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Original Article

Synthesis, *in silico* and Pharmacological Activity of 1, 3-Benzothiazol Derivatives

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ABSTRACT

Background: Benzothiazole and its derivatives serve as a unique and versatile moiety for experimental drug design, especially in pharmaceutical chemistry, because of their potent pharmacological activities. Hence, the present study is planned to develop benzothiazole-containing benzohydrazide derivatives to determine what modifications result in a better corrective benzothiazole shift moiety that permits the achievement of a novel pharmacological profile, action and toxicity reduction. **Materials and Methods:** Five novel derivatives of benzothiazole containing benzohydrazide were synthesized and evaluated for their anti-inflammatory and analgesic activities through *in silico* and *in vivo* studies. **Results:** The molecular docking analysis displayed a strong binding affinity of the newly synthesized compounds with the receptors (PDB ID: 1EQG and 1CX2), with compound 3C exhibiting the highest binding energy of -10.41 and -9.20 kcal/mol. Compounds 3C and 3E prevented rat paw oedema caused by carrageenan at 70.03% and 62. 06%, at 1 hr, 74.38% and 71.04% at 2 hr and 78.11% and 76.14% at 3 hr, correspondingly. Throughout the study evaluating analgesic activity, components 3C, 3E and also 3B had ED_{50} (mM/kg) of 85±4.2 (Mean±SEM; *n*=4), 126±0.5, 95±1.6 after 0.5 hr; 71±4.8, 83±1.0 and 99±1.4, after 1 hr and 68±6.4, 75±5.7 and 87±2.1 mM/kg subsequent 2 hr, accordingly, it is comparably equated with 155±4.2, 70±1.4 and 69±0.9 mM/kg for celecoxib. **Discussion:** The Structure-Activity Relationship (SAR) analysis highlighted whether the replacement in the ortho/para position of benzohydrazide, despite furtherance of being part of halogen groups (I_2) and electron-rich -NH or -OH groups, played a crucial role in enhancing the anti-inflammatory and analgesic properties of the synthesized compounds. **Conclusion:** These findings underscore the potential of these derivatives as promising candidates for further development in the field of drug discovery.

Keywords: Analgesic, Anti-inflammatory, Benzothiazole, Molecular Docking, Synthesis.

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INTRODUCTION

Benzothiazole is a bicyclic ring system that holds a special status due to its wide range of applications across the pharmaceutical field. In order to consider benzothiazole derivatives potential as muscle focused on 2-aminobenzothiazoles during the 1950s. Over time, benzothiazole analogous have been discovered to exhibit a diverse range of pharmacological effects, expanding their potential applications in the field of medicine and pharmaceuticals. A significant class of compounds with a variety of biological properties, such as anticancer, antidiabetic, anti-inflammatory, anticonvulsant, antimalarial and more, are benzothiazole and its analogues.[1] While benzothiazole is not an extensively utilized compound, its derivatives are frequently encountered in many commercial products. Substitutions at the methyne centre of the thiazole ring result in most of the identified benzothiazole derivatives today. For instance, Riluzole (6-trifluoromethoxy-2-benzothiazolamine, PK-26124, RP-25279, Rilutek), which is used for the management of amyotrophic lateral sclerosis[2] and pramipexole, a dopamine agonist commonly employed to handle Parkinson's disease,[3] sexual dysfunction,[4] bipolar disorder[5] and clinical depression.[6] In contrast, ethoxzolamide, also known as benzothiazol-2-ylthiomethyl Thiocynate (TCMTB)] and dimazole are the acknowledged antimicrobial medications that contain a benzothiazole moiety.[7,8] These two prominent examples of benzothiazoles have generated interest in finding out more concerning the pharmacological applications of benzothiazole derivatives. Derivatives of benzothiadiazole showed antimicrobial,[9] anti-inflammatory,[10]

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antitumor,[11] anti-tubercular,[12] anti-malarial,[13] anticonvulsant,[14] anti-helminthic,[15] antioxidant[16] and analgesic activities.[17] In this research article, we have described the pharmacological activities which includes benzothiazole molecules and the common techniques of synthesising the benzothiazole scaffold are being comprehensive. The scientific justification for the activities resolves structural changes associated to the anti-inflammatory and analgesic actions. Investigation on benzothiazole derivatives demonstrates that the variety of biological activities may constitute explained by substitution at the C-2 and C-6 locations. Hence, the present study is planned to develop benzothiazole-containing benzohydrazide derivatives to determine what modifications result in a better corrective benzothiazole shift moiety that permits the achievement of a novel pharmacological profile, action and toxicity reduction.

MATERIALS AND METHODS

Chemistry

All the synthetic-grade chemicals and reagents were purchased from SD Fine Chemical Lid., Mumbai, India for synthesis and pharmacological evaluation. Using open capillary tubes, the melting points of the components were determined and the values obtained were uncorrected. The purity of all the synthesized compounds was achieved by using the Thin Layer Chromatography technique (TLC), with silica gel G plates of 0.2mm thickness being used. The visualization of the spots was conducted in an iodine chamber. The solvents employed in the experiment were purified using standard methods. The k_{max} values of the compounds were measured using an FT-IR8400S Fourier Transform Infrared spectrophotometer (Shimadzu) through the KBr disk method. The 1H NMR spectra were recorded on a Bruker 400 MHz instrument in CDCl3, with TMS as the internal standard. Lastly, the mass spectra were recorded using a Shimadzu 2010A LC-MS spectrophotometer.

Process for making of 2-Mercapto benzothiazole (1)

2-Mercapto benzothiazole was prepared from the reaction of commercially available aniline with potassium ethyl xanthate by using a literature procedure.[18]

Synthesis of 2-hydrazinylbenzo[d]thiazole (2)

Dissolved 3.0 g (17.9 mmol) of 2-mercaptobenzothiazole in 65 mL of absolute ethanol and added with 10 mL of hydrazine hydrate. The reaction mixture was refluxed in the water bath for 5 hr. The progression of the reaction was monitored by TLC using Alcohol: Acetone (9:1) as eluent. After cooling the combination, the solvent vacuously evaporated. After being removed from the ethanol, the product was filtered and re-crystallized again, yielding a pale-yellow crystal (3.10 g, 89% yield); melting point 191-193°C.[19]

Synthesis of N'-(1,3-benzothiazol-2-yl)-4- substituted benzohydrazide (3A-3E)

A mixture of 0.242 g (0.0014 mol) of 2-Hydrazino-1,3-benzothiazole and 0.450 g (0.0029 mol) of hydroxybenzotriazole was progressively added to 15 mL of dimethylformamide comprising 0.2 g (0.0014 mol) of substitute benzoic acid. In an ice bath, the mixture was cooled to 0-5°C while being stirred and 0.563 g (0.00294 mol) of 1-ethyl-3-(3- dimethylaminopropyl)-carbodiimide hydrochloride was introduced. Following an additional hr of gradual room temperature rise, the reaction mixture continued to stir consistently at this temperature until the reaction had finished. The reaction's Progression has been recorded with TLC using n-hexane: ethylacetate (1.5:3.5) as eluent and the derivatives of compounds were obtained with 80-93% yield (Figure 1: Scheme).[20,21]

Compound 3A: N'-(1,3-benzothiazol-2-yl)- benzohydrazide

MF:$C_{14}H_{11}N_3OS$; M.Wt.:269.3216; Rf:0.80; Colourless crystal; yield: 82.45%. M.P: 3298-300°C; FT-IR(cm^{-1}): 3312(NH-NH), 3040(Ar C-H), 1602(C=O), 1630(C=N),1531(Ar C=C),670 (C-S-C); ^{1}HNMR(400 MHz, $CDCl_3$) δ (ppm): 8.66-8.48 (m, 2H), 8.10(d, 2H), 7.69(t,1H), 7.39(t, 1H), 7.21(t, 2H), 5.01(s,2H, NHNH);[13] CNMR(125 MHz, CDCl3) δ (ppm): 170.65(C=O), 139.42(C=N), 132.44, 131.67, 127.63, 127.81, 124.23; HRMS (ESI-TOF) (m/z):[M+H]+ Calculated for $C_{14}H_{11}N_3OS$ $[M+H]^+$ 269.3216; Found 269.3200.

Compound 3B: N'-(1,3-benzothiazole-2-yl) -4-fluorobenzohydrazide

MF: $C_{14}H_{10}FN_3OS$; M.Wt.: 287.3121; Rf:0.71; Colourless powder; yield: 79.23%. M.P: 3 12-315°C; FT-IR(cm^{-1}): 3354, 3287(NH-NH), 3017(Ar C-H), 1596(C=O), 1613(C=N),1543, 1521(Ar C=C); ^{1}HNMR(400 MHz, $CDCl_3$) δ (ppm): 8.13-7.93 (m,2H), 7.81(d, 2H), 7.49(t, 1H), 7.23(t, 1H), 7.10(t, 2H), 4.98(s, 2H, NHNH);[13] CNMR(125 MHz, CDCl3) δ (ppm): 172.12(C=O), 132.22 (C=N), 129.33, 129.01, 128.43, 127.75, 126.54; HRMS (ESI-TOF) (m/z):[M+H]+ Calculated for $C_{14}H_{10}FN_3OS$ $[M+H]^+$ 287.3121; Found 287.3121

Compound 3C: N'-(1,3-benzothiazol-2-yl) -2-iodobenzohydrazide

MF: $C_{14}H_{10}IN_3OS$; M.Wt.: 395.2181; Rf:0.81; Pale yellow powder; yield: 87.10%. M.P: 301-303°C; FT-IR(cm^{-1}): 3300,3275(NH-NH), 3021(Ar C-H), 1667(C=O),1621(C=N), 1513,1500(Ar C=C), 678(C-S-C); ^{1}HNMR(400 MHz, $CDCl_3$) δ (ppm): 7.79 (d,3H), 7.38(d, 2H), 7.27(td,2H), 7.15(td, 1H), 4.91(s, 2H, NHNH);[13] CNMR(125 MHz, CDCl3) δ (ppm): 170.21(C=O), 162.65(C=N), 140.32, 139.15, 128.73, 125.64, 124.43, 120.27,90.14; HRMS (ESI-TOF) (m/z):[M+H]+ Calculated for $C_{14}H_{10}IN_3OS$ $[M+H]^+$ 395.2181; Found 395.2184

Table 1: **Binding energies of ligands on 1EQG and 1CX2 protein.**

	Compound3A		Compound 3B		Compound 3C		Compound 3D		Compound 3E		Celecoxib	
Protein	1EQG	1CX2	1EQG	1CX2	1EQG	1CX2	1EQG	1CX2	1EQG	1CX2	1EQG	1CX2
Rank	1	1	1		1	1	1	1	1	1	1	1
Sub- Rank	1	1	1		1	1	1	1	1	1	1	1
Run	8	9	2	6	5	4	7	6	4	9	2	3
Binding Energy	-7.30	-7.26	-7.39	-7.33	-10.41	-9.20	-6.82	-7.52	-6.97	-7.59	-8.52	-8.26
Cluster RMSD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reference RMSD	186.01	17.18	186.86	15.65	195.39	40.94	197.04	15.54	187.51	21.32	186.56	27.63

Table 2: **Interacting Amino acids on 1EQG and 1CX2 protein.**

Compound	1EQG	1CX2
Compound 3A	ILE89, LEU92, LEU93, TRP100, VAL1031 H-Bond UNL 1 (O1)	VAL291, HIS207, GLN289, LYS211, PHE210, ASN382, HIS3861 H-Bond ASN382 HD 21 1
Compound 3B	LYS468, CYS41, ILE46, GLN44, ARG469, TYR130, ILE151	LYS473, LEU472, LYS468
Compound 3C	MET391, LEU390, HIS388, HIS207, THR206, PHE210, TYR3851 H-Bond UNL 1 (N1)	LEU408, GLN203, TRP387, LEU390, LEU391, PHE404, HIS388
Compound 3D	ARG83, ARG79, THR80, HIS43, TYR64, PRO721H-Bond ARG79, HH 12 1	TRP387, TRP385, ASN382, HIS386, THR212, THR206, HIS2071H-Bond THR212 (HG 1 1)
Compound 3E	VAL119, ASN122, ARG79, GLN44, THR80, HIS43, TYR641H-Bond ARG79, HH 12 1	TYR475, LYS511, ASN87, ARG5131 H-Bond UNL O1, UNL 1 N1
Celecoxib	LEU152, ILE151, PRO153, ARG469, GLN44, GLY45, CYS47, ILE46	LYS468, ASN43, LEU472, ARG44, LYS473

Compound 3D: N'-(1,3-benzothiazol-2-yl) -4-nitrobenzohydrazide

MF: $C_{14}H_{10}N_4O_3S$; M.Wt.: 314.3186; Rf: 7.9; Brown powder; yield: 91% M.P: 290-292°C; FT-IR(cm^{-1}): 3296, 3271(NH-NH), 3220(Ar C-H),1670(C=O), 1625(C=N), 1498, 1504(Ar C=C) 1320(NO_2), 689(C-S-C); ^{1}HNMR(400 MHz, $CDCl_3$) δ (ppm):8.85-8.67(m,1H),8.43-8.30(m, 1H), 8.20(d, 1H), 7.89(d, 2H), 7.62(t, 1H), 5.01(s, 2H, NH-NH); ^{13}CNMR(125 MHz, CDCl3) δ (ppm): 169.08(C=O),145.91(C=N),130.23,129.12, 129.43, 127.67, 125.23, 125.10, 124.35, 122.67; HRMS (ESI-TOF) (m/z):$[M+H]^+$ Calculated for $C_{14}H_{10}N_4O_3S$ $[M+H]^+$ 314.3186; Found 314.3081.

Compound 3E:N'-(1,3-benzothiazol-2-yl)-4-hydroxy benzohydrazide

MF: $C_{14}H_{11}N_3O_2S$; M.Wt.: 285.3210; Rf: Pale brown crystal; yield: 89.34%; MP320-322°C; FT-IR(cm^{-1}): 3312(OH), 3299(NH-NH), 3009 (Ar C-H), 1645(C=O), 1610(C=N), 1580,1576(Ar C=C), 690(C-S-C); ^{1}HNMR(400MHz, $CDCl_3$) δ (ppm): 8.02-7.97(m,1H),7.95-7.91(m, 1H),7. 89 (dd, 1H), 7.69 (d, 1H), 7.51 (dd, 1H), 6.99 (d, 1H), 5.89 (s,2H, NH-NH); ^{13}CNMR(125MHz, CDCl3) δ (ppm):170.67(C=O),168.43(C=N),160.65,150.38, 142.81, 133.23, 131.99, 127.56, 122.65, 116.62; HRMS (ESI-TOF) (m/z):$[M+H]^+$ Calculated for $C_{14}H_{11}N_3O_2S$ $[M+H]^+$ 285.3210; Found 285.2211.

In silico Docking Study

Autodock 4.2 software was utilized to conduct docking simulation studies, attempting to assess the produced compound's anti-inflammatory effects against two human protein targets, such as 1CX2 and 1EQG. The proteins were allowed to remain rigid while the ligands were permitted to be flexible to acquire the listed amount of energy structure and the most precise conformation of the ligands. After the docking procedure, the ligand's binding interaction was thoroughly investigated to identify the most beneficial conformation.[22-24]

Biological studies

Both genders of adult Swiss albino mice (20-25 g) and Albino rats (150-200 g) were selected for the animal study. Groups of 4-8 per cage were kept at a temperature of 25±10°C and a relative humidity of 45-55%. Throughout the studies, 12-hr cycles of dark and light were observed. Animals have unlimited access to food and water as a necessity. The care of animals during the research period was carried out in compliance with the CPSEEA (Committee for Control and Supervision of Experiments on Animals) and IAEC (Institutional Animals Ethics Committee).

Acute toxicity studies

Six Swiss albino mice, weighing 20-25 g each were used in groups for acute toxicity experiments. The mice given oral dosage (ranging from 100-1000 mg/kg body weight) were fasted overnight. With the agreement of the Assam downtown University and the Institutional Animal Ethics Committee, all animal studies were carried out in Assam India.

Anti-inflammatory activity

Anti-inflammatory activity was performed using the carrageenan-induced rat paws oedema method. Albino male rats weighing 300 g were procured from the Faculty of Pharmaceutical Science at Assam Down Town University, Assam and housed in a light-controlled animal facility at room temperature. Before the experiments, the rats underwent a fasting period of at least 12 hr with *ad libitum* access to water. The compounds under investigation were formulated as a suspension in a vehicle consisting of 0.5% Methylcellulose (CMC), with celecoxib serving as the conventional drug. Celecoxib was given to the positive control group, whereas simply the vehicle was administered to the negative control group. To induce oedema, an injection of 0.2 mL of 1% carrageenan was injected into the hind paws of the rats. Subsequently, the rats were intraperitoneally injected with a 1 mL suspension of the test compounds and reference drug in 0.5% methylcellulose. Paw volume was assessed using a plethysmometer (UGO BASILE) through water displacement measurements conducted before treatment, as well as at 0.5 hr,

Table 3: **The analgesic activities of all the compounds.**

Compound No	Mean Number of writhes±SEM (*n*=4)		
	0.5 hr	1 hr	2 hr
Compound 3A	253±1.5	165±1.9	149±1.2
Compound 3B	95±1.6	99±1.4	87±2.1
Compound 3C	85±4.2	71±4.8	68±6.4
Compound 3D	209±1.1	186±3.1	124±5.1
Compound 3E	126±0.5	83±1.0	75±5.7
Celecoxib	155±4.2	70±1.4	69±0.9

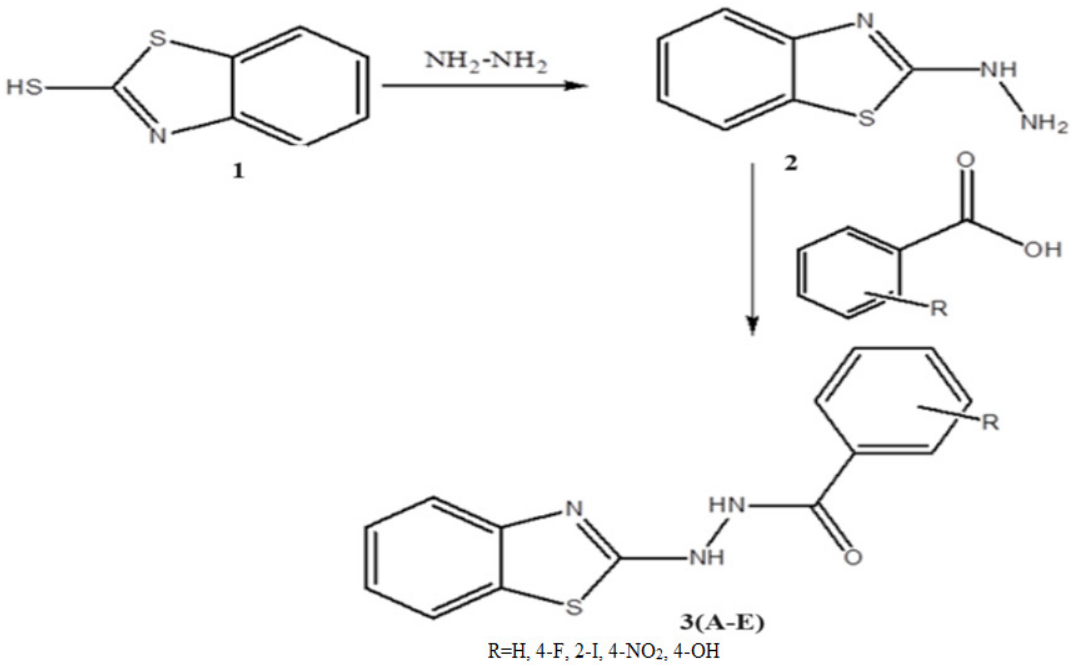
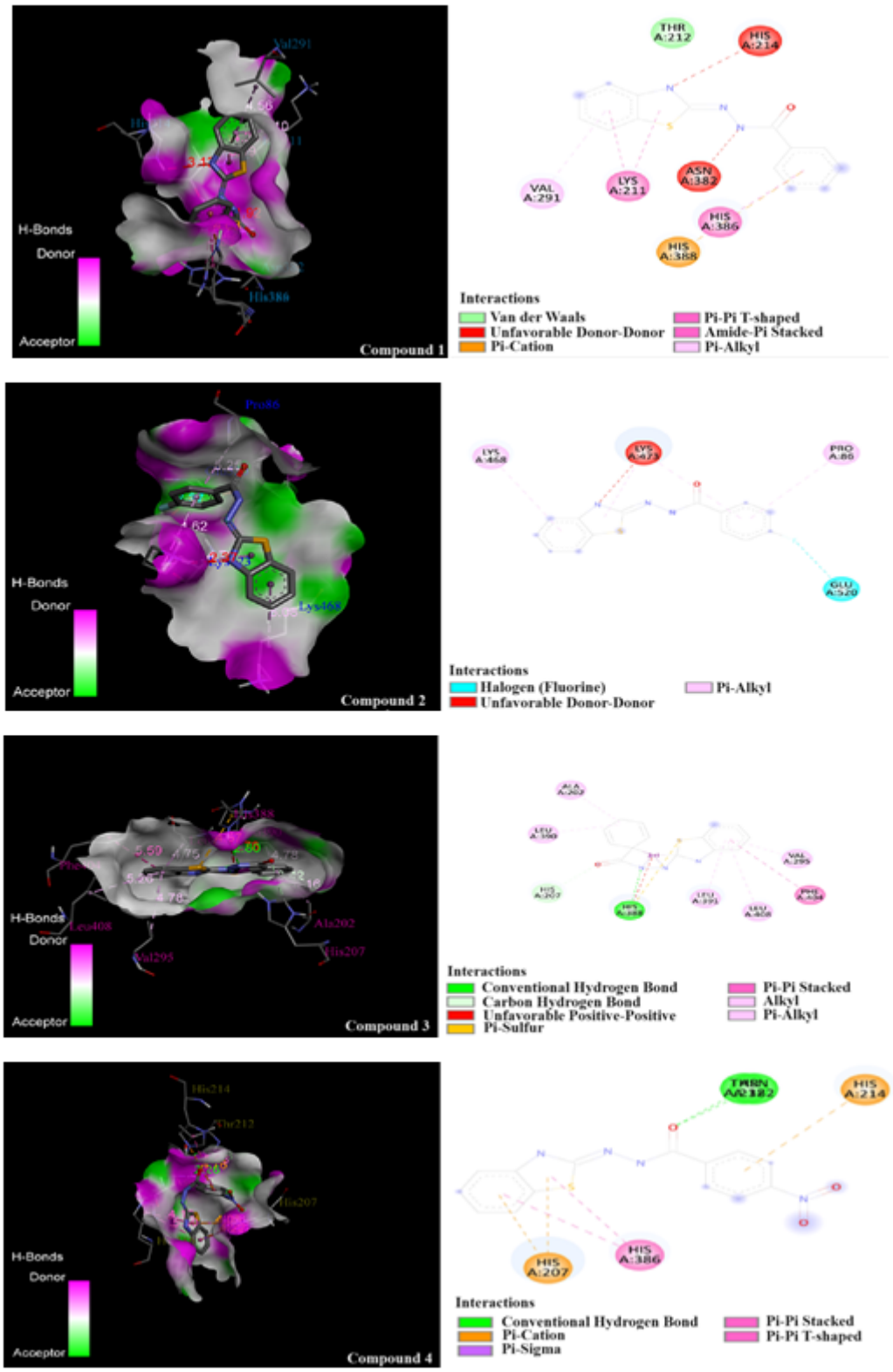


Figure 1: Scheme.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His386
H-Bonds
Donor
Acceptor
Compound 1
THR A:212
HIS A:214
VAL A:291
LYS A:231
ASN A:382
HIS A:386
HIS A:388
Interactions
Van der Waals
Unfavorable Donor-Donor
Pi-Cation
Pi-Pi T-shaped
Amide-Pi Stacked
Pi-Alkyl
Pro86
Lys468
H-Bonds
Donor
Acceptor
Compound 2
LYS A:468
LYS A:473
PRO A:86
GLU A:520
Interactions
Halogen (Fluorine)
Unfavorable Donor-Donor
Pi-Alkyl
His388
Leu408
Val295
Ala202
His207
H-Bonds
Donor
Acceptor
Compound 3
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Positive-Positive
Pi-Sulfur
Pi-Pi Stacked
Alkyl
Pi-Alkyl
His214
Thr212
His207
H-Bonds
Donor
Acceptor
Compound 4
THR A:212
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HIS A:386
HIS A:207
Interactions
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped

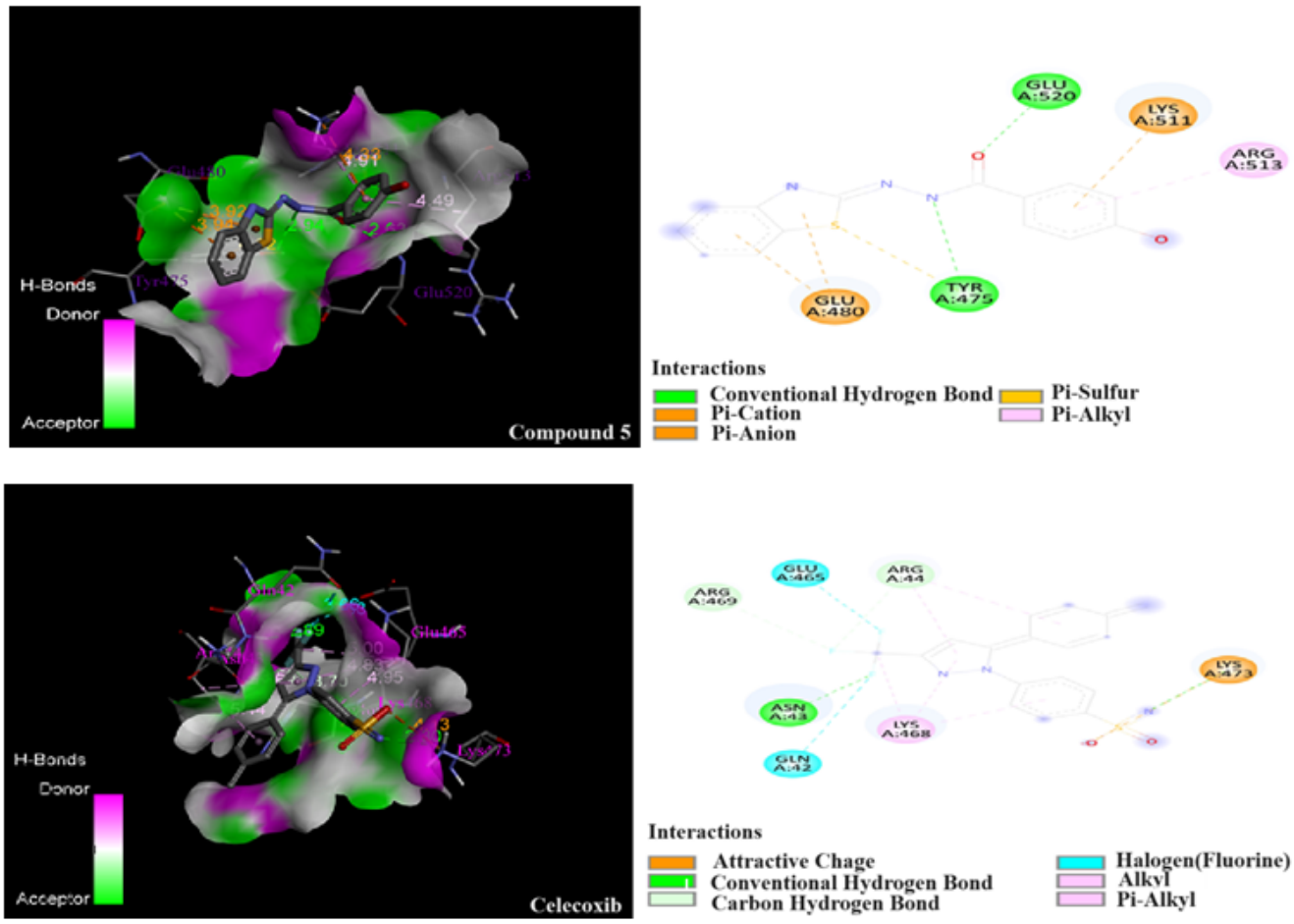


Figure 2a: The amino acid residues of the proteins 1EQG interact with the compound 1 to Compound-5 along with Standard Celocoxib in both 3D and 2D ligand configurations.

1 hr, 2 hr and 3 hr post-treatment.[25] The following equation calculated the percentage of inhibition:

$$\text{Anti-inflammatory activity (\%)} = (1\text{-}D/C) \times 100$$

Where C is the variation in size in the control group, D is the difference in paw volume before and after the drugs were given to them and the outcomes are provided in Figure 2.

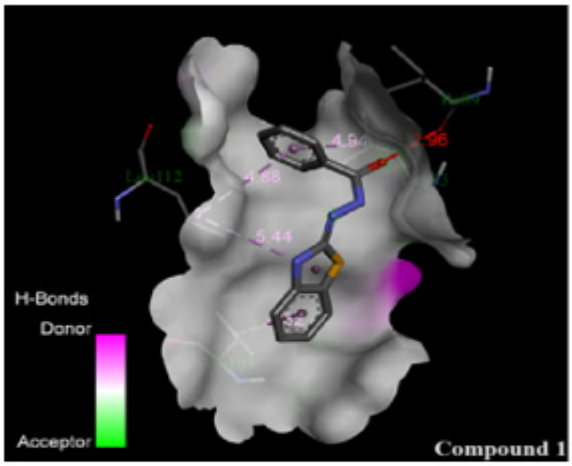
Analgesic activity

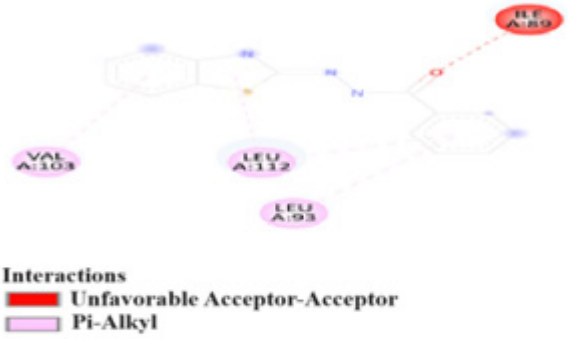
Male albino Swiss mice weighing 25 g were categorized into different groups, each group consisted of 4 mice. Initially, each mouse was first put on a hot plate and maintained at an optimal 58°C temperature via a thermostatic gadget. The duration it took the mice to act out nociceptive reactions including licking or blowing (fanning) their front paws, if they were undergoing close oversight is displayed in Table 3. This was the response's baseline control time used. The 60 sec duration was set aside to prevent accidental damage to the paws. Each set of mice got a single intraperitoneal dosage (5-200 mg/kg i.p.) of the test compounds to evaluate their analgesic efficacy. Then, with each mouse acting as a control, the reaction time was evaluated again at 15-, 30-, 60- and 120-min following injection. Then, the percentage changes in reaction time were computed. And lastly, linear regression analysis was used to find the ED_{50} value for each chemical.[26]

RESULTS

Docking study

Typically, a high binding energy does not serve as a potent inhibitor. Inhibitors are substances that attach to and impede the function of another substance, resulting in a reduction in its activity. This is commonly achieved by creating a complex between the inhibitor and its target molecule. The greater the stability of the inhibitor in terms of energy, the more challenging it becomes for the target molecule to replace or remove it. The Results obtained by docking on protein PDB: 1EQG and 1CX2 were compared with the standard Celecoxib and compound 3C was found to possess less binding energy than the standard drug. Hence, compound 3C may be used for Analgesic and Anti-inflammatory activity. The autodock 4.2 softwares were used to conduct docking simulation studies to evaluate the anti-inflammatory activity of newly synthesized compounds against human protein targets


H-Bonds
Donor
Acceptor
Compound 1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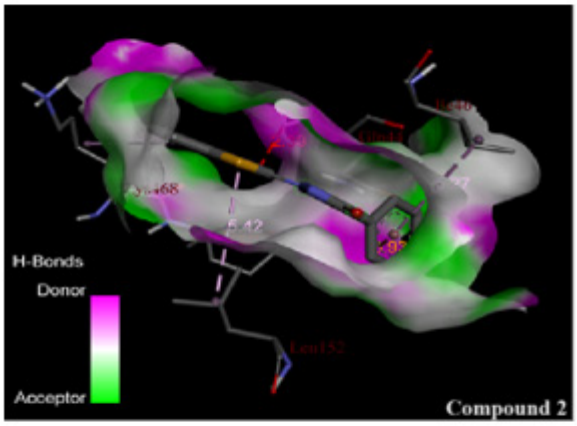
LEU A:132
LEU A:93
Interactions
Unfavorable Acceptor-Acceptor
Pi-Alkyl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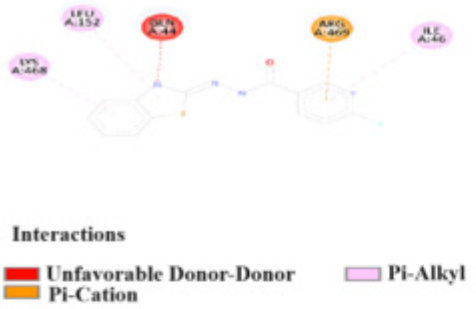
Donor
Acceptor
Compound 2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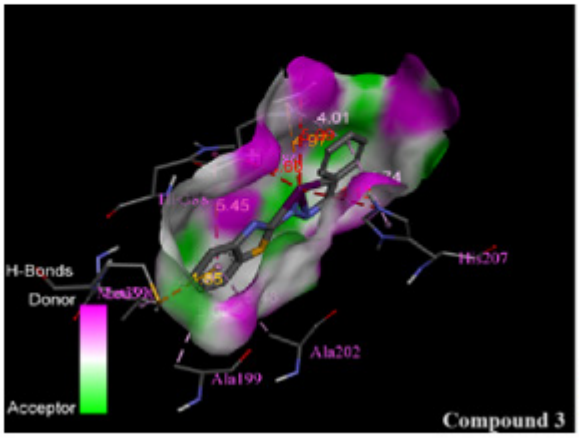
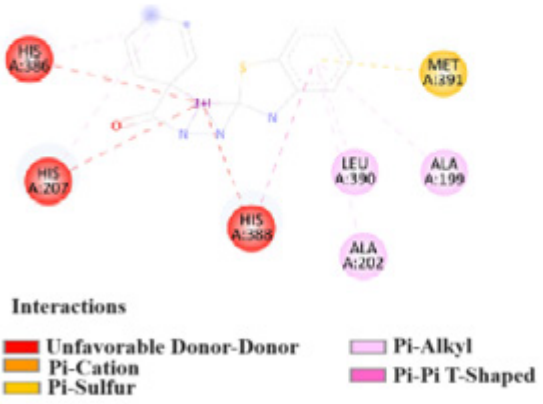
ARG A:469
ILE A:46
Interactions
Unfavorable Donor-Donor
Pi-Cation
Pi-Alkyl


H-Bonds
Donor
Acceptor
Ala202
Ala199
Compound 3


HIS A:386
MET A:391
HIS A:207
LEU A:390
ALA A:199
HIS A:388
ALA A:202
Interactions
Unfavorable Donor-Donor
Pi-Cation
Pi-Sulfur
Pi-Alkyl
Pi-Pi T-Shaped

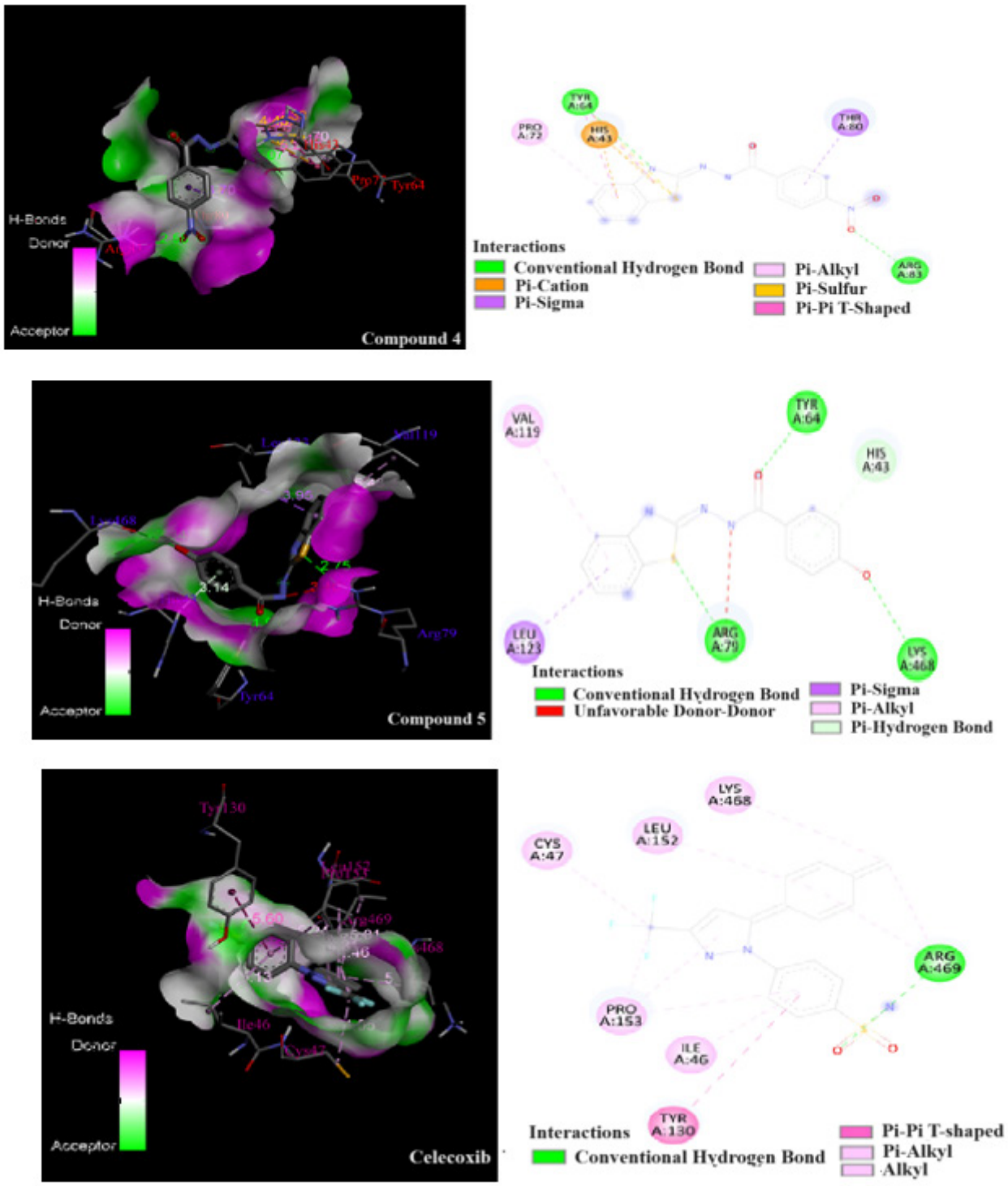


Figure 2b: The amino acid residues of proteins 1CX2 react with compounds 1 to Compound-5 and Standard Celocoxib in both 3D and 2D ligand interactions.

1CX2 and 1EQG. The most efficient molecule, 3C, as well as the co-crystallized ligand, was simultaneously docked into the active sites of 1 CX2 and 1 EQG to test the dock program's dependability. 3C demonstrated a strong fit within the binding cavity, just like the co-crystallized ligand did.

The RMSD values of 40.94 Å and 195.35 for 1CX2 and 1EQG, respectively, demonstrated the applicability of the docking technique for the examined inhibitors, proving the reliability of the MOE-Dock method for docking these substances. The six synthesized substances satisfactorily engaged the active binding sites of 1CX2 and 1EQG. The relevant binding energies of these

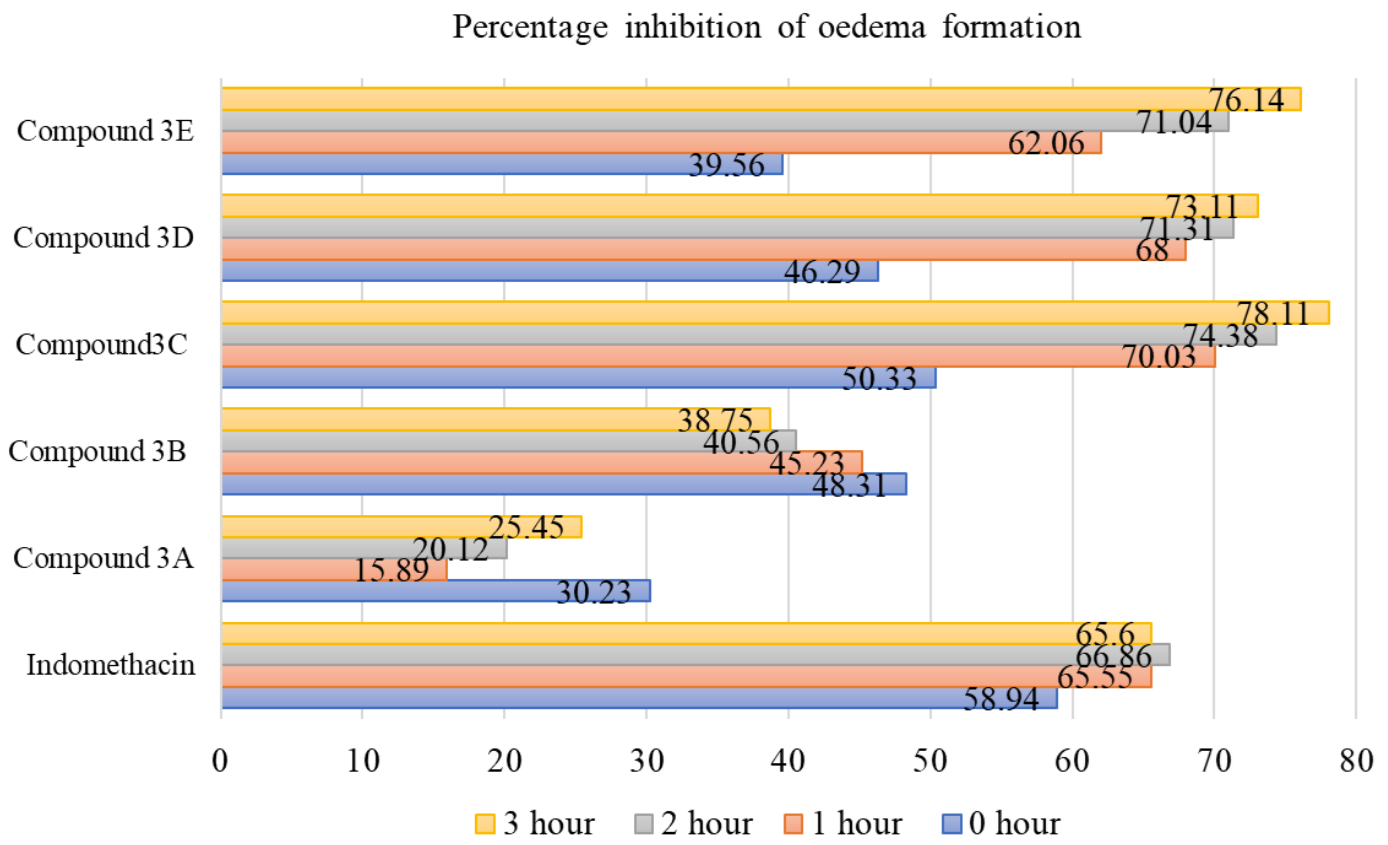


Figure 3: Anti-inflammatory effect of test compounds on rats' acute paw oedema. [*n*=6 animals in each group; Control: 0.5% Carboxy Methyl Cellulose (CMC); * $p<0.05$ vs Control; ** $p<0.01$ vs Control].

compounds appear in Tables 1 and 2. Because 3C's energy of binding to both targets was the highest, more research was necessary to determine how it bound to the targets in order to understand the different kinds of interactions that occur in protein-ligand complexes. The binding interaction, 2D and 3D ligand interaction with receptors was illustrated in Figure 2a and 2b, highlighting 15 active amino acid residues present in the active binding site of the 1CX2-3 and 1EQG-3 complex, including LEU408, GLN203, TRP387, LEU390, LEU391, PHE404, HIS388, MET391, HIS388, HIS207, THR206, PHE210 and TYR385, along with 1 H-Bond UNL 1 (N1).

Anti-Inflammatory Activity

The anti-inflammatory activity data (Figure 3) demonstrates that at 1 hr, 2 hr and 3 hr, all compounds-aside from 3C and 3E-caused less than a 50% decrease of oedema. 3C showed the highest level of anti-inflammatory properties of all the compounds under evaluation. At 1-3 hr, 3C and 3E showed a greater percentage drop than indomethacin. The ortho-iodo benzene ring of compound 3C was shown to be more efficient in decreasing oedema than the unsubstituted benzene ring of compound 3A, according to the Structure-Activity Relationship (SAR). Within the benzothiadiazole derivatives (3A-3E), compound 3E exhibited the highest activity among all derivatives, demonstrating superior anti-inflammatory properties compared to indomethacin. It was noted that para hydroxy benzene had a positive impact on enhancing the anti-inflammatory activity of the compounds. The trend in anti-inflammatory activity indicated that compound 3E had the highest efficacy, followed by 3D and 3B. Among the amide chain with benzothiazole derivatives, it was observed that the absence of any functional group on the carboxamide resulted in lower activity levels. The trend in this scenario showed that 3C had the highest activity, followed by 3E, 3D, 3B and finally 3A. Compounds 3E and 3D exhibited lower activity levels than compound 3C, indicating that the existence at the para position of the electron-withdrawing group of the 1,3-benzothiazol-2-yl-benzohydrazide caused a drop in anti-inflammatory action. Compounds containing electron-withdrawing groups, such 3A, nevertheless demonstrated greater activity than those with an unsubstituted ring.

Analgesic activities

The analgesic activities of all the compounds were evaluated and found to be significant, as indicated in Table 3. However, only compounds 3C and 3E showed comparable results to celecoxib. Among these compounds, compound 3C exhibited the highest analgesic activity with an ED_{50} of 71 and 68 mM/kg after 1 hr and 2 hr, respectively, compared to celecoxib (ED_{50} of

70 and 69 mM/kg). The Structure-Activity Relationship (SAR) investigation revealed that each derivative exhibiting potent analgesic activity impacted both an NHC=O group (amide) and an extra Iodo group (2-Iodo benzene). Notably, compound 3E which belonged to the 4-hydroxy benzene derivatives, exhibited significant analgesic activity compared with celecoxib. These results highlight the importance of the ring activating group (-OH) in the benzene of benzothiazole moiety's para position. The results indicated that compound 3A exhibited lower analgesic activity than compound 3D, highlighting the significance of the ($-NO_2$) substitution in enhancing analgesic effects at the para position of the benzene molecule. These findings suggest promising avenues for the creation of new analgesic drugs and provide valuable insights into the structure-activity relationship of the compounds being studied.

DISCUSSION

This study investigated the potential of a series of newly synthesized compounds (3A-3E) for analgesic and anti-inflammatory activity. Docking simulations and biological assays were employed to evaluate their efficacy. A synthetic process outlined in Scheme 1 was implemented to synthesize the title compounds 3A-3D successfully. The structures and purity of the synthesized compounds have been characterized by using mass spectrum data, proton NMR and infrared imaging. Commercially accessible aniline was initially utilized to prepare 2-Mercapto benzothiazole by reacting it with potassium ethyl xanthate at regulated low temperatures. The mercapto stretching band of Mercapto benzothiazole in compound 1 vanished and the NH-NH stretching band for the amine group appeared in the IR spectra, indicating the production of compound 2. The apparent appearance of NH and C=O stretching bands in the IR spectra pointed out that substituted 2-hydrazinylbenzo[d]thiazole (2) had been modified with substituted aromatic aldehyde to generate substituted benzo hydrazide (3A-3E). Proton NMR study demonstrated the emergence of NH-NH protons and the elimination of NH2 protons of hydrazinylbenzo[d]thiazole for compound 2, further confirming the production of products 3A-3E. In addition, the IR spectra of compounds and 3A-3E confirmed C-S-C stretching and the NMR spectra of compound 2 showed the lack of the NH_2 proton, which further supported the successful synthesis of the desired compound. he docking software (MOE-Dock) successfully predicted the binding modes of the compounds within the active sites of target proteins (1CX2 and 1EQG). Interestingly, compound 3C exhibited a strong fit despite possessing lower binding energy compared to the standard drug, Celecoxib. This suggests that factors beyond binding energy may influence inhibitor efficacy. Further investigation into the specific interactions between 3C and the target proteins is warranted. Among the tested compounds, 3C displayed the most potent anti-inflammatory effect; exceeding indomethacin at all time points (1, 2 and 3 hr). Structure-Activity Relationship (SAR) analysis revealed that the presence of an ortho-iodo benzene ring in 3C contributes significantly to its anti-inflammatory properties compared to the unsubstituted benzene ring observed in 3A. Similar to the anti-inflammatory activity, compound 3C demonstrated the strongest analgesic effect, with results comparable to Celecoxib at both 1 and 2 hr. The SAR analysis suggests that the combination of an NHC=O group (amide) and an additional Iodo group (2-Iodo benzene) plays a crucial role in potent analgesic activity. Notably, 3E, possessing a para hydroxy benzene ring, also exhibited significant analgesic activity. This highlights the importance of a ring activating group in the para position of the benzothiazole moiety. This study identified compound 3C as a promising candidate for further development as an analgesic and anti-inflammatory drug. Its superior activity compared to other synthesized compounds and its comparable efficacy to Celecoxib warrant further investigation. The findings also provide valuable insights into the structural features that contribute to the analgesic and anti-inflammatory properties of these benzothiadiazole derivatives. Further in-depth studies are required to elucidate the specific mechanism of action of compound 3C for both analgesic and anti-inflammatory effects. *In vivo* experiments are necessary to confirm the observed *in vitro* activity of compound 3C. Optimization of the structure of compound 3C can be explored to potentially improve its potency and selectivity. By addressing these aspects, researchers can determine the suitability of compound 3C for clinical development as a novel analgesic and anti-inflammatory drug.

CONCLUSION

The article introduces a fresh method for the synthesis of substituted benzothiazole chemicals, emphasizing efficiency and sustainability. Benzothiazole is known for its diverse pharmacological effects, with the addition of an iodine atom enhancing its pharmacological properties significantly. The inclusion of an iodine atom in the benzothiazole structure results in improved pharmacological attributes, such as increased receptor affinity and enhanced lipophilic nature. Iodine, due to its unique characteristics like a short atomic radius, high electron-attracting power and low polarizability, plays a crucial role in the drug discovery field and influences the development of new pharmaceuticals. Substrates 3C and 3E exhibited anti-inflammatory effects surpassing those of commonly used drugs like indomethacin, as demonstrated through tests evaluating their anti-inflammatory properties. Additionally, *in silico* docking analyses were conducted to investigate the molecular interactions between compounds 3C and 3E and proteins 1CX2 and 1EQG, revealing similarities in anti-inflammatory properties to celecoxib. The enhanced anti-inflammatory activities of compounds 3C and 3E are attributed to the substitution of an ortho iodobenzene ring on the benzothiazole moiety and the presence of electron-rich -NH or -OH (Compound 3E) groups, as indicated by the Structure-Activity Relationship (SAR) study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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