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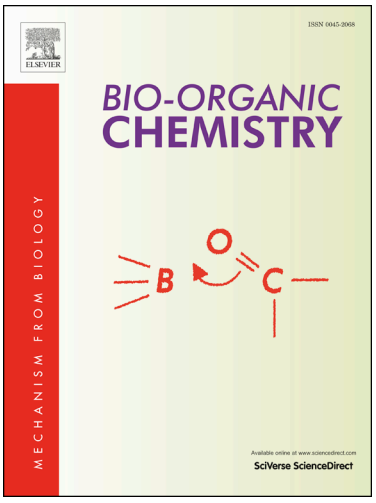

Selective Cyclooxygenase inhibition and ulcerogenic liability of some newly prepared anti-inflammatory agents having thiazolo[4,5-*d*]pyrimidine scaffold

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Selective Cyclooxygenase inhibition and ulcerogenic liability of some newly prepared anti-inflammatory agents having thiazolo[4,5-*d*]pyrimidine scaffold

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Key words: Thiazolo[4,5-*d*]pyrimidine; Thiazolidine; Anti-inflammatory; Cox-2 inhibitors.

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Abstract

Novel candidates of thiazolo[4,5-*d*]pyrimidines (**9a-l**) were synthesized and their structures were elucidated by spectral and elemental analyses. All the novel derivatives were screened for their cyclooxygenase inhibitory effect, anti-inflammatory activity and ulcerogenic liability. All the new compounds exhibited anti-inflammatory activity, especially 1-(4-[7-(4-nitrophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl)ethanone **(9g)** was the most active derivative with 57%, 88% and 88% inhibition of inflammation after 1, 3 and 5 h, respectively. Furthermore, this derivative **9g** recorded higher anti-inflammatory activity than celecoxib which showed 43%, 43% and 54% inhibition after 1, 3 and 5 h, sequentially. Moreover, the target derivatives **9a-l** demonstrated moderate to high potent inhibitory action towards COX-2 (IC_{50} = 0.87–3.78 μM), in particular, the derivatives **9e** (IC_{50} = 0.92 μM), **9g** (IC_{50} = 0.87 μM) and **9k** (IC_{50} = 1.02 μM) recorded higher COX-2 inhibitory effect than the selective COX-2 inhibitor drug celecoxib (IC_{50} = 1.11 μM). The *in vivo* potent compounds (**9e**, **9g** and **9k**) caused variable ulceration effect (ulcer index = 5–12.25) in comparison to that of celecoxib (ulcer index = 3). Molecular docking was performed to the most potent COX-2 inhibitors (**9e**, **9g** and **9k**) to explore the binding mode of these derivatives with Cyclooxygenase-2 enzyme.

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1. Introduction

Cyclooxygenase enzyme (COX) is responsible for the formation of prostaglandins (PGs), and thromboxanes (TXA2) from arachidonic acid [1, 2]. Cyclooxygenase enzyme exists in two distinct isoforms, i) a constitutive form (COX-1) which is essential for the physiological production of (PGs) and maintenance functions such as cytoprotection in the stomach, and ii) an inducible form (COX-2) which is induced in inflammatory cells [3, 4]. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) were recorded to suppress both COX-1 and COX-2 producing adverse side effects as ulceration, and gastrointestinal bleeding [5-7]. Coxibs were introduced in market as selective COX-2 inhibitors that inhibit COX-2 isoform achieving the same anti-inflammatory effect as traditional NSAIDs with minimized risk of ulcers [8, 9]. But some selective COX-2 inhibitors as valdecoxib exhibited cardiovascular side effects as myocardial infarction and hypertension leading to their withdrawal from markets [10, 11]. Thiazolidine derivatives revealed great attention due to their biological activity as anticancer [12, 13], antimicrobial [14, 15], hypoglycemic [16, 17] and anti-inflammatory [18, 19]. Zarghi *et al* [20] designed new derivatives of 2,3-diaryl-1,3-thiazolidin-4-ones which recorded COX-2 inhibitory activity, in particular thiazolidino derivative **1** (Fig. 1) which was the most active derivative (IC_{50} = 0.12 μM), and selective COX-2 inhibitor (SI > 833). Moreover, this compound **1** was more COX-2 selective than the standard drug celecoxib (SI > 403). In addition, (*Z*)-*N*-(3-chlorophenyl)-2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2,6-dimethoxyphenoxy)acetamide (**2)** (Fig. 1) was detected to suppress the iNOS activity (IC_{50} = 25.2 μM) and LPS-induced NO production (IC_{50} = 45.6 μM) in RAW264.7 [21]. Furthermore, pyrimidine ring was recorded in literature as an important scaffold for anti-inflammatory activity [22-24]. For example, compound **3** was prepared and evaluated for its anti-inflammatory activity using carrageenan-induced paw oedema method in rats [25]. Results of this study showed that this compound **3** exhibited percentage inhibition of oedema = 52.9% after 5 hours. Also,

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7-amino-5-(3,4,5-trimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (**4**) (Fig. 1) demonstrated better COX-2 inhibitory activity in a range (IC_{50} = 0.25 μM) than the standard drug celecoxib (IC_{50} = 1.11 μM) [26].

In the view of the aforesaid studies and in continuation of our previous researches on the design of anti-inflammatory agents [26-29], we have carried out synthesis of thiazolo[4,5-*d*]pyrimidine ring system having both pyrimidine ring and thiazolidine moiety in one hybrid structure attempting to obtain more potent and COX-2 selective anti-inflammatory agents with fewer gastric side effects.

Insert Fig.1 here

2. Results and discussion

2.1. Chemistry

A set of thiazolo[4,5-*d*]pyrimidine derivatives (**9a-l**) were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, stirring 4-aminoacetophenone, 3-aminoacetophenone and/or benzocaine (**5a-c**) with chloroacetyl chloride in dimethylformamide at room temperature yielded the corresponding chloroacetamide derivatives **6a-c** in high yields (54–78%), which upon cyclization with ammonium thiocyanate in ethanol provided thiazolidinone derivatives **7a-c** in 60-82% yield. Condensation of the latter **7a-c** with different aldehydes in glacial acetic acid and sodium acetate produced 5-arylidenethiazolidin-4-one derivatives **8a-l** which upon refluxing with thiourea in pyridine afforded the target compounds **9a–l** in (54–78%) yield. The chemical structures of all the novel synthesized compounds were proved by IR, ^{1}H NMR,^{13}C NMR, mass spectra and elemental analyses.

Insert Scheme.1 here

2.2. Pharmacological screening

2.2.1. In vitro Cyclooxygenase inhibition assay

The target derivatives **9a-l** were screened for their COX inhibitory activity by determining IC_{50} (the concentration causing 50% Inhibition) using an enzyme immunoassay (EIA) kit for ovine COX enzyme. COX-2 selectivity indexes (SI values) were recorded using celecoxib as a standard drug (Table 1). The

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results showed that the target compounds (**9a-l**) demonstrated a moderate to weak potency towards COX-1 (IC_{50} = 4.21–10.87 μM), and moderate to high potency towards COX-2 (IC_{50} = 0.87–3.78 μM) (Table 1). The target compounds **9e** (IC_{50} = 0.92 μM, S.I. = 10.05), **9g** (IC_{50} = 0.87 μM, S. I. = 8.68) and **9k** (IC_{50} = 1.02 μM, S.I. = 8.06) showed higher COX-2 inhibitory effect and higher COX-2 selectivity than celecoxib (IC_{50} = 1.11 μM, S.I. = 6.61). Furthermore, the target compound **9h** (IC_{50} = 1.21 μM, S.I. = 7.26) demonstrated comparable COX-2 inhibition activity with better selectivity to that shown by celecoxib (IC_{50} = 1.11 μM, S.I. = 6.61). On the other side, 4-nitrophenylthiazolo[4,5-*d*]pyrimidine derivative **9g** was the most potent inhibitor of COX-2 (IC_{50} = 0.87 μM), while the fluoro derivative **9e** was most COX-2 selective (COX-2 S.I. = 10.05). Structure activity relationship studies revealed that derivatives incorporating electron withdrawing groups at the *para* position of phenyl ring attached to pyrimidine moiety (**9a-c, 9e, 9g** and **9i-k**) exhibited higher COX-2 inhibition activity than the candidates with electron donating groups (**9d** and **9I**). Furthermore, the acetyl group at position 4 on the phenyl ring of compound **9e, 9g-h** afforded higher COX-2 inhibitory activity than at position 3 of the new candidates **9a-d**. In addition, compounds **9e**, **9g-h** having acetyl group at C4 of phenyl group showed higher COX-2 inhibitory activity than derivatives incorporating ethyl ester moiety **9i-l**.

Insert Table1 here

2.2.*2. In vivo anti-inflammatory activity*

The novel thiazolo[4,5-*d*]pyrimidines were evaluated for their anti-inflammatory activity **(9a-l)** by using the model of formalin-induced rat paw oedema in male rats. Each target compound was given orally (50 mg/kg) immediately before stimulating inflammation by formalin subcutaneous injection. The anti-inflammatory activity was recorded according to change in the volume of paw after 1h, 3h and 5h from formalin injection (Table 2). The obtained data demonstrated that compounds **9c**, **9f**, **9g** and **9k** (55%, 45%, 57% and 52%, respectively) revealed better anti-inflammatory activity than the standard drug celecoxib (43%) after 1h. 1-(4-[7-(4-Nitrophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl)ethanone **(9g)** was the most active candidate showing 88% inhibition of

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inflammation after 3 h and 5 h. In addition, the trimethoxyphenylthiazolo[4,5-*d*]pyrimidine benzoic acid ethyl ester **(9l)** showed the lowest activity between all the tested derivatives. Structure activity relationship studies demonstrated that compounds incorporating electron withdrawing groups at the *para* position of phenyl ring attached to pyrimidine moiety (**9e-g** and **9k**) exhibited better oedema inhibition than the candidates with electron donating groups (**9d**, **9h** and **9l**). Moreover, the acetyl group at position 4 on the phenyl ring of compound **9e-h** afforded higher inhibitory activity than at position 3 of the new candidates **9a-d.** Furthermore, replacing the acetyl group at C4 of phenyl group with ethyl ester moiety markedly reduced the anti-inflammatory activity, this is clear in comparing compounds **9e-h** with **9i-I**.

Insert Table 2here

2.2.3. Ulcerogenic liability

The most potent prepared compounds **9e, 9g** and **9k** were tested for their gastric ulcerogenic action in male albino rats (Table 3). Ulcerogenic results of the tested derivatives were compared with celecoxib and indomethacin. Celecoxib was chosen as a low ulcerogenic reference drug, which was reported to be about seven folds less ulcerogenic than ibuprofen as traditional NSAID [27, 30]. From the obtained data, it is clear that all the tested targets showed less ulcerogenic effect than indomethacin. 1-(4-[7-(4-Fluorophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl)ethanone **(9e)** (Ulcer index = 5) recorded the lowest ulcerogenic effect due to its high COX-2 selectivity index (COX-2 S.I. = 10.05).

Insert Table 3 here

2.3. Molecular docking study

The most potent COX-2 inhibitors **9e**, **9g** and **9k** had been docked within COX-2 enzyme binding site to demonstrate the mode of action of these target compounds. The X-ray crystal structure of COX-2 was obtained from the protein data bank with code (PDB: ID 1CX2) and MOE.2010 software (Molecular Operating Environment) was used for performing this study.

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The ligand of COX-2 bromocelecoxib (S-58) had been redocked within the active site with a score energy (S) = -11.93 kcal/mol. Arg513 and His90 amino acids interacted with $-SO_2$ group *via* two hydrogen bonding interactions with in a distance equal to 2.41 and 2.30 Å (Table 4).

Compound **9e** showed higher docking score (S) = -13.76 kcal/mol than revealed by bromocelecoxib (S) = -11.93 kcal/mol, in addition, this target **9e** exhibited five hydrogen bonding interactions; i) Thiazole N with Tyr355 (3.12 A°), ii) Pyrimidine N with Tyr355 (2.34 A°), iii) Pyrimidine N with Arg120 (2.47 A°), iv) C=O with Tyr385 (2.76 A°), and v) C=O with Ser530 (2.74) (Fig. 2) (Table 4).

Insert Fig.2 here

In addition, the candidate **9g** revealed one hydrogen interaction between His90 and the acetyl C=O with energy score S = -12.65 kcal/mol (Fig. 3) (Table 4).

Insert Fig.3 here

Compound **9k** recorded docking score (-13.21 Kcal/mol) forming two hydrogen bonding interactions: i) C=O with His90 (2.77 A°), and ii) CH=N with Ser 530 (2.31 A°) (Fig. 4).

Insert Fig.4 here

Insert Table 4 here

3. Conclusion

Novel derivatives of thiazolo[4,5-*d*]pyrimidines (**9a-l**) were synthesized and screened for their Cyclooxygenase inhibitory effect, ulcerogenic and anti-inflammatory activities. The results of this study showed that the thiazolo[4,5-*d*]pyrimidine having a nitrophenyl moiety in position 7 and p-acetylphenyl ring in position 2 (**9g**) was the most active candidate with edema inhibitory percent = 88% after three and five hours, while the derivative having a fluorophenyl moiety in position 7 (**9e**) was the least ulcerogenic (S.I= 5). In addition, these novel targets **9a-l** were noticed to be selective inhibitors to COX-2 than COX-1, in particular compounds **9e**, **9g** and **9k** revealed better COX-2 inhibitory activity in a range (IC_{50} = 0.87-1.02 μM) than the standard drug celecoxib (IC_{50} = 1.11 μM). The most active candidates inhibiting

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COX-2 (**9e, 9g** and **9k**) were subjected to molecular modeling study within COX-2 binding site. Docking studies showed that the target compounds (**9e, 9g** and **9k**) exhibited better score energy S = -12.65- -13.76 than the cocrystallized ligand (S) = -11.93 kcal/mol and they also good fitted with the active site of COX-2 enzyme. So docking studies confirmed the *in vitro* COX-2 assay since **9e**, **9g** and **9k** exhibited better COX-2 inhibitory activity than the standard drug. Therefore, mixing the thiazole scaffold with pyrimidine moiety in one hybrid structure yields a drug design concept for the development of NSAIDs that have a good anti-inflammatory activity with low ulcerogenic side effect.

4. Experimental

4.1. Chemistry

Melting points had been measured on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were measured on NaCl plates using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet, Faculty of Pharmacy, Mansoura University, Egypt in DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz) and chemical shifts were recorded in ppm on δ scale. Mass spectra (MS) were detected on Hewlett Packard 5988 spectrometer. Microanalyses for C, H and N were performed on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Micro analytical unit of Cairo University, Egypt and all compounds were within ± 0.4% of the theoretical values. All other chemicals, purchased from the Aldrich Chemical Company (Milwaukee, WI), had been used without further purification. 2-Chloro-*N*-(aryl)acetamides (**6b,c**) [31] 2-(arylimino)thiazolidin-4-one derivatives (**7b,c**) [32] and 2-arylimino-5-arylidenethiazolidin-4-one derivatives (**8e-l**) [28, 32] had been synthesized as literature procedures.

*4.1.1. N-(3-Acetyl-phenyl)-2-chloroacetamide (***6a**).

A mixture of 3-aminoacetophenone (**5a**) (1.35 gm, 0.01 mol), chloroacetyl chloride (0.8 mL, 0.01 mol) and anhydrous potassium carbonate (1.38 gm, 0.01 mol) in dry dimethylformamide was stirred at room

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temperature for 24 h. The reaction mixture was poured into ice-cold water and the separated product was filtered, dried and crystallized from benzene to give **6a** (1.5 g, 71%) as a buff powder: mp 188-200°C; IR (film) 3447 (NH), 3043 (CH aromatic), 2957 (CH aliphatic), 1706, 1672 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 4.28 (s, 2H, CH_2), 7.47-7.51 (m, 1H, phenyl H-5), 7.69 (d, J = 7.5 Hz, 1H, phenyl H-4), 7.86 (d, J = 7.5 Hz, 1H, phenyl H-6), 8.17 (s, 1H, phenyl H-2), 10.51 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 27.14 (CH_3), 43.98 (CH_2), 119.01 (acetylphenyl C-2), 124.31 (acetylphenyl C-4), 124.34 (acetylphenyl C-6), 129.76 (acetylphenyl C-5), 137.70 (acetylphenyl C-3), 139.29 (acetylphenyl C-1), 165.44 (NHC=O), 198.00 (C=O); EIMS (m/z) 211 ($M^{+\cdot}$, 100%). Anal. Calcd for $C_{10}H_{10}ClNO_2$: C, 56.75; H, 4.76; N, 6.62. Found: C, 56.66; H, 4.60; N, 6.50.

*4.1.2. 2-(3-Acetyl-phenylimino)thiazolidin-4-one (***7a**).

A solution of *N*-(3-acetyl-phenyl)-2-chloroacetamide (**6a**) (2.11 gm, 0.01 mol) and ammonium thiocyanate (1.14 gm, 0.15 mol) in ethanol (25 mL) was refluxed for 7 h. After cooling, the precipitated product was filtered off, and then recrystallised from acetic acid to give **7a** (1.4 gm, 60%) as yellow powder: mp 215-216°C; IR (film) 3445 (NH), 3042 (CH aromatic), 2959 (CH aliphatic), 1705, 1670 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.55 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 7.42-7.46 (m, 1H, phenyl H-5), 7.75 (d, J = 7.5 Hz, 1H, phenyl H-4), 7.97 (d, J = 7.5 Hz, 1H, phenyl H-6), 8.24 (s, 1H, phenyl H-2), 11.61 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 25.10 (CH_3), 40.23 (CH_2), 120.11 (acetylphenyl C-2), 126.36 (acetylphenyl C-6), 127.06 (acetylphenyl C-4), 129.75 (acetylphenyl C-5), 138.69 (acetylphenyl C-3), 148.25 (acetylphenyl C-1), 163.40 (N=CH), 173.21 (NHC=O), 196.10 (C=O); EIMS (m/z) 234 ($M^{+\cdot}$, 100%). Anal. Calcd for $C_{11}H_{10}N_2O_2S$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.38; H, 4.55; N, 12.00.

4.1.3. General procedure for synthesis of 2-arylimino-5-arylidenethiazolidin-4-one derivatives ***(8a–d)***

A mixture of thiazolidin-4-one derivatives **7a-c** (0.005 mol), the aromatic aldehyde (0.005 mol) and sodium acetate (0.01 mol) in acetic acid (20 ml) was refluxed for 18 hr. after cooling, the reaction mixture

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was poured into ice-cold water and the precipitated solid was filtered off and crystallized from dimethylformamide to produce the target compounds **8a–l**. Physical and spectral data are listed below.

4.1.3.*1. 2-(3-Acetylphenylimino)-5-(4-fluorobenzylidene)thiazolidin-4-one* (**8a**) Buff powder; Yield 62%; mp 274–275◦C; IR (KBr) 3327 (NH), 3076 (CH aromatic), 2955 (CH aliphatic), 1763, 1675 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 7.52–7.57 (m, 4H, fluorophenyl H-3, H-5 and acetylphenyl H-4, H-6), 7.61 (s, 1H, olefinic CH), 7.81–7.89 (m, 4H, fluorophenyl H-2, H-6 and acetylphenyl H-2, H-5), 11.87 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 26.15 (CH_3), 117.30 (fluorophenyl C-3, C-5), 120.46 (acetylphenyl C-2), 122.58 (thiazolidine C-5), 126.22 (acetylphenyl C-6), 127.12 (acetylphenyl C-4), 127.15 (fluorophenyl C-2, C-6), 129.55 (acetylphenyl C-5), 130.12 (fluorophenyl C-1), 138.72 (acetylphenyl C-3), 141.98 (benzylidene CH=), 148.95 (acetylphenyl C-1), 161.34 (fluorophenyl C-4), 163.21 (thiazolidine C-2), 168.10 (NHC=O), 197.11 (C=O); EIMS (m/z) 340 (M+., 54%). Anal. Calcd for $C_{18}H_{13}FN_2O_2S$: C, 63.52; H, 3.85; N, 8.23. Found: C, 63.55; H, 3.81; N, 8.00.

4.1.3.2. *2-(3-Acetylphenylimino)-5-(4-chlorobenzylidene)thiazolidin-4-one* **(8b**) Buff powder; Yield 65%; mp 292–293°C; IR (KBr) 3437 (NH), 3041 (CH aromatic), 2957 (CH aliphatic), 1761, 1677 (2CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.55 (s, 3H, CH_3), 7.10 (s, 1H, olefinic CH), 7.55–7.59 (m, 4H, chlorophenyl H-3, H-5 and acetylphenyl H-4, H-6), 7.83-7.91 (m, 4H, chlorophenyl H-2, H-6 and acetylphenyl H-2, H-5), 12.10 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 23.13 (CH_3), 120.10 (acetylphenyl C-2), 122.18 (thiazolidine C-5), 126.23 (acetylphenyl C-6), 127.21 (acetylphenyl C-4), 127.56 (chlorophenyl C-3, C-5), 128.82 (chlorophenyl C-2, C-6), 129.66 (acetylphenyl C-5), 132.65 (chlorophenyl C-4), 133.01 (chlorophenyl C-1), 138.54 (acetylphenyl C-3), 142.21 (benzylidene CH=), 148.21 (acetylphenyl C-1), 163.25 (thiazolidine C-2), 168.22 (NHC=O), 196.45 (C=O); EIMS (m/z) 356 (M+., 78%). Anal. Calcd for $C_{18}H_{13}ClN_2O_2S$: C, 60.59; H, 3.67; N, 7.85. Found: C, 60.35; H, 3.50; N, 7.65.

4.1.3.3. *2-(3-Acetylphenylimino)-5-(4-nitrobenzylidene)thiazolidin-4-one* **(8c)** Yellow powder; Yield 80%; mp 260–261◦C; IR (KBr) 3442 (NH), 3049 (CH aromatic), 2849 (CH aliphatic), 1789, 1680 (2CO)

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cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.60 (s, 3H, CH_3), 7.31 (s, 1H, olefinic CH), 7.54–7.68 (m, 4H, m, 4H, nitrophenyl H-2, H-6 and acetylphenyl H-4, H-6), 7.82 (d, *J* = 8.2 Hz, 2H, acetylphenyl H-2, H-5), 8.10 (d, *J* = 8.2 Hz, 2H, nitrophenyl H-3, H-5), 12.06 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 27.39 (CH_3), 120.20 (acetylphenyl C-2), 122.16 (thiazolidine C-5), 126.41 (acetylphenyl C-6), 127.23 (acetylphenyl C-4), 127.46 (nitrophenyl C-3, C-5), 128.72 (nitrophenyl C-2, C-6), 129.79 (acetylphenyl C-5), 132.64 (nitrophenyl C-4), 134.90 (nitrophenyl C-1), 138.51(acetylphenyl C-3), 142.00 (benzylidene CH=), 148.93 (acetylphenyl C-1), 163.04 (thiazolidine C-2), 168.22 (NHC=O), 198.13 (C=O); EIMS (m/z) 367 (M+., 100 %). Anal. Calcd for $C_{18}H_{13}N_3O_4S$: C, 58.85; H, 3.57; N, 11.44. Found: C, 58.50; H, 3.20; N, 11.49.

4.1.3.4. *2-(3-Acetylphenylimino)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one* (**8d**) Yellow powder; Yield 64%; mp >300◦C; IR (KBr) 3429 (NH), 3043 (CH aromatic), 2935 (CH aliphatic), 1715, 1671 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.57 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 6.81 (s, 1H, olefinic CH), 6.93 (s, 2H, trimethoxyphenyl H-2, H-6), 7.58–7.74 (m, 2H, and acetylphenyl H-4, H-6), 8.02 (m, 2H, acetylphenyl H-2, H-5); 12.00 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 27.24 (CH_3), 56.33 ($2OCH_3$), 60.69 (OCH_3), 107.60 (trimethoxyphenyl C-2, C-6), 119.98 (acetylphenyl C-2), 122.23 (thiazolidine C-5), 125.58 (acetylphenyl C-6), 127.84 (acetylphenyl C-4), 129.62 (trimethoxyphenyl C-1), 130.16 (acetylphenyl C-5), 131.61 (trimethoxyphenyl C-4), 138.07 (acetylphenyl C-3), 142.85 (benzylidene CH=), 148.23 (acetylphenyl C-1), 153.64 (trimethoxyphenyl C-3, C-5), 163.02 (thiazolidine C-2), 168.92 (NHC=O), 196.08 (C=O); EIMS (m/z) 412 (M+. , 58%). Anal. Calcd for $C_{21}H_{20}N_2O_5S$: C, 61.15; H, 4.89; N, 6.79.Found: C, 61.05; H, 4.65; N, 6.66.

4.1.4. General procedure for synthesis of **9a-l**

A mixture of the appropriate 2-arylimino-5-arylidenethiazolidin-4-one derivatives **(8a–l)** (0.01 mol) and thiourea (0.76 g, 0.01 mol) in the presence of few drops of piperidine was fused at 180 ºC for 5h. After cooling, the product was poured onto ice and the solid was filtered off and recrystallized from dioxane to afford compounds **9a-l**. Physical and spectral data are listed below.

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4.1.4.1. **1-{3-[7-(4-Fluorophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9a**) Buff powder; Yield 60%; mp 297-298◦C; IR (KBr) 3429-3265 (2NH), 3053 (CH aromatic), 2922 (CH aliphatic), 1710 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 7.14–7.21 (m, 4H, fluorophenyl H-3, H-5 and acetylphenyl H-4, H-6), 7.30–7.41 (m, 4H, fluorophenyl H-2, H-6 and acetylphenyl H-2, H-5), 11.57 (s, 1H, NH, D_2O exchangeable), 12.26 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 22.85 (CH_3), 84.56 (thiazolo[4,5-*d*]pyrimidin C-7a), 116.30 (fluorophenyl C-3, C-5), 122.32 (acetylphenyl C-2), 126.24 (acetylphenyl C-6), 127.02 (acetylphenyl C-4), 127.56 (fluorophenyl C-2, C-6), 129.72 (acetylphenyl C-5), 131.11 (fluorophenyl C-1), 138.63 (acetylphenyl C-3), 148.95 (acetylphenyl C-1), 157.53 (thiazolo[4,5-*d*]pyrimidin C-7), 161.32 (fluorophenyl C-4), 163.92 (thiazolo[4,5-*d*]pyrimidin C-2), 164.23 (thiazolo[4,5-*d*]pyrimidin C-3a), 184.25 (C=S), 197.26 (C=O); EIMS (m/z) 396 (M+., 5.12%). Anal. Calcd for $C_{19}H_{13}FN_4OS_2$: C, 57.56; H, 3.31; N, 14.13.Found: C, 57.50; H, 3.22; N, 14.00.

4.1.4.2. **1-{3-[7-(4-Chlorophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9b**)

White powder; Yield 54%; mp 288–289˚C; IR (KBr) 3437-3211 (2NH), 3051 (CH aromatic), 2953 (CH aliphatic), 1760 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.54 (s, 3H, CH_3), 7.52–7.60 (m, 4H, chlorophenyl H-3, H-5 and acetylphenyl H-4, H-6), 7.71-7.79 (m, 4H, chlorophenyl H-2, H-6 and acetylphenyl H-2, H-5), 12.10 (s, 1H, NH, D_2O exchangeable), 12.65 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 22.92 (CH_3), 85.01 (thiazolo[4,5-*d*]pyrimidin C-7a), 122.19 (acetylphenyl C-2), 126.33 (acetylphenyl C-6), 127.12 (acetylphenyl C-4), 127.34 (chlorophenyl C-3, C-5), 128.7 (chlorophenyl C-2, C-6), 129.69 (acetylphenyl C-5), 132.65 (chlorophenyl C-4), 133.11 (chlorophenyl C-1), 138.62 (acetylphenyl C-3), 148.89 (acetylphenyl C-1), 157.36 (thiazolo[4,5-*d*]pyrimidin C-7), 163.15 (thiazolo[4,5-*d*]pyrimidin C-2), 164.52 (thiazolo[4,5-*d*]pyrimidin C-3a), 182.32 (C=S), 196.23 (C=O); EIMS (m/z) 412 (M+., 100%). Anal. Calcd for $C_{19}H_{13}ClN_4OS_2$: C, 55.27; H, 3.17; N, 13.57. Found: C, 55.35; H, 3.20; N, 13.50.

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4.1.4.3. **1-{3-[7-(4-Nitrophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9c)**

Yellow powder; Yield 72%; mp 295–296°C; IR (KBr) 3446-3232 (2NH), 3043 (CH aromatic), 2952 (CH aliphatic), 1762 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.61 (s, 3H, CH_3), 7.63–7.81 (m, 4H, m, 4H, nitrophenyl H-2, H-6 and acetylphenyl H-4, H-6), 7.84 (d, *J* = 8.2 Hz, 2H, acetylphenyl H-2, H-5), 8.16 (d, *J* = 8.2 Hz, 2H, nitrophenyl H-3, H-5), 11.93 (s, 1H, NH, D_2O exchangeable), 12.11 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 22.89 (CH_3), 85.23 (thiazolo[4,5-*d*]pyrimidin C-7a), 122.42 (acetylphenyl C-2), 123.51 (nitrophenyl C-3, C-5), 126.47 (acetylphenyl C-6), 127.16 (acetylphenyl C-4), 127.29 (nitrophenyl C-2, C-6), 129.73 (acetylphenyl C-5), 138.72 (acetylphenyl C-3), 141.53 (nitrophenyl C-1), 147.61 (nitrophenyl C-4), 148.92 (acetylphenyl C-1), 157.01 (thiazolo[4,5-*d*]pyrimidin C-7), 163.22(thiazolo[4,5-*d*]pyrimidin C-2), 164.42 (thiazolo[4,5-*d*]pyrimidin C-3a), 182.09 (C=S), 196.15 (C=O); EIMS (m/z) 423 (M+., 4.07%). Anal. Calcd for $C_{19}H_{13}N_5O_3S_2$: C, 53.89; H, 3.09; N, 16.54. Found: C, 54.00; H, 3.20; N, 16.60.

4.1.4.4. **1-{3-(5-Thioxo-3-[7-(3,4,5-trimethoxyphenyl)-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9d)**

Yellow powder; Yield 78%; mp > 300°C; IR (KBr) 3422-3254 (2NH), 3061 (CH aromatic), 2931 (CH aliphatic), 1760 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.55 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 3.69 (s, 6H, $2OCH_3$), 6.88 (s, 2H, trimethoxyphenyl H-2, H-6), 7.20–7.32 (m, 2H, and acetylphenyl H-4, H-6), 7.49-7.61 (m, 2H, acetylphenyl H-2, H-5); 11.85 (s, 1H, NH, D_2O exchangeable), 12.11 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 27.29 (CH_3), 56.24 ($2OCH_3$), 56.49 (OCH_3), 83.85 (thiazolo[4,5-*d*]pyrimidin C-7a), 106.58 (trimethoxyphenyl C-2, C-6), 122.13 (acetylphenyl C-2), 126.32 (acetylphenyl C-6), 127.35 (acetylphenyl C-4), 129.01 (trimethoxyphenyl C-1), 129.63 (acetylphenyl C-5), 132.35 (trimethoxyphenyl C-4), 141.00 (acetylphenyl C-3), 146.96 (acetylphenyl C-1), 148.93 (thiazolo[4,5-*d*]pyrimidin C-7), 153.26 (trimethoxyphenyl C-3, C-5), 163.29 (thiazolo[4,5-*d*]pyrimidin C-2), 164.52

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(thiazolo[4,5-*d*]pyrimidin C-3a), 184.27 (C=S), 196.06 (C=O); EIMS (m/z) 468 (M+., 32.34%). Anal. Calcd for $C_{22}H_{20}N_4O_4S_2$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.55; H, 4.55; N, 12.00.

4.1.4.5. **1-{4-[7-(4-Fluorophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9e**) Buff powder; Yield 58%; mp 291-292◦C; IR (KBr) 3411-3262 (2NH), 3051 (CH aromatic), 2932 (CH aliphatic), 1715 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.54 (s, 3H, CH_3), 7.60 (d, *J* = 7.4 Hz, 2H, fluorophenyl H-3, H-5), 7.72–7.80 (m, 4H, fluorophenyl H-2, H-6 and acetylphenyl H-2, H-6), 7.93 (d, *J* = 8.2 Hz, 2H, acetylphenyl H-3, H-5), 11.59 (s, 1H, NH, D_2O exchangeable), 12.13 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 29.51 (CH_3), 84.42 (thiazolo[4,5-*d*]pyrimidin C-7a), 115.70 (fluorophenyl C-2, C-6), 122.01 (acetylphenyl C-2, C-6), 126.39 (acetylphenyl C-3, C-5), 130.29 (fluorophenyl C-1), 130.68 (fluorophenyl C-3, C-5), 135.92 (acetylphenyl C-4), 153.21 (acetylphenyl C-1), 157.36 (thiazolo[4,5-*d*]pyrimidin C-7), 161.20 (fluorophenyl C-4), 163.87 (thiazolo[4,5-*d*]pyrimidin C-2), 168.17 (thiazolo[4,5-*d*]pyrimidin C-3a), 184.15 (C=S), 197.17 (C=O); EIMS (m/z) 396 (M+. , 8.98%). Anal. Calcd for $C_{19}H_{13}FN_4OS_2$: C, 57.56; H, 3.31; N, 14.13.Found: C, 57.64; H, 3.53; N, 14.32.

4.1.4.6. **1-{4-[7-(4-Chlorophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9f)**

White powder; Yield 57%; mp 293–294°C; IR (KBr) 3435-3231 (2NH), 3048 (CH aromatic), 2951 (CH aliphatic), 1759 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.54 (s, 3H, CH_3), 7.48–7.55 (m, 4H, chlorophenyl H-3, H-5 and acetylphenyl H-2, H-6), 7.89-7.95 (m, 4H, chlorophenyl H-2, H-6 and acetylphenyl H-3, H-5), 12.10 (s, 1H, NH, D_2O exchangeable), 12.65 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 27.53 (CH_3), 84.31 (thiazolo[4,5-*d*]pyrimidin C-7a), 115.67 (chlorophenyl C-3, C-5), 122.21 (acetylphenyl C-2, C-6), 126.37 (chlorophenyl C-2, C-6), 130.12 (chlorophenyl C-1), 130.59 (acetylphenyl C-4), 131.22 (chlorophenyl C-4), 135.86 (acetylphenyl C-3, C-5), 153.32 (acetylphenyl C-1), 157.42 (thiazolo[4,5-*d*]pyrimidin C-7), 161.06 (thiazolo[4,5-*d*]pyrimidin C-2), 163.87 (thiazolo[4,5-

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d]pyrimidin C-4a), 168.16 (C=O), 184.15 (C=S), 197.16 (C=O); EIMS (m/z) 412 (M+., 100%). Anal. Calcd for $C_{19}H_{13}ClN_4OS_2$: C, 55.27; H, 3.17; N, 13.57. Found: C, 55.01; H, 3.32; N, 13.60.

4.1.4.7. **1-{4-[7-(4-Nitrophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9g)**

Yellow powder; Yield 68%; mp > 300°C; IR (KBr) 3449-3212 (2NH), 3065 (CH aromatic), 2949 (CH aliphatic), 1757 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.53 (s, 3H, CH_3), 7.49–7.56 (m, 4H, nitrophenyl H-2, H-6 and acetylphenyl H-2, H-6), 7.88-7.97 (m, 4H, nitophenyl H-3, H-5 and acetylphenyl H-3, H-5), 11.89 (s, 1H, NH, D_2O exchangeable), 12.46 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 26.84 (CH_3), 85.32 (thiazolo[4,5-*d*]pyrimidin C-7a), 121.15 (phenyl C-2,C-6), 123.64 (nitrophenyl C-3, C-5), 127.22 (phenyl C-4), 129.56 (nitrophenyl C-2, C-6), 130.88 (acetylphenyl C-3, C-5), 135.43 (nitrophenyl C-1), 149.35 (nitrophenyl C-4), 157.32 (acetylphenyl C-1), 162.52 (thiazolo[4,5-*d*]pyrimidin C-7), 164.58 (thiazolo[4,5-*d*]pyrimidin C-3a), 168.12(thiazolo[4,5-*d*]pyrimidin C-2), 182.32 (C=S), 196.72 (C=O); EIMS (m/z) 423 (M+., 100%). Anal. Calcd for $C_{19}H_{13}N_5O_3S_2$: C, 53.89; H, 3.09; N, 16.54. Found: C, 53.50; H, 3.25; N, 16.50.

4.1.4.8. **1-{5-Thioxo-4-[7-(3,4,5-trimethoxyphenyl)-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]phenyl}ethanone (9h)**

Yellow powder; Yield 69%; mp > 300°C; IR (KBr) 3432-3276 (2NH), 3053 (CH aromatic), 2930 (CH aliphatic), 1758 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.56 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 3.73 (s, 6H, $2OCH_3$), 6.50 (s, 2H, trimethoxyphenyl H-2, H-6), 7.52 (d, J = 8.4 Hz, 2H, and acetylphenyl H-2, H-6), 7.74 (d, J = 8.4 Hz, 2H, acetylphenyl H-3, H-5); 11.54 (s, 1H, NH, D_2O exchangeable), 12.45 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 21.53 (CH_3), 56.26 (OCH_3), 56.49 (OCH_3), 84.5 (thiazolo[4,5-*d*]pyrimidin C-7a), 105.99 (trimethoxyphenyl C-2, C-6), 121.85 (acetylphenyl C-2, C-6), 129.23 (trimethoxyphenyl C-1), 130.24 (acetylphenyl C-4), 132.22 (trimethoxyphenyl C-4), 135.62(acetylphenyl C-3, C-5), 148.83 (thiazolo[4,5-*d*]pyrimidin C-7), 153.67 (trimethoxyphenyl C-3, C-5), 157.22 (acetylphenyl C-1), 163.52 (thiazolo[4,5-*d*]pyrimidin C-2), 164.21(thiazolo[4,5-*d*]pyrimidin C-

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3a), 182.26 (C=S), 196.43 (C=O); EIMS (m/z) 468 (M+., 19.88%). Anal. Calcd for $C_{22}H_{20}N_4O_4S_2$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.35; H, 4.15; N, 11.82.

4.1.4.9. **Ethyl 4-[7-(4-fluorophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]benzoate (9i**) white powder; Yield 61%; mp 283-284◦C; IR (KBr) 3410-3259 (2NH), 3051 (CH aromatic), 2932 (CH aliphatic), 1719 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31 (t, J = 7.2 Hz, 3H, CH_3), 4.25 (q, J = 7.2 Hz, 2H, CH_2), 7.13 (d, J = 7.8 Hz, 2H, fluorophenyl H-3, H-5), 7.82–7.98 (m, 4H, fluorophenyl H-2, H-6 and benzoyl H-2, H-6), 8.03 (d, J = 8.6 Hz, 2H, benzoyl H-3, H-5), 11.93 (s, 1H, NH, D_2O exchangeable), 12.39 (s,1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 19.06 (CH_3), 59.1 (CH_2), 84 (thiazolo[4,5-*d*]pyrimidin C-7a), 115.6 (fluorophenyl C-2, C-6), 121.93 (benzoyl C-2, C-6), 127.53 (fluorophenyl C-1), 129.21(fluorophenyl C-3, C-5), 130.61 (benzoyl C-4), 131.23 (benzoyl C-3, C-5), 153.61(benzoyl C-1), 157.21 (thiazolo[4,5-*d*]pyrimidin C-7), 161.22 (fluorophenyl C-4), 163.25 (thiazolo[4,5-*d*]pyrimidin C-2), 164.32 (thiazolo[4,5-*d*]pyrimidin C-3a), 167.32 (C=O), 182.21 (C=S); EIMS (m/z) 426 (M+., 70.91%). Anal. Calcd for $C_{20}H_{15}FN_4O_2S_2$: C, 56.32; H, 3.55; N, 13.14. Found: C, 56.50; H, 3.60; N, 13.25.

4.1.4.10. **Ethyl 4-[7-(4-chlorophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]benzoate (9j**)

White powder; Yield 59%; mp > 300◦C; IR (KBr) 3445-3265 (2NH), 3098 (CH aromatic), 2926 (CH aliphatic), 1713 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31 (t, J = 7.2 Hz, 3H, CH_3), 4.32 (q, J = 7.2 Hz, 2H, CH_2), 7.52–7.61 (m, 4H, chlorophenyl H-3, H-5 and benzoyl H-2, H-6), 7.65–7.98 (m, 4H, chlorophenyl H-2, H-6 and benzoyl H-3, H-5), 11.71 (s, 1H, NH, D_2O exchangeable), 12.30 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 13.51 (CH_3), 59.25 (CH_2), 84.62 (thiazolo[4,5-*d*]pyrimidin C-7a), 121.32 (benzoyl C-2, C-6), 127.22 (chlorophenyl C-2, C-6), 128.32 (chlorophenyl C-3, C-5), 129.65 (benzoyl C-4), 131.62 (benzoyl C-3, C-5), 133.21 (chlorophenyl C-1), 133.64 (chlorophenyl C-4), 153.26 (benzoyl C-1), 157.23 (thiazolo[4,5-*d*]pyrimidin C-7), 163.82 (thiazolo[4,5-*d*]pyrimidin C-2), 167.53

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(thiazolo[4,5-*d*]pyrimidin C-3a), 164.35 (C=O), 182.41 (C=S); EIMS (m/z) 442 (M+., 15%). Anal. Calcd for $C_{20}H_{15}ClN_4O_2S_2$: C, 54.23; H, 3.41; N, 12.65. Found: C, 54.50; H, 3.62; N, 12.56.

4.1.4.11. **Ethyl 4-[7-(4-nitrophenyl)-5-thioxo-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]benzoate (9k)**

Yellow powder; Yield 69%; mp >300◦ C; IR (KBr) 3432 (2NH), 3093 (CH aromatic), 2972 (CH aliphatic), 1721 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.34 (t, *J* = 7.6 Hz, 3H, CH_3), 4.33 (q, *J* = 7.6 Hz, 2H, CH_2), 7.73–7.80 (m, 4H, nitrophenyl H-2, H-6 and benzoyl H-2, H-6), 7.68 (d, *J* = 8.2 Hz, 2H, benzoyl H-3, H-5), 8.27 (d, *J* = 8.2 Hz, 2H, nitrophenyl H-3, H-5), 11.36 (s, 1H, NH, D_2O exchangeable), 12.38 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 14.50 (CH_3), 61.16 (CH_2), 84.35 (thiazolo[4,5-*d*]pyrimidin C-7a), 120.97 (benzoyl C-2,C-6), 121.98 (nitrophenyl C-3, C-5), 126.67 (benzoyl C-4), 129.25 (nitrophenyl C-2, C-6), 131.36 (benzoyl C-3, C-5), 141.35 (nitrophenyl C-1), 153.61 (nitrophenyl C-4), 157.25 (benzoyl C-1), 163.29 (thiazolo[4,5-*d*]pyrimidin C-7), 164.01 (thiazolo[4,5-*d*]pyrimidin C-3a), 165.12 (thiazolo[4,5-*d*]pyrimidin C-2), 165.63 (C=O), 182.31 (C=S); EIMS (m/z) 453 (M+., 100%). Anal. Calcd for $C_{20}H_{15}N_5O_4S_2$: C, 52.97; H, 3.33; N, 15.44. Found: C, 52.65; H, 3.62; N, 15.22.

4.1.4.12. **Ethyl 4-[5-thioxo-7-(3,4,5-trimethoxyphenyl)-5,6-dihydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ylideneamino]benzoate (9l)**

Yellow powder; Yield 69%; mp >300◦ C; IR (KBr) 3446 (2NH), 3071 (CH aromatic), 2932 (CH aliphatic), 1725 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 (t, *J* = 7.2 Hz, 3H, CH_3), 4.30 (q, *J* = 7.2 Hz, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 6.81 (s, 2H, trimethoxyphenyl H-2, H-6), 7.93 (d, J = 8.6 Hz, 2H, benzoyl H-3, H-5), 8.11 (d, J = 8.6 Hz, 2H, benzoyl H-2, H-6), 11.35 (s, 1H, NH, D2O exchangeable), 12.21 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 14.66 (CH_3), 56.51 (CH_2), 60.67 (OCH_3), 61.16 (OCH_3), 84.32 (thiazolo[4,5-*d*]pyrimidin C-7a), 106.85 (trimethoxyphenyl C-2, C-6), 121.63 (benzoyl C-2, C-6), 123.25 (benzoyl C-4), 129.30 (trimethoxyphenyl C-1), 131.01 (benzoyl C-3, C-5), 132.12 (trimethoxyphenyl C-4), 148.83 (thiazolo[4,5-*d*]pyrimidin C-7), 153.67 (trimethoxyphenyl C-3, C-5), 157.64 (benzoyl C-1), 162.91 (thiazolo[4,5-*d*]pyrimidin C-2), 164.32

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(thiazolo[4,5-*d*]pyrimidin C-3a), 166.65 (C=O), 179.34 (C=S); EIMS (m/z) 498 (M+., 15.32%). Anal. Calcd for $C_{23}H_{22}N_4O_5S_2$: C, 55.41; H, 4.45; N, 11.24. Found: C, 55.50; H, 4.30; N, 11.31.

4.2. Pharmacological studies

4.2.1. In vitro COX-1/COX-2 inhibition assay:

The inhibition of ovine COX-1/COX-2 was measured using an enzyme immuno assay (EIA) kit as a reported procedure (27). Various concentrations of the tested compounds and positive control (celecoxib) were incubated with the enzymes for a period of 5 min at 25 ◦C. After the incubation period and the addition of colorimetric substrate and arachidonic acid, absorbance was measured at 590 nm using plate reader.

4.2.2. Formalin induced rat paw edema

The newly prepared target compounds **9a–l** were evaluated for their *in vivo* anti-inflammatory activity by the use of formalin-induced paw edema method in male rats (32). Wister albino male rats were divided into fourteen groups of four animals each. The first group was given a vehicle 0.5 % carboxymethylcellulose (CMC) and considered as a control. Celecoxib was administered orally to the second group as a reference standard in (50 mg/kg) dose. The rest of the groups were given orally the tested target compounds **9a–l** in a dose of (50 mg/kg). Thickness of the left hind paw of each rat was measured in millimeters, at the beginning of the experiment. Induction of paw edema was performed by subcutaneous injection of formalin 2.5% (0.05 ml) into the right hind paw of each rat, one hour after administration of vehicle, test compounds or celecoxib. Paw thickness of each rat was measured after 1, 3 and 5 h of formalin injection using plethysmometer. Then the change in thickness and % inhibition of paw edema were calculated.

4.2.3. Ulcerogenic liability study

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The most potent COX-2 inhibitors (**9e**, **9g** and **9k**) as well as celecoxib were tested for their ulcerogenic liability using the previously reported method [27]. The ulcerogenic effects of compounds **9e**, **9g**, **9k**, celecoxib, and indomethacin were evaluated. Twenty four rats were used in this study, divided into 6 groups and fasted for 18 h before drug administration. The control group received the vehicle, while other groups received test compounds, celecoxib or indomethacin at a dose of 50 mg/kg, then animals were fed after 2 h. Rats were given the specified dose orally for three successive days. Rats were sacrificed after 2 h of the last dose, then the stomach of each rat was removed and opened along the greater curvature for determination of the ulcer number and ulcer index.

4.2.4. Molecular docking

The crystal structures of bromocelecoxib bound at COX-2 isoform (Protein Data Bank; PDB: ID 1CX2) [28]. Docking was performed using London dG force and sophistication of the results was done using force field energy. Preparation of the synthesized compounds for docking was attained via their 3D structure built by Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc., QC, Canada). Definite procedures were in use before docking which include: 3D protonation of the structures, running conformational analysis using systemic search, selecting the least energetic conformer and applying the same docking protocol used with ligands. Docking for the synthesized compounds was applied. Amino acid interactions and the hydrogen bond lengths were summarized in (Table 4).

4.2.5. Statistical analysis

The presented data are mean ± SD, and the statistical analysis was performed using one way ANOVA followed by Dunnett multiple comparisons test. Differences were considered significant at $p < 0.05$. Statistical analysis was performed using SPSS for Windows (SPSS, Inc., Chicago, IL).

Acknowledgement

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Figure, schemes and table captions

Fig. 1: Chemical Structures of some reported thiazolidine derivatives (**1**, **2**) and pyrimidine derivatives (**3**, **4**) with anti-inflammatory activity.

Fig.2: Binding of the **9e** inside COX-2. (A) 2D of the binding mode of **9e** inside the active site of COX resulting from docking, the most important amino acids are shown It forms five H bonds with Tyr355, Arg120, Tyr385 and Ser530; (B) 3D binding of **9e**.

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Fig.3: Binding of the **9g** inside COX-2. (A) 2D of the binding mode of **9g** inside the active site of COX resulting from docking, the most important amino acids are shown it forms one H bonds with His90 (B) 3D binding of **9g**.

Fig.4: Binding of the **9k** inside COX-2. (A) 2D of the binding mode of **9k** inside the active site of COX resulting from docking, the most important amino acids are shown It forms two H bonds with His90, and Ser530; (B) 3D binding of **9k**.

Scheme 1. Synthesis of the compounds **9a-l**. Reagents and conditions: a) $ClCH_2COCl$, DMF, K_2CO_3, stirring, 24h; b) NH_4SCN, C_2H_5OH, reflux, 7h; c) ArCHO, CH_3COOH, CH_3COONa, 18h; d) NH_2CSNH_2, C_6H_5N, 180 ºC, 5h.

Table 1. *In vitro* COX-1 and COX-2 inhibition of the novel candidates **9a-l** and celecoxib.

Table 2. Results of the anti-inflammatory activity of control, celecoxib and the novel targets **9a-l**.

Table 3. Ulcerogenic effect of the targets **9e, 9g, 9k,** indomethacin and celecoxib.

Table 4**:** Molecular modeling data for compounds **9e, 9g, 9k** and **S-58** during docking in the active site of COX-2 enzyme (PDB:ID 1CX2).

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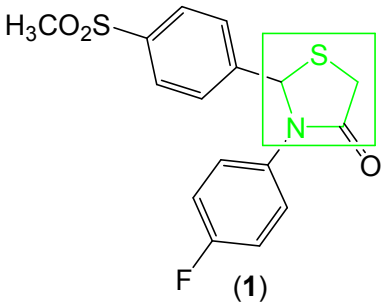

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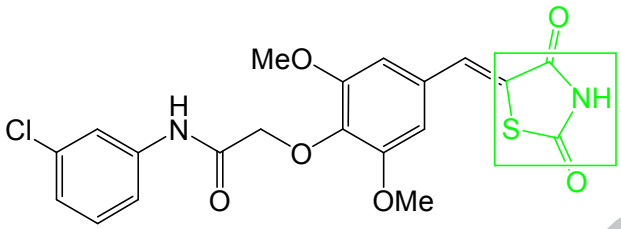

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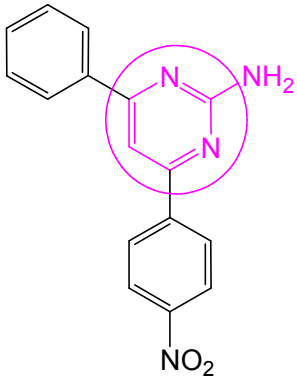

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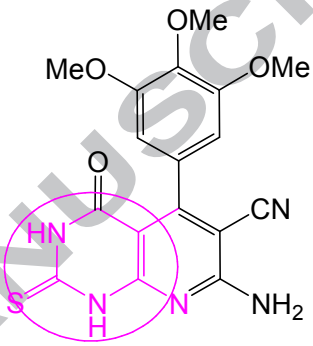

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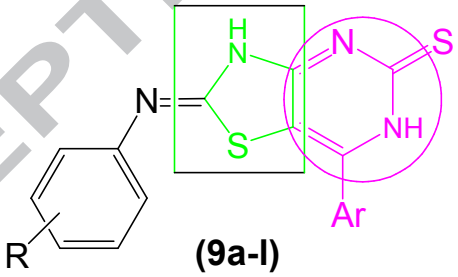

(9a-l)

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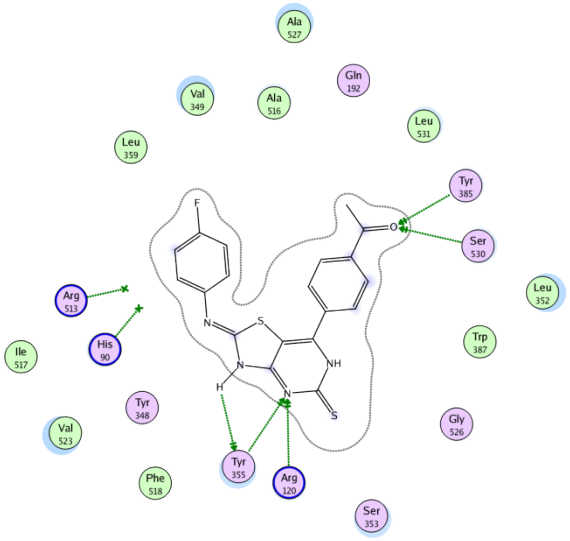

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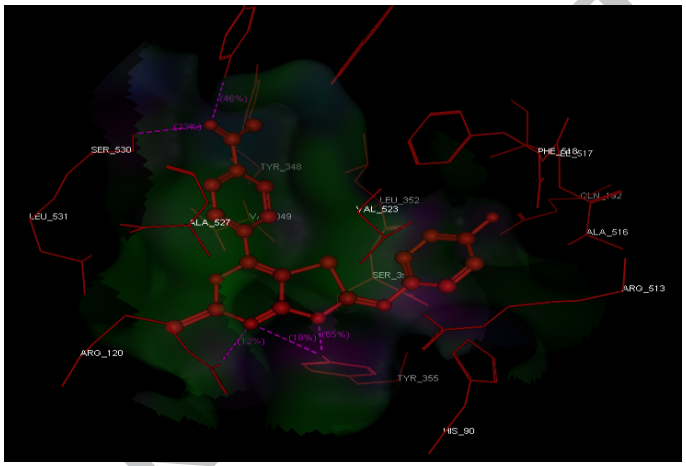

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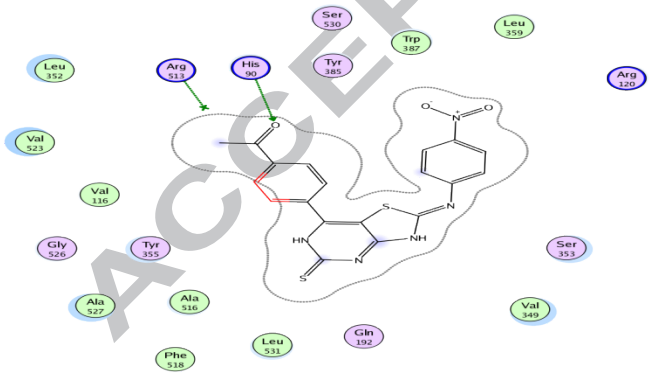

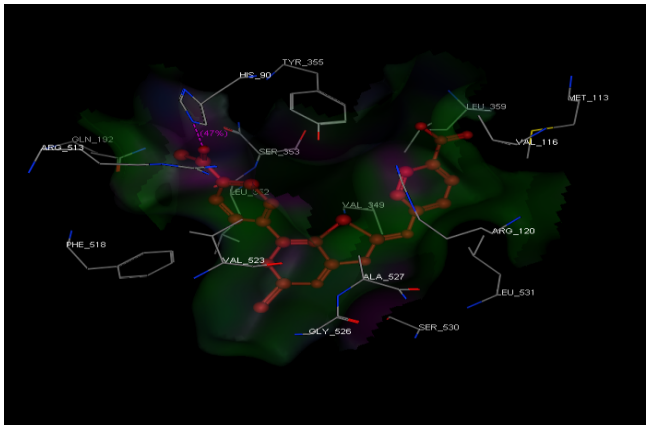

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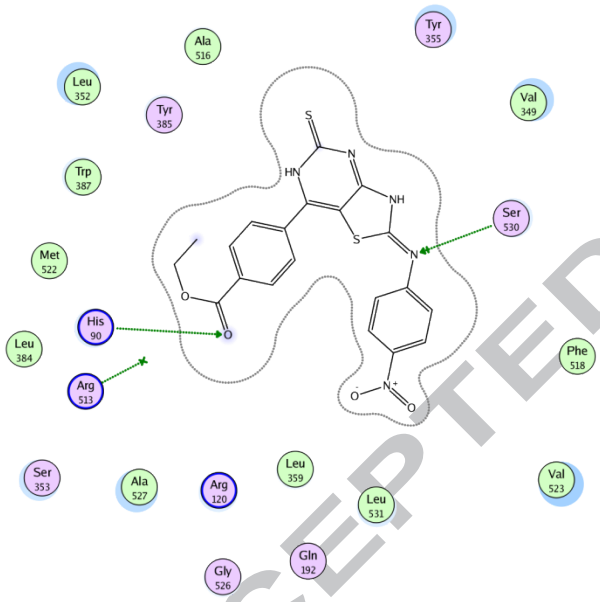
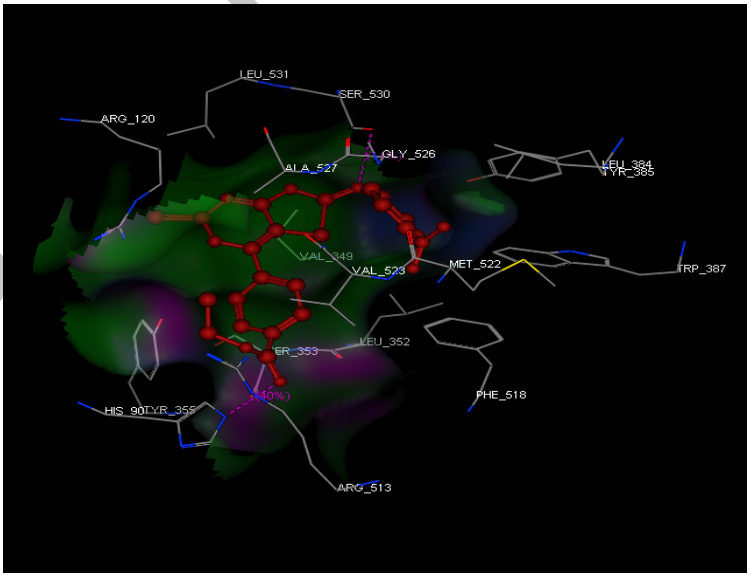
(A) **(B)**





(A) **(B)**

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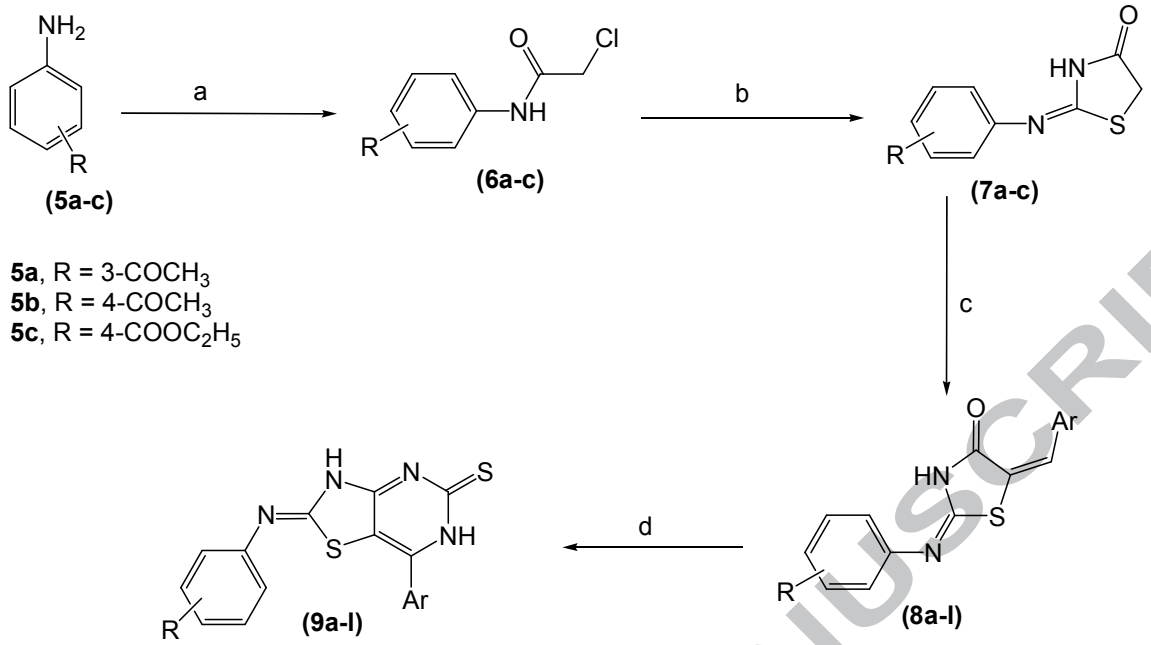

5a, R = 3-$COCH_3$
5b, R = 4-$COCH_3$
5c, R = 4-$COOC_2H_5$

	R	Ar
9a	3-$COCH_3$	4-F-C_6H_4
9b	3-$COCH_3$	4-Cl-C_6H_4
9c	3-$COCH_3$	4-NO_2-C_6H_4
9d	3-$COCH_3$	3,4,5-$(OCH_3)_3$-C_6H_2
9e	4-$COCH_3$	4-F-C_6H_4
9f	4-$COCH_3$	4-Cl-C_6H_4
9g	4-$COCH_3$	4-NO_2-C_6H_4
9h	4-$COCH_3$	3,4,5-$(OCH_3)_3$-C_6H_2
9i	4-$COOC_2H_5$	4-F-C_6H_4
9j	4-$COOC_2H_5$	4-Cl-C_6H_4
9k	4-$COOC_2H_5$	4-(NO_2)-C_6H_4
9l	4-$COOC_2H_5$	3,4,5-$(OCH_3)_3$-C_6H_2

Scheme 1. Synthesis of the compounds **9a-l**. Reagents and conditions: a) $ClCH_2COCl$, DMF, K_2CO_3, stirring, 24h; b) NH_4SCN, C_2H_5OH, reflux, 7h; c) ArCHO, CH_3COOH, CH_3COONa, 18h; d) NH_2CSNH_2, C_6H_5N, 180 ºC, 5h.

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Compound Number	IC_{50} (μM)[a]		COX-2 S.I.
	COX-1 (μm IC_{50})	COX-2 (μm IC_{50})	
9a	4.21	1.45	2.90
9b	4.65	1.65	2.81
9c	6.32	1.28	4.94
9d	4.21	2.52	1.67
9e	9.25	0.92	10.05
9f	10.87	3.78	2.88
9g	7.55	0.87	8.68
9h	8.78	1.21	7.26
9i	5.25	1.83	2.87
9j	7.21	2.03	3.55
9k	8.23	1.02	8.06
9l	8.52	2.13	4.00
Celecoxib	7.34	1.11	6.61

[a]IC_{50} represents the compound concentration that causes 50% suppression of COX-1 or COX-2.

[b]Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

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Compound number	Percentage of anti-inflammatory activity (AI)[a]		
	1h	3h	5h
9a	1.00 ± 0.14* (31%)	0.80 ± 0.15** (34%)	0.73 ± 0.17** (48%)
9b	0.98 ± 0.15* (33%)	0.95 ± 0.17 (32%)	0.78 ± 0.17** (45%)
9c	0.65 ± 0.03***(55%)	0.90 ± 0.09* (36%)	0.88 ± 0.09* (50%)
9d	1.05 ± 0.05 (26%)	0.88 ± 0.12*(38%)	0.70 ± 0.12** (38%)
9e	0.88 ± 0.13** (40%)	0.93 ± 0.09 (43%)	0.58 ± 0.10*** (59%)
9f	0.80 ± 0.04***(45%)	0.83 ± 0.05*(54%)	0.70 ± 0.07** (57%)
9g	0.63 ± 0.15***(57%)	0.18 ± 0.06***(88%)	0.18 ± 0.10*** (88%)
9h	1.08 ± 0.09 (28%)	0.65 ± 0.13***(41%)	0.60 ± 0.15*** (50%)
9i	1.10 ± 0.14 (24%)	0.80 ± 0.15**(43%)	0.68 ± 0.13** (52%)
9j	0.90 ± 0.09** (38%)	0.93 ± 0.09 (34%)	0.73 ± 0.06** (48%)
9k	0.70 ± 0.11***(52%)	0.65 ± 0.13*** (54%)	0.43 ± 0.14*** (70%)
9l	1.20 ± 0.09 (17%)	1.23 ± 0.19 (13%)	1.03 ± 0.20 (27%)
Control	1.45 ± 0.06 (0%)	1.40 ± 0.09 (0%)	1.40 ± 0.09 (0%)

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Celecoxib	0.83 ± 0.09**(43%)	0.80 ± 0.11** (43%)	0.65 ± 0.10*** (54%)

Values represent mean±SEM (n = 3), Significance levels

*p < 0.05,

**p < 0.01 and

***p < 0.001.as compared to the control group.

[a]Inhibitory activity in a formalin-induced rat paw edema assay using a dose of 50 mg/kg.

Compound	Ulcer number	Ulcer index
9e	2.75	5.00
9g	7.25**	12.25*
9k	4.75	6.00
Celecoxib	3.25	3.00
Indomethacin	14.25	22.50

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Compound No.	Affinity Kcal/mol	No.of hydrogen bonds	Distance (A°) from main residue		Functional Group
9e	-13.76	5	3.12	Tyr355	-Thiazole N
			2.34	Tyr355	-Pyrimidine N
			2.47	Arg120	- Pyrimidine N
			2.76	Tyr385	C=O
			2.74	Ser530	C=O
9g	-12.65	1	2.82	His 90	C=O
9k	-13.21	2	2.77	His90	C=O
			2.31	Ser530	CH=N
S-58	-11.93	2	2.41	Arg513	$-SO_2$
			2.30	His90	$-SO_2$

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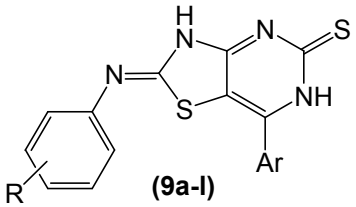
Graphical Abstract



(9a-l)

	R	Ar
9a	3-$COCH_3$	4-F-C_6H_4
9b	3-$COCH_3$	4-Cl-C_6H_4
9c	3-$COCH_3$	4-NO_2-C_6H_4
9d	3-$COCH_3$	3,4,5-$(OCH_3)_3$-C_6H_2
9e	4-$COCH_3$	4-F-C_6H_4
9f	4-$COCH_3$	4-Cl-C_6H_4
9g	4-$COCH_3$	4-NO_2-C_6H_4
9h	4-$COCH_3$	3,4,5-$(OCH_3)_3$-C_6H_2
9i	4-$COOC_2H_5$	4-F-C_6H_4
9j	4-$COOC_2H_5$	4-Cl-C_6H_4
9k	4-$COOC_2H_5$	4-(NO_2)-C_6H_4
9l	4-$COOC_2H_5$	3,4,5-$(OCH_3)_3$-C_6H_2

New thiazolo[4,5-*d*]pyrimidines (**9a-l**) were prepared and screened for their cyclooxygenase inhibitory effect, anti-inflammatory activity and ulcerogenic liability. All the new compounds exhibited anti-inflammatory activity; especially **9g** was the most active derivative with 57%, 88% and 88% inhibition of inflammation after 1, 3 and 5 h, respectively**.**

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Highlights

1- Novel candidates of thiazolo[4,5-*d*]pyrimidines (9a-l) were synthesized

2- The target compound 9g was the most active derivative with 57%, 88% and 88% inhibition of inflammation after 1, 3 and 5 h, respectively.

3- The target derivatives **9a-l** demonstrated moderate to high potent inhibitory action towards COX-2 (IC_{50} = 0.87–3.78 μM).

4- The target compound **(9e**) recorded the lowest ulcerogenic effect (Ulcer index = 5).

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