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DESIGN, SYNTHESIS AND EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SCHIFF BASE CONTAINING 2,4-DISUBSTITUTED THIAZOLE RING

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ABSTRACT

A series of 2-(arylidene)-1-(3-nitrophenyl) thiazol-2-yl) hydrazine(2a-j) were synthesized by the Reaction of Schiff Base Thiosemicarbazone with substituted phenacyl bromide few drop of glacial acetic acid. The structures of synthesized compounds were established by chemical analysis (FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass). The synthesized compounds 2b, 2h, and 2i were screened for Anti-inflammatory Activity by the carrageenan-induced paw edema method. The synthesized compounds showed significant anti-inflammatory activity against target macromolecule Cyclooxygenase when compared with standard celecoxib. As a result, these substances can be seen as prospective lead compounds for the creation of fresh anti-inflammatory medications with novel mechanisms of action.

KEYWORDS: Schiff Base, 2,4-Disubstituted thiazoles, Anti-inflammatory, Thiosemicarbazone.

INTRODUCTION

Inflammation is a biological response to any adverse stimulus, such as tissue damage and infection. The transport of blood components to the illness or injury site causes vasodilation and enhanced vascular permeability.[1] Acute inflammation is an element of the body's natural response to infection or injury and may justly be thought about as an adjustive response, as long as it remains at intervals healthy limits. The response usually begins domestically and represents an extremely evolutionarily preserved program of reactions associated with

immunity, that area unit comparatively "hard-wired" (i.e., modifiable however not preventable). The acute response to infection and inflammation is closely associated with immune defense, wound healing, and tissue repair.[2]

Chronic inflammation is additionally noted as slow, long-run inflammation lasting for prolonged periods of many months to years. Generally, the extent and effects of chronic inflammation vary with the reason for the injury and therefore the ability of the body to repair and overcome the injury.[3] Nonsteroidal anti-inflammatory drugs (NSAIDs), which are effective in treating pain, fever, redness, and edema brought on by the release of inflammatory mediators, are the most frequently used medications for inflammatory disorders.[4-7] The amino acid cascade includes two essential enzymes: cyclooxygenases (COX) and lipoxygenases (LOX). COX is made up of two isoenzymes, COX-1 and COX-2, which convert amino acids into prostaglandins (PGs), prostacyclin (PGI2), and thromboxane.[8] While the COX-2 is involved in the pain caused by inflammation and plays a significant role in prostaglandin biosynthesis in inflammatory cells and the central nervous system, the COX-1 is involved in the production of vital biological mediators like prostanoids, including prostaglandins, prostacyclin, and thromboxane, and is involved in pain causing, blood clotting, and protecting the stomach.[9]

Schiff bases are compounds containing associate degree imine or azomethine cluster (-RC=N-) shaped by the condensation of carbonyl compounds with primary amines with a full-of-life carbonyl;(Fig-1). they were named when Hugo Schiff World Health Organization initially reported them in 1864.[10] Thiazole is a five-membered aromatic heterocyclic chemical molecule with the molecular ring formula C3H3NS. Hantzch and Weber were the first to describe thiazole in 1887. In 1889, Prop verified its structure.[11] Huckel's criteria are satisfied because it's an aromatic compound. The 6 electrons are completed by delocalization of a lone pair of electrons from the sulfur atom.[12] Thiazole and its derivatives are helpful compounds in varied fields of chemistry as well as drugs and agriculture. Also, thiazoles are artificial intermediates and customary substructures in a very variety of biologically active compounds like varied derivatives of antibiotics.[13] Only a few are 2,5-disubstituted or 2,4,5-trisubstituted thiazoles, while the majority of 1,3-thiazole compounds are 2,4-disubstituted thiazole derivatives. We planned to synthesize novel 2,4-disubstituted Thiazole derivatives as anti-inflammatory lead candidates in the present work.

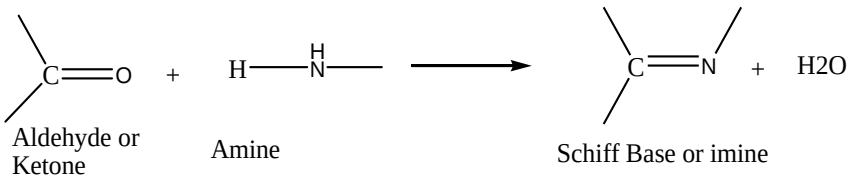


Fig. 1: Formation of Schiff base.

MATERIAL AND METHODS

Chemicals

The reagents and solvents for synthesis were purchased from Loba-Chemie, Alorich, and Analab fine, and were used as received: 4-triflurobenzaldehyde, 2-nitrobenzaldehyde, thiosemicarbazide, acetic acid, ethyl acetate, chloroform, 3-nitro phenacyl bromide.

Instrumentation

The melting point of the synthesized derivatives were determined by open capillary tube method. The structures of the synthesized derivatives were elucidated by Shimadzu, IR Affinity-1, Japan FTIR spectrophotometer With KBR pellets. ^{1}H-NMR spectra were recorded by Bruker AVANCE III HD NMR Spectrometer 500 MHz using DMSO as internal standard. Mass spectra were recorded on Impact II UHR-TOF Mass Spectrometer at Sophisticated Analytical Instrument Facility (SAIF). The assigned structures were supported by the ^{1}H-NMR and IR spectra. The purity of the compounds was checked by Using TLC on pre-coated aluminium sheets (Silica gel 60 F254 Merck-Germany) and the mobile phases (6:4 chloroform: ethyl acetate and iodine vapours as indicator.

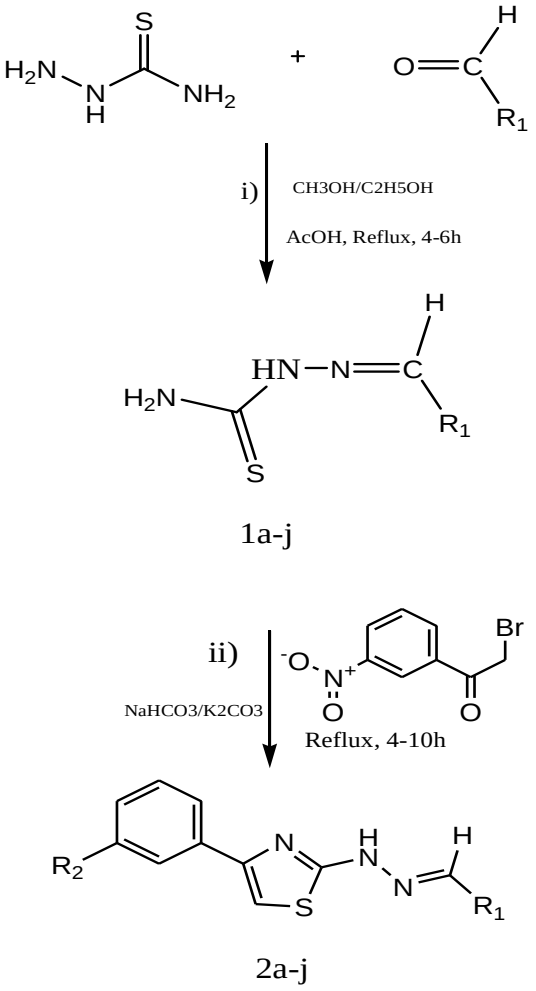
General Method for the Synthesis of Schiff bases thiosemicarbazone(1a-j)

Equimolar mixtures of substituted aldehyde (0.01 mol) in ethanol/methanol (20 ml) and thiosemicarbazide (0.01 mol) in ethanol (20 ml) were heated in a water bath for 4-6 hours while being catalysed by a few drops of glacial acetic acid. TLC checked the status of the reaction at regular intervals. When the reaction was finished, the solution was cooled, the solid that had been separated was rinsed with ice-cold water (3×50 ml), and it was then dried. The final step was to recrystallize ethanol from the product thusly produced. The synthetic scheme of the title compounds is outlined in Scheme 1.

General procedure for the 2-(arylidene)-1-(3-nitrophenyl) thiazol-2-yl) hydrazine(2a-j)

Thiosemicarbazone(1a-j) (0.01 mol) and substituted phenacyl bromide (0.01 mol) in ethanol/methanol (20 ml) were combined and refluxed over a water bath for 4–10 hours. At

the proper intervals, TLC checked on the reaction's development. In order to obtain the desired product, the excess of solvent was removed, and the solid that separated was collected by filtering, suspended in water, and neutralized using NaHCO3. Methanol was used to recrystallize the final product.



Scheme 1: Synthetic scheme of arylidene-2-(4-(3-nitrophenyl) thiazol-2-yl) hydrazine.

Molecular Docking[14-15]

A crucial tool in structural molecular biology and computer-aided drug design is molecular docking. Ligand-protein docking aims to foretell the main mode(s) of ligand-protein interaction with proteins with known three-dimensional structures. Effective docking algorithms examine high-dimensional spaces and make use of a scoring system that ranks potential dockings appropriately. The study of 2,4-disubstituted Thiazole derivatives and COX-2 interaction was evaluated by using molecular docking methods like PyRx 0.8. We used the X-ray crystal structure of the COX-2 in which the celecoxib bound at the active site (PDB ID: 3LN1) as the target protein taken from http://www.pdb.org.

The structures of 2,4-disubstituted Thiazole derivatives such as 2a, 2b, 2c, and 2d, were haggard via Chem Draw Ultra 8.0 software from Cambridge Soft. The 2D structures of the proposed ligand were converted to the 3D structure using Chem 3D Ultra 8.0 tool. The optimization of the proposed ligand and energy minimization of the proposed ligands were accomplished using the semi-empirical PM3 technique and applying a termination RMS gradient of 0.001 KCal/mol for extreme up to 1000 iterations and saved as PBD arrangement, to be read by the AutoDock vina software. During the docking, the grid dimension was 33.40, 36.73, and 35.23 A˚. Then X, Y Aand Z Coordinates were specified as 29.83, -20.77, and -10.71 respectively. After that, the drug-likeness property is determined by the Swiss ADME tool **(Table 2)**.

Discovery studio 3.1visualizer

This software is mostly used to display how docked images interact with conformational search. Along with the surface image, the docking analysis's 2D and 3D interactions will be shown. The van der Waals interaction and hydrogen bonding in the ligand interactions with amino acid coding will be seen in the 2D interactions. Additionally, surface pictures that demonstrate the confirmations of structure-based ligand docking against bacterial enzymes were observed.

Pharmacological Evaluation

Acute Toxicity Study[16]

Acute oral toxicity studies were performed as per the Institutional Animal Ethics Committee (IAEC) Guidelines project proposal no. DYPCOP/IAEC/2022/16. Albino Wistar female rats (9) Weigh between 150-200 gm(10), grouped three in each and fasted overnight. The dosage ranged from 1000 to 100 mg per kg1 of body weight. For 24 hours, the animals were watched for any indications of acute toxicity, including altered motor function, convulsions, sedation, lacrimation, and other symptoms None of the dead of the animals was seen even 24 hours later. hence the test substances' LD50 cut off value was set as 1000 mg kg-1. In order for 100 mg kg-1, or 1/10 of the cut off amount, was consumed as a screening dose to assess the drug's anti-inflammatory activity.

Carrageenan induced Paw Oedema[17-19]

Anti-inflammatory potency was analyzed using carrageenan-induced paw oedema in rats. The rats were divided into 06 groups consisting six in each group. The first control group was given vehicle DMSO per oral 1ml/100gm body weight whereas third group served as

reference standard received Celecoxib 20 mg/kg orally. The test compounds (2b, 2h, 2i) 2,4-disubstituted Thiazole derivatives were administered to groups 4th to 6th at the dose of 100 mg/kg per oral. The vehicle used for the preparation of the test compound was DMSO. After thirty minutes of above treatment, carrageenan solution 0.1 ml (1% w/v carrageenan dissolved in normal saline) injected into sub-plantar region of rat's left hind paw to induce inflammation. The group 2nd received only carrageenan injection and served as induction control (Negative control) group. Digital Plethysmometer was used to record the paw volume of control, reference standard and test compound treated groups and degree of paw oedema measured at the interval of 1, 2, 3, 4 hours after carrageenan injection. The percentage inhibition of oedema in each group compared to the control group served as a measure of the anti-inflammatory activity. Table 2 provides the specified value. The formula below was used to determine the % inhibition of oedema.

$$\% \text{ Inhibition of edema} = \frac{V_o - V_t}{V_o} X100$$

Where, V_o and Vt are the paw volume of control and test groups at respective time interval.

Statistical Analysis

The results are expressed as the mean ± SEM per group, and the data were statistically analyzed by one-way analysis of Variance (ANOVA) followed by Tukey test. Values of *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 were considered statistically significant. All statistical calculations were performed using the evaluation version of Graph Pad® Instat version 3.1 statistical software.

Table 2: Anti-inflammatory activity of title (2b, 2h, 2i) compounds.

Compounds	volume of edema (ml) (Mean±SEM) ml				% Inhibition			
	1h	2h	3h	4h	1h	2h	3h	4h
Control	0.805±0.06$^{###}$	0.761±0.04$^{#}$	1.23±0.0.1$^{###}$	1.485±0.107$^{###}$	-	-	-	-
Celecoxib	0.453±0.06***	0.586±0.04*	0.3±0.02***	0.325±0.02***	43.72	22.99	75.60	78.11
2b	0.403±0.03***	0.516±0.04**	0.378±0.02***	0.308±0.02***	49.93	32.19	69.26	79.25
2h	0.316±0.02***	0.417±0.04***	0.319±0.006***	0.22±0.02***	60.74	45.20	74.06	85.18
2i	0.352±0.03***	0.438±0.05***	0.385±0.04***	0.3±0.02***	56.27	42.44	68.69	79.79

RESULT AND DISCUSSION

The title compounds (2a-j) were synthesized as per the scheme. In the first step, thiosemicarbazone[20-21] derivatives (1a-j) were synthesized from condensation followed by reacting with hydrazide. The absence of absorption band at 1700–1750 cm-1 also confirms the conversion of –CHO group to –CH=N– group. In the second step, 2,4-disubstituted[22]

Thiazole (2a-j) derivatives were synthesized by cyclization of thiosemicarbazone (1a-j) with phenacyl bromide. The structures were confirmed by IR spectra with appearance of C=S peak. The Chemical structure of all synthesized compounds confirmed by H-NMR, C-NMR and mass spectroscopy.

Spectral data of synthesized compounds

(z)-2-(4-dimethylaminobenzylidene)-1-(4-(3-nitrophenyl) thiazol-2-yl) hydrazine (2a)

IR (KBr; ν cm-1): 3292(-NH), 1602 (C=N Azomethine), 1514 (aromatic; C=C), 1571 (NO2); 1H NMR (DMSO) δ(ppm): 2.81 (m, 6H; dimethylamine), (s, 1H; thiazole H), 7.6 (s, 1H; -N=CH), 7.5, 7.8, 8.2, 8.8 (m,4H; o-nitrophenyl), 6.7 (d, 2H; Ph), 7.3(d, 2H; Ph); 13C NMR (DMSO-d6) δ (ppm): 40.3 (dimethylamine), 139.5 146.5(-N=CH), 145.3, 123.3, 129, 125, 126.8, 132 (aryl-CH), 165.7(thiazole-C-2), 148.5 (thiazole-C-4), 102.2 (thiazole-C-5); MS (m/z, %): Calculated: 367.42, Found: 367.23.

(z)-2-(2-nitrobenzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2b): IR (KBr; ν cm-1): 3265(-NH), 1614 (C=N Azomethine), 1506 (aromatic; C=C),1558 (NO2); 1H NMR (DMSO) δ(ppm): 7.45 (s, 1H; thiazole H), 7.9 (s, 1H; -N=CH), 7.4, 7.5, 7.7, 8.1 (m,4H; o-nitrophenyl), 7.59,7.89, 8.2, 8.8 (m,4H; m-nitrophenyl); 13C NMR (DMSO-d6) δ (ppm): 145.3, 123.3, 125, 129, 126.8, 132 (aryl-CH),146.5 (-N=CH), 165.7(thiazole-C-2), 148.5 (thiazole-C-4), 102.2(thiazole-C-5); MS (m/z, %): Calculated: 369.35, Found: 370.0608.

(z)-2-(4-nitrobenzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2c): IR (KBr; ν cm-1): 32694(N-H), 1606 (C=N), 1514 (aromatic; C=C), 1566 (NO2); 1H NMR (DMSO) δ(ppm): 7.43 (s, 1H; thiazole H), 7.9 (s, 1H; -N=CH), 7.5, 7.8, 8.2, 8.8 (m,4H; m-nitrophenyl); 13C NMR (DMSO-d6) δ (ppm): 139.5 (-N=CH), 123,125,126, 129,132, 145, (aryl-CH), 165.7 (thiazole-C-2), 148.5 (thiazole-C-4), 102.2 (thiazole-C-5); MS (m/z, %): Calculated: 369.35, Found: 370.0608.

(z)-2-(4-methylbenzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2d): IR (KBr; ν cm-1): 3296(-NH), 1602 (C=N Azomethine), 1517 (aromatic; C=C), 1566 (NO2), 702 (aromatic; C-H); 1H NMR (DMSO) δ(ppm): 7.4 (s, 1H; thiazole H), 7.7 (s, 1H; -N=CH), 2.2 (s, 3H; CH3), 7.1 (d, 2H; aryl), 7.3 (d, 2H; aryl) 7.5, 7.8, 8.2, 8.8 (m,4H; m-nitrophenyl); 13C NMR (DMSO-d6) δ (ppm):, 139.5 (-N=CH), 21.3(CH3), 145.3, 123.3, 129, 125, 126.8, 132 (aryl-CH), 165.7(thiazole C-2), 148.5 (thiazole-C-4), 102.2 (thiazole-C-5); MS (m/z, %): Calculated: 338.38, Found: 339.35.

(z)-2-(4-isopropylbenzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2e): IR (KBr; ν cm-1): 3273 (-NH), 1606 (C=N Azomethine), 1512 (aromatic; C=C), 1529 (NO2), 717 (aromatic; C-H), 2866 (C-H); 1H NMR (DMSO) δ(ppm): 7.4 (s, 1H; thiazole H), 7.5 (s, 1H; -N=CH), 7.5, 7.8, 8.2, 8.8 (m,4H; m-nitrophenyl), 1.18 (d, 6H; isopropane), (sept, 1H; isopropane); 13C NMR (DMSO-d6) δ (ppm): 140.9 (-N=CH), 23.85 (isopropane (CH3)2), 33.9 (-CH-), 145.3, 123.3, 129, 125, 126.8, 132 (aryl-CH), 165.7 (thiazole-C-2), 148.5 (thiazole-C-4), 102.2 (thiazole-C-5); MS (m/z, %): Calculated: 366.44, Found:366.85.

(z)-2-(4-fluorobenzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2f): IR (KBr; ν cm-1): 3250 (N-H), 1600 (C=N Azomethine), 1506 (aromatic; C=C), 1566 (NO2), 1346 (C-F); 1H NMR (DMSO) δ(ppm): 6.9 (d, 2H; (CH)2), 7.4 (d, 2H; (CH)2), 7.4 (s, 1H; thiazole H), 7.6 (s, 1H; -N=CH), 7.5, 7.8, 8.2, 8.8 (m,4H; m-nitrophenyl), 13C NMR (DMSO-d6) δ (ppm): 139.5 146.5(-N=CH), 145.3, 123.3, 129, 125, 126.8, 132 (aryl-CH), 165.7 (thiazole-C-2), 148.5 (thiazole-C-4), 102.2(thiazole-C-5); MS (m/z, %): Calculated: 342.35, Found:342.56.

(z)-2-(4-ethylbenzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2g): IR (KBr; ν cm-1): 3294 (N-H), 1627 (C=N Azomethine), 1527 (aromatic; C=C), 1554 (NO2), 732 (aromatic; C-H); 1H NMR (DMSO) δ(ppm): 7.4 (s, 1H; thiazole H), 7.7 (s, 1H; -N=CH), 7.5, 7.8, 8.2, 8.8 (m,4H; m-nitrophenyl), 2.53 (q, 2H; CH2), 1.10 (t, 3H; CH3); 13C NMR (DMSO-d6) δ (ppm): 139.5 (-N=CH), 28.7 (ethyl CH2), 14.6 (ethyl CH3), 145.3, 123.3, 129, 125, 126.8, 132 (aryl-CH), 165.7(thiazole-C-2), 148.5 (thiazole-C-4), 102.2 (thiazole-C-5); MS (m/z, %): Calculated: 352.41, Found: 352.33.

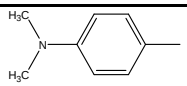
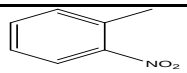
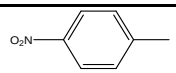
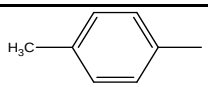
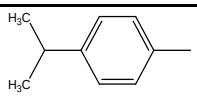
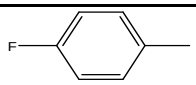
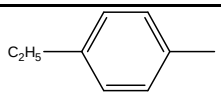
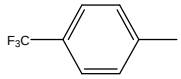
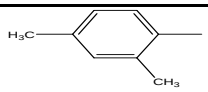
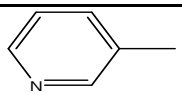
(z)-2-(4-(trifluoromethyl)benzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2h): IR (KBr; ν cm-1): 3253 (N-H), 1616 (C=N), 1514 (aromatic; C=C), 1568 (NO2), 1327 (C-F), 713 (aromatic; C-H); 1H NMR (DMSO) δ(ppm): 7.4 (s, 1H; thiazole H), 7.6 (s, 1H; -N=CH), 7.5, 7.8, 8.2, 8.8 (m,4H; m-nitrophenyl), 7.5, 7.6 (dd, 2H; trifluoromethyl benzyl); 13C NMR (DMSO-d6) δ (ppm): 123.8 (CF3), 139.5 (-N=CH),125 (aryl (CH)2), 128 (aryl (CH)2), 145.3, 123.3, 129, 125, 126.8, 132 (aryl-CH), 165.7(thiazole-C-2), 148.5 (thiazole-C-4), 102.2(thiazole-C-5); MS (m/z, %): Calculated: 392.36, Found: 393.0633.

(z)-2-(2,4-dimethylbenzylidene)1-(3-nitrophenyl)thiazol-2-yl)hydrazine (2i): IR (KBr; ν cm-1): 3259 (N-H), 1614 (C=N), 1508 (aromatic; C=C), 1570 (NO2), 704,735 (aromatic; C-H); 1H NMR (DMSO) δ(ppm): 7.4 (s, 1H; thiazole H), 7.6 (s, 1H; -N=CH), 7.5, 7.8, 8.2, 8.8

(m,4H; m-nitrophenyl), 2.17 (CH3 ;benzyl), 2.19 (CH3; benzyl); 13C NMR (DMSO-d6) δ (ppm):, 146.5 (-N=CH), 20 (benzyl CH3), 21 (benzyl CH3), 145.3, 123.3, 129, 125, 126.8, 132 (aryl-CH), 165.7(thiazole-C-2), 148.5 (thiazole-C-4), 102.2(thiazole-C-5); MS (m/z, %): Calculated: 352.41, Found: 353.1074.

(z)-1-(4-(3-nitrophenyl)thiazol-2-yl)-2-((pyridine-3-yl)methylene)hydrazine (2j): IR (KBr; ν cm-1): 3266 (N-H), 1604 (C=N), 1506 (aromatic; C=C), 1568 (NO2); 1H NMR (DMSO) δ (ppm): 7.4(s, 1H; thiazole H), 7.70(s, 1H; -N=CH), 7.41-8.7(m, 4H; Pyridine), 7.5-8.8 (m,4H; m-nitrophenyl); 13C NMR (DMSO-d6) δ (ppm): 145.3, 123.3, 125, 129, 126.8, 132 (aryl-CH), 165.7(thiazole-C-2), 148.5 (thiazole-C-4), 102.2(thiazole-C-5); MS (m/z, %): Calculated: 325.35, Found:326.0533.

Table 1: Analytical and Physiochemical data of synthesized compounds.

Compound	R_1	R_2	M.p.(^{0}C)	Yield (%)	Rf value	Dock Score (kcal/mol)
2a		NO2	211-214	82	0.69	-8.5
2b		NO2	232-233	72	0.83	-9.2
2c		NO2	252-254	64	0.86	-8.7
2d		NO2	187-188	40	0.65	-8.9
2e		NO2	155-160	79	0.45	-9.1
2f		NO2	180-184	46	0.76	-8.8
2g		NO2	125-130	80	0.63	-8.6
2h		NO2	213-215	85	0.79	-9.3
2i		NO2	178-180	52	0.54	-9.1
2j		NO2	243-245	35	0.59	-8.8
Celecoxib (Std)	-	-	-	-	-	-8.2

Molecular docking

The molecular docking of ligands into the active site of COX-2. Compound 2h demonstrated the highest docking score -9.3 kcal/mol which suggests the highest affinity for the enzyme as compared to other compounds in the series. The compound interacted strongly with the amino acid residues (Figure 4,5,6). Numerous alkyl interactions were displayed by thiazole (with ARG29), phenyl (with PHE49) moieties. The Phenyl moiety displayed a strong alkyl Fluorine interaction with GLN447, GLU451. The Nitro group on phenyl and thiazole -SH groups of 2b, 2h and 2i form hydrogen bonds with TRY116 and ASN28, respectively. The pi-alkyl interaction on thiazole ring exhibited with LYS454 and alkyl interaction with three amino acid residues; LEU65, PHE49, and LEU138, respectively. The molecule 2h expressed a highest docking score than the standard drug, Celecoxib (-8.2 kcal/mol). The analogue 2b exhibited good affinity for the enzyme with docking score -9.2 kcal/mol. Compound 2i, although displayed exactly similar interaction like 2b, however, the affinity was found to be low as compared to both 2h and standard drug. The other derivatives 2a-2k displayed the dock score more than -8.2 kcal/mol, representing moderate affinity for COX-2. This may be the probable reason for good anti-inflammatory activity towards the target. The docking score of the synthesized compounds shown in Table 1.

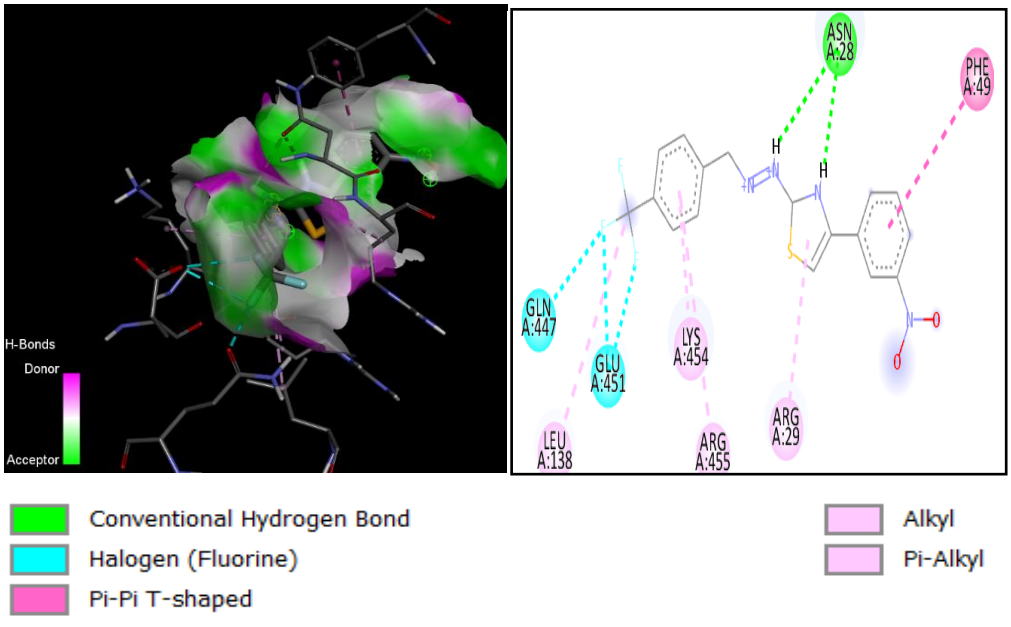


Fig 4. 3D and 2D figure about interaction of 2h with COX-2 target protein

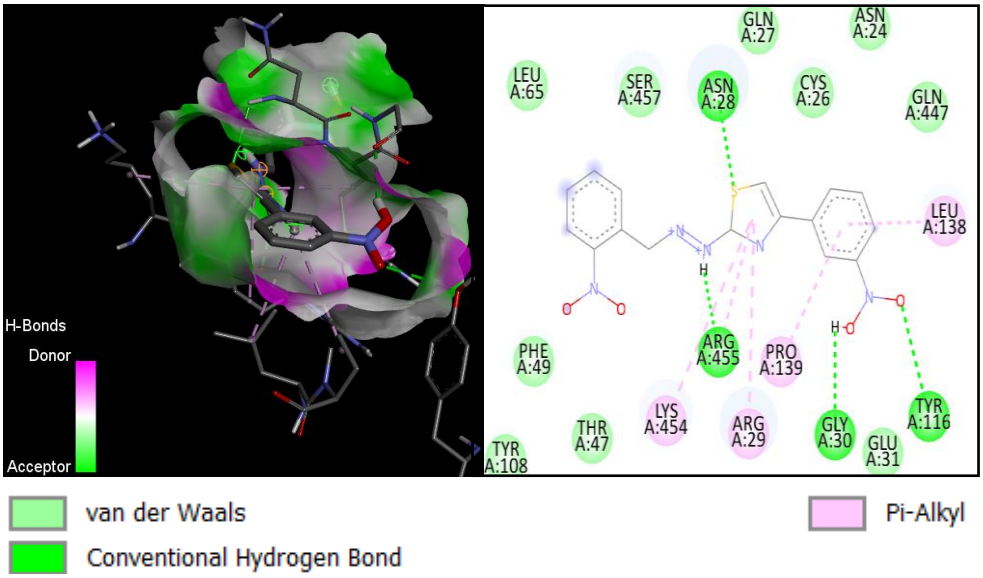


Fig. 5: 3D and 2D figure about interaction of 2b with COX-2 target protein.

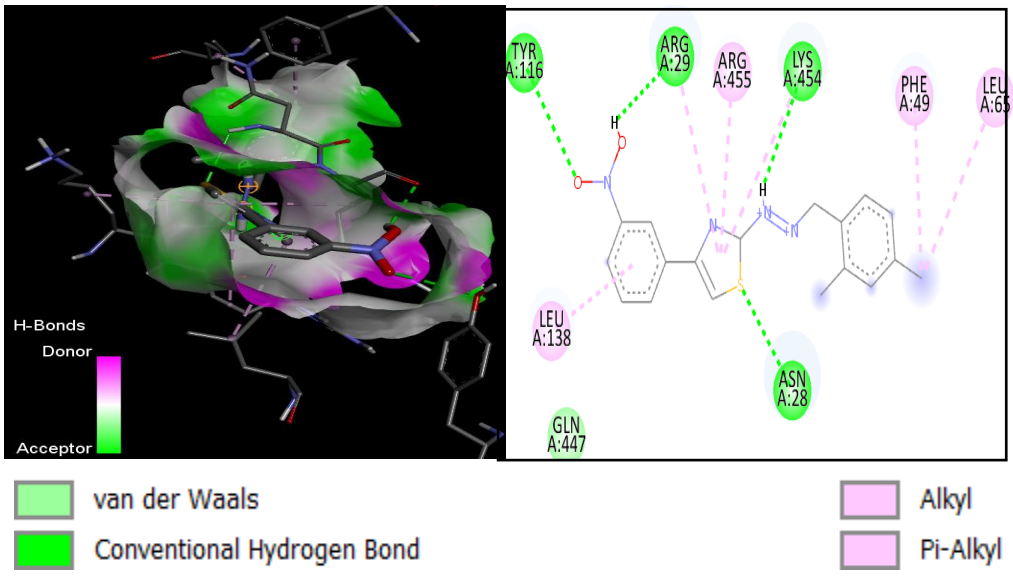


Fig. 6: 3D and 2D figure about interaction of 2i with COX-2 target protein.

In vivo Anti-inflammatory Activity

Anti-inflammatory activity of synthesized compounds evaluated by carrageenan-induced rat paw oedema model. 100mg/kg Subplanar injection 0.1ml, 1% carrageenan produced an increase in paw volume(oedema) of all the animals of various groups. The onset of action starts from 1 hour in various test group. a reduction in rat paw oedema was observed by A significant reduction in rat paw oedema was observed by most test compounds at 4 hours compared to the control group. celecoxib was used as reference standard. Close examination of the outcomes of in vivo experiments led us to the conclusion that chemicals that were substituted at 3h Significant anti-inflammatory action was shown by 2h. Compared to the

standard drug Celecoxib, whereas Compounds 2b, & 2i showed significant anti-inflammatory effects after 4 hours.

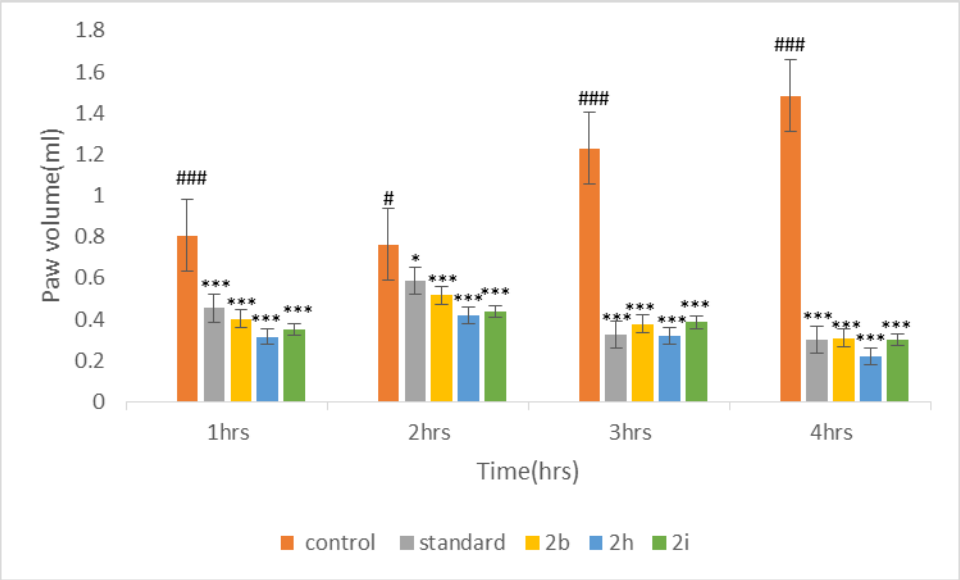


Fig. 2: Volume of edema of title (2b, 2h, 2i) compounds.

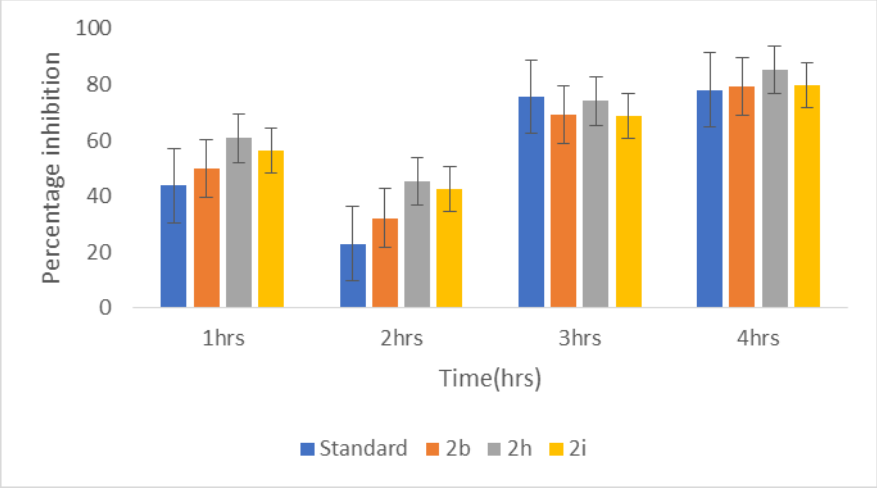


Fig. 3: Percentage inhibition of title (2b, 2h, 2i) compound.

CONCLUSION

Newly synthesized 2,4-disubstituted thiazole derivatives were screened for their preliminary *in vivo* anti-inflammatory activity using a carrageenan-induced paw edema model. The current study reported the anti-inflammatory activity of newly synthesized compounds, having electrons released and withdrawing groups on the phenyl ring. The NO2, F, $(CH_3)_2$, and CF3 substitution on the phenyl ring showed significant anti-inflammatory activity. The study provided potential derivatives exhibiting significant anti-inflammatory activity with

fast onset and extended duration of action, which is the most promising expectation of any anti-inflammatory agent, especially when administered along with complaint-specific therapy.

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REFERENCES

1. Abdelgawad MA, Al-Sanea MM, Musa A, et al. Docking Study, Synthesis, and Anti-Inflammatory Potential of Some New Pyridopyrimidine-Derived Compounds. Journal of Inflammation, 2022; 15: 451. DOI: 10.2147/JIR.S343263.
2. Catharine Ross A. Impact of chronic and acute inflammation on extra- and intracellular iron homeostasis. The American Journal Clinical Nutrition, 2017; 106: 1581-1587. DOI: https://doi.org/10.3945/ajcn.117.155838.
3. Pahwa R, Goyal A, Jialal I. Chronic Inflammation. Pathobiology of Human Disease. A Dynamic Encyclopedia of Disease Mechanisms, 2022; 300-314. DOI: https://doi.org/10.1016/j.foodres.2022.111928.
4. Brune K, Hinz B. The discovery and development of anti-inflammatory drugs. Arthritis & Rheumatism. Official Journal of the American College of Rheumatology, 2004; 50(8): 2391-2399. DOI 10.1002/art.20424.
5. Vane JO, Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs: The FASEB journal, 1987; 1(2): 89-96. DOI: https://doi.org/10.1096/fasebj.1.2.3111928.
6. Rainsford KD. Ibuprofen: from invention to an OTC therapeutic mainstay. International Journal of Clinical Practice, 2013; 67: 9-20. DOI: https://doi.org/10.1111/ijcp.12055.
7. Lucas, SM, Rothwell, NJ, Gibson RM. The role of inflammation in CNS injury and disease. British journal of pharmacology, 2006; 147(S1): 232-240. DOI: https://doi.org/10.1038/sj.bjp.0706400.
8. Jaismy Jacob P, Manju SL. Novel approach of multi-targeted thiazoles and thiazolidenes toward anti-inflammatory and anticancer therapy—dual inhibition of COX-2 and 5-LOX enzymes. Medicinal Chemistry Research, 2021; 30(1): 236-257. DOI: https://doi.org/10.1007/s00044-020-02655-9.

9. Jarapula R, Gangarapu K, Manda S, Rekulapally S. Synthesis, In Vivo Anti-Inflammatory Activity, and Molecular Docking Studies of New Isatin Derivatives. International Journal of Medicinal Chemistry, 2016; 1-9. DOI: https://doi.org/10.1155/2016/2181027.
10. Nagajothi D, Maheswari J. Synthesis, Characterization and Antimicrobial Activity of Schiff Base Ligand Metal Complexes. Gis Science Journal, 2021; 8: 1314-1326.
11. Morris GM, Lim-Wilby M. Molecular Docking, 443(1): 365-382.
12. Qureshi A, Pradhan A. Qureshi A, Pradhan A, Short Review on Thiazole Derivative: Journal of Drug Delivery and Therapeutics, 2019; 9(A): 842. DOI: https://doi.org/10.22270/jddt.v9i4-A.3701.
13. TH N, Nair SS, Kumar Dr. A, B Dr. V, P A DrD. A Review on Synthesis and Biological Activity of Thiazole and its Derivatives. International Journal Pharmaceutical Science and Research, 2021; 70(1). DOI: 10.47583/ijpsrr. 2021.v70i01.029.
14. Ibrahim SA, Rizk HF. Synthesis and Biological Evaluation of Thiazole Derivatives, 2020; 13-51. DOI: 10.5772/intechopen.93037.
15. R AB, Pazhamalai V. A Structure-Based Drug Designing of Bioactive Compounds of Gracilaria edulis against Virulent Bacterial Enzyme Aureolysin. International Journal of Pharmaceutical Sciences and Drug Research, 2019; 11(05). DOI: 10.25004/IJPSDR.2019.110512.
16. Vaibhav Saxena, Chandra Shekhar Sharma*. Synthesis and Analgesic, Anti-inflammatory Activities of Some Novel Thiadiazole Derivatives. International Journal of Pharmaceutical Sciences and Drug Research, 2021; 13(1): 87-92. DOI https://doi.org/10.25004/IJPSDR.2021.130113.
17. Kalkhambkar RG, Kulkarni GM, Shivkumar H, Rao RN. Synthesis of novel triheterocyclic thiazoles as anti-inflammatory and analgesic agents. European Journal of Medicinal Chemistry, 2007; 42(10): 1272–1276. DOI: https://doi.org/10.1016/j.ejmech.2007.01.023.
18. Holla BS, Rao S, Shivarama Holla B, Malini K v, Rao BS, Sarojini BK, et al. Synthesis of some new 2, 4-disubstituted thiazoles as possible antibacterial and anti-inflammatory agents. European Journal of Medicinal Chemistry, 2003; 313-318. DOI: https://doi.org/10.1016/s0223-5234(02)01447-2.
19. Ovais S, Yaseen S, Bashir R, Rathore P, Samim M, Singh S, et al. Synthesis and anti-inflammatory activity of celecoxib like compounds. Taylor & Francis, 2013; 28(5): 1105–12. DOI: https://doi.org/10.3109/14756366.2012.710847.

20. Rajasekaran N. Synthesis and Biological Activity of 2-(Thiazolidin-4-One) Phenyl]-1h-Phenylbenzimidazoles and 2-[4-(Azetidin-2-One)-3-Chloro-4-Phenyl]-1h-Phenyl Benzimidazoles. International Journal of Pharmaceutical Sciences and Drug Research, 2010; 2(2): 115-119.
21. Sujatha K, Vedula RR. Novel one-pot expeditious synthesis of 2,4-disubstituted thiazoles through a three-component reaction under solvent-free conditions. An international journal for rapid communication of organic chemistry, 2018; 48(3): 302–308. DOI: https://doi.org/10.1080/00397911.2017.1399422.
22. Bharti SK, Nath G, Tilak R, Singh SK. Synthesis, anti-bacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubstituted thiazole ring. European Journal of Medicinal Chemistry, 2010; 45(2): 651–660. DOI: https://doi.org/10.1016/j.ejmech.2009.11.008.