that how our newly synthesized molecules behave virtually in a putative binding site, we have executive the newly synthesized molecules on to molecular docking platform for virtual screening.

Docking scores of the synthesized ligand 1C-10C against Oxidoreductase/ Oxidoreductase inhibitor (PDB ID: 3NT1) and Glide energies (kcal/mol) as obtained through ligand docking are represented in Table 1.

Table 1: Docking scores of synthesized compounds

Protein	**Oxidoreductase/ Oxidoreductase inhibitor (PDB ID: 3NT1)**				
Ligands	**DockingScore**	**GlideEvdw**	**Glide Energy (kcal/mol)**	**Glide Emodel**	**XP Hbond**
1C	-7.533	-33.013	-37.136	-50.868	-0.457
2C	**-8.617**	**-32.996**	**-40.449**	**-56.013**	**-1.143**
3C	-6.798	-36.594	-36.565	-51.544	-0.871
4C	-7.586	-40.669	-45.692	-66.038	-0.469
5C	-8.274	-30.028	-40.967	-55.72	-1.444
6C	-7.515	-36.547	-37.662	-43.162	-0.072
7C	-7.539	-43.356	-45.971	-56.291	-0.484
8C	-7.812	-39.241	-41.883	-52.723	-0.857
9C	-6.568	-35.431	-35.918	-47.447	-0.871
10C	-8.091	-29.841	-34.805	-48.716	-0.435
Indomethacin	-6.242	-21.609	-26.068	-33.395	-0.876

The docked results were visualized and analyzed using Discovery studio visualizer and results are represented in Figure 1.

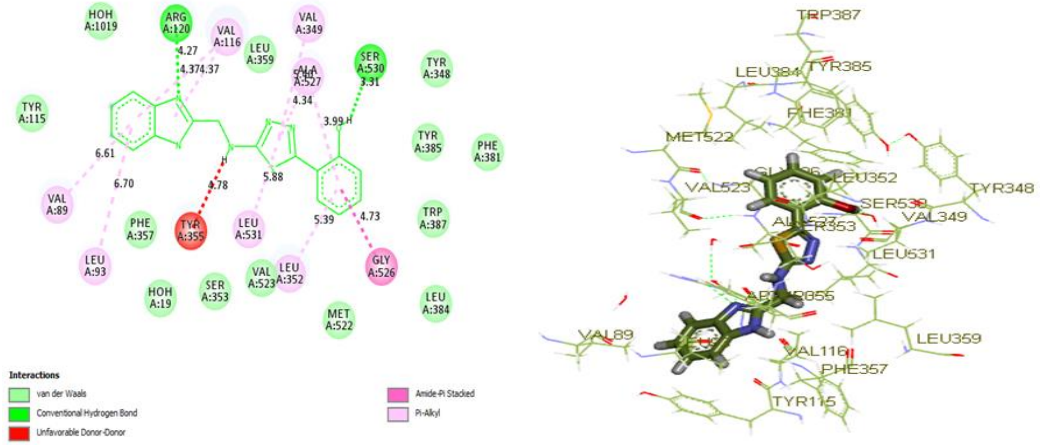


Figure 1: 2D and 3D Docking Interactions for Ligand 2C against 3NT1

According to the results generated by the molecular docking studies, express that the ligands possess high affinity towards the active binding site of oxidoreductase inhibitors of protein 3NT1 protein. 2D and 3D interactions of ligand 2C against

protein 3NT1 to various amino acids are mentioned in the Figure 1, because compound 2C shows good docking score with protein 3NT1, and their interactions for various bonds are **Van der Waals:** TYR A:115, HOH A:1019, LEU A:359, TYR A:348, TYR A:385, PHE A:381, TRP A:387, LEU A:384, MET A:522, VAL A:523, SER A:353, HOH A:19, PHE A:357. **Unfavorable Donor-Donor:** TYR A:355, **Conventional Hydrogen Bond:** ARG A:120, SER A:530, **Amide- Pi Stacked:** GLY A:526**, Alkyl/Pi-Alkyl:** LEU A:352, LEU A:531, LEU A:93, VAL A:89, VAL A:116, VAL A:349, ALA A:527.

Table 2: Drug likeliness properties of synthesized ligands generated from molecular docking are represented below.

Derivative	Lipinski rule of five (RO5)						Docking Score Kcal/mol (3NT1)	No of violations
	Mol.Wt (≤500 g/mol)	No. of H-ba (≤10)	No. of H-bd (≤5)	LogP value (≤5)	TPSA (Å²)	Molar Refractivity		
1C	307.37	03	02	2.34	94.73	88.79	-7.533	0
2C	325.39	03	04	2.37	110.34	98.56	-8.617	0
4C	354.39	04	03	2.21	135.93	105.36	-7.586	0
5C	325.39	03	04	2.37	110.34	98.56	-8.274	0
6C	339.41	03	03	2.77	99.34	103.03	-7.515	0
7C	399.47	05	03	2.76	117.80	116.02	-7.539	0
9C	352.46	02	03	2.79	93.35	110.75	-6.568	0
10C	343.83	02	03	3.31	90.11	101.55	-8.091	0

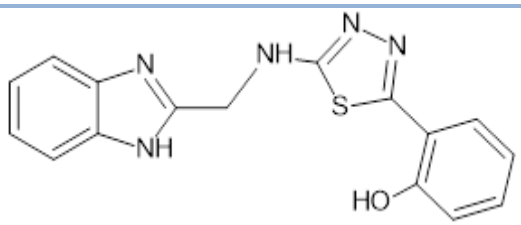
3.2. Physico-chemical characterization data of synthesized derivatives.

Synthesized derivatives physico-chemical properties are listed in Table 3.

Table 3. Physico-chemical characterization data for final compounds 1C-10C

Compound code	R	Molecular formula	Molecular weight	M.P (C^0)	% Yield	Physical state (colour)	Solubility
1C	H	$C_{16}H_{13}N_5S$	307.37	110	64.17	Brick red	DMSO, DMF
2C	2-OH	$C_{16}H_{13}N_5OS$	323.37	114	76.78	Brownish black	DMSO, DMF
3C	4-NO_2	$C_{16}H_{12}N_6O_2S$	352.37	124	88.35	Brown	DMSO, DMF
4C	3-NO_2	$C_{16}H_{12}N_6O_2S$	352.37	138	75.56	Black	DMSO, DMF
5C	3-OH	$C_{16}H_{13}N_5OS$	323.37	178	92.56	Dark brick red	DMSO, DMF
6C	4-OCH_3	$C_{17}H_{15}N_5OS$	337.39	110	78.27	Brown	DMSO, DMF
7C	3,4,5- OCH_3	$C_{19}H_{19}N_5O_3S$	397.45	120	67.75	Brown	DMSO, DMF
8C	4-OH,3-OCH_3	$C_{17}H_{15}N_5O_2S$	353.39	132	93.36	Dark brick red	DMSO, DMF
9C	4-$N(CH_3)_2$	$C_{18}H_{18}N_6S$	350.44	120	93.12	Dark brick red	DMSO, DMF
10C	4-Cl	$C_{16}H_{12}N_5ClS$	341.81	132	85.29	Yellowish brown	DMSO, DMF

Table 4. Physico-chemical characterization data of synthesized compound 2C.

COMPOUND CODE	2C
Structure	
Chemical name	2-(5-{[(1*H*-benzimidazol-2-yl) methyl] amino}-1,3,4-thiadiazol-2-yl) phenol
Solubility	DMSO & DMF
Molecular Formula	C16H13N5OS
Molecular Weight	323.37
Melting Point	114^0 C
Percentage yield	76.78%
TLC Solvent system	Methanol: Chloroform: Acetic acid glacial (v/v/v 7:2:1)
R_f value	

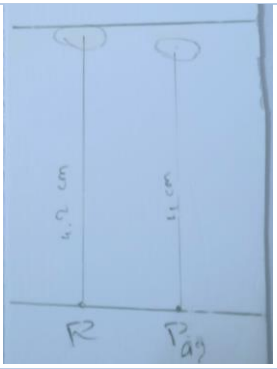
	$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$ R_f of Reactant = 4.2/4.8=0.87 R_f of Product = 4.0/4.8=0.83
Physical state	Brownish black in colour

Table 5: Spectral characterization data of the final compounds 1C-10C

Sr.No	Compound code	UV (λmax) in nm	FTIR (KBr, cm^{-1})	^1H NMR (DMSO-D6, δ in ppm)	Mass (m/e) $[M+H]^+$
1.	1C	312	3422.46 (N-H), 3152.76(C-H) 1591.44(C=C), 869.88 (C-N) 1368.98(C=N), 693.05 (C-S) 2375.76(C-H)	3.21(s,2H), 4.41(s,2H), 5.68(s,1H), 7.141-8.232 (m,9H)	308.15
2.	2C	331	3441 (O-H), 3319.79(N-H) 3174.33(C-H), 1537.26(C=C) 829.95 (C-N), 1611.41(C=N) 745.81(C-S), 2377.18(C-H)	4.63 (s, 1H), 3.19 (s, 2H) 4.42 (s, 1H), 6.14 (s, 1H) 6.81-8.39 (m, 9H)	315.20
3.	3C	354	1340.46 (NO_2), 2375.76 (C-H) 690.20 (C-S), 1587.17 (C=N) 848.48 (C-N), 1515.86 (N-H) 1433.16 (C=C), 2315.86 (N-H)	3.22 (s, 2H) 4.32 (s, 1H) 6.002 (s, 1H) 7.11-8.428 (m, 8H)	354.19
4.	4C	317	1525.85 (NO_2), 3423.89 (N-H) 3152.94 (C-H), 1597.15 (C=C) 841.35 (C-N), 1431.73 (C=N) 673.11 (C-S), 2375.76 (C-H)	3.408 (s, 2H) 4.44 (s, 1H) 6.55 (s, 1H) 7.13-8.67 (m, 8H)	351.16
5.	5C	321	3418.18 (O-H), 1600 (C=C) 1508.73 (N-H), 8332.8 (C=N) 742.96 (C-S), 2375.76 (C-H)	5.12 (s, 1H), 3.39 (s, 2H) 4.55 (s, 1H), 5.9 (s, 1H) 6.86-8.07 (m, 8H)	324.17
6.	6C	324	1252.05 (O-CH_3), 3428.16 (N-H) 2836.36 (C-H), 1604.28 (C=C) 1433.16 (C=N), 744.39 (C-S) 2374.33 (C-H), 1510.16 (N-H)	3.75-3.88 (m, 3H) 2.52 (s, 2H), 4.36 (s, 1H) 6.08 (s, 1H) 7.01-8.21 (m, 8H)	338.16
7.	7C	323	1125.13 (O-CH_3), 2937.61 (N-H) 2834.94 (C-H), 1418.89 (C=C) 1503.03 (C=N), 627.45 (C-S) 2374.33 (C-H), 1584.31 (N-H)	3.35-3.88 (m, 9H) 2.52 (s, 2H) 4.3 (s, 1H), 5.94 (s, 1H) 6.93-7.88 (m, 6H)	399.19
8.	8C	328	1274.87 (O-CH_3), 2375.76 (C-H) 742.96 (C-S), 1431.73 (C=N) 1511.59 (N-H), 1591.44 (C=C) 3058.82 (C-H)	3.195 (s, 2H) 5.07 (s, 1H), 3.91 (m, 3H) 4.22 (s, 1H) 6.53-8.13 (s, 7H)	355.24
9.	9C	355	1272.01 (N-C), 3426.74 (N-H) 1604.28 (C=C), 1434.58 (C=N) 1522.99 (N-H), 742.96 (C-S) 2375.76 (C-H)	2.77-2.98 (m, 6H) 2.52 (s, 2H), 4.28 (s, 1H) 5.9 (s, 1H) 6.77-8.01 (m, 8H)	352.13
10.	10C	343	742.96 (C-Cl), 3054.55 (C-H) 2844.92 (C-H), 2676.65 (N-H) 1592.87 (C=C), 1426.03 (C=N) 1487.34 (N-H), 683.07 (C-S)	2.52 (s, 2H) 4.22 (s, 1H) 5.57 (s, 1H) 7.20-7.98 (m, 8H)	336.70

Chemical characterization data of compound 2C are represented in Figure 2.

UV Spectra of Compound 2C has absorbance 0.371 at wavelength 331 nm.

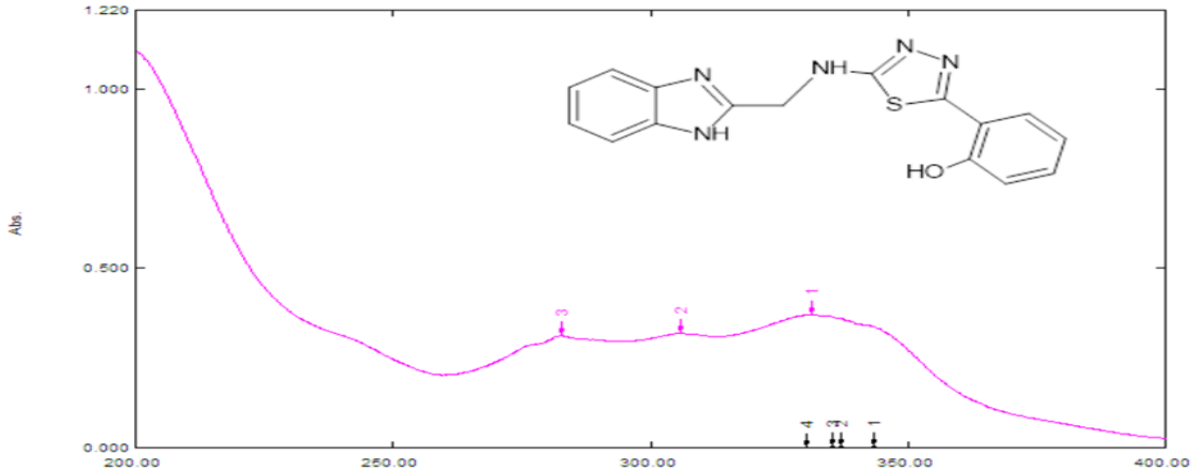


Figure 2: UV Spectra of Compound 2C

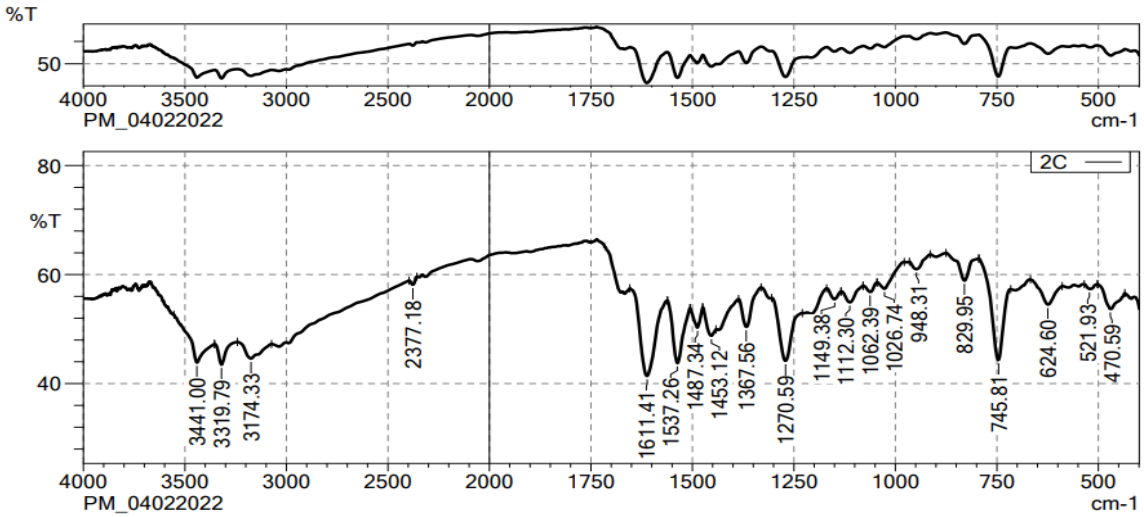


Figure 3: FTIR Spectra of 2C

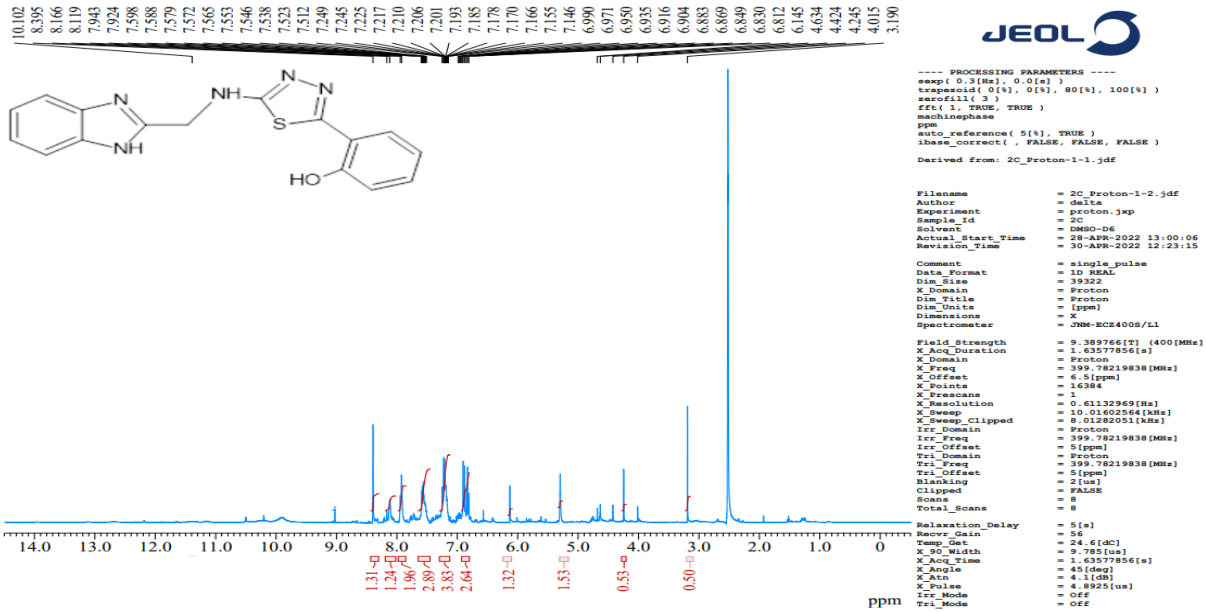


Figure 4: ^{1}H-NMR Spectra of 2C

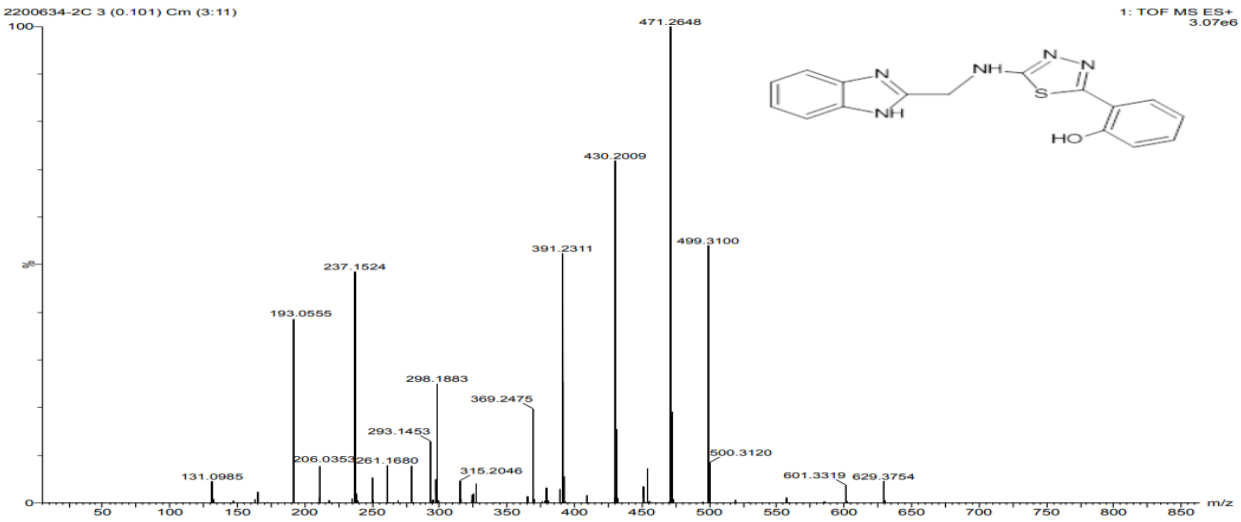
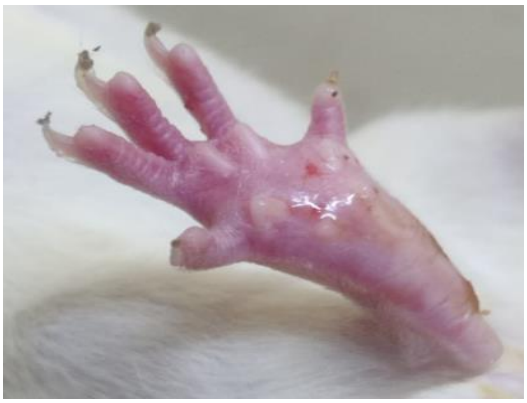
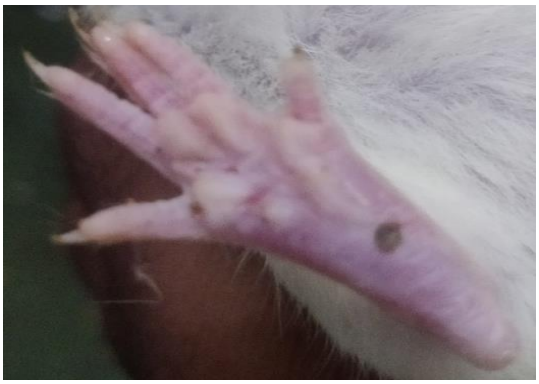


Figure 5: Mass Spectra of 2C

Group I Diseased control at 0 hr

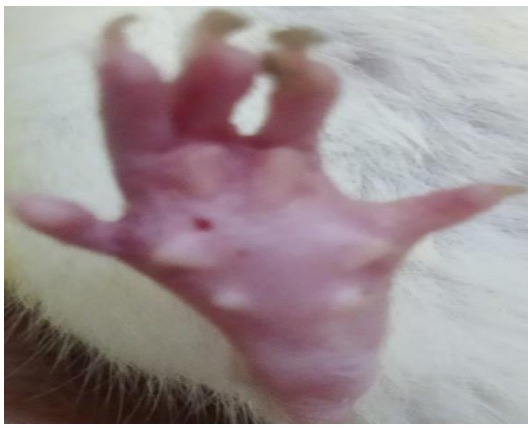


Group I Diseased control at 4th hr



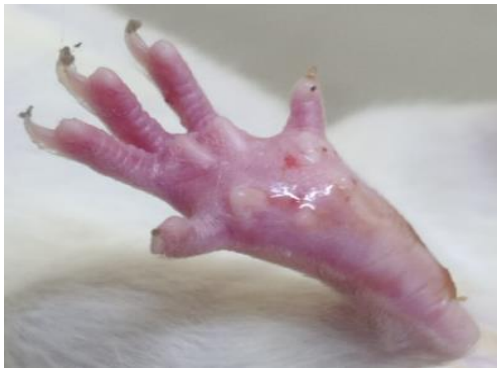
Group II Standard at 0 hr

Group II Standard at 4th hr

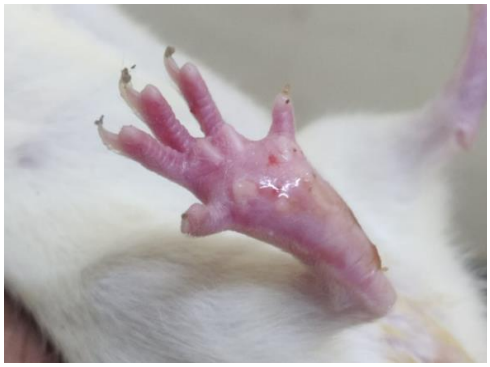


Group III 100 mg/kg b.w at 0 hr

Group III 100 mg/kg b.w at 4th hr

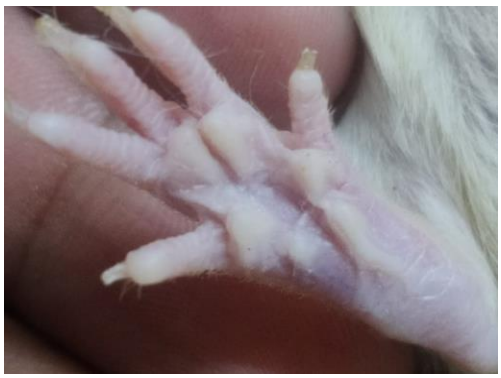


Group IV 200 mg/kg b.w at 0 hr



Group IV 200 mg/kg b.w at 4th hr

Group V 400 mg/kg b.w at 0 hr



Group V 400 mg/kg b.w at 4th hr

Figure 6: Animals showing decreases in paw volume.

Table 6: Anti-inflammatory activity of compound 2C on carrageenan induced paw edema in rats.

Group	Paw thickness in mm					%Inhibition at 3 hr
	0 hr	1 hr	2 hr	3 hr	4 hr	
Group-I Carrageenan (Control)	1.71± 0.168	3.53± 0.183**	4.88± 0.0542*	6.11± 0.1869**	4.55± 0.117**	------
Group-II Indomethacin (10mg/kg b.w)	1.23± 0.066	2.3± 0.085	2.966± 0.0714	3.333± 0.15202**	2.4± 0.05774*	45.5
Group-III (100mg/kgb.w)	1.58± 0.13	3.15± 0.0763	4.65± 0.1258	4.1333± 0.088**	3.86± 0.0714**	32.4
Group-IV (200mg/kgb.w)	1.56± 0.142	3.066± 0.0881	4.45± 0.1384	4.0166± 0.116**	3.966± 0.0918**	34.3
Group-V (400mg/kgb.w)	1.5± 0.115	3.066± 0.0881	4.3± 0.0447	3.7± 0.0856**	3.85± 0.0885**	39.5

Values were mean ± SEM, (n=6), *P<0.05, **P<0.01 Vs control. Data were analyzed by using Two-way ANOVA (Graph pad prism 8.0 version) software followed by Dunnett's multiple comparisons test.

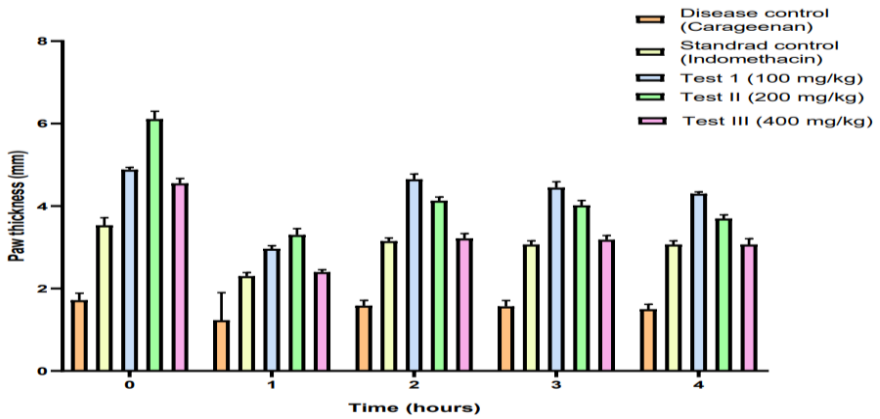


Figure 7: Anti-inflammatory activity of Benzimidazole derivative (2C), on carrageenan induced paw edema method in Wistar rats.

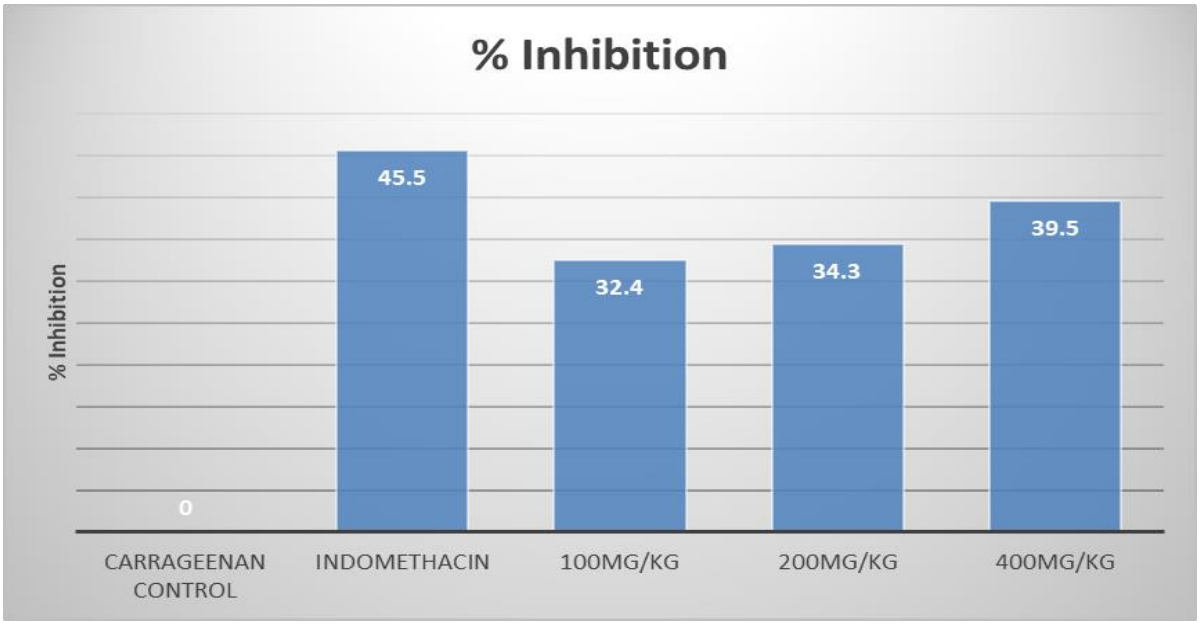


Figure 8: Anti-inflammatory activity of Benzimidazole derivative (2C), on carrageenan induced paw edema method in Wistar rats. Results are expressed as a percentage inhibition.

The test drug 2C at the dose of 100, 200, 400 mg/kg b.w p.o showed significant reduction in paw edema ($P<0.001$) after carrageenan administration. It was observed that 2C at the dose of 400 mg/kg b.w p. o produced 39.5% inhibition of paw edema (Table 6) at the 3rd hr of the drug administration, whereas, 45.5% was produced by Indomethacin.

3.3. Anti-Inflammatory Activity

Anti-Inflammatory agent having a wide range of importance in present scenario because plenty of the NSAID's present in the market are resistant to the available synthetic COX-2 inhibitors necessitating the discovery and development of potent and novel anti-inflammatory agents. In this regard, in the present study 10 benzimidazole derivatives were docked, synthesized. Among them one compound (2C) screened for anti-inflammatory activity. Indomethacin was used as standard drug. Results of the anti-inflammatory activity is given in the below Table 6 and Figure 6. The % inhibition is shown in graph and it was compared with the standard. Figure 6 shows Decrease in animal's paw volume of Group I to V at 0 hr and 4th hr.

4. SUMMARY

The present work is to design and synthesize certain derivatives of Benzimidazole fused to Phenyl-1,3,4-Thiadiazolamine with the help of methylamine bridge by using different substituted aromatic aldehyde and were studied for their Anti-inflammatory activity. Docking study of designed molecules was carried out by using Autodock Tools-1.5.6 software. Molecular docking studies gives potent molecule interacting in a putative binding site. The ligands are docked against protein Oxidoreductase/ oxidoreductase inhibitors (PDB ID 3NT1). 2C has shown good docking score -8.617 against protein 3NT1. The ADME/Tox result indicates that all these molecules possess pharmaceutical properties in the range of 95% of drugs without the Lipinski's rule violation. The synthesized compounds were characterized by spectral studies, which include UV, IR, ^{1}H NMR and Mass spectroscopy. UV data showing absorbance 0.371 at wavelength 331nm. The characteristic peak at 3441.00 cm^{-1} O-H of phenolic group moiety, 3319.79 cm^{-1} N-H(aliphatic) str of methylamine chain, 3174.33 cm^{-1} C-H (aromatic) of aromatic ring, 1537.26 cm^{-1} C=C (aromatic) of aromatic ring, 2377.18 cm^{-1} C-H (aliphatic) of a cyclic carbon 1611.41cm^{-1} C=N of cyclic ring, 1125.13 cm^{-1} O-CH_3 of aromatic ring, 742.96 cm^{-1} of C-Cl of aromatic ring, 1272.01 cm^{-1} N-C of tertiary amine, in IR confirm the formation of the targeted compounds. Further, the reaction was ascertained by detailed ^{1}H NMR study of products. A peak at δ 6.812-8.395 ppm of 9 aromatic hydrogens, peak at δ 4.634 ppm of 1 H of OH, peak at δ 3.190 ppm of 2 H of CH2, peak at δ 4.424 ppm of 1 H of aliphatic NH, peak at δ 6.145 ppm of 1 H of Imidazole NH. The molecular ion peak in the mass spectral studies confirms the synthesized compounds. The synthesized compound 2C was subjected for Anti-inflammatory activity by Carrageenan-induced rat hind paw edema method. Anti-inflammatory studies done with the reference of standard drug such as Indomethacin, Activity was done using rats at different concentrations 100, 200 and 400 mg/kg body weight by using plethysmometer. Among the synthesized derivatives, compound 2C is chosen for anti-inflammatory activity based on docking studies, it showed significant anti-inflammatory activity at 100, 200 and 400 mg/kg. By comparing all three doses 400 mg/kg showed maximum anti-inflammatory activity at reaction time 3rd hour (39.5% of Inhibition) is slightly lower than the standard drug Indomethacin (45.5% of Inhibition) in this anti-inflammatory testing model. Molecular docking helps to predict the potency of ligand before the synthesis of molecules. These results were encouraging and extend our research to design and synthesize a greater number of derivatives which having

potent by molecular docking using auto dock software and subjected to other biological and pharmacological studies with comparison with molecular docking score.

5. CONCLUSION

With the above results the designed and synthesized novel Benzimidazole -Methylamine Bridged Phenyl- 1,3,4-Thiadiazolamine Derivatives 1C-10C, among them 2C was subjected for evaluation of anti-inflammatory activity and it shows maximum activity by comparing with standard Indomethacin drug, with the above results it can be concluded that Benzimidazole -Methylamine Bridged Phenyl- 1,3,4-Thiadiazolamine derivative containing hydroxy substituents enhanced the activity.

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