Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[12] November 2020 : 52-59

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Design, Synthesis, And Molecular Docking Studies of Novel Isothiazole Derivatives as Potent Anti-inflammatory and Analgesic Agents

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ABSTRACT
*A novel series of benzo[d]isothiazole derivatives **(3a-3n)** were synthesized in a single step condensation reaction between 6-bromo benzo[d]isothiazole with various substituted phenyl boronic acid. Insilco structure-based analysis of designed molecules with COX-2 enzyme were investigated using molecular docking tool, GLIDE. The final products were characterized by detailed spectral analysis using Mass, H^1-NMR, C^{13}- NMR and IR spectroscopy. The title products were assessed for their anti-inflammatory and analgesic activities on Swiss albino rats. All the compounds **(3a-3n)** exhibited noteworthy defence against inflammation and nociception. Among all the compounds **3d** (61.83%), **3n** (60.96%) and **3h** (59.65%) displayed good anti-inflammatory and compounds **3n**(61.88%), **3l**(60.89%)and **3h** (59.78%) exhibited analgesic activities with respect to the activity of standard drugs. Molecular docking scores of the compounds against the COX-2 ranges from -46.659to -60.928.*
Key Words: *Benzo[d]isothiazole, Molecular docking, Anti-inflammatory, Analgesic.*

Received 21.08.2020 Revised 20.09.2020 Accepted 21.10.2020

INTRODUCTION

Heterocyclic frameworks provide enormous potential in the discovery of new chemotherapeutics in drug discovery and development programs. They form the core elements of natural biomolecules and are also present in clinically used drugs. In drug discovery, the heterocycles are well known to modulate the properties in a drug such as potency, lipophilicity, polarity, solubility and so on and are widely investigated in the search of a drug with desired properties. Benzothiazole is one of the classes of privileged fused heterocycles found in several marine and terrestrial bioactive natural products. In recent decades, it has been established as a promising pharmacophore possessing diverse biological properties such as anticonvulsant [1,2], anthelmintic [3], neuroprotective [4], anti-glutamate [5], antimalarial [6], antitubercular [7], antimicrobial [8], analgesic, anti-inflammatory [9] and anticancer activities [10-13]. Interestingly, benzo[d]isothiazoles are present in bioluminescent luciferin and are used as fluorophores for two photon excitations as well as absorption processes [14,15]. Several successful clinical drugs such as ethoxzolamide (1), frentizole (2), riluzole (3), zopolrestat (4) and so on and the well-known amyloid imaging agent thioflavin T (5) contain benzo[d]isothiazole nucleus (Figure 1) [16]. These drugs have applications in the treatment of various diseases/disorders, which are outcome of impaired physiological processes in humans. Ethoxzolamide, the sulphonamide drug with a core benzothiazole ring serves as diuretic and frentizole, the urea derivative of benzothiazole is used as antiviral as well as immunosuppressive agent. On the other hand, riluzole is a glutamate receptor antagonist, which is used to treat amyotrophic lateral sclerosis. In addition, it is also known to possess antidepressant activity and have clinical significance in the treatment of anxiety disorders [17]. Zopolrestat is another important drug containing benzothiazole core with antidiabetic effects [18].

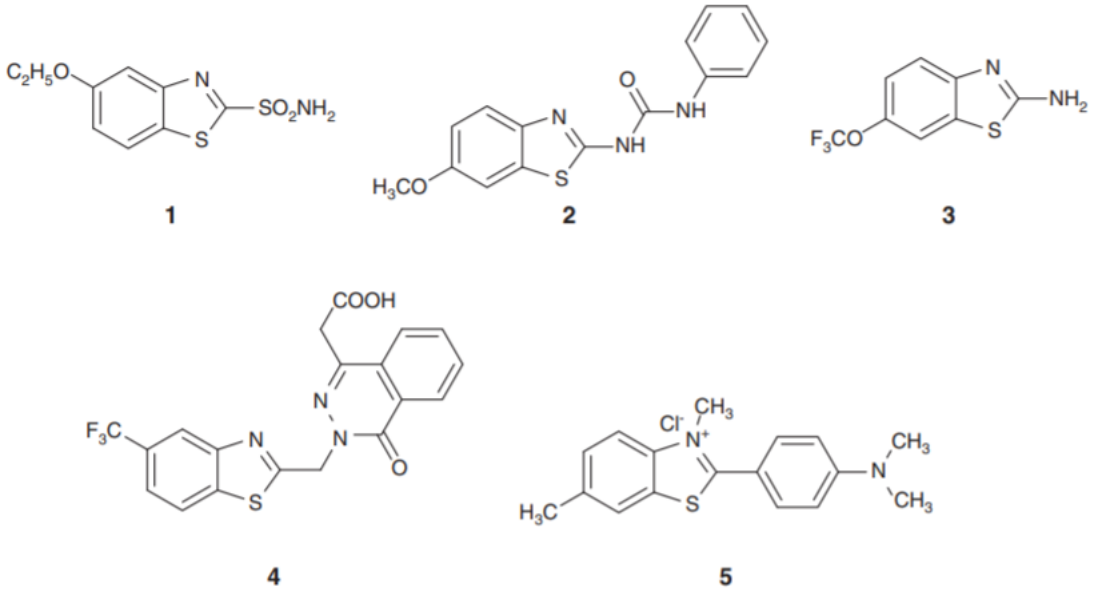


Figure 1: Ethoxzolamide (1), frentizole (2), riluzole (3), zopolrestat (4) and so on and the well-known amyloid imaging agent thioflavin T (5) contain benzo[d]isothiazole nucleus

The advent of computational chemistry provided the comprehensive understanding of target enzymes and their interaction with various investigational ligands [19]. Molecular docking is a rational drug designing approach that facilitate the discovery of lead molecules for the targeted enzymes and proteins by simulating the interaction energies of drug and target binding sites *in silico* [20]. In a quest of this objective, our research endeavours have been focused on the direction of the rational designing of new chemical molecules that are effective as anti-inflammatory agents. In this regard, an endeavour was made to integrate a novel series of benzo[d]isothiazole derivatives to screen for anti-inflammatory (carrageenan induced rodent paw oedema model), analgesic (acetic acid induced writhing model and hot plate method) in Swiss albino rats.

MATERIAL AND METHODS

All chemicals and solvents used in the experimental study were purchased from S.D fine, Merck, HIMEDIA and Sigma Aldrich. On thin layer chromatography, the progression of the reaction, purity of the intermediate and final compounds was tested using silica gel pre-coated plates as stationary phase in the solvent method- Dichloromethane (DCM): methanol (MeOH) (7:3 v / v). Spot identification is achieved by observing under UV light and the spectral experiments were used to classify the compounds. The title compound IR spectra have been reported on the Bruker FTIR spectrophotometer and are given in KBr in cm^{-1}. The ^1H NMR was measured in chloroform and deuterated DMSO on the Bruker AM-400 NMR spectrometer. The chemical shifts are reported in δ (ppm) relative to tetramethyl silane as internal standard. Mass spectra analyses was performed with an Agilent 6400 Series equipped with an electrospray ionization source (capillary voltage at 4000V, nebulizing gas temperature at 300 °C, nebulizing gas flow at 12 L/min). The elemental analysis of the title compounds was performed on Perkin Elmer C, H and N model 240 C analyser.

The use of the animals for pharmacological experiments was performed in compliance with WHO ethical guidelines. Swiss albino rats of 180-200g were procured from an inbred colony from Sri Venkateshwara Enterprises, Bangalore. The animals were provided with standard feed & water and were maintained under control conditions of temperature and light. Paw oedema of the experimental Albino rats was measured by BASILE 7140 Plethysmometer. All the experimental protocols were approved by the institutional ethical committee. Acute toxicity studies for the novel compounds were carried out in mice (500mg/kg, 1000mg/kg, 1500mg/kg p.o) [21]. Before the biological evaluation of the compounds the animals were kept hungry for food until 4h but fed with water.

Synthesis

General procedure for the synthesis of benzo[d]isothiazole derivatives

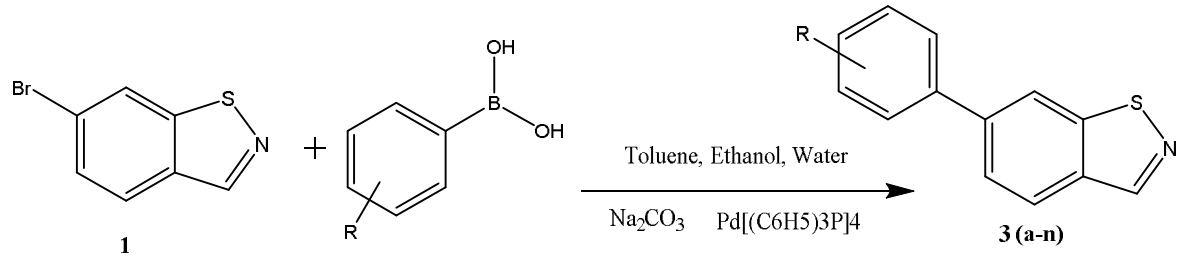
To a stirred solution of 6-bromo benzo[d]isothiazole1 (100 mg, 0.46 mmol,1eq) in the mixture of Toluene (7 ml), ethanol (2 ml) and water (1 ml), Na_2CO_3 (124 mg, 1.17 mmol, 2.5 eq) and phenylboronicacid**(2a-n)**

(85.9 mg, 0.70 mmol, 1.5 eq) were added and degassed with nitrogen for 15 min. Then Tetrakis (triphenylphosphine) palladium (0) (27 mg, 0.023 mmol, 0.05 eq) was added and the reaction mixture was stirred at 100^{0}C for 3h. Completion of the reaction was monitored by TLC and the reaction mixture was concentrated under reduced pressure. The residue obtained was purified by silica gel column chromatography using 10% ethyl acetate and petroleum ether, finally the pure fractions were concentrated to get the title compounds**3(a-n).**

***Physical and Spectral data of the compounds*:**

Compound a: Yield: 82%; M.W: 211.28; M.P: 208.43 ^{0}C; IR (cm^{-1}): 2727 (NH Stretching), 2352 (CN Stretching), 830 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.40-7.48 (m, 3H, benzene); 7.68 -7.69 (m, 3H, benzene) 8.11-8.14 (m, 2H, benzene), 8.93 (s, 1H, isothiazole); Mass (m/z): 211.91 (M+1); Anal. Calcd for $C_{13}H_9NS$: C: 73.90; H: 4.29; N: 6.63; S: 15.18; Found: C: 73.86; H: 4.22; N: 6.66; S: 15.20.

Compound b: Yield: 80%; M.W: 241.31; M.P: 264.45^{0}C; IR (cm^{-1}): 2730 (NH Stretching), 2348 (CN Stretching), 826 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.36-7.40 (m, 3H,benzene); 7.70 -7.72 (m, 3H, benzene) 8.14-8.16 (m, 2H, benzene), 8.93 (s, 1H, isothiazole); Mass (m/z): 241.91 (M+1); Anal. Calcd for $C_{14}H_{11}NOS$: C: 69.66; H: 4.54; N: 5.76; O:6.60; S: 13.26; Found: C: 69.68; H: 4.59; N: 5.80; O: 6.63; S: 13.29.



	R		R		R
3a	H	**3f**	4 - CH_3	**3k**	3-CF_3
3b	4 - OCH_3	**3g**	3- CH_3	**3l**	4-OH
3c	3- OCH_3	**3h**	4 -F	**3m**	4 - NH_2
3d	4 -NO_2	**3i**	2 -F	**3n**	4 – Cl
3e	2 -NO_2	**3j**	4-CF_3		

Figure.2. Scheme of synthesis for the benzo[d]isothiazolederivatives.

Compound c: Yield: 72%; M.W: 241.31; M.P: 264.45^{0}C; IR (cm^{-1}): 2732 (NH Stretching), 2348 (CN Stretching), 824 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.36-7.38 (m, 3H, benzene); 7.70 -7.72 (m, 3H, benzene) 8.12-8.14 (m, 2H, benzene), 8.94 (s, 1H, isothiazole); Mass (m/z): 241.91 (M+1); Anal. Calcd for $C_{14}H_{11}NOS$: C: 69.66; H: 4.54; N: 5.7'6; O:6.60; S: 13.26; Found: C: 69.68; H: 4.59; N: 5.80; O: 6.63; S: 13.29.

Compound d : Yield: 74%; M.W: 256.28; M.P: 269.45^{0}C; IR (cm^{-1}): 2730 (NH Stretching), 2346 (CN Stretching), 828 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.38-7.40 (m, 3H, benzene); 7.74 -7.76 (m, 3H, benzene) 8.14-8.16 (m, 2H, benzene), 8.96 (s, 1H, isothiazole); Mass (m/z): 257.03 (M+1); Anal. Calcd for $C_{13}H_8N_2O_2S$: C: 60.93; H: 3.15; N: 10.93; O:12.49; S: 12.51; Found: C: 60.94; H: 3.18; N: 10.90; O: 12.46; S: 12.54.

Compound e: Yield: 78%; M.W: 256.26; M.P: 269.45^{0}C; IR (cm^{-1}): 2732 (NH Stretching), 2346 (CN Stretching), 826 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.38-7.40 (m, 3H, benzene); 7.74 -7.76 (m, 3H, benzene) 8.14-8.16 (m, 2H, benzene), 8.96 (s, 1H, isothiazole); Mass (m/z): 257.03 (M+1); Anal. Calcd for $C_{13}H_8N_2O_2S$: C: 60.92; H: 3.16; N: 10.93; O:12.49; S: 12.51; Found: C: 60.94; H: 3.18; N: 10.90; O: 12.46; S: 12.54.

Compound f: Yield: 71%; M.W: 225.31; M.P: 242.22 ^{0}C; IR (cm^{-1}): 2734 (NH Stretching), 2342 (CN Stretching), 826 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.36-7.38 (m, 3H, benzene); 7.70 -7.72 (m, 3H, benzene) 8.10-8.12 (m, 2H, benzene), 8.92 (s, 1H, isothiazole), 2.36-2.40 (m,3H, methyl); Mass (m/z): 226.06 (M+1); Anal. Calcd for $C_{14}H_{11}NS$: C: 74.63; H: 4.92; N: 6.22; S: 14.23; Found: C: 74.60; H: 4.88; N: 6.20; S: 14.20.

Compound g: Yield: 69%; M.W: 225.31; M.P: 242.22^{0}C; IR (cm^{-1}): 2733 (NH Stretching), 2340 (CN Stretching), 826 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.38-7.40 (m, 3H, benzene); 7.70 -7.72 (m, 3H, benzene)8.10-8.12 (m, 2H, benzene), 8.92 (s, 1H, isothiazole), 2.38-2.40 (m,3H, methyl); Mass (m/z): 226.06 (M+1); Anal. Calcd for $C_{14}H_{11}NS$: C: 74.63; H: 4.92; N: 6.22; S: 14.23; Found: C: 74.60; H: 4.88; N: 6.20; S: 14.20.

Compound h: Yield: 67%; M.W: 229.27; M.P: 231.54 ^{0}C; IR (cm^{-1}): 2734 (NH Stretching), 2342 (CN Stretching), 826 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.38-7.40 (m, 3H, benzene); 7.70 -7.72 (m, 3H, benzene)8.10-8.12 (m, 2H, benzene), 8.92 (s, 1H, isothiazole); Mass (m/z): 230.04 (M+1); Anal. Calcd for $C_{13}H_8FNS$: C: 68.10; H: 3.52; N: 8.29; S: 13.99; Found: C: 68.06; H: 3.48; N: 8.28; S: 13.97.

Compound i: Yield: 65%; M.W: 229.27; M.P: 232 ^{0}C; IR (cm^{-1}): 2730 (NH Stretching), 2338 (CN Stretching), 828 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.40-7.42 (m, 3H, benzene); 7.74 -7.76 (m, 3H, benzene)8.14-8.162 (m, 2H, benzene), 8.90 (s, 1H, isothiazole); Mass (m/z): 230.08 (M+1); Anal. Calcd for $C_{13}H_8FNS$: C: 68.10; H: 3.52; N: 8.29; S: 13.99; Found: C: 68.04; H: 3.50; N: 8.26; S: 13.98.

Compound j: Yield: 61%; M.W: 279.28; M.P: 236.54 ^{0}C; IR (cm^{-1}): 2734 (NH Stretching), 2342 (CN Stretching), 826 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.38-7.40 (m, 3H, benzene); 7.70 -7.72 (m, 3H, benzene)8.10-8.12 (m, 2H, benzene), 8.92 (s, 1H, isothiazole); Mass (m/z): 280.08 (M+1); Anal. Calcd for $C_{14}H_8F_3NS$: C: 60.21; H: 2.81; F:20.41; N: 5.02; S: 11.48; Found: C: 60.21; H: 2.80; F: 20.39; N: 5.04; S: 11.46.

Compound k : Yield: 64%; M.W: 279.29; M.P:228.96^{0}C; IR (cm^{-1}): 2734 (NH Stretching), 2342 (CN Stretching), 830 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.38-7.40 (m, 3H, benzene); 7.70 -7.72 (m, 3H, benzene)8.10-8.12 (m, 2H, benzene), 8.92 (s, 1H, isothiazole); Mass (m/z): 280.08 (M+1); Anal. Calcd for $C_{14}H_8F_3NS$: C: 60.21; H: 2.81; F:20.41; N: 5.02; S: 11.48; Found: C: 60.21; H: 2.80; F: 20.39; N: 5.04; S: 11.46.

Compound l : Yield: 78%; M,W: 227.28; M.P: 330.15 ^{0}C; IR (cm^{-1}): 2724 (NH Stretching), 2350 (CN Stretching), 828 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.40-7.42 (m, 3H, benzene); 7.70 -7.68 (m, 3H, benzene) 8.08-8.12 (m, 2H, benzene), 8.90 (s, 1H, isothiazole); Mass (m/z): 228.02 (M+1); Anal. Calcd for $C_{13}H_9NOS$: C: 68.70; H: 3.99; N: 6.16; O: 7.04; S: 14.12; Found: C: 68.68; H: 3.98; N: 6.12; O: 7.02; S: 14.16.

Compound m : Yield: 70%; M,W: 226.30; M.P: 314.21 ^{0}C; IR (cm^{-1}): 2720 (NH Stretching), 2348 (CN Stretching), 826 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.38-7.40 (m, 3H,benzene); 7.64 -7.66 (m, 3H, benzene) 8.08-8.12 (m, 2H, benzene), 8.90 (s, 1H, isothiazole), 4.24 (s, 2H, aromatic NH_2); Mass (m/z): 226.91 (M+1); Anal. Calcd for $C_{13}H_{10}N_2S$: C: 69.10; H: 4.45; N: 12.38; S: 14.17; Found: C: 69.08; H: 4.42; N: 12.40; S: 14.20.

Compound n : Yield: 66%; M.W: 245.73; M.P: 260.87 ^{0}C; IR (cm^{-1}): 2732 (NH Stretching), 2344 (CN Stretching), 834 (C=C Stretching); ^{1}H NMR ($CDCl_3$): 7.40-7.420 (m, 3H, benzene); 7.72 -7.74 (m, 3H, benzene)8.1-8.14 (m, 2H, benzene), 8.94 (s, 1H, isothiazole); Mass (m/z): 247.48 (M+1); Anal. Calcd for $C_{13}H_8ClNS$: C: 63.54; H: 3.28; Cl: 14.43; N: 5.07; S: 11.48; Found: C: 60.21; H: 2.80; F: 20.39; N: 5.04; S: 13.05.

Pharmacological Evaluation:

Anti-inflammatory activity:

The synthesized compounds were screened for their anti-inflammatory activity using carrageenan-induced paw oedema method [22]. The experiment was performed on Albino rats, randomly divided into groups of six, one group was kept as control and received only 0.5% carboxymethyl cellulose (CMC) solution. The other groups received the test compounds and the standard drug. The rats were administered orally with the test compounds (100mg/kg), 100mg/kg Diclofenac sodium 1h before injection of 0.05mL of 1% suspension of Carrageenan into the sub plantar region of the rat hind paw. The volume of the injected paw was measured by water displacement in a plethysmometer before and after 3h of carrageenan treatment. Average oedema volumes of the rats for test group and positive control group were compared statistically with the vehicle control group and expressed as percent oedema inhibition, calculated by using the formula,

$$\text{Percentage oedema inhibition} = 100\ (1\text{-}V_t/\ V_c)$$

where, V_t = volume of oedema in treated group and V_c= volume of the oedema in the control group. Statistical significance of the results was tested by Anova and Dunnett's t-test.

Analgesic activity:

Analgesic activity was evaluated by acetic acid-induced writhing method in mice [23,24]. For this experiment, Swiss albino mice were divided into groups consisting of six animals each, weighing about 20-25 g, of either sex were used. Mice were kept individually in the test cage before the administration of the acetic acid injection and habituated for 30 min. The test groups were dosed with the test compounds and the standard group with ibuprofen, at a dose of 100 mg/kg po. All the compounds were dissolved in 1% CMC solution. One group was kept as control and received p.o 1%CMC. After 1 hr of drug administration, 0.1 ml of 1% acetic acid was given to mice intraperitoneally. In this test, the mice showed stretching movements involving arching of the back, elongation of the body and extension of hind limbs which were counted for 5-15 min after acetic acid injection.

Percent inhibition %= $100(1\text{-}W_t/W_c)$

Where, W_c no. of writhes for the control group, and W_t no. of writhes for the treated group. Statistical significance of the results was tested by Tukey-Kramer multiple comparisons test.

***In Silico* Evaluation/ Molecular docking:**

The molecular docking study plays a pivotal role in drug design process in predicting and establishing the predominant binding modes of the ligands or the target compounds with a specific protein of known three-dimensional structure. This also assists us in the interpretation of the structural features of the binding site, binding mode of analogues and in turn the key fragments in the title compound responsible for the interactions [25]. The crystal structure of COX-2 (PDB ID:5F19) of Homosapiens was retrieved from protein data bank (PDB). The protein preparation wizard to upgrade and underrate the protein structure by executing the OPLS force field until the RMSD constraint reached to 0.2 A^0. The bound water and ligands were excluded from the protein and the receptor grid for COX-2 active site was generated using XP- GLIDE. The 3D structure of the aforesaid compounds was drawn by employing chem3D ultra 12.0 software. The ligands and the standard drugs into the human COX-2 grid created to attain their binding affinities. The best pose per each ligand was selected and rest of the poses with RMS deviation less than $0.5A^0$ and atomic displacement less than $1.3A^0$ were rejected. The docking studies reveal the orientation and interactions of the ligand within the environment of target.

RESULTS AND DISCUSSION

Chemistry

The synthesis route of 6-phenyl benzoisothiazole and its derivatives is designed as shown in the figure 2. The title compounds **3**(**a-n**) were obtained by the reaction between 6-bromo benzoisothiazole (**1**) and substituted phenyl boronic acid**2(a-n).** The chemical structures of the synthesized compounds **3**(**a-n**) were established by IR, NMR and Mass spectroscopic techniques. the yield of the compound ranges from 61%-82%. The IR spectra of the compounds showed NH stretching in the region of 2728-2736 cm^{-1}, CN stretching at 2340 -2346 cm^{-1}, C=C stretching in the range of 828-836 cm^{-1}. ^1H NMR spectra of title compounds**3**(**a-n**) showed aromatic protons in the range of δ 7.36 -8.16, singlet proton due to isothiazole proton is observed around δ 8.94. The mass spectra of all the synthesized compounds **(3a - n)**are inaccordance with their molecular weights. Detailed spectral data is given in experimental protocols.

Pharmacological Evaluation

All the synthesized compounds **(3a-3n)** were subjected to anti-inflammatory and analgesic activity screening. The average percentage inhibition values of anti-inflammatory and analgesic activities obtained from the experimental results were enumerated in Table 1.

Compound	Anti-inflammatory Activity % of inhibition	Analgesic Activity % of inhibition	Docking Score
3a	55.36***	59.02***	-54.026
3b	52.99***	58.00***	-51.986
3c	52.12***	56.98***	-49.156
3d	61.83***	58.02***	-60.923
3e	54.04***	59.06***	-52.047
3f	51.49***	54.88***	-48.656
3g	51.03***	52.96***	-47.123
3h	59.65***	59.78***	-57.653.
3i	56.12***	56.48***	-55.065
3j	59.26***	55.06***	-56.656
3k	54.76***	55.01***	-53.789
3l	58.88***	60.89***	-56.567
3m	45.69***	54.12***	-46.659
3n	60.96***	61.88***	-59.223
Std drug Indomethacin	63.90 ***	NA	NA
Std drug Aspirin	NA	66.96***	NA
celecoxib	65.56 ***		-66.511

Significance Levels *p < 0.05, **p < 0.01 and ***p < 0.001 by Dunnet test. At 100 mg/ kg (p.o) edema volume was measured 3h after carrageenan and activity is presented as % inhibition of inflammation.

Anti-inflammatory Activity:

The acute anti-inflammatory activity of the synthesized compounds (3a – 3n) was determined following the carrageenan induced paw edema method in swiss albino rats against the standard drug indomethacin

at 100mg/kg p.o and the edema were measured after 3h. The experimental results revealed that majority of the compounds tested displayed moderate to good potential in anti-inflammatory screening when compared with the activity of the standard drug standard drug, Indomethacin. The compounds 3d, 3n and 3hdisplayed exceptional activity, with percentage inhibition of 61.83%, 60.96%and 59.65%respectively, that are almost in par with the percentage inhibition of standard drugwhich is at 62.70%. Compounds 3j, 3l, and 3i showed significant activity with 58.88%, 58.26%, and56. 16% inhibitory values, respectively. Compound 3m disclosed lowest inhibitory potential among all the compounds with average inhibition of 45.69%. From the results, it is apparent that phenyl ring substitution on the 6th position of benzimidazole ring might play an important role in the anti-inflammatory activity. Highest activity was observed with 4-nitro phenyl group (3f),4-fluoro phenyl (3n)and 4- chloro phenyl substitutions, followed by 4-trifluoromethyl phenyl (3j),4-hydroxy phenyl (3l), and 2- fluoro phenyl (3i) substitutions.

Analgesic activity:

Analgesic activity was evaluated by acetic acid-induced writhing method in mice and the activity of the test compounds was compared against the activity of standard drug. Results revealed that all the compounds disclosed moderate to excellent potential for analgesic activity in relation with the activity displayed by the standard drug Aspirin. Among the title compounds, majority of them significantly inhibited writhing compared to the control group treated with acetic acid alone. The compounds 3n, 3l, 3hand 3e displayed excellent activity with 61.88%, 60.89%, 59.78% and 59.06% inhibitions respectively, which were similar with the percentage inhibition displayed by standard drug, Aspirin(66.96%). The compounds 3aand 3dshowed notable analgesic activity with percentage inhibitions 59.02%, and 58.02% respectively. Highest analgesic activity was observed with 4-chlorophenyl substitution (3n) followed by 4-hydroxy(3l), 4- fluoro phenyl (3h)and 2-nitro phenyl(3e) substituents.

Molecular docking

Based on the molecular docking studies, the binding free energies obtained clearly outlines the affinity of the designed ligands with the COX-2 protein. Based on the score obtained, the compounds were graded, and this information can be used for further structural optimization and the results are tabulated in Table 1. When docked to COX-2 protein, the compounds **3n, 3l** found to exhibit the highest fitness score. Binding interactions of **3n,**were displayed in Figure **2**. Docking of standard drug, celecoxib with COX-2 displayed that amino acids SER 353, 533 were participating with polar interaction, ARG 513is critical for hydrogen bonding interactions and ALA516, 517, 518, MET522, VAL523,residues are instrumental in hydrophobic interaction at active site. The docking score for the designed compounds was in the range of -46.659 to -60.923 Kcal/mole, whereas for standard drug it was from -66.511 Kcal/mole. In the light of these observations, the title compounds have similar affinity and interactions as the standard drug. These findings give an idea not only of the level of anti-inflammatory activity but also of the structural components responsible for the interactions with COX-2's active site.

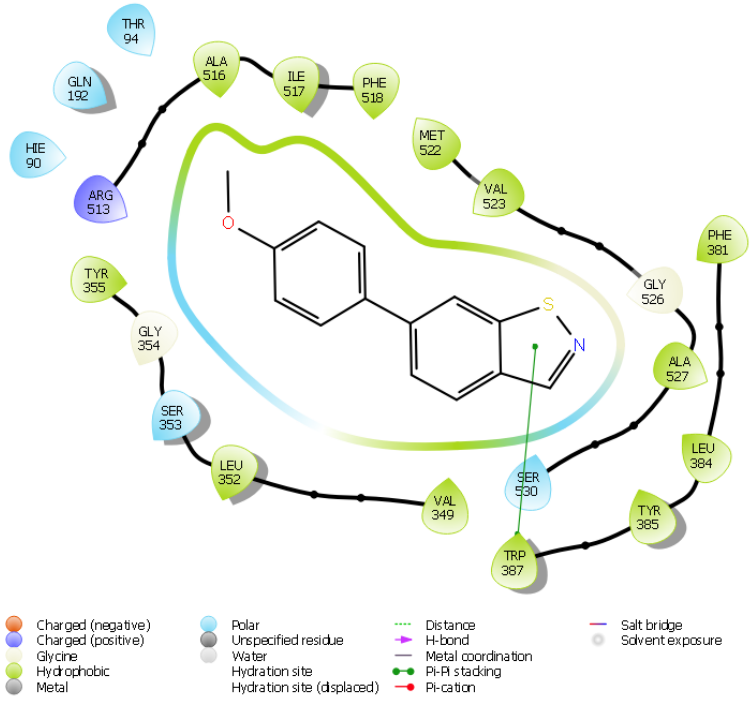


Figure 3. Docking interactions of the designed ligand 3b with the cox 2 receptor

CONCLUSION

The synthesis of novel 6-phenyl benzoisothiazole derivatives was performed and structures of the compounds were established by means of IR, ^{1}NMR and mass spectral data as well as elemental analysis. All the compounds were screened for antiinflammatory and analgesic activities. All the tested compounds elicited moderate to strong anti-inflammatory and analgesic activities. In addition to animal experiments, molecular docking studies was performed to depict the essential structural components contributing to their biological activity.Compound **3d** with 4-nitrophenyl substituent exhibited potent anti-inflammatory activity when compared with the standard drug indomethacin and celecoxib. The designed ligands **3n**, **3h** and **3j** also demonstrated maximum antiinflammatory activity. When the title compounds are screened for analgesic activity, compounds **3n** with 4- chlorophenyl, **3l** with 4-hydroxyphenyl and **3f** with 4-flouro substituents showed significant analgesic activity when compared to the standard drug, aspirin. From the present study, it may be concluded that the isothiazole derivatives can potentially be developed into potent antiinflammatory and analgesic agents and prompt future researchers to develop a series of novel isothiazole derivatives with wide variety of substituents with the goal of obtaining novel heterocyclic systems with enhanced activity.

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CITATION OF THIS ARTICLE

K. Varalakshmi Devi, Galla Rajitha. Design, Synthesis, And Molecular Docking Studies of Novel Isothiazole Derivatives as Potent Anti-inflammatory and Analgesic Agents. Bull. Env. Pharmacol. Life Sci., Vol 9[12] November 2020 : 52-59