RESEARCH PAPER

Synthesis in Vitro Anti-inflammatory Activity and Molecular Docking of Some New Thiazole-Linked Thiopyrimidine Derivatives

Samadhan S. More[1], Shivcharan L. Rathod[1], Kadir. R. Khan[2], Achut S. Munde[1], Rahul A. Waghmare[1*]

[1]Department of Chemistry, Milind College of Science, Nagsenvana, Chhatrapati Sambhajinagar - 431002, Maharashtra, India.

[2]Department of Chemistry, Sir Sayyed College of Arts, Commerce and Science, Chhatrapati Sambhajinagar - 431002, Maharashtra, India.

*Corresponding author's email address: moresamadhan384@gmail.com

Received:- 10-05-2026 , Accepted :- 20-05-2026 , Revised:- 02-06-2026 , Available Online:- 23-06-2026

Abstract:N-(4-(((4-(6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11a-j) were synthesized by convenient synthetic protocols and characterized by FT-IR, ^{1}H NMR, Mass Spectroscopy techniques and elemental analyses. Molecular docking study of the synthesized compounds at the DNA cleavage site of Cyclooxygenase-2 (PDB ID: 1CX2) was done. The interactions of all compounds with the Cyclooxygenase-2 complex were analyzed and found that most compounds showed similar binding patterns with amino acid residues and DNA fragments at the binding site. All the synthesized compounds (11a-j) were screened for in vitro anti-inflammatory activity by protein denaturation assay (Egg albumin), by the modified Williams et al method, with 50µg/ml drug concentration. The most active compounds were 11f and other derivatives 11a, 11c, 11g and 11j were found to be the most potent as compared to the standard drug Ibuprofen

Keywords: Heterocyclic compounds, thiopyrimidine, thiazole, molecular docking and in vitro antiinflammatory

How to cite this article: More SS, Rathod SL, Khan KR, Munde AS, Waghmare RA. Synthesis in Vitro Anti-inflammatory Activity and Molecular Docking of Some New Thiazole-Linked Thiopyrimidine Derivatives. Int J Drug Deliv Technol. 2026;16(63s):768-777. DOI: 10.25258/ijddt.16.63s.76

Source of support: Nil.

Conflict of interest: None

Introduction:

Heterocyclic compounds form a major part of medicinal and organic chemistry and are comprised of a large number of different structures; in all cases, at least one of the atoms in the ring of atoms is a heteroatom (that is, an atom that is not carbon or hydrogen, such as N, O, S). The wide range of structural possibilities and the numerous biological properties of these compounds have fostered innovation and discovery throughout pharmaceutical science and drug discovery. Of all heterocyclic compounds, the heterocycles based on pyrazole, thiazole, or carboxamide have been the subject of considerable research in terms of their anti-inflammatory properties, and research on the biological activity of these compounds is increasingly being supported by molecular docking studies to better understand how molecular interactions occur at the target [1,2]. There is also a great deal of interest in 5-membered heterocycles with two heteroatoms due to their documented biological activity; for example, thiazole amino derived rings are well represented in this subtype of heterocyclics [3]. The pharmacologically active thiazole substructures represent a large, diverse group of molecules with pharmacological potential. The wide range of compounds containing thiazole substructures exhibit a variety of biologically relevant pharmacological activities; e.g., they are antioxidants, analgesics, and have antibacterial, anticancer, antiallergic, and antihypertensive

properties as well as being anti-inflammatory, antimalarial, antifungal, and antipsychotic in nature[4]. Numerous commercial agents containing a thiazole ring structure can be found on the market further highlighting their relevance to drug discovery. Many compounds that contain a thiazole nucleus and are combined with many other drug types have shown to possess potent antiinflammatory activity [5-7]. The use of thiazole derivatives as selective cyclooxygenase-2 (COX-2) inhibitors was demonstrated by Therien et al. [8] as well as Roy et al. [9] which establishes a model for using thiazole-containing agents to control inflammation.

Pharmacologically diverse, thiopyrimidines represent a subclass of the heterocyclic compound family that have shown efficacy against a variety of pathological conditions, including cancer, chronic HIV / AIDS infections, tuberculosis, and inflammatory conditions [10]. The chemical reactivity of thiopyrimidines is noticeably enhanced by the presence of a sulfur atom in the 2-position of the pyrimidine ring, which increases the number of potential chemical reactions that can occur between these compounds and their biological targets. No other element on the periodic table can be substituted for the sulfur atom in this position without affecting how a compound will interact with a biological target; therefore, it is expected that the thiopyrimidine class will continue to be the subjects of ongoing synthetic and biological research activities [11]. The construction of hybrid frameworks composed of fused thiazole and thiopyrimidine units provides an appealing rational design strategy, where the complementary pharmacological profiles of each of these moieties might be combined to afford hybrid compounds having improved and more selective biological activity.

This study builds off of earlier studies on developing new substances that contain thiopyrimidines and thiazoles connected by a linkage made of nitrogen atoms, which can help us to assess whether these new compounds have the very useful property of being able to reduce inflammation using laboratory assessments in conjunction with computersupported modeling techniques to simulate how they would interact with egg albumin during the denaturation process associated with the albumin's denaturation test. Additionally, to result in finding new drug candidates to be developed that would have desirable characteristics that are common among the two classes of substances (thiazole & thiopyrimidine) using simple synthetic methods, while also increasing the number of new antiinflammatory drugs available

EXPERIMENTAL: Experimental Section: All the Melting points were determined in open capillaries. (Bruker Avance) Cryo-magnet Spectrometer in DMSO Solvent using TMS as an internal standard. IR spectral data were recorded on an FT Infra-Red Spectrophotometer Model RZX Perkin Elmer. The synthesized products were confirmed by the comparison of their Mass, IR, and ^{1}H NMR spectral data. TLC was carried out on Silica gel G (Merk) plates with an n-Hexane/Ethyl Acetate system. Chemical and Material

Procedure for the synthesis of 2-Amino-4(chloromethyl) thiazole hydrochloride (3).

Thiourea (7.4 mmol) was added to a solution of 1, 3-dichloropropanone (7.4 mmol) in absolute ethanol (40 ml). The mixture was stirred at room temperature for 24 h and then kept at 5 °C for 12 h. The crystalline material was collected by filtration and recrystallized from ethanol to yield (70%) of hydrochloride with m. p. 143–144 °C. Lit.. 9 mp. 144–145 °C. [12]

Procedure for the synthesis of 2-methylsulphinamino-4-chloromethyl thiazole (5).

Triethyl amine (7.4 mmol) was added to a solution of 2-Amino-(4 chloromethyl) thiazole hydrochloride (7.4 mmol) in DCM (40 ml). The mixture was stirred at room temperature for 30 minutes until a pink colour was observed, which indicates the generation of free 2-amino-4chloromethyl thiazole. Then the reaction mass is cooled to 0-5 °C, and to it, cooled mesyl chloride (50mmol) was added in one lot. Keeping the temperature and stirring constant, triethylamine (55mmol) was added dropwise to the reaction mass. After complete addition, the reaction mass was stirred at r.t. overnight. The progress of the reaction was monitored by thin-layer chromatography, using n-hexane/ethyl acetate as a solvent system.[18] On completion of the reaction, the product was isolated by pouring the reaction mass into 40 ml water in a

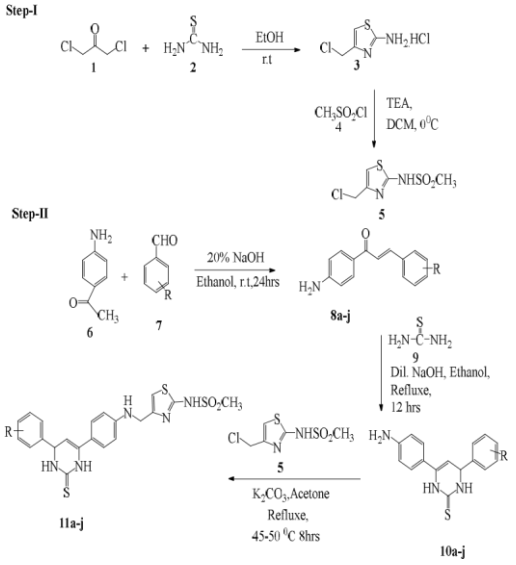
[1] H NMR spectral data were recorded at 500 MHz

separating funnel, followed by separation, drying on sodium sulfate, and finally removing DCM on Rota evaporator to give white crystalline material, which was further recrystallized in ethanol to yield (62%).

General procedure for the preparation of 1-(4aminophenyl)-3-phenylprop-2en-1-ones (8a-j): Equimolar quantity (0.01mol) of 4-Amino acetophenone and respective aryl aldehyde were mixed and dissolved in 30 ml of alcohol. To this, add aqueous potassium hydroxide (KOH) 20% solution, then it was continuously stirred for 24 hours at room temperature. The reaction progress was monitored on Thin Layer Chromatography (n-hexane /Ethyl Acetate, 8:2). After completion of the reaction, it was poured on crushed Ice and neutralized with dilute. HCl and the obtained product were filtered, dried and recrystallised from alcohol. The physical data is recorded and correlated with the reference [19].

General procedure for the preparation of 6-(4aminophenyl)-4-phenyl-3,4-dihydropyrimidine2(1H)-thione (10a-j). A Mixture of Chalcones (0.01mol) and Thiourea (0.02mol) was taken in Ethanol (30ml) and Dil. NaOH (3ml) was added slowly with constant stirring, and the reaction mixture was refluxed for 15 hours. The reaction's progress was monitored on Thin Layer Chromatography (n-Hexane/Ethyl Acetate). After completion of the reaction, it was poured on crushed Ice, filtered, dried and recrystallized from alcohol. The physical data is recorded and correlated with reference [13,14].

Synthesis of N-(4-(((4-(6-phenyl-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11a-j) To the stirred solution (0.001mol) of compound 5 in 20 ml acetone add Compound 10a-j (0.001mol), and stirred the reaction mixture for 8 hrs, maintaining the temp 4550ºC the pH was kept neutral by the appropriate addition of 10% K_2CO_3 Solution. The temperature was steadily raised to 45 °C and maintained for 6 hours. The reaction progress was monitored on Thin Layer Chromatography (n-Hexane/Ethyl Acetate, 7:2). After completion of the reaction, it was poured on crushed Ice. The solid obtained product was filtered and dried. The crude was purified and recrystallized from Acetone.



Where: R=H, 2-Cl, 4-Cl, 2,4-Cl, 2-F, 4-F, 4-Br, 2-CH_3, 4-CH_3, 4-OCH_3

Scheme: N-(4-(((4-(6-phenyl-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide

Spectral data of intermediate, 2-methylsulphonyl amino -4-chloromethyl thiazole(5): Yield-62%, mp-178-180°C IR (cm $^{-}$1): 3256 (NH stretch), 3246 (aromatic CH), 3110 (aromatic CH), 2927 (aliphatic CH), 2857 (aliphatic CH), 1717(C=N stretch) 1606 (C=C), 1552 (NH bend), 1293 (S=O assy.) and 1119 (S=O symm.). 1H NMR (DMSOd6, 300 MHz), δ(ppm) : 3.87 (s, 3H,SO_2CH_3), 4.61(s, 2H,CH_2Cl) and 6.91(s, 1H,thizolyl) and12.50 (s,1H,NH, exchangable with D_2O). ^{13}C NMR (DMSOd6, 75 MHz) δ(ppm): 43.07, 56.24, 107.93, 124.81 and 168.05. MS (ESI+ mode): m/z (% intensity): 226. 96 (M+,100), 228.96 (M+2,30). Elemental Analysis : M.F-$C_5H_7ClN_2O_2S_2$: Found% (Calculated %): C, 26.23 (26.49); H, 3.09 (3.11); N, 12.31 (12.36); S, 28.25 (28.29).

N-(4-(((4-(6-phenyl-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11a)

Yield 74%, m.p. 103-105°C. IR (KBr, Vmax,cm1):3440,3120 (NH-Stretch), 2893 (-C-H-stretch in Ar-H), 1177(C=S stretch in Thiocarbonyl group), 1562 (C=N amide), 1302 (C-N amine). ^{1}H

NMR (DMSO, 500MHz, δ ppm): 13.11 (s, 1H, -NH),
10.90 (s,1H,-NH), 9.13 (s,1H,-NH), 7.65(d,2H), 7.20-7.25 (m5H), 6.72(s,1H NH), 6.68 (d,1H), 6.66(d,2H), 6.35 (s,1H), 4.56(d,2H) 4.45(d,1H), 3.18(s 3H) Mass (m/z): 471.61[m+1] Chemical Formula: $C_{21}H_{21}N_5O_2S_3$ Elemental Analysis: C, 53.48; H, 4.49; N, 14.85; O, 6.78; S, 20.39 Found:C, 53.31; H, 4.34; N, 14.61; O, 6.56; S, 20.16

N-(4-(((4-(6-(2-chlorophenyl)-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11b)Yield 80%, m.p. 9294°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1178 (C=S stretch in Thiocarbonyl group), 1562 (C=N amide), 1298 (C-N amine), 758 (C-Cl). ^{1}H NMR (DMSO,
500MHz, δ ppm): 13.14 (s, 1H, -NH), 10.91 (s,1H,NH), 9.15(s,1H NH), 7.62(d,2H), 7.60(dd,1H),
7.28 (m,1H), 7.24(dd,1H), 7.22 (m,1H), 6.74(d,1H), 6.69 (d,2H) 6.72(s,1H NH), 6.30(s,1H), 4.56 (d,2H), 4.39 (d,1H), 3.16(s 3H) Mass (m/z): 506.05[m+1] Chemical Formula: $C_{21}H_{21}N_5O_2S_3$ Elemental Analysis: C, 49.84; H, 3.98; Cl, 7.01; N, 13.84; O, 6.32; S, 19.01 Found : C, 49.69; H, 3.82; Cl, 6.93; N, 13.71; O, 6.17; S, 18.91

N-(4-(((4-(6-(4-chlorophenyl)-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11c) Yield 79%, m.p. 7880°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1177 (C=S stretch in Thiocarbonyl group), 1562 (C=N amide),1295 (C-N amine), 770 (C-Cl). ^{1}H NMR (DMSO, 500MHz, δ ppm): 13.15(s,1H NH) 10.90 (s, 1H, -NH), 9.13 (s,1H,-NH), 7.60(d,2H),7.44 (d,2H), 7.22(d,2H), 6.72(d,1H), 6.67 (d2H), 6.74 (s,1H coupling NH), 6.38(s,1H), 4.59 (d,2H), 4.45(d,1H), 3.19(s 3H), Mass (m/z): 506.05[m+1] Chemical Formula: $C_{21}H_{20}ClN_5O_2S_3$ Elemental Analysis: C, 49.84; H, 3.98; Cl, 7.01; N, 13.84; O, 6.32; S, 19.01 Found : C, 49.69; H, 3.78; Cl, 6.91; N, 13.64; O, 6.18; S, 18.90

N-(4-(((4-(6-(2,4-dichlorophenyl)-2-thioxo1,2,3,6-tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11d) Yield 81%, m.p. 8991°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1174(C=S stretch in Thiocarbonyl group), 1562 (C=N amide), 1297 (C-N amine), 776 (C-Cl). ^{1}H NMR (DMSO, 500MHz, δ ppm): 13.16(s, 1H NH), 10.88 (s, 1H, NH), 9.10 (s,1H,-NH), 7.62(d,2H), 7.60(d,1H),
7.32(dd,1H), 7.19 (d,1H), 6.69(d,1H), 6.67 (d,2H),6.73 (s,1H NH), 6.38(s,1H), 4.53 (d,2H), 4.51(d,1H), 3.17(s,3H), Mass (m/z): 540.503
[m+1] Chemical Formula: $C_{21}H_{19}Cl_2N_5O_2S_3$ Elemental Analysis: C, 46.67; H, 3.54; Cl, 13.12; N, 12.96; O, 5.92; S, 17.79 Found : C, 46.56; H, 3.33; Cl, 13.02; N, 12.81; O, 5.80; S, 17.68

N-(4-(((4-(6-(2-fluorophenyl)-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11e) Yield 77%, m.p. 7173°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1180 (C=S stretch in Thiocarbonyl group), 1298 (C-N amine), 1562 (C=N amide), 962(C-F). ^{1}H NMR (DMSO,
500MHz, δ ppm): 13.15 (s,1H NH), 10.86 (s, 1H, NH), 9.09 (s,1H,-NH), 7.71(d,2H), 7.69 (m,1H), 7.60(dd,1H), 7.30 (dd,1H), 7.16(m,1H), 6.73 (d,1H), 6.71 (s,1H NH), 6.68(d,2H), 6.39(s,1H) 4.58(d,2H), 4.45(d,1H), 3.16(s,3H), Mass (m/z): 489.60[m+1] Chemical Formula: $C_{21}H_{20}FN_5O_2S_3$ Elemental Analysis: C, 51.52; H, 4.12; F, 3.88; N, 14.30; O, 6.54; S, 19.64 Found : C, 51.32; H, 4.01; F, 3.65; N, 14.07; O, 6.36; S, 19.46

N-(4-(((4-(6-(4-fluorophenyl)-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11f) Yield 77%, m.p. 8283°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1176 (C=S stretch in Thiocarbonyl group), 1562 (C=N amide),1299 (C-N amine), 960(C-F). ^{1}H NMR (DMSO, 500MHz, δ ppm): 13.10 (s,1H NH), 10.84 (s, 1H, -NH), 9.14 (s,1H,-NH), 7.62(d,2H), 7.31 (d,2H), 7.22(d,2H), 6.74 (s,1H coupling NH), 6.73(d,1H), 6.69 (d,2H), 6.31(s,1H), 4.50 (d,2H), 4.38(d,1H), 3.21(s 3H), Mass (m/z): 489.60[m+1] Chemical Formula: $C_{21}H_{20}FN_5O_2S_3$ Elemental Analysis: C, 51.52; H, 4.12; F, 3.88; N, 14.30; O, 6.54; S, 19.64 Found : C, 51.31; H, 4.01; F, 3.67; N, 14.09; O, 6.44; S, 19.49

N-(4-(((4-(6-(4-bromophenyl)-2-thioxo-1,2,3,6tetrahydropyrimidin-

4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11g) Yield 91%, m.p. 99101°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1175 (C=S stretch in Thiocarbonyl group), 1562 (C=N amide), 1298 (C-N amine), 1124(C-Br). ^{1}H NMR (DMSO, 500MHz, δ ppm):13.16 (s,1H NH), 10.92 (s, 1H, NH), 9.13 (s,1H,-NH), 7.76(d,2H), 7.65 (d,2H), 7.21(d,2H), 6.74 (s,1H NH), 6.68(d,1H), 6.66 (d,2H), 6.31(s,1H), 4.51(d,2H), 4.40(d,1H) 3.13(s 3H), Mass (m/z): 550.51 [m+1] Chemical Formula: $C_{21}H_{20}BrN_5O_2S_3$ Elemental Analysis: C, 45.82; H, 3.66; Br, 14.51; N, 12.72; O, 5.81; S, 17.47 Found :C, 45.62; H, 3.43; Br, 14.39; N, 12.59; O, 5.66; S, 17.31

N-(4-(((4-(2-thioxo-6-(o-tolyl)-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11h) Yield 76%, m.p. 7981°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1178 (C=S stretch in Thiocarbonyl group),1294 (C-N amine), 1300 (C-N amine), 1562 (C=N amide). ^{1}H NMR (DMSO, 500MHz, δ ppm):13.11 (s,1H NH), 10.91 (s, 1H, -NH), 9.16 (s,1H,-NH), 7.71(d,2H), 7.35(dd,1H), 7.26 (dd,1H), 7.24(m,1H), 7.15 (m,1H), 6.78(s,1H NH), 6.73 (d,1H), 6.69 (d,2H), 6.31(s,1H) 4.59(d,2H), 4.38(d,1H), 3.21(s,3H), 2.40 (s,3H), Mass (m/z): 485.64[m+1] Chemical Formula: $C_{22}H_{23}N_5O_2S_3$ Elemental Analysis: C, 54.41; H, 4.77; N, 14.42; O, 6.59; S, 19.80 Found: C, 54.29; H, 4.58; N, 14.26; O, 6.44; S, 19.71

N-(4-(((4-(2-thioxo-6-(p-tolyl)-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11i) Yield 78%, m.p. 7778°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1178 (C=S stretch in Thiocarbonyl group), 1296 (C-N amine), 1562 (C=N amide). ^{1}H NMR (DMSO, 500MHz, δ ppm): 13.17 (s,1H NH), 10.91 (s, 1H, -NH), 9.14(s,1H,-NH), 7.72(d,2H), 7.25 (d,2H), 7.14(d,2H), 6.73(d,1H), 6.71(s,1H NH), 6.70 (d, 2H) 6.38(s,1H), 4.60 (d,2H), 4.41(d,1H), 3.22(s,3H), 2.41(s 3H), Mass (m/z): 485.64[m+2] Chemical Formula: $C_{22}H_{23}N_5O_2S_3$ Elemental Analysis: C, 54.41; H, 4.77; N, 14.42; O, 6.59; S, 19.80 Found: C, 54.31; H, 4.66; N, 14.33; O, 6.39; S, 19.69

N-(4-(((4-(6-(4-methoxyphenyl)-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11j) Yield 79%, m.p. 6971°C. IR (KBr, Vmax,cm-1):3440,3120 (NHStretch), 2893 (-C-H-stretch in Ar-H), 1180 (C=S stretch in Thiocarbonyl group), 1302 (C-N amine), 1562 (C=N amide), 1120 (C-O-C). ^{1}H NMR (DMSO, 500MHz, δ ppm):13.09 (s,1H NH), 10.87 (s, 1H, -NH), 9.14 (s,1H,-NH), 7.70(d,2H), 7.21 (d,2H), 6.81(d,2H), 6.74 (s,1H NH), 6.72(d,1H), 6.68 (d,2H), 6.40(s,1H), 4.60(d,2H), 4.42 (d,1H), 3.79(s 3H), 3.22(s 3H), Mass (m/z): 501.64 [m+1] Chemical Formula: $C_{22}H_{23}N_5O_3S_3$ Elemental Analysis: C, 52.68; H, 4.62; N, 13.96; O, 9.57; S, 19.17 Found: C, 52.53; H, 4.47; N, 13.74; O, 9.38; S, 19.02

Molecular docking:

The molecular docking study of compounds was carried out at the DNA cleavage site of Cyclooxygenase -2 (PDB ID: 1CX2) [15]. The main purpose of docking studies was to investigate the possible interactions of synthesized compounds with the above enzymes in order to support the analgesic activity [16]. Docking algorithms provide essential structural information about protein-drug interactions, which play a pivotal role in drug development [17]. Molecular docking tools were used to predict the orientation of the newly designed novel N-(4-(((4-(6-(4-fluorophenyl)-2-oxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide (11f) of hybrids within the constraints of protein binding pockets [18]. The Cyclooxygenase -2 inhibition results of these hybrids (11a-j) encouraged us to perform molecular docking studies and compare the results with the Cyclooxygenase -2 inhibitor [16].

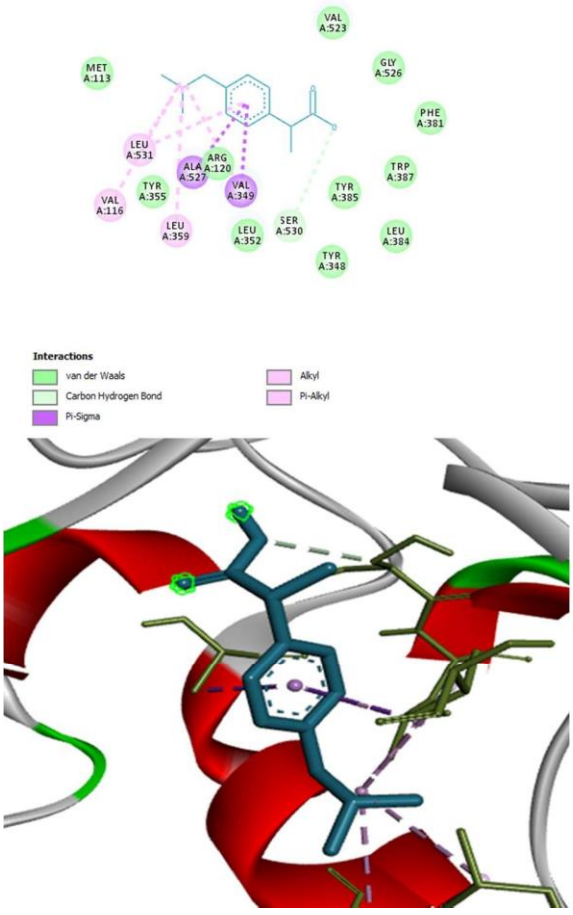


Fig. No.1 Docking pose of Ibuprofen with 1COX2

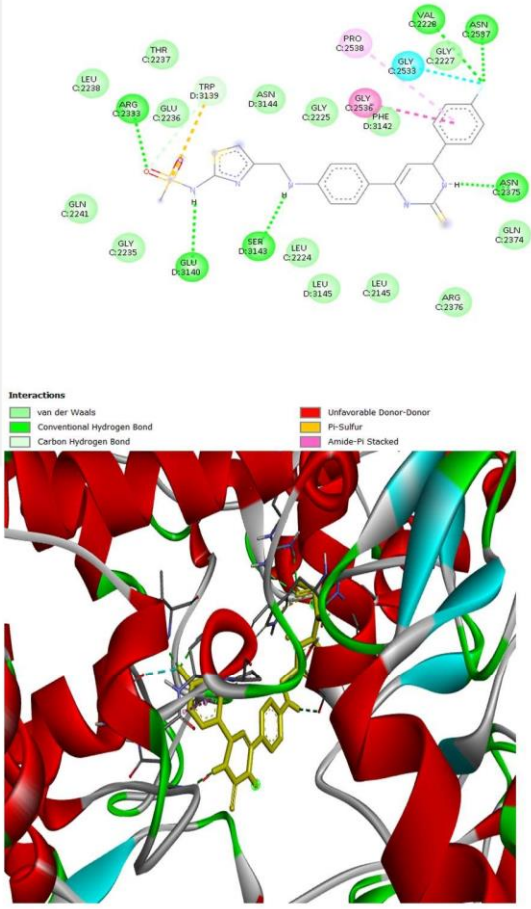


Fig. No.1 Docking pose of 11f with 1CX2

The docking of the standard Ibuprofen showed that Ibuprofen bind at the DNA cleavage site with binding affinity of–7.5 kcal/mol by establishing pisigma bond with amino acid residue ALA-527 and VAL-349 [19] The docking study of synthesized compound showed that compound 11f was able to interact perfectly within the active site of enzyme with binding affinity of -11.5 kcal/mol by developing hydrogen bond with amino acid residue ASN-2537, SER-3143, GLU-3140, ARG-2333, PRO-2538 and GLY-2536 The interaction of all compounds with Cyclooxygenase -2 complex were analyzed and it was revealed that most compounds exhibited identical binding patterns with amino acid residues and DNA fragments at the binding site [16] These docking findings suggested that the synthesized compounds were found to be secured within the Cyclooxygenase -2 hydrophobic DNA cleavage site [18]

Table no. 1 Docking score

Target Molecule: 1CX2 (Cyclooxygenase-2)

Sr. No.	Drug code	Binding affinity kcal/mol
1	11a	-10.9
2	11b	-10.7
3	11c	-10.5
4	11d	-10.6
5	11e	-10.5
6	11f	-11.5
7	11g	-10.8
8	11h	-11.0

9	11i	-10.8
10	11j	-6.6
11	Ibuprofen	-7.5

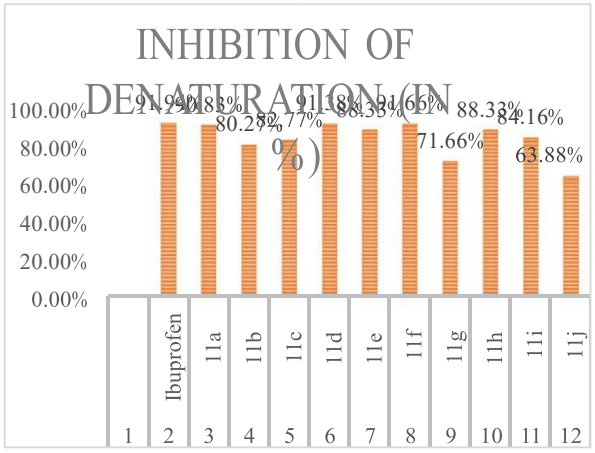
Anti-inflammatory activity:

By using the protein Denaturation Assay. (Hen egg albumin)

The anti-inflammatory activities of synthesized compounds were determined using a modified version of the BSA assay reported by Williams et al. [20]. The in vitro anti-inflammatory activity by the protein denaturation assay using egg albumin was determined. The reaction mixture (5ml) was prepared, containing 2ml of diluted egg albumin (2% v/v in DI water), 1.0 ml of phosphate buffer saline (PBS, pH 6.4) and 2ml of samples (50µg/ml, prepared using DMF and water and DMF percentage is less than 0.2%) and in the case of the control, 2 ml of DI water. Then the mixtures were incubated for 10 minutes at 37°C and then heated at 60°C in a water bath for an additional 10 minutes to induce denaturation of egg albumin. After cooling the mixture, the absorbance was measured at 660 nm (UV-1700 PharmaSpec UV-visible Shimadzu Spectrophotometer). Standard Clinical Drugs Ibuprofen were used as a positive control for the study [21]. The mean absorbance values were noted after the trials were carried out in triplicate. The following formula was used to calculate the percentage inhibition of precipitation (protein denaturation) in comparison to the negative control [22, 23].

Table 2 screened for in vitro anti-inflammatory activity, by (hen Egg albumin) protein denaturation assay

Sr. No.	Compounds	Mean absorbance value	Inhibition of denaturation (in %)
1	control	0.036	-
2	Ibuprofen	0.0691	91.94%
3	11a	0.0687	90.83%
4	11b	0.0649	80.27%
5	11c	0.0658	82.77%
6	11d	0.0689	91.38%
7	11e	0.0678	88.33%
8	11f	0.0690	91.66%
9	11g	0.0618	71.66%
10	11h	0.0678	88.33%
11	11i	0.0663	84.16%
12	11j	0.0599	63.88%



The percentage of protein denaturation was determined using the following equation

% of Inhibition = (Vt / Vc – 1) × 100

Where,

Vt = Mean absorbance value of test group.
Vc = Mean absorbance value of the control group
* % Anti-Denaturation Activity = % Inhibition of Protein Denaturation = % Anti-inflammatory Activity

RESULT AND DISCUSSION

Literature survey reveals that there are no reports of N-(4-(((4-(6-phenyl-2-thioxo-1,2,3,6tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide, hence it was planned to synthesize these compounds. In the present study, step-I, 1,3-dichloropropanone (1) is reacted with thiourea (2) in the presence of ethanol to yield 2amino-4-(chloromethyl) thiazole hydrochloride (3) in good yield. 2-amino-4-(chloromethyl) thiazole hydrochloride reacted with methyl sulphonyl chloride (4) in the presence of TEA in DCM as a solvent to yield 2-methylsulphinamio-4chloromethyl thiazole (5). The IR spectra of compound (5) show a strong absorption band at 3256 cm^{-1}, indicating the stretching frequency of the NH functional group. 1293 cm^{-1} and 1119 cm^{-1} show stretching frequency of S=O asymmetrical and S=O symmetry, which conform to the thiazole (5) functional groups. ^1H NMR spectrum of compound (5) shows a singlet at 12.50 ppm for NH, again a singlet at 6.91 ppm confirms the thiazolyl H. Mass spectral shows the molecular ion peak at 226.96 [M+]. All the spectral analysis confirmations of the thiazole (5) compound. Step-II, 4-amino acetophenone (6), is reacted with the substituted aryl aldehydes (7) in the presence of NaOH, followed by a condensation reaction to yield 1-(4-aminophenyl) 3-phenylprop-2-en-1-one compound derivatives (8a-j) with good yield. Further synthesis, Compounds (8a-j) reacted with the compound (9), which yielded compounds (10a-j). In the final step, compound (10a-j) reacted with compound (5) in the presence of K_2CO_3 and Acetone as solvent to yield (11a-j) with excellent yield. The IR spectra of (11a) show a strong absorption band at 3440 cm-1 and 3120 $cm^{-1,}$ indicating the Stretching frequency of the -NH- functional group. 1562 cm-1 is the stretching of (-C=N in stretch), 1177cm^{-1} is the value for (C=S) stretching in pyrazoline, confirming the synthesis of coupling of thiopyrimidine derivatives with thiazole. ^1H NMR Spectrum of (11a) show that a singlet at δ 13.11 ppm for (-NH-) confirms secondary amine, and another NH singlet at δ 10.90 ppm for (-NH-) confirms secondary amine, again a singlet at δ 6.72 ppm confirms the (-NH-) coupling of thiazole. The Mass Spectra shows the molecular Ion peak at 471.61 [m+1]. All these Spectral analyses show the Confirmation of the synthesis of (11a-j) compounds. The molecular docking investigation of the compounds was performed at the DNA cleavage site of Cyclooxygenase -2 (PDB ID: 1CX2). The docking of the standard Ibuprofen indicated the binding of Ibuprofen at the DNA cleavage site with a binding affinity of -7.5 kcal/mol by establishing a pi-sigma bond with amino acid residues ALA-527 and VAL-349. The docking study

of synthesized compound showed that the compound (11f) was able to interact perfectly with the active site of the enzyme with a binding affinity of -11.5 kcal/mol by developing hydrogen bonds with amino acid residues ASN-2537, SER-3143, GLU-3140, ARG-2333, PRO-2538 and GLY-2536. Screening of the biological activities of synthesized compounds revealed that compounds (11a-j) show good anti-inflammatory activities. Compounds (11a, 11d, 11e and 11f), having electron-withdrawing, and 11h is electron donating, show good antiinflammatory activity using the protein denaturation assay (egg albumin). The Investigation of antiinflammatory activity data revealed that the compound, which has substituted Chloro, fluoro, Bromo and methyl, shows good anti-inflammatory activity compared to other substituents compared with the standard ibuprofen drug.

CONCLUSION

In the present research, we have reported the synthesis of a new series of N-(4-(((4-(6-phenyl-2thioxo-1,2,3,6-tetrahydropyrimidin-4yl)phenyl)amino)methyl)thiazol-2yl)methanesulfonamide derivatives (11a-j). The molecular docking study of compounds was carried out at the DNA cleavage site of Cyclooxygenase -2 (PDB ID: 1CX2). The interaction of all compounds with the Cyclooxygenase-2 complex was analyzed, and it was revealed that most compounds exhibited identical binding patterns with amino acid residues and DNA fragments at the binding site. All the compounds show promising Anti-inflammatory activities as compared to the standard ibuprofen drug. All synthesized compound shows potent Antiinflammatory activity against the standard ibuprofen drug. Compounds with chlorine, fluorine, bromine and substituents have more Anti-inflammatory

activity compared to other substituents. All the synthesized series of compounds show excellent to moderate activity using denaturation methods (egg albumin) and are very promising core molecules as potent anti-inflammatory agents; further investigation is needed.

ACKNOWLEDGEMENT:

The Authors gratefully acknowledge SAIF and CIL, Chandigarh, India, for ^{1}H NMR, Elemental Analysis, and Mass Spectral Analysis. The authors are very much thankful to the Principal of Milind College of Science, Nagsenvana, Chhatrapati Sambhajinagar, and also, we are appreciative of the IR facilities provided by the Chemistry Department of Dr Babasaheb Ambedkar Marathwada University in Chhatrapati Sambhajinagar. The authors are very thankful to Dr Babasaheb Ambedkar Research and Training Institute (BARTI), Pune, Government of Maharashtra, for providing fellowship during this research.

References:

[1] M. Hawash, N. Jaradat, M. Abualhasan, M. K. Şüküroğlu, M. T. Qaoud, D. C. Kahraman, H. Daraghmeh, L. Maslamani, M. Sawafta, A. Ratrout, and L. Issa, "Design, synthesis, molecular docking studies and biological evaluation of thiazole carboxamide derivatives as COX inhibitors," BMC Chem., vol. 17, no. 1, p. 11, Mar. 2023, doi: 10.1186/s13065-023-00924-3.

[2] M. A. M. Abdel Reheim, H. S. Abdel Rady, O. A. Mohamed, A. Hassan, I. S. Abdel Hafiz, H. M. Reffat, F. G. Elsaid, M. Eldesoqui, D. S. Alshaya, A. A. Badawy, E. Fayad, and A. H. Abdelmonsef, "Synthesis, anti-inflammatory, and molecular docking studies of new heterocyclic derivatives comprising pyrazole, pyridine, and/or pyran moieties," Pharmaceuticals, vol. 18, no. 3, p. 335, Feb. 2025, doi: 10.3390/ph18030335.

[3] O. Ebenezer, M. A. Jordaan, G. Carena, T. Bono, M. Shapi, and J. A. Tuszynski, "An overview of the biological evaluation of selected nitrogencontaining heterocycle medicinal chemistry compounds," Int. J. Mol. Sci., vol. 23, no. 15, p. 8117, Jul. 2022, doi: 10.3390/ijms23158117.

[4] Z. Xu, S. Zhao, and G. Lv, "Thiazole ring—A biologically active scaffold," Molecules, vol. 26, no. 11, p. 3166, May 2021, doi: 10.3390/molecules26113166.

[5] M. Abdelgawad, R. B. Bakr, and A. H. A. Abdelazeem, "Multicomponent reaction for synthesis, molecular docking, and antiinflammatory evaluation of novel indole-thiazole hybrid derivatives," Mol. Diversity, vol. 29, no. 1, pp. 201–215, Aug. 2024, doi: 10.1007/s11030-02410969-8.

[6] N. A. Elkanzi and A. M. Alasiri, "Synthesis and biological evaluation of coumarin appended thiazole hybrid heterocycles: Antibacterial and antifungal study," J. Mol. Struct., vol. 1294, p. 136496, Nov. 2023, doi: 10.1016/j.molstruc.2023.136496.

[7] A. Al-Harbi, I. Saleh, and N. M. El-Metwaly, "Antimicrobial activity of novel substituted 1,2,4triazole and 1,3-thiazole derivatives," J. Mol. Struct., vol. 1296, p. 136874, Feb. 2024, doi: 10.1016/j.molstruc.2023.136874.

[8] M. A. Therien, W. C. Black, C. S. Chan, F. Cromlish, M. Ethier, and D. Riendeau, "Synthesis of selective COX-2 inhibitors based on thiazole derivatives," J. Med. Chem., vol. 44, no. 20, pp. 3231–3245, 2001, doi: 10.1021/jm0100873.

[9] S. Roy, R. Roy, and D. Bhattacharya, "Thiazole derivatives as selective cyclooxygenase-2 inhibitors: Synthesis and biological evaluation," Bioorg. Med. Chem. Lett., vol. 16, no. 7, pp. 1957– 1962, 2006, doi: 10.1016/j.bmcl.2005.12.061.

[10] S. F. Mohamed, E. M. Flefel, A. E. E. Amra, and D. N. A. El-Shafy, "Anti-HSV-1 activity and mechanism of action of some new synthesized substituted pyrimidine, thiopyrimidine and thiazolopyrimidine derivatives," Eur. J. Med. Chem., vol. 45, no. 4, pp. 1494–1501, 2010, doi: 10.1016/j.ejmech.2010.01.011.

[11] R. Keshk, M. A. Keshk, and E. Abuelhana, "Synthesis, antimicrobial, anti-inflammatory, antioxidant and cytotoxicity of new pyrimidine and pyrimidopyrimidine

derivatives," Sci. Rep., vol. 15, no. 1, p. 9152, Mar. 2025, doi: 10.1038/s41598025-92066-w.

[12] Synthesis and in vitro Anti-inflammatory Activity of Some 2-(Methylsulphonyl Amino) 4(Arylthio) methyl Thiazoles Rahul A. Waghmare1, Dinesh L. Lingampalle2, Vasant B. Jagrut3 and Ashish Asrondkar4 doi.org/10.14233/ajomc.2018.AJOMC-P92

[13]Synthesis and Anti-Microbial Activity of 2(Coumarinyl-4-Oxy)-4, 6 Dichloro-1,3,5-Triazine Pyrazole-1-Carboxamide Derivatives. Rahul. R. Dhavse, Santosh. R. Kshirsagar, Rahul. A. Waghmare, Achut. S. Munde* Afr. J. Bio. Sc. 6(6) (2024) 9206-9214

[14] A. Ozdemir, G. TuranZitouni, Z.A. Kaplanciklien, G. Revial, K. Guven, Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2thienyl)-5-aryl-2-pyrazoline derivatives, Eur. J. Med. Chem. 42 (2007) 403–409.

[15] Cyclization of Chalcone Derivatives: Design, Synthesis, In Silico Docking Study, and Biological Evaluation of New Quinazolin-2,4-diones Incorporating Five-, Six-, and Seven-Membered Ring Moieties as Potent Antibacterial Inhibitors Mohamed El-Naggar, Huda R. M. Rashdan, and Aboubakr H.A bdelmonsef*: ACS Omega 2023,8,27216−2723

[16]. Rasayan Journal. (n.d.). In-silico screening of some hydroxyquinoline analogues through ADMET and molecular docking studies approach using Cyclooxygenase2 (1CX2). https://rasayanjournal.co.in/admin/php/uploads/216 8_pdf.pdfrasayanjournal

[17]. BioMed Pharma Journal. (2024). In-silico investigation and development of Cyclooxygenase2 (1CX2) selective inhibition as a possible antiinflammatory activity. https://biomedpharmajournal.org/vol17no3/insilico-investigation-and-development-ofcyclooxygenase-2-1cx2-selective-inhibition-as-apossible-anti-inflammatoryactivity/biomedpharmajournal

[18]. PMCID: PMC8630655. (2021). Benchmarking different docking protocols for predicting the binding poses of ligands complexed with cyclooxygenase enzymes and screening chemical libraries. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8630655/pmc.ncbi.nlm.nih

[19]. MDPI. (2022). Enlarging the NSAIDs Family: Molecular Docking of Designed Pyrazole and Oxadiazole Derivatives as Novel Anti-Inflammatory Agents. https://www.mdpi.com/1424-8247/15/11/1349mdpi [20]. PMC. (2025). Aspirin vs. ibuprofen: Unveiling the distinct cyclooxygenase-1/2 behaviour and dual efficacy of their synthesized analogues via molecular modelling and in vitro biological assessment. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10556345/pmc.ncbi.nlm.nih

[21]. Williams, LAD., Connar, AO, Latore, L, Dennis O, Ringer S, Whittaker, JA, Conrad J, Vogler B, Rosner H, Kraus, W. The in vitro antidenaturation effects induced by natural products and non-steroidal compounds in heat-treated (immunogenic) Bovine Serum Albumin (BSA) is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals in the early stages of the drug discovery process. West Indian Med. J., 57(4), 2008, 327-331.

[22]. Das P, Ghosal K, Jana NK, Mukherjee A, Basak P: Green synthesis and characterization of silver nanoparticles using belladonna mother tincture and its efficacy as a potential antibacterial and anti-inflammatory agent. Mater Chem Phys. 2019, 228:310-7. 10.1016/j.matchemphys.2019.02.064

[23]. Yvonne A. Bailey-Shaw*1, Lawrence A. D. Williams1,2, Cheryl E. Green1, Shawntae Rodney1, Ann Marie Smith1 Int. J. Pharm. Sci. Rev. Res., 47(1), November - December 2017; Article No. 27, Pages: 145-153