Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Design, Synthesis, Biological and Docking Studies of Novel 6-fluorobenzothiazole Substituted 1,2,4-Triazole Analogues as Prospective Anti-inflammatory Agent

ShaikKhadarYazdan[a], Kurni Lakshmi Deepthi[b], Laxmi Devi BN[c], Sajida Afreen[d], Subramanyam Sibbala[e], Banothu Bhadru[f], Madhu Gudipatii[g], Vijay Kishore Kanakaraju[h], NareshPodila[*]

[a]Department of Pharmaceutical Sciences, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, Andhra Pradesh, India

[b]Department of Pharmaceutical Chemistry, Sreedattha Institute of Pharmacy, Sheriguda, Ibrahimpatnam, Hyderabad, Telangana, India

[c]Department of Pharmaceutical Chemistry, Joginapally B.R. Pharmacy College, Yenkapally, Moinabad, Hyderabad, Telangana, India

[d]Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad, Telangana, India

[f]Department of Pharmaceutical Analysis, CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India

[g]Department of Pharmaceutics, KITS College of Pharmacy for Women, Divili, Tirupathi (V), Peddapuram (M), Kakinada (Dt)-533 433, Andhra Pradesh

[h]Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

[e,I] DepartmentofPharmaceutical Sciences,Vignan's Foundation for Science, Technology and Research, Guntur, Andhra Pradesh, India

Abstract

The Part of the body's immunological reaction to an external stimulus is inflammation. It is helpful initially because it starts with the mending process. It is concerning, though, because inflammation has the ability to self-replicate, causing new inflammation to arise in reaction to pre-existing inflammation. In this study, a novel series of 6-Fluoro Benzothiazole substituted 1,2,4-Triazole derivatives were synthesized as prospective anti-inflammatory agents. The newly created substances were examined using mass spectrometry, ^{1}H-NMR, ^{13}C-NMR, and FTIR. First, the compounds underwent rigorous in vivo testing for acute toxicity and anti-inflammatory activity.To further validate these findings, an in silico docking study was carried out against COX-2 (PDB ID: 1pxx). The in vivo results revealed that three compounds-**TZ9**, **TZ2**, and **TZ1**, displayed no acute toxicity and significant anti-inflammatory activity, surpassing the efficacy of the standard drug, diclofenac sodium. Notably, **TZ9**, which featured diphenyl amino substitution, emerged as the most potent anti-inflammatory agent among the screened compounds. The computational analysis demonstrated that **TZ9**, and **TZ2**, exhibited substantial binding affinity, with the highest binding energies (-11.6 and -10.2, Kcal/mol) compared to diclofenac (-8.4 Kcal/mol). This alignment between in vivo and in silico data supported the robust anti-inflammatory potential of these derivatives. According to this work, the anti-inflammatory action of benzothiazole substituted with diphenyl amine, tyrosine, ortho phenylene diamine and 4-amino benzoic acid at the seventh position is enhanced. The synthesised compounds were also characterised by solubility, TLC, analytical data, IR, ^{1}HNMR, and mass spectrum examinations.

Keywords:Benzothiazole, COX-2, Anti-inflammatory activity, Binding affinity, Diclofen

1. Introduction

Inflammation is a common feature of both acute and chronic illnesses, such as lupus and rheumatoid

*Corresponding author e-mail:nareshtrcp10@gmail.com (Naresh Podila)
EJCHEM use only: Received date 18 March 2024; revised date 18 June 2024;accepted date 19 July 2024
DOI: 10.21608/ejchem.2024.277247.9460

arthritis, cardiovascular diseases, neurological illnesses including Alzheimer's disease, and several cancers [1]. The creation of novel anti-inflammatory medications may offer more potent therapies for various illnesses, enhancing the prognosis and quality of life of affected individuals [2]. When taken over an extended period of time, existing anti-inflammatory medications such corticosteroids and nonsteroidal anti-inflammatory medicines (NSAIDs) can have serious side effects [3-5]. By creating new drugs with better safety profiles, these side effects can be lessened and patients' treatments can be more bearable. Researchers have been able to pinpoint particular inflammatory pathways and molecules implicated in a variety of diseases because to advancements in molecular biology and genetics.By focusing on these particular pathways, new anti-inflammatory drugs can be created that provide more accurate and tailored treatments with fewer side effects [6]. Treatment for chronic inflammatory diseases is frequently ongoing. Reducing the frequency of hospitalizations and interventions and improving disease management are two benefits of developing new anti-inflammatory drugs with improved efficacy and tolerability.

Advantageous heterocyclic rings with atoms of sulfur and nitrogen are essential tools in the search for new medications, such as anti-inflammatory ones. Several research published in scientific journals have demonstrated that artificial chemicals with heterocyclic structures can reduce acute and chronic inflammation and may even aid in healing. Because of their biological activity, target selectivity, structural variety, and therapeutic relevance, they are useful building blocks for developing safer and more effective therapies for inflammatory disorders [7-8]. The 6-fluoro-benzothiazole ring is one of the scaffolds that medicinal chemists frequently utilize to construct and alter compounds with desired pharmacological properties, among other things.This approach enables the development of novel drugs and the improvement of existing ones. Consequently, the 6-fluoro-bezothiazole ring has become a promising heteroaryl ring to be considered for the creation and manufacturing of new compounds with noteworthy biological properties. Due to their low bioavailability, the majority of new bioactive compounds are never commercialized. By lengthening the molecules' in vivo lifetime and boosting tissue bioavailability, the addition of fluorine to the molecule enhances its lipophilicity [9] and inhibits metabolic detoxification [10].

The incorporation of the chlorine group of the 6-fluoro benzothiazole moiety into drug molecules, for example, has the benefit of acting as a Bioisosteres for the benzene ring. The substitution of this group with diphenyl amine, tyrosine, ortho phenylene diamine, 4-amino benzoic acid, anisidine, morpholine, and pyrrolidine will alter the physicochemical properties, affecting the interactions between the drug and the receptor[11]. Additionally, benzothiazole derivatives are a subject of ongoing study in several areas of chemical research, such as polymer chemistry, dyes, and medications[12]. A wide range of biological activities, such as antituberculosis, antiproliferative, antibacterial, anthelmintic, antioxidant, and antimicrobial properties, have led to the patenting of numerous benzothiazoles [13, 14]. Benzothiazole scaffolds are also found in several medications, such as2-amino-6-trifluoromethoxybenzothiazole (Riluzole), which is used to treat amyotrophic lateral sclerosis, and 2-thiocyanomethylthio-benzothiazole (TCMTB-60), an antimicrobial agent; 1- (6-methoxy-2-benzothiazolyl) -3-phenyl urea (frentizole), a fungicide also used to prevent fungal attacks on the skin; 2-(4-amino-3-methylphenyl) benzothiazole, an anticancer agent; and ethoxzolamide, a diuretic used to treat glaucoma and duodenal ulcers [15]. Moreover, numerous benzothiazole compounds are presently undergoing varying phases of clinical testing **Figure 1** [16].

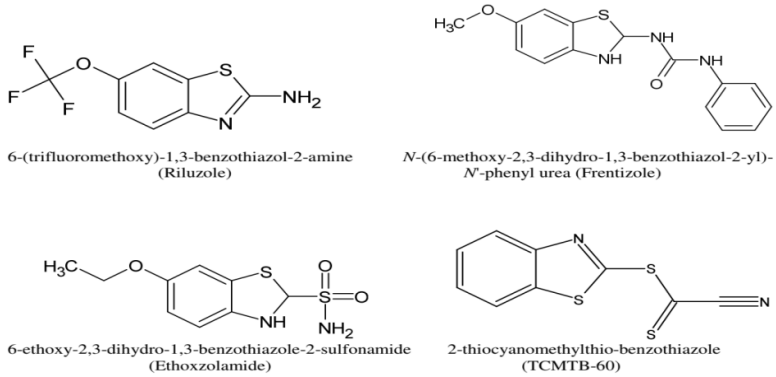
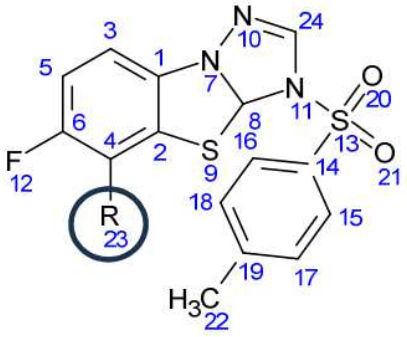


Figure 1: Structures of selected drug molecules decorated with benzothiazole substituted with 1,2,4-triazole scaffolds

Moreover, in the recent past, the tested anti-inflammatory lead compounds were decorated with 6-Fluoro benzothiazole and triazole rings [17-22]. In view of the above facts, we contemplated that incorporating diphenyl amine, ortho phenyl diamine, pyrrolidine, diethyl amine, tyrosine, phenyl ethyl amine, and morpholine groups at 7th position of the chlorine moiety and 1,3,4-thiadiazole nucleus into the same molecule will help in the development of new class of anti-inflammatory agents. Hence, we designed, synthesized and tested a series of novel linked 6-Fluoro benzothiazole substituted 1,2,4-triazole **Figure 2**.



R- Diphenyl amine, Ortho phenyl diamine, Pyrrolidine, Diethyl amine, Tyrosine, Phenyl ethyl amine, Morpholine

Figure 2: 6-fluoro-triazolo-benzothiazolescaffold

2. Experimental

2.1. MaterialsandMethods

The produced compounds were characterised using the following experimental techniques. The synthesised compounds' uncorrected melting points were measured in open capillary tubes. Using potassium bromide pellets, the ELICIO FTIR spectrometer recorded the infrared spectrum. Using a BRUKER Av 400 spectrometer, the ^{1}H-NMR spectra of the chemicals in deutiriated dimethylsulfoxide were recorded. The mass spectrum was captured using the Shimadzu GCMS QP 5000. Utilising precoated aluminium plates coated with silica gel GF254 [E. Merck], thin layer chromatography was carried out. N-hexane: theeluent employed was ethylacetate. In the chamber with ultraviolet light, the spots were visible.

2.2. Chemistry

Synthesis of 2-amino-6-fluoro-7-chloro (1, 3) Benzothiazole[17]

1.45gm (0.01 mol) of 3-chloro 4-fluoroaniline and 8gm (0.08 mol) of potassium thiocyanate were added to 20ml of glacial acetic acid that had been chilled below room temperature. A dropping funnel was used to add 1.6 ml of bromine to 6 ml of glacial acetic acid at a rate that ensured the mixture never heated above room temperature. The liquid was then placed in a water bath and agitated with a magnetic stirrer. After adding all of the bromine, the mixture was shaken for ten hours at a temperature below room temperature. After standing overnight, during which time an orange precipitate settled at the bottom, water (6 ml) was rapidly added, the slurry was heated at 850°C, and it was filtered hot. 10mlof glacial acetic acid, heated at 850°C, were added to the orange residue in a reaction flask before it was filtered while still hot. After cooling and neutralizing the combined filtrate with an ammonia solution to a pH of 6.0, a dark yellow precipitate was recovered. Recrystalised from benzene, ethanol of (1:1) after treatment with animal charcoal gave yellow crystals of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole. After drying in an oven at 80^{0}C, the dry material (1gm 51.02%) melted at 210–212°C (**Figure 3**).

Synthesis of 7- chloro- 6- fluoro-2-hydrazinyl- 1, 3- Benzothiazole[17]

In 500ml of round bottom flask and added 10 ml of concentrated hydrochloric acid was added drop wise with stirring of hydrazine hydrate 12 ml(0.02mol) at 5-10°C drop wise cool the mixture and add 20.2gm (0.1 mol) of 7- chloro- 6- fluoro 2-amino benzothiazole i.e. slowly added then added 40ml to 60ml of ethylene glycol and the resulting mixture refluxed for 3hrs and poured into crushed ice then residue settle down to the beaker then filter the product and dry the product and recrystalised from ethanol.

Synthesis of 8-chloro-7-fluoro-1,9a-dihydro [1,2,4] triazolo [3,4-b] [1,3] Benzothiazole[17]

The mixture of 0.01mol, or 2.19gm of 7-chloro-6-fluoro-2-hydrazinyl-1, 3-benzothiazole and 1gm of anhydrous potassium carbonate, was added to a 250 ml round-bottom flask. The mixture was then refluxed with formic acid for two hours and poured into crushed ice, where the residue settled down to a beaker. The product was then filtered and dried to separate it from the ethanol to obtain the pure final product.

Synthesis of 8-chloro-7-fluoro-1-[4-methylphenyl] sulphonyl-1,9a-dihydro [1,2,4] triazolo [3,4-b] [1,3] Benzothiazole[17]

8-chloro-7-fluoro-1,9a-dihydrol (0.013mol), or 2.2gm, was added to a 500ml round-bottom flask [1, 2, 4] triazole [3,4-b] [1,3]. The final product was obtained by treating benzothiazole with 0.01mol, or 1.71gm, of p-toluene sulphonamide in the presence of pyridine (1-2ml), refluxing the mixture for two hours, and then pouring the mixture into crushed ice. The residue settled and the product was drained and recrystallized from ethanol. [4-methylphenyl]-8-chloro-7-fluoro-1-1,2,4sulphonyl-1,9a-dihydrotriazolo[3,4-b] Benzothiazole [1,3].

Synthesis of 8-chloro-7-fluoro-1-[4-methylphenyl] sulphonyl-1,9a-dihydro [1,2,4] triazolo [3,4-b] [1,3] Benzothiazole derivatives (TZ 1-9)[17]

Equimolar amounts of different primary and secondary aromatic amines were added to a 100ml round-bottom flask containing 2.7gm(0.14988 moles) of 8-chloro-7-fluoro-1-[4-methylphenylsulphonyl-1,9 dihydro [1,2,4] triazolo [3,4b] [1,3]benzothiazole. The mixture was refluxed for two hours while N, N'-dimethyl formamide (DMF) was present. After cooling, the mixture was added to crushed ice. Using a pinch of activated charcoal, the separated solid was filtered out, dried, and recrystallized from alcohol and benzene.

2-amino-6-fluoro-7-chloro (1, 3) Benzothiazole,mf:$C_7H_{14}ClFN_2S$; mw: 202.63 Da;yield: 94%; Brown crystalline; mp: 214°C;R_f = 0.95 (EtOAc: n-hexane), FTIR (KBr, cm^{-1}): 667.22, 827.01, 1021.14, 1635.21, 1227.12; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 5.99 (2H NH_2), 7.27 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 114.0, 116.2, 122.3, 125.7, 145.8, 158.3, 166.1; MS m/z: 203.12 (M^{+1}).

7-chloro-6-fluoro-2-hydrazinyl-1,3-Benzothiazole, m. f: $C_7H_5ClFN_3S$,mw: 219.35 Da; yield: 78%; yellow crystalline; mp: 242°C, mw: 219.63; R_f = 0.78 (n-hexane: EtOAc); FTIR (KBr, cm^{-1}): 682.02, 798.21, 1048.34, 1662.38, 1214.75; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 3.80 (1H, NH), 3.24 (s, 2H, NH_2), 7.37-7.98 (m, 2H, aromatic); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 114.5, 117.8, 121.5, 123.6, 145.8, 173.2; m/z: 219.35.

8-chloro-7-fluoro-1,9a-dihydrol [1, 2, 4] triazole [3, 4-b] [1, 3] Benzothiazole,mf: $C_8H_5ClFN_3S$; mw: 229.65 Da; Yield: 42%; brown solid; mp: 284-286°C;R_f = 0.75, (n-hexane: EtOAc); FTIR (KBr, cm^{-1}): 714.82, 1064.62, 1321.01, 1589.97; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 7.0 (1H, CH), 7.33-8.04 (m, 2H, Ar); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 67.8, 112.8, 113.6, 118.2, 121.8, 133.7, 152.2, 154.8; MS m/z: 229.87.

8-chloro-7- fluoro- 1-tosyl-1,9a-dihydro Benzo [4,5]thiazolo [2, 3-c] [1, 2, 4] triazole, mf: $C_{15}H_{11}ClFN_3O_2S_2$, mw: 383.85 Da; Yield: 80%; Green solid; mp: 304-306°C; R_f = 0.91 (EtOAc: n-hexane); FTIR (KBr, cm^{-1}): 699.54, 1144.35, 1381.24, 1681.84; 2191.38; ^{1}H- NMR (DMSO, 300 MHz) δ ppm: 7.81-7.6 (m, 6H, Ar), 7.03-7.38 (m, 2H, Ar), 3.02 (m, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 25.3, 58.9, 1135, 114.1, 118.2, 120.2, 125.4, 127.8, 129.6, 129.9, 133.7, 142.8, 151.8, 154.2; MS m/z: 383.14.

6-fluoro-N, N-diphenyl-3,3a-dihydrobenzo [4,5] thiazolo [3,2-b] [1,2,4] triiazol-5-amine (1), mf: $C_{21}H_{21}FN_5O_2S_2$; mw: 458.27 Da; Yield: 83%; white powder; mp: 114-116°C;R_f = 0.68 (EtOAc: n-Butanol: $CHCl_3$ 2:1:1); FTIR (KBr, cm^{-1}): 1272.18, 1427.50, 1662.45, 1044.24, 1438.87, 1107.51, 1184. 31; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 6.44-6.67 (m, 10H, Ar),7.45-7.68 (m, 4H ArPh) 7.29-7.8 (s, 1H, CH), 4.14 (s, 2H, NH_2) 3.01 (m, 3H, CH_3), 8.17 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 25.4, 58.3, 103.7, 112.7, 115.2, 119.4, 123.1, 124.2, 124.9, 126.5, 126.8, 128.2, 129.1, 129.4, 129.9, 131.2, 133.5, 137.8, 140.5, 148.1, 152.3; MS m/z: 457.50.

6-fluoro-3-tosyl-3,3a-dihydrobenzo [4,5] thiazolo [3,2-b] [1,2,4] triaiazol-5-yl)amino)-3-(4-hydroxyphenyl) propanoic acid (2),mf: $C_{24}H_{21}FN_4O_5S_2$, mw: 527.12 Da; Yield: 68%, Brown solid, mp: 120°C;R_f = 0.72 (EtOAc: n-Bu: $CHCl_3$ 2:1:1); FTIR (KBr, cm^{-1}): 1346.52, 1137.41, 1380.21, 1145.42, 1156.53, 1525.04, 1588.28; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 6.13-6.57 (m, 12H, Ar), 9.27 (s, 1H, NH), 1.89 (m, 3H, CH_3) 2.18(s, 1H, CH), 2.98 (s, 2H, CH_2), 9.65, 12.90 (d, 2H, OH); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 26.5, 34.6, 61.5, 66.4, 67.3, 83.5, 101.3, 103.6, 111.2, 115.1, 116.9, 126.1, 128.6, 129.1, 129.4, 130.2, 132.5, 134.6, 135.7, 140.8, 141.5, 152.8, 156.2, 173.4 MS m/z: 527.58.

6-fluoro-3-tosyl-3, 3a-dihydrobenzo [4,5] thiazolo [3,2-b] [1,2,4] triazol-5-yl) benzene-1,2-diamine (3),mf: $C_{21}H_{18}FN_5O_2S_2$, mw: 457.80 Da; Yield: 75.5%; orange solid; mp: 167°C;Rf= 0.77 ($CHCl_3$: n-Bu: EtOAc 1:2:1); FTIR (KBr, cm^{-1}): 1019.26, 1152.86, 1224.26, 1278.17, 1478.40, 1530.71, 1620.37; ^{1}HNMR (300 MHz) DMSO)) δ ppm: 7.04 -7.19 (m, 8H, Ar), 9.08 (s, 1H, NH), 2.50 (m, 3H, CH_3) 2.99 (s, 2H, CH_2), 9.70, 10.98 (d, 2H, OH), 3.21 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 26.6, 61.4, 101.2, 111.4, 113.7, 115.4, 116.9, 121.2, 122.6, 125.2, 127.7, 128.3, 129.5, 129.9, 131.3. 134.7, 142.5, 149.7, 149.6, 153.6, 161.6; MS m/z: 457.15.

N-(4-methoxy phenyl amino)-6-fluoro-3-[(4-methyl phenyl) sulfonyl]-3, 3a-dihydro [1, 2, 4] triazolo [5, 1-b] [1, 3] benzothiazol-5-amine (4), mf: $C_{22}H_{19}FN_4O_3S_2$, mw: 469.31 Da; Yield: 68.6%; pink solid; mp: 178°C;R_f = 0.62 ($CHCl_3$: n-But: EtOAc: 1:2:1); FTIR (KBr, cm^{-1}): 1095.53, 1121.68, 1128.12, 1301.51, 1465.24, 1528.07, 1587.46. ^{1}H-NMR (DMSO, 300 MHz)) δ ppm: 7.02 -7.46 (m, 10H, Ar), 9.21 (s, 1H, NH), 2.98 (m, 3H, CH_3), 2.92 (s, 2H, CH_2), 3.27, 2.45 (s, 2H, CH_2); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 25.7, 56.2, 62.5, 106.8, 110.7, 114.1, 114.8, 115.2, 120.5, 120.9, 125.3, 126.9, 128.6, 129.3, 129.9, 130.5, 132.3, 140.5, 148.3, 149.8, 153.8, 155.1; MS m/z: 469.25.

6-fluoro-3-[(4-methyl phenyl) sulfonyl]-morphonyl-3, 3a-dihydro [1, 2, 4] triazolo [5,1-b] [1, 3] benzothiazol-5-amine (5),mf: $C_{19}H_{20}FN_5O_3S_2$, mw: 448.81 Da; Yield: 71.14%, milkfish; mp: 175°C;; R_f = 0.75 (EtOAc:n-Bu1: $CHCl_3$: 2:1:1); FTIR (KBr, cm^{-1}): 1195.14, 1444.14, 1659.07, 1135.35, 1500.28, 1181.44, 1221.04; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 7.45-7.68 (m, 5H, Ar), 1.89 (m, 3H, CH_3) 3.18 (s, 2H, CH_2), 3.01-3.47 (m, 4H, CH_2); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 23.4, 42.3, 54.3, 56.2, 62.4, 65.8, 67.2, 105.4, 107.3, 113.5, 123.3, 125.3, 127.5, 127.1, 128.9, 130.5, 132.4, 141.2, 143.3; MS m/z: 448.12.

6-fluoro- (4-pyrrolidinyl) 3-[(4-methyl phenyl) sulphonyl]-3, 3a-dihydro [1, 2, 4] triazolo [5, 1-b] [1, 3] benzthiazol-5-amine (6),mf: $C_{19}H_{19}FN_4O_2S_2$, mw: 421.20 Da; Yield: 63.8%; cream; mp: 184°C;R_f = 0.74 (EtOAc: n-But:$CHCl_3$ 2:1:1); FTIR (KBr, cm^{-1}): 1304.00, 1548.21, 1621.08, 1078.51, 1490.09, 1142.71, 1212.10; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 6.42-6.59 (m, 6H, Ar), 3.00 (m, 3H, CH_3) 1.99 (m, 2H, CH_2), 2.96-3.47 (m, 4H, CH_2); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm:23.5, 24.8, 25.3, 50.5, 51.4, 60.4, 104.7, 104.8, 105.4, 112.3, 126.3, 126.9, 127.8, 129.3, 129.8, 132.5, 136.2, 140.5, 142.4; MS m/z: 420.27.

6-fluoro-N-diethyl amino-3-[(4-methyl phenyl) sulfonyl]-3, 3a-dihydro [1, 2, 4] triazolo [5, 1- b] [1, 3] benzothiazol-5-amine (7),mf: $C_{19}H_{21}FN_4O_2S_2$, mw: 428.28 Da; Yield: 68.5%, blue; mp: 112°C; R_f = 0.46 (EtOAc : n-But: $CHCl_3$ 2:1:1);); FTIR (KBr, cm^{-1}): 1298.21, 1531.27, 1658.21, 1115.37, 1534.42, 1092.00, 1272.28; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 7.14 -7.52 (m, 5H, Ar), 2.00 - 2.94 (m, 6H, CH_3) 2.97 (m, 4H, CH_2); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 22.50, 42.2, 50.2, 53.5, 62.4, 63.5, 65.8, 102.3, 105.3, 113.8, 121.9, 123.4, 125.3, 127.3, 128.8, 130.1, 132.6, 141.3, 143.8; MS m/z: 428.02.

1-[6-fluoro-7- (4-phenethyl amino)-3-[4-methyl phenyl] sulphonyl]-3, 3a dihydro [1, 2, 4] triazole [5, 1-b] [1, 3] benzothiazole (8),mf: $C_{23}H_{21}FN_4O_2S_2$, mw: 481.26 Da; Yield: 70.9%; green; mp: 134°C; R_f = 0.68 (EtOAc: n-But: $CHCl_3$: 2:1:1); FTIR (KBr, cm^{-1}): 1300.14, 1512.21, 1601.20, 1099.17, 1428.04, 1202.21, 1241.54; ^{1}H-NMR (DMSO, 300 MHz) δ ppm: 7.21-7.40 (m, 11H, Ar), 3.15-3.59 (m, 4H, CH_2) 7.08 (s, H, NH) 3.51 (m, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 20.4, 22.3, 41.0, 51.5, 60.4, 65.6, 69.1, 101.2, 104.8, 111.2, 115.3, 121.3, 126.5, 127.3, 128.5, 129.9, 130.1, 131.0, 133.5, 134.2, 141.6, 143.2, 147.6; MS m/z: 480.75.

6-fluoro-3-[(4-methyl phenyl) sulfonyl]-5(naph thayl amino)-3, 3a-dihydro [1, 2, 4] triazolo [5, 1-b] [1, 3] Benzothiazole (9),mf: $C_{25}H_{19}FN_4O_2S_2$, mw: 475.70 Da; Yield: 60.02%; violet; mp: 172°C;R_f = 0.83 (EtOAc: n-Bul: $CHCl_3$: 2:1:1); FTIR (KBr, cm^{-1}): 1301.24, 1569.72, 1623.14, 1081.09, 1437.14, 1327.28, 1182.27; ^{1}H-NMR (DMSO, 300 MHz)) δ ppm: 7.03-7.63 (m, 6H, aromatic), 3.23-3.47 (m, 4H, CH_2) 9.38 (s, 4H, NH) 2.52 (m, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ ppm: 23.2, 25.8, 26.2, 27.5, 41.8 43.2, 55.6, 65.3, 82.8, 101.8, 111.4, 115.3, 119.5, 121.3, 127.6, 127.2, 128.6, 129.1, 129.3, 130.2, 131.2, 131.9, 132.7, 133.3, 140.27; MS m/z: 475.30.

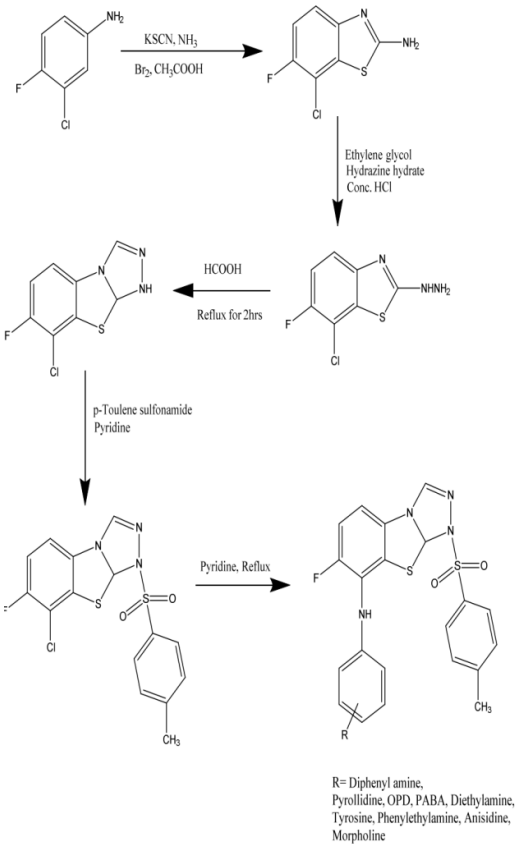


Figure 3: Route of synthesis 6-fluorobenzothiazole analogues

2.3. Evaluation of Anti-inflammatory Activity:

2.3.1. Acute oral toxicity

Following the OECD 423 recommendations, we employed the acute toxic class approach to assess the toxicity of the target chemicals (TZ1-TZ9) in the current study. Swiss albino rats weighing between 200 and 250 grams were used in this investigation. To evaluate the toxicity of the synthesized compounds, three rats of the same gender were included in each group. A starting dose of 200 mg/kg body weight was given. Before receiving any medication, female rats were fasted for three to four hours without food or water. The animals were weighed after the fasting period, and 200 mg of the synthetic derivatives were administered orally for every kilogram of body weight [23]. Following the delivery of the test chemical, the animals were closely monitored for the first four hours at 30-minute intervals, and then every hour for up to twenty-four hours. For a total of 14 days, the animals were observed every day, and during that time, every animal was carefully examined for any strange alterations in its biological, behavioural, or physical state [24].

2.3.2. Carrageenan–Induced Rat Paw Edema Model

We used male or female healthy rats weighing between 100 and 190 grams as subjects in the carrageenan-induced paw edema experiment [25]. Three sets of six test animals each were created out of these rats. The animals fasted for the whole night before the trial started, with access to water given as needed [26]. A normal saline solution was given to the control group (negative control), while NTD1 at a dosage of 20 mg/kg was given to the second group, NTD2 to the third group, NTD3 to the fourth group, and the standard medication Diclofenac sodium at a dosage of 4.5 mg/kg to the fifth group. Using an oral catheter, each test and standard material was given orally after being dissolved in normal saline [27]. A 0.2 mL solution of 1% w/v carrageenan was injected into the rats' right paw's sub plantar area in order to cause edema. The paw was inked up to the level of the lateral malleolus, and then it was immersed in mercury until the indicated point was reached. A Plethysmometer was then used to measure the amount of edema in the rat's paw [28]. Following carrageenan injection, the paw volumes of each group were measured immediately (at 0 hours) and then every hour for the next two hours. The difference between the first and final measurements was used to determine the actual increase in paw size. The average increase in paw size between medication-treated and untreated control rats was measured, and the percentage that edema development was suppressed was computed using a method [29].

$$\% \text{ inhibition} = \frac{1 - Vt}{Vc} \times 100$$

Where,

'Vc' represents edema volume in the control

'Vt' stands for the volume of edoema in the group receiving the test medication. The values are the mean +/- standard error of the mean (SEM) of six animals per group; $p<0.001$% inhibition indicates statistically significant differences from the control. Tuckey's test was run after an ANOVA was used for statistical analysis.

2.3.3. Molecular Docking:

Using the Autodock Vina module of PyRx 0.8 software, Chemoffice 2016 tools, and Discovery Studio 2020 software, Molecular Docking and simulation experiments were carried out [30–32]. It was possible to obtain the co-crystal protein structure via the Protein Data Bank (www.pdb.com or www.rcsb.com) [33]. Downloaded from the protein data bank (www.rcsb.com/www.pdb.com) is the target protein cyclooxygenase 2 (COX-2) X-ray crystal structure, co-crystallized with diclofenac (PDB ID: 1pxx). The protein was created by removing HET atoms, adding polar hydrogens, removing water molecules, and confirming that no amino acid residues were missing using the Discovery Studio Visualizer 2021 program. After that, the file was saved in pdb format. The target compounds' (2D) structures (TZ1–9) were drawn using Chemdraw Professional and saved as.pdb files. Using the macromolecule option in the PyRx Virtual screening program 0.8's Autodock tool, the protein pdb file was converted to pdbqt format. The ligand files were forced to field-off (minimize energy) using the Open Babel tool, which created the conformers (Autodock pdbqt files). The ligands and macromolecule (protein pdbt file) for the docking process (auto dock pdbqt files) were chosen using Vina Wizard. by drawing a grid on a piece of paper. By placing a grid box around the area where the co-crystal ligand exhibits amino acid iterations,
the protein's active binding site for docking ligands was found. The ligands with the lowest binding energies were the likely molecules having a high affinity for the target protein. The Discovery Studio Visualizer 2021 program was used to visualize the binding interactions [34, 35].

.Results and Discussion

3.1. Spectral Studies:

The peak in the infrared spectrum provides information about the compound's likely structure. The infrared spectrum spans 4000–6666 cm^{-1}. This spectrum's radiation quanta are related to the energy

differences between molecules' various vibrational states. Using the KBr pellet technique, the compounds were recorded on the SHIMADZU FTIR-8400Sspectrophotometer, which displays various molecular vibration levels.

The samples were examined using a 300MHz BRUKER spectrometer. We may learn about the various chemical and magnetic environments that protons in molecules relate to thanks to proton NMR spectra.

With extreme precision, the Relative molecular masses (also known as molecular weights) can be calculated using this precise molecular formula. Finding the locations in the molecule where fragmentation is preferred will allow us to infer the existence of identifiable groups. as a technique for detecting analytes by comparing their digitalized mass spectra with libraries of known substances. The title compound's mass spectra are stored on an LCMS. The following is a representation of the synthesized compounds' spectrum data.

3.2. Acute Oral Toxicity Study

The OECD guideline-423 approach was used to carry out the oral acute toxicity study. This methodology was developed to investigate compounds at fixed dosages and provides information for hazard evaluation as well as chemical ranking for hazard classification. A starting dose of 200 mg/kg body weight was administered by floating the synthesized compounds in acacia and water. The animals were observed for 14 days following the sample administration. Careful monitoring was done at least twice a day to check for any effects on the ANS, CNS, salivation, skin colour, motor activity, and other general toxicity indications. The compounds under title had an LD_{50} value of class 5, meaning that at 200 mg/kg body weight of animal weight, there was no evidence of toxicity. Based on data from toxicity studies, five of the nine synthesized compounds—TZ9, TZ2, TZ1, TZ3, and NTD8—were found to be well tolerated by the experimental animals and non-toxic at selected dose levels. Of the nine compounds, four derivatives—TZ5, TZ6, TZ7, and NTD8—were found to be toxic.

3.3. *Invitro* Anti-inflammatory Activity

Rat paw edema (Carrageenan-induced) was used to determine the synthetic compounds' anti-inflammatory efficacy. The activity was examined at doses of 20 mg/kg body weight, and the effects were timed at 0 minutes, 30 minutes, one hour, and 2 h. The results are given in **Table 1**. The anti-inflammatory effects of the four produced substances ranged from slight to strong. For up to 2 h, all three substances showed their peak action. The maximum activity was observed when R was substituted with phenyl, $-CH_3$, and phenyl carboxylate, according to the data from the synthesized compounds at a dose of 20 mg/kg body weight [36].

The anti-inflammatory activity of synthesized derivatives (**TZ1-TZ9**) compared with the standard drug diclofenac sodium is represented in **Figure 4** and the data suggests that these the three compounds have remarkable (p 0.01) anti-inflammatory activity. The high significant action was seen for the synthesized compounds at 60 and 120 minutes. This study demonstrates how the target compounds blocked prostaglandins, particularly during the biphasic response.

Table 1: Effect of selected derivatives on paw edema in rats

S. No.	Name	Paw edema volume (mm)			
		0 min	30 min	60 min	120 min
1	TZ9 (20 mg/kg)	0.56±0.03	0.43±0.02	0.41±0.01	0.21±0.01*
2	TZ2 (20 mg/kg)	0.82±0.08	0.71±0.04	0.40±0.02	0.31±0.01*
3	TZ1 (20 mg/kg)	0.50±0.02	0.41±0.01	0.35±0.02*	0.31±0.01*
4	TZ3 (20 mg/kg)	0.50±0.05	0.40±0.08	0.32±0.04*	0.32±0.02*
5	Diclofenac (4.5mg/kg)	0.51±0.01	0.41±0.01	0.30±0.00*	0.21±0.00*
6	Control	0.33±0.04	0.33±0.04	0.23±0.02	0.31±0.01

N=6, values are expressed in mean ± SEM, *p<0.05, Assessed using one-way ANOVA followed by Tukey's multiple comparison test

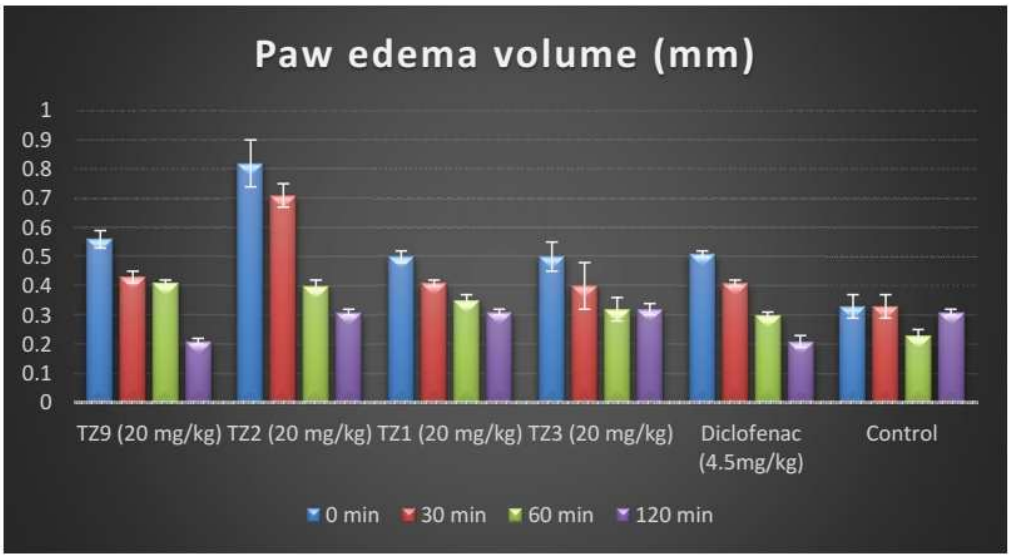


Figure 4: Bar diagram with mean and standard error of the mean at 0-2 hrs

Following carrageenan administration. In addition, these derivatives have the potential to selectively inhibit the cyclooxygenase enzyme. In **Figure 5** it is clearly demonstrated the difference in test animals with paw edema before and after treatment with the tested synthetic compounds.

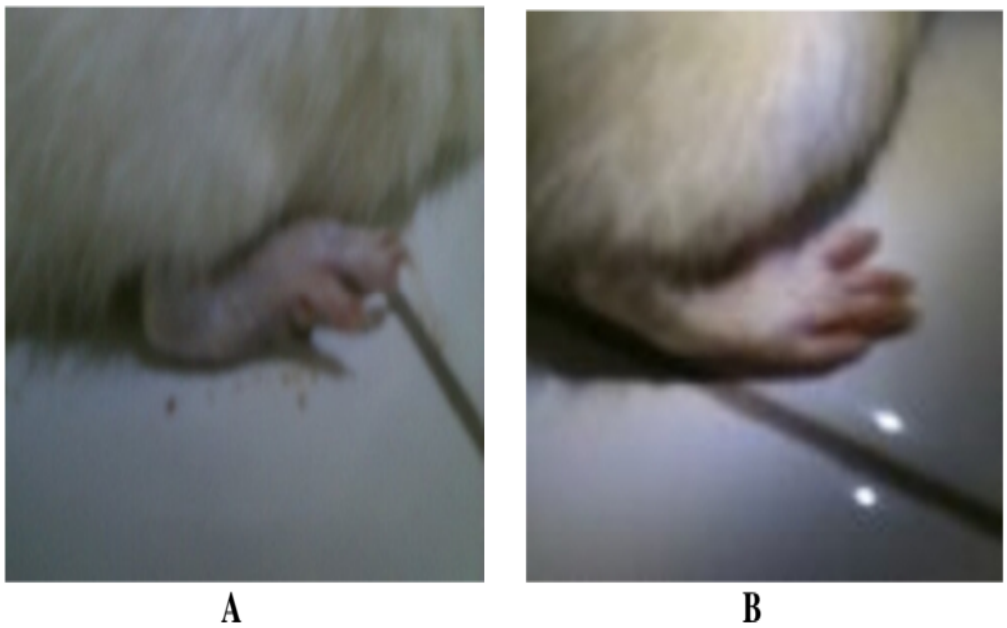


Figure 5: Anti-inflammatory activity in Wistar rats. A: Paw edema before treatment; B: Paw edema after treatment

3.4. Docking Assessment

Because it is essential for the synthesis of inflammatory mediators such as prostaglandins, the cyclooxygenase 2 (COX-2) protein was chosen as the target for in silico anti-inflammatory activity screening [37]. **Table 2** displays the findings of the

title derivatives' docking investigation against the chosen protein (PDB ID: 1pxx). In comparison to the co-crystal ligand diclofenac, which has a binding energy of -8.4 Kcal/mol, the derivatives TZ9 and TZ1 demonstrated substantial binding affinities with the least binding energies of -8.5 and -8.4 Kcal/mol, respectively, according to the data. Table 2 lists all of the compounds' binding energies. The derivatives, on the other hand, exhibit significant binding affinities by interacting with amino acid residues in the target protein's active site through different hydrogen and hydrophobic interactions given in **Table 3** and illustrated in **Figure 6**. These interactions occur because the derivatives have an aryl substitution (TZ9 having diphenyl amino) or a substituted aryl substitution (TZ1 having benzene 1,2-diamine substitution) on the thiadiazole ring. The *in-silico* results seem merely correlated with the *in vivo* rat paw edema test results which conforms to the validation of *in silico* docking results. Moreover, the research findings reported by Redzicka et al. [38-40] Soni et al.,and Veerasamy et al., reported that compounds with aryl or heteroaryl substitutions may have shown potent anti-inflammatory activity.

Table 2: Binding energies of the title derivatives against COX-2

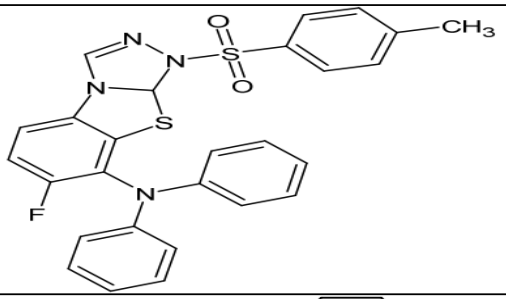
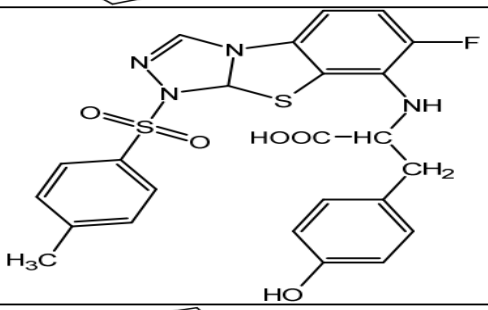
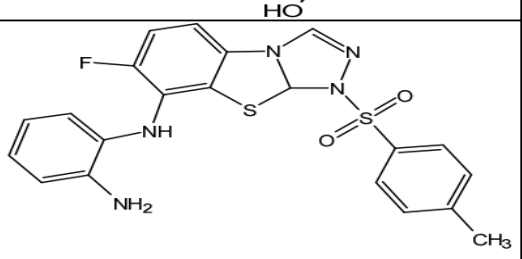
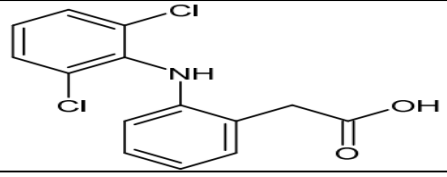
S. No	Compound Code	Chemical Structure	Binding Energy (Kcal/mol)
1	TZ 9		-11.6
2	TZ 2		-10.8
3	TZ 1		-10.2
4	Diclofenac		-8.4

Table 3: Interactions of the selected derivatives with Cox-2 protein active pocket residues

S.No.	Compound	Binding Energy (Kcal/mol)	The type of interaction		No. of bonds	Interacting amino acid residues
1	TZ9	-11.6	H-bond	Conventional H-bond	1	One between His207 and the fluorine atom of Benzothiazole
			Hydrophobic	Pi-Sigma	1	One between HEM682 and the Naphthalene-1-amine
				Pi-Sulfur	1	between His214 and the Sulfur atom of thiazine moiety
				Pi-Alkyl	2	One between Val291 and Benzthiazole, Another one between Leu294 and the Naphthalene ring
2	TZ2	-10.8	H-bond	Conventional	3	One between Gln461 and sulfur, Gly135 to carbonyl group another between Tyr136 and phenol
				Carbon-H bond	1	Between Asn39 and Sulphone group
			Hydrophobic	Pi-Alkyl	1	Between Leu152 and the Toluene ring, Another one between Met48 and Benzothiazole
				Alkyl	1	Between Pro153 and Benzene ring
3	TZ1	-10.2	H-bond	Conventional H Bond	2	One between Arg469and NH_2, Another between Arg44 and Sulphone
				Carbon H Bond	2	One between Arg469 and Sulphone and Another between Cys41 and Triazole ring
			Hydrophobic	Halogen	1	Between Asn43 and F atom
				Pi-Sigma	1	Between Lys468 and Benzothiazole
				Alkyl	1	Between Pro153 and Benzene ring
				Pi-Alkyl	1	Between Cys47 and CH_3
4	Diclofenac	-8.4	H-bond	Conventional	1	Between Ser530 and carboxylic acid hydrogen
			Hydrophobic	Pi-Sulphur	1	Between Met522 and the phenyl ring
				Pi-Pi Stacking	1	Between Trp387 and the phenyl ring
				Pi-Alkyl	3	Each one between Val349, Val523, Ala527

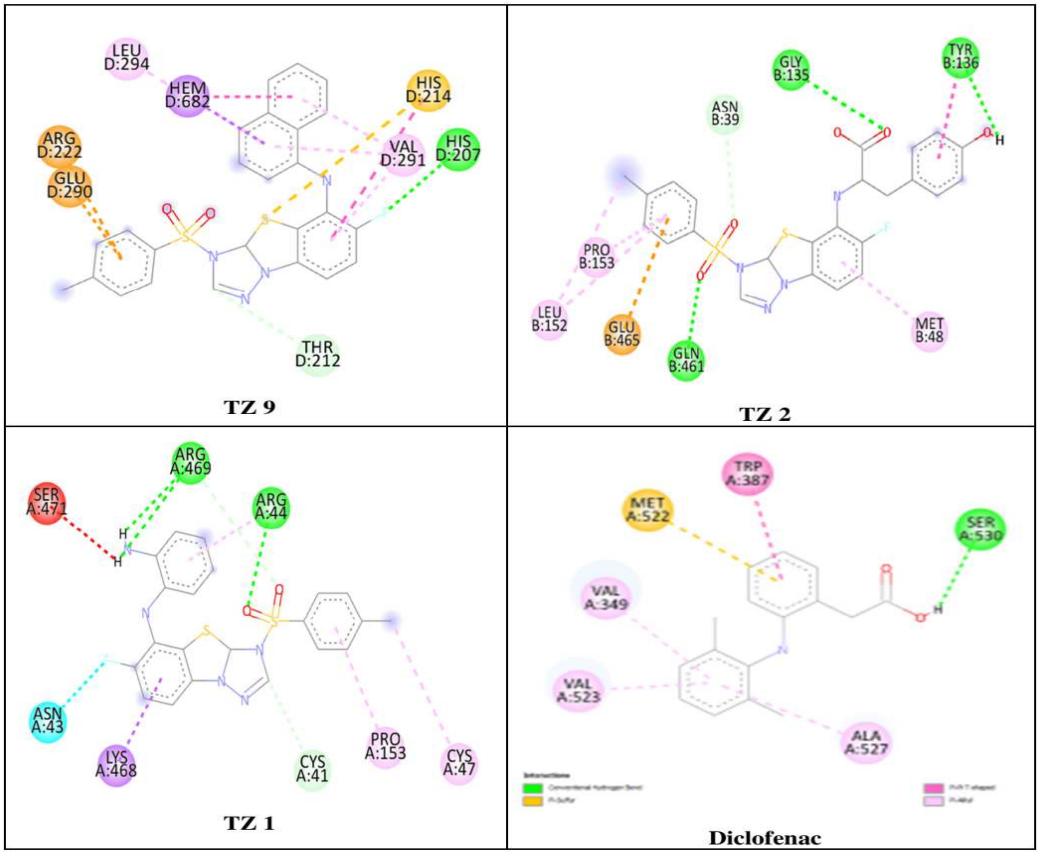


Figure 6: Interactions of selected derivatives (NTD2 and NTD3) with active pocket residues of COX-2

4. Conclusion

In conclusion, this study has successfully synthesized and characterized a series of Benzothiazole linked 1,3,4-thiadiazole derivatives (TZ1-TZ9), through a well-defined synthetic process. These derivatives were subjected to a comprehensive evaluation, encompassing both experimental and computational approaches, to assess their potential as anti-inflammatory agents. Notably, TZ9, featuring a benzoic acid substitution, emerged as a promising lead compound, demonstrating remarkable anti-inflammatory properties in both in vivo and in silico studies. Its binding affinity, stability, and interactions with the target protein COX-2 were found to be superior to the standard drug diclofenac sodium. Additionally, the safety profile of these derivatives was assessed, revealing that TZ9, TZ2, and TZ1 exhibited no toxicity concerns at the tested dose levels, while TZ5 and TZ7 displayed undesirable toxic effects. This highlights the need for careful consideration of toxicity in drug development. The findings from this research offer valuable insights into the potential of TZ9 as a lead compound for the development of anti-inflammatory drugs. However, further investigations are warranted to explore the structure-activity relationship and the precise mechanisms underlying the observed anti-inflammatory activity. Overall, this study providesfoundation for future work in the field of drug development and underscores the potential of TZ9 as a significant candidate in the search for more effective anti-inflammatory therapies.

Conflict of interest

There are no conflicts to declare.

Acknowledgement

The authors would like to thanks whole faculty members and management of the Vignan's Foundation for Science, Technology and Research, Vadlamudi, Guntur, for providing all necessary technical supports and motivating us at every stage.

References

[1] V. Jogpal, M. Sanduja, R. Dutt, V. Garg, Tinku, Advancement of Nanomedicines in Chronic Inflammatory Disorders, Inflammo pharma cology 30 (2022) 355–368.

[2] S.R. Feldman, L.S. Cox, L.C. Strowd, R.A. Gerber, S. Faulkner, D. Sierka, T.W. Smith, J.C. Cappelleri, M.E. Levenberg, The challenge of managing atopic dermatitis in the United States,Am. Health Drug Benefits12(2) (2019) 83.

[3] C. Cadet, E. Maheu, Non-Steroidal Anti-Inflammatory Drugs in the Pharmacological Management of Osteoarthritis in the Very Old: Prescribe or Proscribe,Therapeutic Advances in Musculoskeletal Disease13 (2021) 1759720X2110221.

[4] S. Drożdżal, K. Lechowicz, B. Szostak, J. Rosik, K. Kotfis, A. Machoy-Mokrzyńska, M. Bielecki, K. Ciechanowski, B. Gawrońska-Szklarz, Kidney Damage from Nonsteroidal Anti-Inflammatory Drugs—Myth or Truth? Review of Selected Literature, Pharmacology Research & Perspectives9 (4) (2021).

[5] P. Naresh, R. Mithun, S. Subramanyam, R. Atul, R. Bendale, P. Yanadaiah, R.B. Molakpogu, M. Kiran, D.C. Renzon, Sreelatha Muddisetti. *In vitro* antimitotic activity and *in silico* study of some 6-fluoro-triazolo-benzothiazole analogues, Pharmacia. 70(4) (2023) 887-894.

[6] W. Zhang, Y. Bai, Y. Wang, W. Xiao, Polypharmacology in Drug Discovery: A Review from Systems Pharmacology Perspective, Current Pharmaceutical Design22 (21) (2016) 3171–3181.

[7] S. Sharma, D. Kumar, G. Singh, V. Monga, B. Kumar, Recent Advancements in the Development of Heterocyclic Anti-Inflammatory Agents. European Journal of Medicinal Chemistry200 (2020) 112438.

[8] P. Naresh, R.Rajesh Kumar,H. N. Vishwas, GopalanRajagopal,T.PrabhaandS. Jubie, Larvicidal andhistopathological efficacy of cinnamic acid analogues: anovelstrategytoreducethe denguevectorcompetence, RSC Advances12(16) (2022) 9793–9814.

[9] L. Mouhid, M. Corzo-Martínez, C. Torres, L. Vázquez, G. Reglero, T. Fornari, A. Ramírez de Molina, Improving *In Vivo* Efficacy of Bioactive Molecules: An Overview of Potentially Antitumor Phytochemicals and Currently Available Lipid-Based Delivery Systems, Oncol7 (2017) 7351976.

[10] G. Shabir, A. Saeed, W. Zahid, F. Naseer, Z. Riaz, N. Khalil, F. Albericio, Chemistry and Pharmacology of Fluorinated Drugs Approved by the FDA (2016–2022), Pharmaceuticals16(8) (2023) 1162.

[11] Sumit, Arvind Kumar, Arun Kumar Mishra, Advancement in Pharmacological Activities of Benzothiazole and its Derivatives: An Up-to-Date Review. Mini-Reviews in Medicinal Chemistry 21(3)(2021) 314-335.

[12] Rangappa S. Keri, Mahadeo R. Patil, Siddappa A. Patil, Srinivasa Budagumpi,A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry,European Journal of Medicinal Chemistry,89 (2015) 207-251.

[13] E.N. Djuidje, R. Barbari, A. Baldisserotto, E. Durini, S. Sciabica, J. Balzarini, S. Liekens, S. Vertuani, S. Manfredini, Benzothiazole Derivatives as Multifunctional Antioxidant Agents for Skin Damage: Structure-Activity Relationship of a Scaffold Bearing a Five-Membered Ring System. Antioxidants (Basel). 11(2)(2022) 407.

[14] S. Paliwal, S. Nain, Diverse Biological Activities of 1,3,4-Thiadiazole Scaffold, Chemistry, 4 (4) (2022) 1654–1671.

[15] Obakachi, Babita Kushwaha, Narva Deshwar Kushwaha, Sithabile Mokoena, Majeed Ganai, Tabasum Khan Pathan, Werner, Rajshekhar Karpoormath. Synthetic and Anti-Cancer Activity Aspects of 1, 3, 4-Thiadiazole Containing Bioactive Molecules: A Concise Review, Journal of Sulfur Chemistry42 (6) (2022) 670–691.

[16] Y. Li, Geng, Y. Liu, S. Yu, G. Zhao, Thiadiazole-a Promising Structure in Medicinal Chemistry, Chem Med Chem8 (1) (2012) 27–41.

[17] V. Kamat,R. Santosh, B. Poojary, S.P. Nayak, B.K. Kumar, M. Sankaranarayanan, S. Faheem, Khanapure, D. Barretto, Avilla, Vootla SK. Pyridine- and Thiazole-Based Hydrazides with Promising Anti-Inflammatory and Antimicrobial Activities along with Their in-Silico Studies, ACS Omega5 (39) (2020) 25228–25239.

[18] J.D. Bilavendran, A. Manikandan, Pandiyan Thangarasu, K.K. Sivakumar, Synthesis and Discovery of Pyrazolo-Pyridine Analogs as Inflammation Medications through Pro- and Anti-Inflammatory Cytokine and COX-2 Inhibition Assessments, Bioorganic Chemistry94 (2020) 103484–103484.

[19] M.H. Eslam, M.S. Abdel-Maksoud, R.M. Hassan, K.I. Mersal, U.M. Ammar, S. Choi, H. He-Soo, B.H. Jeong, A. Lee, K.T. Lee, CH. Oh, Design, Synthesis and Anti-Inflammatory Activity of Imidazol-5-Yl Pyridine Derivatives as P38α/MAPK14 Inhibitor, Bioorganic & Medicinal Chemistry31 (2021) 115969–115969.

[20] T. Chaban, V. Ogurtsov, V. Matiychuk, I. Chaban, I. Demchuk, I. Nektegayev. synthesis, Anti-Inflammatory and Antioxidant Activities of Novel 3H-Thiazolo[4,5-b] Pyridines. Acta Chimica Slovenica2019; 103–111.

[21] Y.M. Omar, H.M. Abdu-Allah, S.G. Abdel-Moty, Synthesis, Biological Evaluation and Docking Study of 1,3,4-Thiadiazole-Thiazolidinone Hybrids as Anti-Inflammatory Agents with Dual Inhibition of COX-2 and 15-LOX, Bioorganic Chemistry80 (2018)461–471.

[22] A. Cristina, D. Leonte, L. Vlase, L. Bencze, S. Imre, G. Marc, B. Apan, C. Mogoşan, V. Zaharia. Synthesis, Characterization and Biological Evaluation of Imidazo[2,1-b] [1,3,4] Thiadiazole Derivatives as Anti-Inflammatory Agents. Molecules2018;23 (10): 2425.

[23] Y.M. Omar, S.G. Abdel-Moty, H.M. Hajjaj, Abdu-Allah. Further Insight into the Dual COX-2 and 15-LOX Anti-Inflammatory Activity of 1,3,4-Thiadiazole-Thiazolidinone Hybrids: The Contribution of the Substituents at 5th Positions Is Size Dependent,Bioorganic Chemistry97 (2020) 103657–103657.

[24] R. Roll, T. Höfer-Bosse, D. Kayser, New perspectives in acute toxicity testing of chemicals, Toxicol Lett Suppl 31 (1986) 86.

[25] R. Roll, M. Riebschlaeger, U. Mischke, D. Kayser, New approaches to acute toxicity testing of chemicals, Federal Health Gazette (FR Germany) 32(8) (1989).

[26] C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Anti-inflammatory Drugs,Experimental Biology and Medicine111 (3) (1962) 544–547.

[27] J.S. Bang, D.H. Oh, H.M. Choi, B.J. Sur, S.J. Lim, J.Y. Kim, H.I. Yang, M.C. Yoo, D.H. Hahm, K.S. Kim, Anti-Inflammatory and Antiarthritic Effects of Piperine in Human Interleukin 1β-Stimulated Fibroblast-like Synoviocytes and in Rat Arthritis Models, Arthritis Research & Therapy11(2) (2009)

[28] S. Alqasoumi, G. Abd, E. Soliman, A. Awaad, R.M. Donia, S. Bin, Anti-inflammatory activity safety and protective effects of Leptadenia pyrotechnica, Haloxylon salicornicum and Ochradenus baccatus in ulcerative colitis,Phytopharmacology 2(1) (2012)58-71.

[29] J.N. Sharma, A.M. Samud, M.Z. Asmawi, Comparison between Plethysmometer and Micrometer Methods to Measure Acute Paw Oedema for Screening Anti-Inflammatory Activity in Mice, Inflammo Pharmacology12(1) (2004) 89–94.

[30] V.B. Owoyele, J.O. Adediji, A.O. Soladoye, Anti-Inflammatory Activity of Aqueous Leaf Extract of Chromolaena Odorata,Inflammo Pharmacology 13 (2005) 479–484.

[31] S.K. Konidala, V. Kotra, R.C. Danduga, P.K. Kola, Coumarin-Chalcone Hybrids Targeting Insulin Receptor: Design, Synthesis, Anti-Diabetic Activity, and Molecular Docking, Bioorganic Chem 104 (2020) 104207.

[32] A.K. Subramani,A. Sivaperuman, R. Natarajan, R.R. Bhandar, A.B. Shaik, QSAR and Molecular Docking Studies of Pyrimidine-Coumarin-Triazole Conjugates as Prospective

Anti-Breast Cancer Agents,Molecules 27 (2022) 1845.

[33] B.L. Surendra, P.Y. Rajendra, N. Srinath R.R. Bhandare, V. Sampath, B.V. Subrahmanya Lokesh, M.D. Abdur Rahman, A.B. Shaik, Antitubercular Activity Assessment of Fluorinated Chalcones, 2-Aminopyridine-3-Carbonitrile and 2-Amino-4H-Pyran-3-Carbonitrile Derivatives: In Vitro, Molecular Docking and In-Silico Drug Likeliness Studies,PLOS ONE17 (2022) e0265068–e0265068.

[34] Schrödinger Release 2022-1: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2021. Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY (2021).

[35] PNaresh,A, Selvaraj,P. ShyamSundar,S Murugesan,S. Sathiaanarayanan,p.k. Krishnan, Namboori,SJubie, Targeting aconserved pocket (n-octyl-β-D-glucoside)onthe denguevirusenvelope protein bysmallbioactive molecule inhibitors, Journal of biomolecular structure and dynamics. 40(11) (2022) 4866-4878.

[36] R. Chinnasamy, R. Sundararajan, S. Govindaraj, Synthesis, Characterization, and Analgesic Activity of Novel Schiff Base of Isatin Derivatives, J. Adv. Pharm. Technol. Res 1 (2010) 342.

[37] M.M. Ibrahim, T. Elsaman, M.Y. Al-Nour, Synthesis, Anti-Inflammatory Activity, and In Silico Study of Novel Diclofenac and Isatin Conjugates, Int. J. Med. Chem (2018) 1–11.

[38] A. Redzicka, Z. Czyżnikowska, B. Wiatrak, K. Gębczak, A. Kochel, Design and Synthesis of N-Substituted 3,4-Pyrroledicarboximides as Potential Anti-Inflammatory Agents, Int. J. Mol. Sci 22 (2021) 1410.

[39] J.P. Soni, D.J. Sen, K.M. Modh, Structure activity relationship studies of synthesised pyrazolone derivatives of imidazole, benzimidazole and benzotriazole moiety for anti-inflammatory activity, J. Appl. Pharm. Sci1 (2011) 115-120.

[40] R. Veerasamy, A. Roy, R. Karunakaran, H. Rajak, Structure–Activity Relationship Analysis of Benzimidazoles as Emerging Anti-Inflammatory Agents: An Overview, Pharmaceuticals 14 (2021) 663.