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Original article

# Design, synthesis and biological evaluation of some novel thienopyrimidines and fused thienopyrimidines as anti-inflammatory agents☆

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ABSTRACT

Some new substituted thienopyrimidine derivatives comprising thioxo, thioalkyl and pyrazolyl derivatives as well as fused thienotriazolopyrimidine and thienopyrimidinotriazine ring systems were prepared from 3-benzyl-2-hydrazino-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamide **4**. The designed compounds were evaluated for their anti-inflammatory activity. Compounds **4**, **9**, **10** and **13** showed the highest anti-inflammatory effect compared with the reference drug diclofenac sodium.



## 1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutic agents, primarily for the treatment of pain and inflammation in arthritis. However, long-term clinical usage of NSAIDs is associated with significant side effects including gastrointestinal lesions, bleeding and nephrotoxicity [1–4]. NSAID-induced gastropathy is estimated to affect up to half of chronic NSAID users, with major world health implications [5]. Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area.

In the last few years, thienopyrimidines and fused thienopyrimidines have gained much attention due to their diverse biological activities [6–10]. Recently, a large number of thienopyrimidines and fused thienopyrimidines (Fig. 1) were found to possess promising anti-inflammatory activities [11–13]. Moreover, triazoles, pyrazoles and triazines moieties have been gaining prominence for their wide spectrum of activities especially as anti-inflammatory [14–18].

Based on the above mentioned findings, we decided to synthesize some novel thienopyrimidines and fused thienopyrimidines aiming at developing more active anti-inflammatory agents devoid of the undesirable side effects associated with classical NSAIDs. The newly synthesized compounds were designed so as to comprise a thienopyrimidine moiety directly attached to a pyrazole or fused with a triazole or triazine rings. Such structural assembly was supposed to enhance the biological potential of this class of compounds [13].

## 2. Results and discussion

### 2.1. Chemistry

The synthetic strategies adopted for the synthesis of the intermediate and final compounds are depicted in Schemes 1–3.

In Scheme 1, the starting compound ethyl 2-amino-5-carbamoyl-4-methylthiophene-3-carboxylate **1** [19] was prepared by heating a mixture of acetoacetamide, ethyl cyanoacetate and sulfur in the presence of morpholine as a basic catalyst. Refluxing **1** with benzyl isothiocyanate in acetonitrile using anhydrous potassium carbonate afforded the sulfanyl thienopyrimidine derivative **2** following the method previously used for preparing such analogs compounds [20]. The structure of **2** was confirmed by its elemental analysis and spectral data. $^1$H NMR spectrum indicated the presence of signals assigned to the benzyl moiety and the sulfanyl

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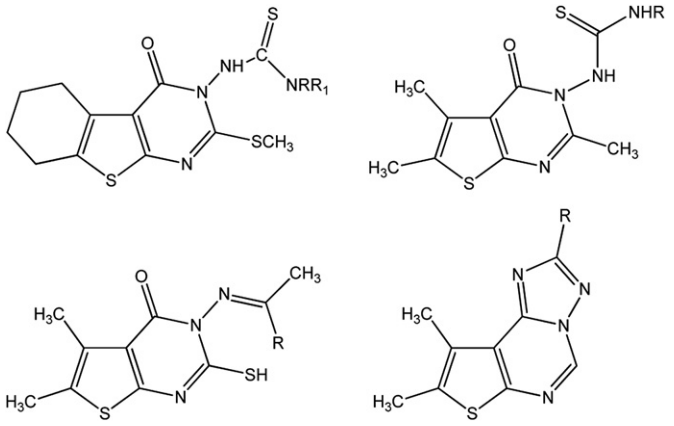
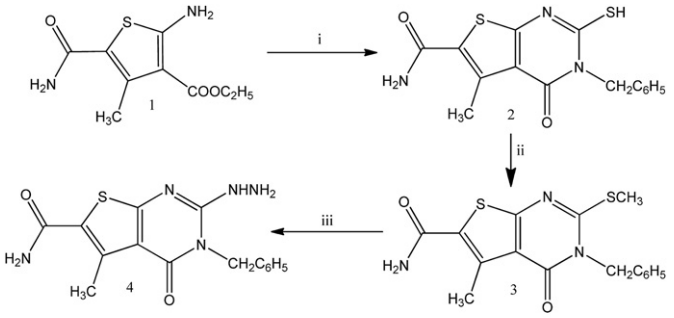


**Fig. 1.** Examples of anti-inflammatory thienopyrimidines and fused thienopyrimidines.

group. Treatment of compound **2** with methyl iodide and anhydrous potassium carbonate in dry acetone yielded the methylsulfanyl derivative **3** according to a literature procedure [21]. Analytical and spectral data of compound **3** are in agreement with the proposed structure, confirmed by the appearance of a methyl singlet at 2.71 ppm in the $^1$H NMR spectrum. Refluxing **3** with 99% hydrazine hydrate in ethanol furnished the corresponding hydrazino derivative **4**. $^1$H NMR showed the absence of the methyl singlet and the appearance of the $D_2O$ exchangeable signals at 9.32 and 11.40 ppm assigned to $NH_2$ and NH respectively.

In Scheme 2, the new hydrazones **5a**,**b** were prepared by condensation of the key intermediate **4** with 4-bromo or 4-nitrobenzaldehyde utilizing a previously reported procedure [22]. Reaction of **5a**,**b** with bromine in glacial acetic acid and anhydrous sodium acetate yielded the cyclized counterparts **6a**,**b**. $^1$H NMR spectra of compounds **6a**,**b** lacked signals characteristic for N=CH and NH protons, while $^{13}$C NMR spectrum for compound **6a** provided further confirmation of the structure. Moreover, heating the hydrazones **5a**,**b** with acetic anhydride yielded **7a**,**b** in good yields. $^1$H NMR spectra of **7a**,**b** showed additional singlets at 2.82 or 2.88 ppm attributed to $COCH_3$ group and lacked signals assigned for N=CH and NH protons. The structure of these compounds was further verified by $^{13}$C NMR spectral data. Refluxing the hydrazino derivative **4** with phenyl isothiocyanate in dry dimethylformamide produced the cyclized tetrahydrothieno[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidine derivative **9** instead of the thiosemicarbazide **8**. Compound **9** was also prepared through heating the hydrazine derivative **4** with carbon disulfide in ethanolic potassium hydroxide. Structure of **9** was substantiated by its $^1$H NMR spectral data which revealed the disappearance of signals attributed to the anilino moiety. The thiol tautomer of compound **9** was S-alkylated with ethyl bromoacetate in absolute ethanol to give the thioalkyl derivative **10** in an excellent yield. $^1$H NMR spectrum of **10** showed a triplet and a quartet signals assigned for the ethyl moiety, while its $^{13}$C NMR spectrum displayed signals at 23.27 and 70.83 ppm due to the ethyl group.

In Scheme 3, the key intermediate **4** was cyclized with aromatic carboxylic acids by refluxing with phosphorous oxychloride to afford compounds **11a**,**b** which were identified by IR and $^1$H NMR spectra. Reacting compound **4** with formic acid gave the corresponding 5-benzyl-3-methyl-4-oxo-4,5-dihydrothieno[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidine-2-carboxamide **12**. Furthermore, heating compound **4** with ethyl chloroformate in dry dioxane induced cyclization to the 5-benzyl-3-methyl-4,8-dioxo-4,5,7,8-tetrahydrothieno[3,2-*e*][1,2,4]triazolo[4,3-*a*]pyrimidine-2-carboxamide **13**. $^1$H NMR spectrum of compound **13** showed a $D_2O$ exchangeable singlet assigned for the triazolo NH whereas; its $^{13}$C NMR spectrum revealed two signals at 146.74 and 157.62 ppm corresponding to triazolothienopyrimidine-$C_{3a}$ and $C_8$ respectively. Synthesis of the triazolo derivative **14** was accomplished by heating a mixture of **4** and diethyl malonate in glacial acetic acid. $^1$H NMR spectrum of compound **14** showed a triplet and a quartet characteristic for the ethyl moiety, while its MS spectrum revealed a molecular ion peak at *m*/*z* 425 (12.51%) which match its molecular weight. Additionally, compound **4** was cyclized with the appropriate phenacyl bromides in boiling absolute ethanol and anhydrous sodium acetate to give the thieno[3′,2′:5,6]pyrimidino[2,1-*c*][1,2,4]triazine derivatives **15a**–**c**. $^1$H NMR spectra of compounds **15a**–**c** showed the presence of singlet at 5.13–5.20 ppm assigned to the triazino C-9 protons. On the other hand, refluxing compound **4** with the appropriate phenacyl cyanides in glacial acetic acid resulted in the formation of the pyrazolyl derivatives **16a**,**b**. Their $^1$H NMR spectrum showed singlets at 10.33 and 10.31 ppm assigned to additional $NH_2$ group and singlets at 3.21 and 2.88 ppm corresponding to the pyrazolyl C-4 protons. The pyrazolyl derivatives **17** and **18** were successfully achieved by heating **4** with acetylacetone and ethyl acetoacetate respectively in glacial acetic acid. $^1$H NMR spectra of compounds **17** and **18** were characterized by pyrazolyl C-4 protons and signals for the methyl groups on the pyrazole ring. $^{13}$C NMR spectrum for compound **17** revealed two signals for the two methyl groups of the pyrazole ring at 23.86 and 49.80 ppm and a signal at 125.01 ppm due to pyrazole C-4. Moreover, the MS spectrum of **17** showed a molecular ion peak at *m*/*z* 393 (7.15%) which matched its molecular weight.

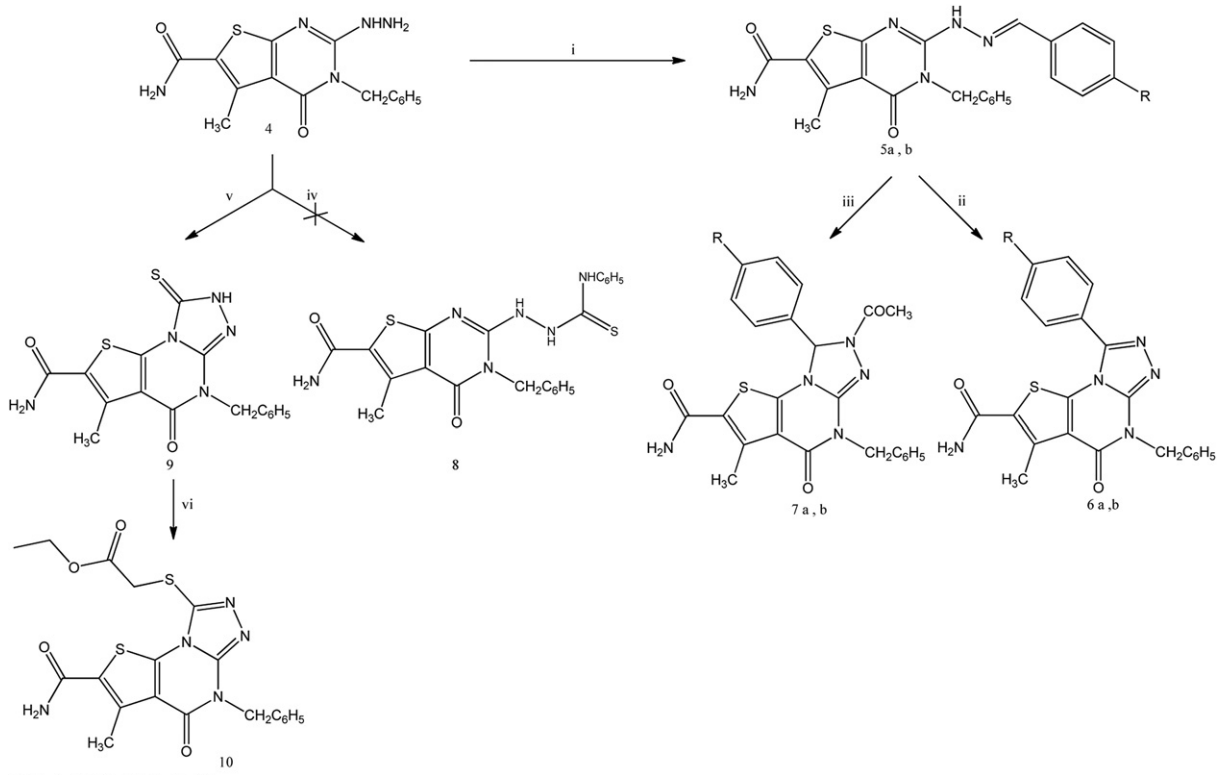


Reagents and conditions: i) $C_6H_5CH_2NCS$ / $K_2CO_3$/ acetonitrile / reflux ; ii) $CH_3I$ / $K_2CO_3$ / dry acetone / reflux ; iii ) $N_2H_4$ 99 % / EtOH / reflux.

**Scheme 1.** Synthetic routes of compounds **2**–**4**.

### 2.2. Anti-inflammatory (AI) activity

To assess the AI activity of the designed compounds, 16 compounds namely; **2**, **3**, **4**, **5a**, **6a**, **7a**, **9**, **10**, **11b**, **12**, **13**, **14**, **15c**, **16b**, **17** and **18** were evaluated by two screening protocols; namely, the formalin-induced paw edema [23] and turpentine oil-induced granuloma pouch [24] bioassays, using diclofenac Na (10 mg/kg) as a reference standard anti-inflammatory agent. The paw edema was employed as a model for acute and sub-acute inflammation, while the turpentine oil-induced granuloma pouch assay was utilized as another model for sub-acute inflammatory condition. The data obtained were presented in Tables 1–3 and expressed as means ± SE. Statistical differences of control and test groups were carried out using the Analysis of Variance (ANOVA) followed by 'Student–Newman–Keuls Multiple Comparison Test. They were performed using computer package of the Statistical Analysis System (SAS, 1987), SAS Incorporation Institute. The difference in results was considered significant when $P < 0.05$.




**Scheme 2.** Synthetic routes of compounds **5**–**10**.

*2.2.1. Formalin-induced paw edema bioassay (acute inflammatory model)*

In this acute inflammatory model [23] each test compound was dosed orally (10 mg/kg body weight) 1 h prior to induction of inflammation by formalin injection. Diclofenac Na was utilized as a reference anti-inflammatory drug at a dose of 10 mg/kg, po. The anti-inflammatory activity was then calculated 1–4 h after induction and presented in Table 1 as the mean paw volume (mL) in addition to the percentage anti-inflammatory activity (AI%).

A comparative study of the anti-inflammatory activity of test compounds relative to the reference drug at different time intervals indicated the following: after 1 h, three compounds, **4** (69%), **5a** (69%) and **13** (75%) showed promising pharmacokinetic profiles as revealed from their potent and rapid onset of action which was higher than diclofenac Na (63%) at a dose of 10 mg/kg, po. Whereas, the rest of the compounds showed less activities. After 2 h interval, the data indicated that compounds **4**, **9**, **10**, **13** and **16b** displayed better anti-inflammatory activity (52–61%) compared to diclofenac Na (43%). Whereas, compounds **3**, **11b**, **12**, **14**, **15c** and **18** were nearly as effective (43–48%) as diclofenac Na. The anti-inflammatory activity after 4 h interval for compounds **10**, **11b**, **13** and **17** were found to be equally effective to diclofenac Na (49%). Whereas, compounds **4**, **9** and **12** proved to be more effective (51%, 51%, and 54%, respectively) than diclofenac Na (49%) at the same time interval.

Some of the most active compounds **4**, **9**, **10**, **13**, **16b** and **18** were further tested at 5, 10, 20, 40 and 50 mg/kg body weight in order to determine their $ED_{50}$ values. Most of these compounds were found to be nearly equipotent ($ED_{50}$ = 8.36–11.53 mg/kg, Table 1). Meanwhile, compounds **4**, **9** and **10** were found to be the most potent ($ED_{50}$ = 8.36–8.72 mg/kg) compared to the reference drug diclofenac Na ($ED_{50}$ = 11.53 mg/kg of body weight).

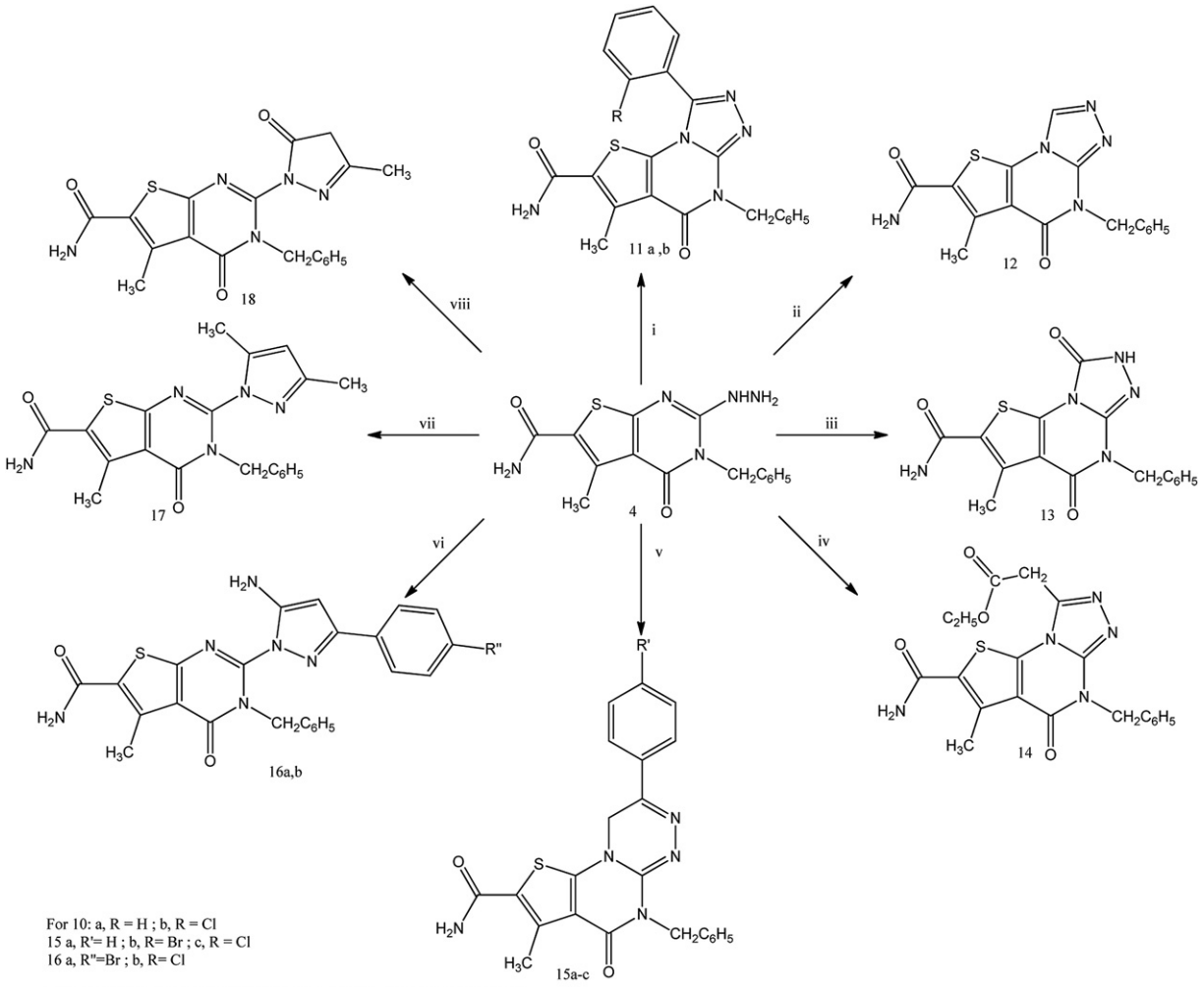
*2.2.2. Formalin-induced paw edema bioassay (sub-acute inflammatory model)*

For this sub-acute inflammatory model [23] inflammation was induced by formalin injection in the first and third days, and test compounds were administered orally (at 10 mg/kg daily) for 7 days. Again, diclofenac Na was used as a reference anti-inflammatory agent in this assay. The anti-inflammatory activity was calculated at 1st and 8th day after induction and presented in Table 2 as the mean paw volume and the percentage anti-inflammatory activity (AI%).

The obtained data revealed that, at the 1st day, compounds **4**, **9**, **10**, **13**, **14** and **15c** showed superior anti-inflammatory activities (41–46%) than diclofenac Na (39%). On the other hand, compounds **5a**, **12**, **17** and **18** displayed anti-inflammatory activities (39%) equal to diclofenac Na. At the 8th day, compound **4** was found to be superior over the reference drug with anti-inflammatory activity (43%), compounds **10**, **13**, **14** and **15c** were nearly effective (36%, 37%) as diclofenac Na (36%).

*2.2.3. Turpentine oil-induced granuloma pouch bioassay (sub-acute inflammatory model)*

In this bioassay [24] each test compound was administered orally (10 mg/kg) 1 h prior to turpentine oil injection and continued for 7 days. At the 8th day, the exudates volume (mL) was measured and the percentage of granuloma inhibition was calculated. Diclofenac Na (10 mg/kg) was used as a reference drug. The results depicted in Table 3 revealed that, while most of the test compounds showed anti-inflammatory activity less than the reference drug, compound **4** was proved to be more effective (44.79%) than diclofenac Na (41.17%). Compounds **6a** and **10** were equipotent (41.17%) with diclofenac Na. While, compounds **7a**, **12**, **14**, **16b**, and **17** showed moderate anti-inflammatory activity in this bioassay with




**Scheme 3.** Synthetic routes of compounds **11a**,**b**–**18**.

**Table 1**
Anti-inflammatory activity (AI) of some selected compounds in formalin-induced rat paw edema bioassay (acute inflammatory model).

| Compound[a] | Volume of edema (mL)[b] | | | | |
|---|---|---|---|---|---|
| | 0 | 1 h | 2 h | 4 h | $ED_{50}$ (mg/kg) |
| Control | 0.27 ± 0.01 | 0.43 ± 0.01 | 0.50 ± 0.01 | 0.66 ± 0.02 | |
| **2** | 0.26 ± 0.01 | 0.38 ± 0.01* (25)[c] | 0.49 ± 0.01 (0) | 0.63 ± 0.02 (5) | |
| **3** | 0.29 ± 0.02 | 0.38 ± 0.01* (44) | 0.42 ± 0.01* (43) | 0.53 ± 0.01* (38) | |
| **4** | 0.30 ± 0.01 | 0.35 ± 0.02* (69) | 0.39 ± 0.01* (61) | 0.49 ± 0.01* (51) | 8.36 |
| **5a** | 0.28 ± 0.03 | 0.33 ± 0.01* (69) | 0.42 ± 0.02* (39) | 0.50 ± 0.01* (44) | |
| **6a** | 0.26 ± 0.01 | 0.37 ± 0.02* (31) | 0.40 ± 0.01* (39) | 0.49 ± 0.02* (41) | |
| **7a** | 0.25 ± 0.01 | 0.36 ± 0.01* (31) | 0.41 ± 0.02* (30) | 0.50 ± 0.01* (36) | |
| **9** | 0.29 ± 0.02 | 0.37 ± 0.01* (50) | 0.39 ± 0.02* (56) | 0.48 ± 0.01* (51) | 8.72 |
| **10** | 0.30 ± 0.01 | 0.37 ± 0.01* (56) | 0.39 ± 0.01* (61) | 0.50 ± 0.02* (49) | 8.36 |
| **11b** | 0.24 ± 0.03 | 0.34 ± 0.03* (37) | 0.37 ± 0.01* (43) | 0.44 ± 0.03* (49) | |
| **12** | 0.27 ± 0.01 | 0.34 ± 0.01* (56) | 0.40 ± 0.02* (43) | 0.45 ± 0.02* (54) | |
| **13** | 0.29 ± 0.02 | 0.33 ± 0.01* (75) | 0.38 ± 0.01* (61) | 0.49 ± 0.03* (49) | 11.53 |
| **15c** | 0.28 ± 0.01 | 0.37 ± 0.02* (44) | 0.41 ± 0.02* (43) | 0.49 ± 0.02* (46) | |
| **16b** | 0.28 ± 0.03 | 0.35 ± 0.01* (56) | 0.39 ± 0.02* (52) | 0.49 ± 0.01* (46) | 9.65 |
| **17** | 0.28 ± 0.02 | 0.37 ± 0.02* (44) | 0.42 ± 0.01* (39) | 0.48 ± 0.01* (49) | |
| **18** | 0.28 ± 0.01 | 0.38 ± 0.01* (37) | 0.40 ± 0.02* (48) | 0.49 ± 0.01* (46) | 10.5 |
| Diclofenac Na | 0.28 ± 0.02 | 0.34 ± 0.01* (63) | 0.41 ± 0.03* (43) | 0.48 ± 0.01* (49) | 11.53 |

*Significantly different compared to corresponding control. $P \leq 0.05$.
[a] Dose levels, po: test compounds (10 mg/kg body weight), diclofenac Na (10 mg/kg body weight).
[b] Values are expressed as mean ± S.E. (Number of animals $N = 5$ rats).
[c] Between parentheses (Percentage anti-inflammatory activity, AI %).

**Table 2**
Anti-inflammatory activity (AI) of some selected compounds in formalin-induced rat paw edema bioassay (sub-acute inflammatory model).

| Compound[a] | Volume of edema (mL)[b] | | |
|---|---|---|---|
| | 0 | 1st day | 8th day |
| Control | 0.27 ± 0.01 | 0.73 ± 0.01 | 0.83 ± 0.01 |
| **2** | 0.26 ± 0.01 | 0.70 ± 0.02 (4)[c] | 0.79 ± 0.02 (5) |
| **3** | 0.29 ± 0.02 | 0.67 ± 0.02* (17) | 0.73 ± 0.02* (21) |
| **4** | 0.30 ± 0.01 | 0.56 ± 0.01* (43) | 0.62 ± 0.01* (43) |
| **5a** | 0.28 ± 0.03 | 0.56 ± 0.01* (39) | 0.65 ± 0.01* (34) |
| **6a** | 0.26 ± 0.01 | 0.55 ± 0.01* (37) | 0.65 ± 0.01* (30) |
| **7a** | 0.25 ± 0.01 | 0.56 ± 0.02* (33) | 0.64 ± 0.01* (30) |
| **9** | 0.29 ± 0.02 | 0.56 ± 0.01* (41) | 0.66 ± 0.01* (34) |
| **10** | 0.30 ± 0.01 | 0.57 ± 0.01* (41) | 0.66 ± 0.01* (36) |
| **11b** | 0.24 ± 0.03 | 0.55 ± 0.01* (33) | 0.63 ± 0.01* (30) |
| **12** | 0.27 ± 0.01 | 0.55 ± 0.01* (39) | 0.65 ± 0.01 (32) |
| **13** | 0.29 ± 0.02 | 0.55 ± 0.02* (43) | 0.64 ± 0.02* (37) |
| **14** | 0.28 ± 0.01 | 0.53 ± 0.01* (46) | 0.63 ± 0.02* (37) |
| **15c** | 0.28 ± 0.01 | 0.53 ± 0.02* (46) | 0.63 ± 0.01* (37) |
| **16b** | 0.28 ± 0.03 | 0.58 ± 0.02* (35) | 0.67 ± 0.02* (30) |
| **17** | 0.28 ± 0.01 | 0.56 ± 0.01* (39) | 0.66 ± 0.01* (32) |
| **18** | 0.28 ± 0.02 | 0.56 ± 0.02* (39) | 0.65 ± 0.02* (34) |
| Diclofenac Na | 0.28 ± 0.02 | 0.56 ± 0.01* (39) | 0.64 ± 0.02* (36) |

*Significantly different compared to corresponding control. $P \leq 0.05$.
[a] Dose levels, po: test compounds (10 mg/kg body weight), diclofenac Na (10 mg/kg body weight).
[b] Values are expressed as mean ± S.E. (Number of animals $N = 5$ rats).
[c] Between parentheses (Percentage anti-inflammatory activity, AI %).

percentage of granuloma inhibition of 39.82%, 39.36%, 39.82%, 40.27% and 40.27%, respectively.

A collective interpretation of the anti-inflammatory activity of the test compounds in pre-mentioned screens (Tables 1–3) revealed that the hydrazinothienopyrimidine **4** and the thienotriazolopyrimidine **10** showed pronounced activity in the formalin-induced paw edema screen (acute inflammatory model), in formalin-induced paw edema and turpentine oil-induced granuloma pouch screens (sub-acute inflammatory models). These facts would suggest that such compounds might be effective in managing acute inflammation and chronic inflammatory conditions. Whereas, the thienotriazolopyrimidines **9** and **13** proved to be highly active in the formalin paw edema screen (acute inflammatory model) and less active in formalin paw edema and turpentine oil-induced granuloma pouch screens (sub-acute inflammatory models). This would indicate that these compounds are effective in acute inflammation and less active in chronic inflammatory conditions.

**Table 3**
Anti-inflammatory activity of some selected compounds in turpentine oil induced granuloma pouch in rats.

| Compound[a] | Volume of exudates (mL)[b] | Percentage of inhibition |
|---|---|---|
| Control | 2.21 ± 0.09 | – |
| **2** | 2.12 ± 0.03 | 4.07 |
| **3** | 1.96 ± 0.04 | 11.31 |
| **4** | 1.22 ± 0.05* | 44.79 |
| **5a** | 1.45 ± 0.17* | 34.38 |
| **6a** | 1.30 ± 0.06* | 41.17 |
| **7a** | 1.33 ± 0.08* | 39.82 |
| **9** | 1.70 ± 0.15* | 23.07 |
| **10** | 1.30 ± 0.08* | 41.17 |
| **11b** | 1.42 ± 0.09* | 35.74 |
| **12** | 1.34 ± 0.06* | 39.36 |
| **13** | 1.36 ± 0.08* | 38.46 |
| **14** | 1.33 ± 0.07* | 39.82 |
| **15c** | 1.36 ± 0.12* | 38.46 |
| **16b** | 1.32 ± 0.09* | 40.27 |
| **17** | 1.32 ± 0.07* | 40.27 |
| **18** | 1.35 ± 0.05* | 38.91 |
| Diclofenac Na | 1.30 ± 0.07* | 41.17 |

*Significantly different compared to corresponding control. $P \leq 0.05$.
[a] Dose levels, po: test compounds (10 mg/kg body weight), diclofenac Na (10 mg/kg body weight).
[b] Values are expressed as mean ± S.E. (Number of animals $N = 5$ rats).

### 2.2.4. Ulcerogenic activity

Six tested compounds namely **4**, **9**, **10**, **13**, **16b** and **18** that exhibited variable anti-inflammatory profiles in the pre-mentioned animal models were further evaluated for their ulcerogenic potential in rats [25]. Gross observation of the isolated rat stomachs showed a normal stomach texture for compounds **13**, **16b** and **18** with no observable hyperemia indicating a superior GI safety profile (no ulceration) in the population of the test animals at an oral dose of 300 mg/kg, when administered twice at 2 h interval in fasted rats. Whereas compounds **4**, **9** and **10** showed weak ulceration effect (10, 10 and 20% respectively) compared to the reference drug diclofenac Na (no ulceration). It is worth-mentioning that, indomethacin; the reference standard anti-inflammatory drug; was found to cause 100% ulceration under the same experimental conditions.

### 2.2.5. Acute toxicity

Six of the selected compounds namely **4**, **9**, **10**, **13**, **16b** and **18** were further evaluated for their approximate acute lethal dose $ALD_{50}$ in male rats using a literature method [26]. The results indicated that all of the tested compounds were proved to be non-toxic and are well tolerated by the experimental animals. The compounds showed a high safety margin when screened at graded doses (0.1–0.3 g/kg, po), where their $ALD_{50}$ values were found to be >0.3 g/kg.

## 2.3. Conclusion

In conclusion, the aim of the present investigation was to synthesize novel thienopyrimidines and fused tricyclic thienopyrimidines as possible anti-inflammatory agents. Among the tested analogs, compounds **4**, **9**, **10** and **13** showed pronounced anti-inflammatory activity comparable with diclofenac Na in the acute inflammatory model and the sub-acute inflammatory models, suggesting that they might be effective in managing acute and chronic inflammatory conditions. On the other hand, compounds **14** and **15c** were nearly effective as diclofenac Na in the sub-acute inflammatory model and compound **6a** was equipotent with diclofenac Na in the turpentine oil-induced granuloma pouch screen. Additionally, all of the tested compounds revealed super GI safety profile and are well tolerated by the experimental animals with high safety margin ($ALD_{50} > 3.0$ g/kg). Consequently, such type of compounds would represent a fruitful matrix for further development of more potent and selective anti-inflammatory agents that deserve further investigation and derivatization in order to explore the scope and limitation of its biological activities.

## 3. Experimental

### 3.1. Chemistry

Melting points were determined in open-glass capillaries using a Gallen-Kamp melting point apparatus and are uncorrected. The IR spectra (KBr) were recorded using a Perkin–Elmer 1430 spectrophotometer. The $^1$H NMR and $^{13}$C NMR spectra were determined on a Varian spectrometer (300 MHz), Faculty of Science, Cairo University using tetramethylsilane (TMS) as internal standard and DMSO-$d_6$ as the solvent (chemical shifts are given in $\delta$ ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Mass spectra were carried out using an

Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV, Faculty of Science, Cairo University. Microanalyses were performed at the Microanalytical Unit, Faculty of Science, Cairo University. The results of the microanalyses were within ±0.4% of the calculated values. Follow up of the reactions and checking the purity of the compounds was made by thin layer chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60 GF254; Merck; Germany) and the spots were detected by exposure to UV lamp at λ 254 nm for few seconds. Compound **1** was synthesized as described in Ref. [19].

*3.1.1. 3-Benzyl-5-methyl-4-oxo-2-sulfanyl-3,4-dihydrothieno[2,3-d] pyrimidine-6-carboxamide (**2**)*

A mixture of **1** (2.28 g, 10 mmol), benzyl isothiocyanate (1.49 g, 1.3 mL, 10 mmol) and anhydrous potassium carbonate (1.4 g, 10 mmol) in acetonitrile (30 mL) was heated under reflux for 15 h. The reaction mixture was left to cool and filtered. The obtained potassium salt was dissolved in water, neutralized with acetic acid then the obtained crude product was filtered, washed with water, dried and crystallized from ethanol.

Yield: 78%, mp: 292–4 °C. IR (KBr, $cm^{-1}$): 3476, 3171 (NH), 1686 (C=O), 1638 (C=N), 1289, 1079 (C–S–C). $^1$H NMR (δ ppm): 2.62 (s, 3H, $CH_3$), 5.59 (s, 2H, $CH_2$), 7.22–7.31 (m, 5H, Ar–H), 7.56 (s, 2H, $NH_2$, $D_2O$ exchangeable), 11.24 (s, 1H, SH, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{13}N_3O_2S_2$ (331.42): C 54.36, H 3.95, N 12.68. Found: C 54.12, H 3.86, N 12.92.

*3.1.2. 3-Benzyl-5-methyl-2-(methylsulfanyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**3**)*

A stirred mixture of **2** (0.33 g, 1 mmol), methyl iodide (2 mmol) and anhydrous potassium carbonate (0.276 g, 2 mmol) in dry acetone (10 mL) was heated under reflux for 4 h. The reaction mixture was cooled, poured into ice-cold water; the precipitate formed was dried and crystallized from DMF.

Yield: 72%, mp: 252–4 °C. IR (KBr, $cm^{-1}$): 3363, 3173 (NH), 1690, 1644 (C=O), 1605 (C=N), 1286, 1077 (C–S–C). $^1$H NMR (δ ppm): 2.56 (s, 3H, $CH_3$–C), 2.71 (s, 3H, $CH_3$–S), 5.3 (s, 2H, $CH_2$), 7.22–7.36 (m, 5H, Ar–H), 7.59 (s, 2H, $NH_2$, $D_2O$ exchangeable). Anal. Calcd. for $C_{16}H_{15}N_3O_2S_2$ (345.44): C 55.63, H 4.38, N 12.16. Found: C 55.41, H 4.27, N 12.08.

*3.1.3. 3-Benzyl-2-hydrazino-5-methyl-4-oxo-3,4-dihydrothieno [2,3-d]pyrimidine-6-carboxamide (**4**)*

To a suspension of **3** (3.45 g, 10 mmol) in ethanol (50 mL), hydrazine hydrate 99% (4 mL, 80 mmol) was added. The reaction mixture was refluxed for 3 h, during which a precipitate was formed. After cooling, the product was filtered, washed with water, dried and crystallized from DMF/EtOH (5:1).

Yield: 62%, mp: 314–6 °C. IR (KBr, $cm^{-1}$): 3364, 3230 (NH), 1673 (C=O), 1614 (C=N), 1266, 1061 (C–S–C). $^1$H NMR (δ ppm): 2.73 (s, 3H, $CH_3$), 5.33 (s, 2H, $CH_2$), 7.26–7.34 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.42 (d, $J$ = 7.2 Hz, 2H, phenyl–$C_{2,6}$–H), 7.76 (s, 2H, $CONH_2$, $D_2O$ exchangeable), 9.32 (s, 2H, $NH_2$, $D_2O$ exchangeable), 11.40 (s, 1H, NH, $D_2O$ exchangeable). Anal. Calcd. for $C_{15}H_{15}N_5O_2S$ (329.38): C 54.70, H 4.59, N 21.26. Found: C 54.63, H 4.45, N 20.98.

*3.1.4. 3-Benzyl-2-[4-substituted benzylidenehydrazino]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**5a**,**b**)*

The title compounds were prepared by refluxing a mixture of the hydrazine **4** (0.329 g, 1 mmol) and the appropriate aryl aldehyde (1 mmol) in absolute ethanol (20 mL) containing 2 drops of acetic acid for 2 h. The reaction mixture was allowed to cool and the obtained product was filtered, dried and crystallized from DMF.

*3.1.4.1. 3-Benzyl-2-[4-bromobenzylidenehydrazino]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**5a**).* Yield: 78%, mp: 290–92 °C. IR (KBr, $cm^{-1}$): 3495, 3381 (NH), 1674, 1639 (C=O), 1613 (C=N), 1253, 1068 (C–S–C). $^1$H NMR (δ ppm): 2.67 (s, 3H, $CH_3$), 5.26 (s, 2H, $CH_2$), 7.22–7.34 (m, 5H, Ar–H), 7.39 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.61 (d, $J$ = 8.4 Hz, 2H, bromophenyl–$C_{2,6}$–H), 7.81 (d, $J$ = 8.4 Hz, 2H, bromophenyl–$C_{3,5}$–H), 8.29 (s, 1H, N=CH), 11.94 (s, 1H, NH, $D_2O$ exchangeable). Anal. Calcd. for $C_{22}H_{18}BrN_5O_2S$ (496.39): C 53.23, H 3.66, N 14.11. Found: C 53.01, H 3.32, N 14.33.

*3.1.4.2. 3-Benzyl-2-[4-nitrobenzylidenehydrazino]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**5b**).* Yield: 75%, mp: 268–70 °C. IR (KBr, $cm^{-1}$): 3382, 3332 (NH), 1686, 1644 (C=O), 1615 (C=N), 1252, 1062 (C–S–C). $^1$H NMR (δ ppm): 2.66 (s, 3H, $CH_3$), 5.26 (s, 2H, $CH_2$), 7.23–7.35 (m, 5H, phenyl–H), 7.47 (s, 2H, $NH_2$, $D_2O$ exchangeable), 8.24 (d, $J$ = 8.2 Hz, 2H, nitrophenyl–$C_{2,6}$–H), 8.32 (d, $J$ = 8.2 Hz, 2H, nitrophenyl–$C_{3,5}$–H), 8.46 (s, 1H, N=CH), 12.05 (s, 1H, NH, $D_2O$ exchangeable). Anal. Calcd. for $C_{22}H_{18}N_6O_4S$ (462.49): C 57.14, H 3.92, N 18.17. Found: C 56.89, H 3.76, N 18.14.

*3.1.5. 5-Benzyl-3-methyl-4-oxo-8-(4-substituted phenyl)-4,5-dihydrothieno[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**6a**,**b**)*

To a stirred mixture of the appropriate hydrazone **5a** or **b** (1 mmol) and anhydrous sodium acetate (0.25 g, 3 mmol) in gl. acetic acid (5 mL), bromine (0.32 g, 0.1 mL, 2 mmol) was added dropwise. The mixture was stirred at room temp. for 12 h then poured on 0.5 N cold NaOH. The obtained precipitate was filtered, washed with water, dried and crystallized from DMF/$H_2O$ (5:1).

*3.1.5.1. 5-Benzyl-8-(4-bromophenyl)-3-methyl-4-oxo-4,5-dihydrothieno[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**6a**).* Yield: 68%, mp: 272–4 °C. IR (KBr, $cm^{-1}$): 3456, 3351 (NH), 1685 (C=O), 1622 (C=N), 1238, 1073 (C–S–C). $^1$H NMR (δ ppm): 2.71 (s, 3H, $CH_3$), 5.38 (s, 2H, $CH_2$), 7.28–7.36 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.48 (d, $J$ = 7.2 Hz, 2H, phenyl–$C_{2,6}$–H), 7.7 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.72 (d, $J$ = 8.2 Hz, 2H, Br–phenyl–$C_{2,6}$–H), 7.86 (d, $J$ = 8.2 Hz, 2H, Br–phenyl–$C_{3,5}$–H). $^{13}$C NMR (δ ppm): 19.55 ($CH_3$), 50.40 ($CH_2$–benzyl), 123.59 (bromophenyl–$C_4$), 129.53 (benzyl–$C_4$), 130.46 (bromophenyl–$C_1$), 132.64 (benzyl–$C_3$ and $C_5$), 133.15 (benzyl–$C_2$ and $C_6$), 133.48 (thienotriazolopyrimidine–$C_2$), 137.00 (bromophenyl–$C_2$ and $C_6$), 137.22 (bromophenyl–$C_3$ and $C_5$), 137.48 (benzyl–$C_1$), 141.70 (thienotriazolopyrimidine–$C_3$), 141.88 (thienotriazolopyrimidine–$C_{3e}$), 147.07 (thienotriazolopyrimidine–$C_{3a}$), 151.14 (thienotriazolopyrimidine–$C_8$), 154.05 (thienotriazolopyrimidine–$C_{2e}$), 161.12 (thienotriazolopyrimidine–$C_4$–C=O), 167.88 ($CONH_2$). Anal. Calcd. for $C_{22}H_{16}BrN_5O_2S \cdot 1/2H_2O$ (503.38): C 52.49, H 3.40, N 13.91. Found: C 52.26, H 3.01, N 13.87.

*3.1.5.2. 5-Benzyl-3-methyl-8-(4-nitrophenyl)-4-oxo-4,5-dihydrothieno[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**6b**).* Yield: 63%, mp: 224–6 °C. IR (KBr, $cm^{-1}$): 3464, 3350 (NH), 1683 (C=O), 1622 (C=N), 1234, 1045 (C–S–C). $^1$H NMR (δ ppm): 2.73 (s, 3H, $CH_3$), 5.41 (s, 2H, $CH_2$), 7.29–7.38 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.49 (d, $J$ = 7.5 Hz, 2H, phenyl–$C_{2,6}$–H), 7.67 (s, 2H, $NH_2$, $D_2O$ exchangeable), 8.09 (d, $J$ = 8.7 Hz, 2H, nitrophenyl–$C_{2,6}$–H), 8.46 (d, $J$ = 8.7 Hz, 2H, nitrophenyl–$C_{3,5}$–H). Anal. Calcd. for $C_{22}H_{16}N_6O_4S$ (460.47): C 57.39, H 3.50, N 18.25. Found: C 57.18, H 3.21, N 18.29.

*3.1.6. 7-Acetyl-5-benzyl-3-methyl-4-oxo-8-(4-substituted phenyl)-4,5,7,8-tetrahydrothieno[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**7a**,**b**)*

The appropriate hydrazone **5a** or **b** (1 mmol) was heated under reflux in $Ac_2O$ (5 mL) for 8 h then the reaction mixture was

concentrated to small volume, poured onto ice cold water, stirred for 2 h and refrigerated for an over night. The separated products were filtered, washed with water, dried and crystallized from DMF.

*3.1.6.1. 7-Acetyl-5-benzyl-8-(4-bromophenyl)-3-methyl-4-oxo-4,5,7,8-tetrahydrothieno[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**7a**).* Yield: 60%, mp: 290–2 °C. IR (KBr, $cm^{-1}$): 3441, 3261 (NH), 1692 (C=O), 1639 (C=N), 1227, 1071 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.66 (s, 3H, $CH_3$), 2.82 (s, 3H, $COCH_3$), 5.39 (s, 2H, $CH_2$), 7.28–7.37 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.48 (d, $J$ = 6.9 Hz, 2H, phenyl–$C_{2,6}$–H), 7.51 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.72 (d, $J$ = 8.1 Hz, 2H, bromophenyl–$C_{2,6}$–H), 7.87–7.91 (m, 3H, bromophenyl–$C_{3,5}$–H and triazolo–$C_8$–H). Anal. Calcd. for $C_{24}H_{20}BrN_5O_3S$ (538.43): C 53.54, H 3.74, N 13.01. Found: C 53.26, H 3.45, N 12.96.

*3.1.6.2. 7-Acetyl-5-benzyl-3-methyl-8-(4-nitrophenyl)-4-oxo-4,5,7,8-tetrahydrothieno [3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**7b**).* Yield: 61%, mp: 252–4 °C. IR (KBr, $cm^{-1}$): 3440, 3243 (NH), 1677 (C=O), 1620 (C=N), 1229, 1047 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.66, 2.71 (2s, 3H, $CH_3$), 2.83, 2.88 (2s, 3H, $COCH_3$), 5.41 (s, 2H, $CH_2$), 7.29–7.38 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.49 (d, $J$ = 7.2 Hz, 2H, phenyl–$C_{2,6}$–H), 7.52 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.95 (s, 1H, triazolo–$C_8$–H), 8.09 (d, $J$ = 8.1 Hz, 2H, nitrophenyl–$C_{2,6}$–H), 8.49 (d, $J$ = 8.1 Hz, 2H, nitrophenyl–$C_{3,5}$–H). $^{13}$C NMR ($\delta$ ppm): 23.59 ($CH_3$), 24.99 ($CO\underline{C}H_3$), 54.98 ($CH_2$–benzyl), 122.17 (nitrophenyl–$C_3$ and $C_5$), 126.75 (benzyl–$C_4$), 133.53 (nitrophenyl–$C_2$ and $C_6$), 133.81 (nitrophenyl–$C_1$), 137.55 (benzyl–$C_3$ and $C_5$), 137.59 (thienotriazolopyrimidine–$C_2$), 137.86 (benzyl–$C_2$ and $C_6$), 141.26 (benzyl–$C_1$), 145.07 (nitrophenyl–$C_4$), 154.46 (thienotriazolopyrimidine–$C_3$), 155.02 (thienotriazolopyrimidine–$C_{3e}$), 158.19 (triazolothienopyrimidine–$C_{3a}$), 158.85 (thienotriazolopyrimidine–$C_8$), 164.66 (thienotriazolopyrimidine–$C_{2e}$), 165.28 (thienotriazolopyrimidine–$C_4$–C=O), 171.71 ($CONH_2$), 172.22 ($\underline{C}OCH_3$). Anal. Calcd. for $C_{24}H_{20}N_6O_5S$ (504.53): C 57.14, H 4.0, N 16.66. Found: C 56.95, H 3.87, N 16.96.

*3.1.7. 5-Benzyl-3-methyl-4-oxo-8-thioxo-4,5,7,8-tetrahydrothieno [3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**9**)*

*3.1.7.1. Method A.* To a solution of **4** (0.329 g, 1 mmol) in dry DMF (5 mL), phenyl isothiocyanate (1 mmol) was added dropwise, the reaction mixture was then refluxed for 6 h and cooled. The precipitated product was filtered, washed with ethanol and crystallized from DMF/EtOH (5:1). Yield: 68%, mp: >300 °C.

*3.1.7.2. Method B.* A mixture of **4** (0.658 g, 2 mmol), potassium hydroxide (0.11 g, 2 mmol) and carbon disulfide (2 mL) in ethanol (20 mL) was heated under reflux for 12 h. The reaction mixture was concentrated, cooled, diluted with water and acidified with dil HCl. The separated solid was filtered, washed with ethanol, dried and crystallized from DMF/EtOH (5:1).

Yield: 72%. IR (KBr, $cm^{-1}$): 3384, 3225 (NH), 1680, 1649 (C=O), 1624 (C=N), 1266, 1069 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.72 (s, 3H, $CH_3$), 5.16 (s, 2H, $CH_2$), 7.26–7.34 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.38–7.42 (m, 3H, phenyl–$C_{2,6}$–H & triazolo NH), 7.69 (s, 2H, $NH_2$, $D_2O$ exchangeable). Anal. Calcd. for $C_{16}H_{13}N_5O_2S_2$ (371.44): C 51.74, H 3.53, N 18.85. Found: C 51.45, H 3.33, N 18.69.

*3.1.8. Ethyl [(5-benzyl-2-carbamoyl-3-methyl-4-oxo-4,5-dihydrothieno [3,2-e][1,2,4]triazolo[4,3-a]pyrimidin-8-yl)sulfanyl]acetate (**10**)*

To a suspension of **9** (0.329 g, 1 mmol) in absolute ethanol (10 mL), ethyl bromoacetate (0.18 g, 0.11 mL, 1 mmol) was added. The reaction mixture was heated under reflux for 9 h. Anhydrous sodium acetate (0.08 g, 1 mmol) was added and the reaction mixture was heated for further 30 min, cooled and poured into ice-cold water. The formed precipitate was filtered, washed with water dried and crystallized from DMF/EtOH (5:1).

Yield: 82%, mp: 238–40 °C. IR (KBr, $cm^{-1}$): 3334, 3171 (NH), 1729, 1678 (C=O), 1596 (C=N), 1250, 1040 (C–O–C), 1225, 1062 (C–S–C). $^1$H NMR ($\delta$ ppm): 1.1 (t, $J$ = 6.9 Hz, 3H, $CH_2\underline{CH_3}$), 2.75 (s, 3H, $CH_3$), 3.29 (s, 2H, $CH_2$–S), 4.04 (q, $J$ = 6.9 Hz, 2H, $\underline{CH_2}CH_3$), 5.33 (s, 2H, $CH_2$), 7.26–7.34 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.42 (d, $J$ = 7.8 Hz, 2H, phenyl–$C_{2,6}$–H), 7.78 (s, 2H, $NH_2$, $D_2O$ exchangeable). $^{13}$C NMR ($\delta$ ppm): 23.27 ($CH_2\underline{CH_3}$), 23.79 ($CH_3$), 49.81 ($CH_2$–benzyl), 54.58 (S–$CH_2$), 70.83 ($\underline{CH_2}CH_3$), 127.84 (benzyl–$C_4$), 136.99 (thienotriazolopyrimidine–$C_2$), 137.37 (benzyl–$C_3$ and $C_5$), 137.82 (benzyl–$C_2$ and $C_6$), 137.78 (benzyl–$C_1$), 145.28 (thienotriazolopyrimidine–$C_3$), 146.11 (thienotriazolopyrimidine–$C_{3e}$), 149.94 (thienotriazolopyrimidine–$C_{3a}$), 150.99 (thienotriazolopyrimidine–$C_8$), 158.85 (thienotriazolopyrimidine–$C_{2e}$), 165.45 (thienotriazolopyrimidine–$C_4$–C=O), 172.45 ($CONH_2$), 177.34 ($\underline{CO}OC_2H_5$). Anal. Calcd. for $C_{20}H_{19}N_5O_4S_2$ (457.53): C 52.50, H 4.19, N 15.31. Found: C 52.36, H 3.99, N 15.18.

*3.1.9. 5-Benzyl-3-methyl-4-oxo-8-(2-substituted phenyl)-4,5-dihydrothieno [3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide(**11a,b**)*

A mixture of **4** (0.329 g, 1 mmol) and the appropriate carboxylic acid (2 mmol) in phosphorous oxychloride (4 mL) was refluxed for 2 h and allowed to cool. The products were poured onto crushed ice while stirring, filtered, washed with sodium bicarbonate solution then with water, left to dry and crystallized from EtOH/$H_2O$ (5:1).

*3.1.9.1. 5-Benzyl-3-methyl-4-oxo-8-phenyl-4,5-dihydrothieno [3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**11a**).* Yield: 72%, mp: 232–4 °C. IR (KBr, $cm^{-1}$): 3438, 3243 (NH), 1687 (C=O), 1624 (C=N), 1235, 1075 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.65 (s, 3H, $CH_3$), 5.39 (s, 2H, $CH_2$), 7.28–7.37 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.5 (d, $J$ = 7.2 Hz, 2H, phenyl–$C_{2,6}$–H), 7.65 (d, $J$ = 7.2 Hz, 2H, 8-phenyl-$C_{2,6}$–H), 7.69 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.72–7.77 (m, 3H, 8-phenyl–$C_{3,4,5}$–H). Anal. Calcd. for $C_{22}H_{17}N_5O_2S\cdot 1/2H_2O$ (424.48): C 62.25, H 4.27, N 16.50. Found: C 62.05, H 3.99, N 16.38.

*3.1.9.2. 5-Benzyl-8-(2-chlorophenyl)-3-methyl-4-oxo-4,5-dihydrothieno[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**11b**).* Yield: 74%, mp: 114–6 °C. IR (KBr, $cm^{-1}$): 3439, 3238 (NH), 1685 (C=O), 1620 (C=N), 1237, 1075 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.65 (s, 3H, $CH_3$), 5.4 (s, 2H, $CH_2$), 7.3–7.39 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.52 (d, $J$ = 7.2 Hz, 2H, phenyl–$C_{2,6}$–H), 7.6–7.81 (m, 4H, chlorophenyl–$C_{3,4,5,6}$–H), 7.83 (s, 2H, $NH_2$, $D_2O$ exchangeable). Anal. Calcd. for $C_{22}H_{16}ClN_5O_2S\cdot 1/2H_2O$ (458.92): C 57.58, H 3.73, N 15.26. Found: C 57.31, H 3.34, N 15.15.

*3.1.10. 5-Benzyl-3-methyl-4-oxo-4,5-dihydrothieno[3,2-e][1,2,4] triazolo[4,3-a]pyrimidine-2-carboxamide (**12**)*

A solution of **4** (0.329 g, 1 mmol) in formic acid (5 mL) was heated under reflux for 2 h, then concentrated to small volume and diluted with ice-cold $H_2O$. The obtained precipitate was filtered, washed with $H_2O$, dried and crystallized from dioxane.

Yield: 69%, mp: 312–4 °C. IR (KBr, $cm^{-1}$): 3363, 3192 (NH), 1673 (C=O), 1600 (C=N), 1267, 1061 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.73 (s, 3H, $CH_3$), 5.33 (s, 2H, $CH_2$), 7.26–7.41 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.42 (d, $J$ = 7.5 Hz, 2H, phenyl–$C_{2,6}$–H), 7.75 (s, 2H, $NH_2$, $D_2O$ exchangeable), 9.31 (s, 1H, triazolo–$C_8$–H). Anal. Calcd. for $C_{16}H_{13}N_5O_2S$ (339.38): C 56.63, H 3.86, N 20.64. Found: C 56.43, H 3.59, N 20.39.

*3.1.11. 5-Benzyl-3-methyl-4,8-dioxo-4,5,7,8-tetrahydrothieno[3,2-e] [1,2,4]triazolo[4,3-a]pyrimidine-2-carboxamide (**13**)*

A suspension of **4** (0.329 g, 1 mmol), and ethyl chloroformate (0.165 g, 0.145 mL, 1.5 mmol) in dry dioxane (5 mL) was heated under reflux for 4 h, then cooled. The obtained precipitate was filtered, washed with ethanol, dried and crystallized from DMF.

Yield: 71%, mp: >300 °C. IR (KBr, $cm^{-1}$): 3362, 3231 (NH), 1674 (C=O), 1600 (C=N), 1267, 1061 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.73 (s, 3H, $CH_3$), 5.33 (s, 2H, $CH_2$), 7.26–7.34 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.42 (d, $J$ = 8.1 Hz, 2H, phenyl–$C_{2,6}$–H), 7.76 (s, 2H, $NH_2$, $D_2O$ exchangeable), 9.32 (s, 1H, triazolo NH, $D_2O$ exchangeable). $^{13}$C NMR ($\delta$ ppm): 24.02 ($CH_3$), 54.94 ($CH_2$–benzyl), 127.53 (benzyl–$C_4$), 136.89 (thienotriazolopyrimidine–$C_2$), 137.30 (benzyl–$C_3$ and $C_5$), 137.79 (benzyl–$C_2$ and $C_6$), 137.88 (benzyl–$C_1$), 145.46 (thienotriazolopyrimidine–$C_3$), 145.75 (thienotriazolopyrimidine–$C_{3e}$), 146.74 (thienotriazolopyrimidine–$C_{3a}$), 151.27 (thienotriazolopyrimidine–$C_{2e}$), 157.62 (thienotriazolopyrimidine–$C_8$–C=O), 165.61 (thienotriazolopyrimidine–$C_4$–C=O), 172.36 ($CONH_2$). Anal. Calcd. for $C_{16}H_{13}N_5O_3S$ (355.38): C 54.08, H 3.69, N 19.71. Found: C 53.94, H 3.66, N 19.43.

*3.1.12. Ethyl (5-benzyl-2-carbamoyl-3-methyl-4-oxo-4,5-dihydrothieno[3,2-e][1,2,4]triazolo[4,3-a]pyrimidin-8-yl)acetate (**14**)*

A mixture of the hydrazino **4** (0.329 g, 1 mmol) and diethyl malonate (0.32 g, 0.226 mL, 2 mmol) in gl. acetic acid (3 mL) was heated under reflux for 4 h, and then cooled. The obtained precipitate was filtered, washed with ethanol and crystallized from DMF.

Yield: 78%, mp: 314–6 °C. IR (KBr, $cm^{-1}$): 3344, 3138 (NH), 1742, 1682 (C=O), 1619 (C=N), 1250, 1050 (C–O–C), 1237, 1066 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.6 (t, $J$ = 6.9 Hz, 3H, $CH_2\underline{CH_3}$), 2.73 (s, 3H, $CH_3$), 2.89 (s, 2H, $CH_2$–CO), 3.55 (q, $J$ = 6.9 Hz, 2H, $\underline{CH_2}CH_3$), 5.3 (s, 2H, $CH_2$), 7.25–7.39 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.41 (d, $J$ = 7.8 Hz, 2H, phenyl–$C_{2,6}$–H), 7.76 (s, 2H, $NH_2$, $D_2O$ exchangeable). MS, $m/z$ (relative abundance %): 425 [$M^+$, (12.51)], 352 (42.92), 248 (12.54), 91 (100), 65 (17.17). Anal. Calcd. for $C_{20}H_{19}N_5O_4S$ (425.47): C 56.46, H 4.50, N 16.46. Found: C 56.32, H 4.22, N 16.25.

*3.1.13. 5-Benzyl-3-methyl-4-oxo-8-(substituted phenyl)-4,5-dihydro-9H-thieno[3′,2′:5,6]pyrimidino[2,1-c][1,2,4]triazine-2-carboxamide (**15a–c**)*

To a suspension of **4** (0.329 g, 1 mmol) in absolute ethanol (10 mL), 4-substituted phenacyl bromide (1 mmol) was added. The reaction mixture was heated under reflux for 8 h, then anhydrous sodium acetate (0.08 g, 1 mmol) was added and the reaction mixture was heated for further 30 min, cooled and poured into ice-cold water. The formed precipitate was filtered, washed with water and crystallized from $CHCl_3$/EtOH (5:1).

*3.1.13.1. 5-Benzyl-3-methyl-4-oxo-8-phenyl-4,5-dihydro-9H-thieno[3′,2′:5,6] pyrimidino[2,1-c][1,2,4]triazine-2-carboxamide (**15a**).* Yield: 69%, mp: 232–4 °C. IR (KBr, $cm^{-1}$): 3429, 3290 (NH), 1690, 1668 (C=O), 1616 (C=N), 1270, 1045 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.65 (s, 3H, $CH_3$), 5.16 (s, 2H, triazino–$C_9$–H), 5.34 (s, 2H, $CH_2$), 7.16–7.29 (m, 5H, phenyl–H), 7.47 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.56–7.79 (m, 5H, 8-phenyl–H). Anal. Calcd. for $C_{23}H_{19}N_5O_2S$ (429.5): C 64.32, H 4.46, N 16.31. Found: C 64.21, H 4.27, N 16.10.

*3.1.13.2. 5-Benzyl-8-(4-bromophenyl)-3-methyl-4-oxo-4,5-dihydro-9H-thieno[3′,2′:5,6]pyrimidino[2,1-c][1,2,4]triazine-2-carboxamide (**15b**).* Yield: 75%, mp: 223–5 °C. IR (KBr, $cm^{-1}$): 3350, 3210 (NH), 1680, 1670 (C=O), 1618 (C=N), 1275, 1035 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.67 (s, 3H, $CH_3$), 5.13 (s, 2H, triazino–$C_9$–H), 5.42 (s, 2H, $CH_2$), 7.18–7.36 (m, 5H, phenyl–H), 7.48 (d, $J$ = 8.4 Hz, 2H, Br–phenyl–$C_{2,6}$–H), 7.89 (s, 2H, $NH_2$, $D_2O$ exchangeable), 8.36 (d, $J$ = 8.7 Hz, 2H, Br–phenyl–$C_{3,5}$–H). Anal. Calcd. for $C_{23}H_{18}BrN_5O_2S$ (508.4): C 54.34, H 3.57, N 13.78. Found: C 54.01, H 3.44, N 13.38.

*3.1.13.3. 5-Benzyl-8-(4-chlorophenyl)-3-methyl-4-oxo-4,5-dihydro-9H-thieno[3′,2′:5,6]pyrimidino[2,1-c][1,2,4]triazine-2-carboxamide (**15c**).* Yield: 73%, mp: 244–6 °C. IR (KBr, $cm^{-1}$): 3434, 3310 (NH), 1692, 1664 (C=O), 1612 (C=N), 1266, 1033 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.66 (s, 3H, $CH_3$), 5.20 (s, 2H, triazino–$C_9$–H), 5.39 (s, 2H, $CH_2$), 7.14–7.3 (m, 5H, phenyl–H), 7.49 (d, $J$ = 8.7 Hz, 2H, Cl–phenyl–$C_{2,6}$–H), 7.87 (s, 2H, $NH_2$, $D_2O$ exchangeable), 8.3 (d, $J$ = 8.7 Hz, 2H, Cl–phenyl–$C_{3,5}$–H). Anal. Calcd. for $C_{23}H_{18}ClN_5O_2S$ (463.95): C 59.54, H 3.91, N 15.10. Found: C 59.24, H 3.65, N 14.97.

*3.1.14. General procedure for the synthesis of compounds **16–18***

A mixture of the hydrazino **4** (0.329 g, 1 mmol) and 4-substituted phenacylcyanide, acetylacetone or ethyl acetoacetate (1 mmol) in gl. acetic acid (5 mL) was refluxed for 12, 6 and 10 h respectively, and then cooled. The obtained precipitates were filtered, washed with ethanol and crystallized from DMF to give compounds **16a**,**b**, **17** or **18** respectively.

*4.1.14.1. 3-Benzyl-2-[5-amino-3-(4-bromophenyl)-1H-pyrazol-1-yl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxa-mide (**16a**).* Yield: 68%, mp: 256–8 °C. IR (KBr, $cm^{-1}$): 3332, 3165 (NH), 1678 (C=O), 1594 (C=N), 1260, 1033 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.67 (s, 3H, $CH_3$), 3.21 (s, 1H, pyrazolyl–$C_4$–H), 5.35 (s, 2H, $CH_2$), 7.26–7.38 (m, 5H, phenyl–H), 7.43 (d, $J$ = 8.7 Hz, 2H, bromophenyl–$C_{2,6}$–H), 7.78 (d, $J$ = 8.7 Hz, 2H, bromophenyl–$C_{3,5}$–H), 7.96 (s, 2H, $NH_2$, $D_2O$ exchangeable), 10.33 (s, 2H, pyrazolyl–$C_5$–$NH_2$, $D_2O$ exchangeable). Anal. Calcd. for $C_{24}H_{19}BrN_6O_2S$ (535.43): C 53.84, H 3.58, N 15.70. Found: C 53.64, H 3.39, N 15.35.

*3.1.14.2. 3-Benzyl-2-[5-amino-3-(4-chlorophenyl)-1H-pyrazol-1-yl]-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**16b**).* Yield: 65%, mp: 270–2 °C. IR (KBr, $cm^{-1}$): 3342, 3150 (NH), 1681 (C=O), 1596 (C=N), 1257, 1031 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.68 (s, 3H, $CH_3$), 2.88 (s, 1H, pyrazolyl–$C_4$–H), 5.25 (s, 2H, $CH_2$), 7.24–7.34 (m, 5H, phenyl–H), 7.41 (d, $J$ = 8.7 Hz, 2H, chlorophenyl–$C_{2,6}$–H), 7.68 (d, $J$ = 8.7 Hz, 2H, chlorophenyl–$C_{3,5}$–H), 7.77 (s, 2H, $NH_2$, $D_2O$ exchangeable), 10.31 (s, 2H, pyrazolyl–$C_5$–$NH_2$, $D_2O$ exchangeable). Anal. Calcd. for $C_{24}H_{19}ClN_6O_2S$ (490.98): C 58.71, H 3.90, N 17.12. Found: C 58.49, H 3.74, N 16.97.

*3.1.14.3. 3-Benzyl-2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**17**).* Yield: 77%, mp: >300 °C. IR (KBr, $cm^{-1}$): 3342, 3137 (NH), 1682 (C=O), 1617 (C=N), 1237, 1067 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.69 (s, 3H, $CH_3$), 2.76 (s, 6H, pyrazolyl–$CH_3$), 5.3 (s, 2H, $CH_2$), 7.25–7.33 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.39–7.42 (m, 3H, phenyl–$C_{2,6}$–H & pyrazolyl–$C_4$–H), 7.76 (s, 2H, $NH_2$, $D_2O$ exchangeable). $^{13}$C NMR ($\delta$ ppm): 21.06 ($CH_3$), 23.86 (pyrazole–$C_5$–$CH_3$), 49.80 (pyrazole–$C_3$–$CH_3$), 54.51 ($CH_2$–benzyl), 125.01 (pyrazole–$C_4$), 127.06 (benzyl–$C_4$), 133.11 (thienopyrimidine–$C_6$), 136.88 (benzyl–$C_3$ and $C_5$), 137.28 (benzyl–$C_2$ and $C_6$), 137.53 (benzyl–$C_1$), 137.77 (pyrazole–$C_5$), 145.48 (thienopyrimidine–$C_5$), 146.75 (thienopyrimidine–$C_{3d}$), 151.78 (pyrazole–$C_3$), 154.08 (thienopyrimidine–$C_2$), 157.74 (thienopyrimidine–$C_{2d}$), 165.50 (thienopyrimidine–$C_4$–C=O), 172.29 ($CONH_2$). MS, $m/z$ (relative abundance %): 393 [$M^+$, (7.15)], 353 (96.39), 345 (24.03), 315 (100), 299 (20.21), 182 (20.64), 147 (24.12), 130 (15.11), 60 (20.64), 57 (33.37). Anal. Calcd. for $C_{20}H_{19}N_5O_2S$ (393.47): C 61.05, H 4.87, N 17.80. Found: C 60.94, H 4.56, N 17.60.

*3.1.14.4. 3-Benzyl-5-methyl-2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide (**18**).* Yield: 72%, mp: 292–4 °C. IR (KBr, $cm^{-1}$): 3343, 3141 (NH), 1681 (C=O), 1597 (C=N), 1236, 1067 (C–S–C). $^1$H NMR ($\delta$ ppm): 2.63 (s, 2H, pyrazolyl–$C_4$–H), 2.68 (s, 3H, $CH_3$), 2.75 (s, 3H, pyrazolyl–$CH_3$), 5.3 (s, 2H, $CH_2$), 7.23–7.33 (m, 3H, phenyl–$C_{3,4,5}$–H), 7.39–7.42 (d, $J$ = 6.6 Hz, 2H, phenyl–$C_{2,6}$–H), 7.76 (s, 2H, $NH_2$, $D_2O$ exchangeable). Anal. Calcd. for $C_{19}H_{17}N_5O_3S$ (395.44): C 57.71, H 4.33, N 17.71. Found: C 57.35, H 4.01, N 17.41.

### 3.2. Anti-inflammatory (AI) activity

#### 3.2.1. Formalin-induced paw edema bioassay (acute inflammatory model)

Male albino rats weighing 180–200 g were used throughout the assay. They were kept in the animal house under standard condition of light and temperature with free access to food and water. The animals were randomly divided into groups each of five rats. One group of five rats was kept as a control and another group received the standard drug diclofenac Na (at a dose of 10 mg/kg body weight, po). A solution of formalin (2%, 0.1 mL) was injected into the subplanter region of the left hind paw under light ether anesthesia 1 h after oral administration (po) of the test compound (at a dose level of 10 mg/kg body weight). The paw volume (mL) was measured by means of water plethysmometer and re-measured again 1, 2 and 4 h after administration of formalin. The edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition for each rat and each group was obtained as follows:

$$\%\ \text{Inhibition} = (V_t - V_0)\ \text{control} - (V_t - V_0)\ \text{tested compound}/(V_t - V_0)\ \text{control} \times 100$$

where $V_t$ = volume of edema at specific time interval and $V_0$ = volume of edema at zero time interval.

#### 3.2.2. Formalin-induced paw edema bioassay (sub-acute inflammatory model)

Rats in the first experiment were given the same test compounds at a dose level of 10 mg/kg body weight daily for 7 consecutive days. A solution of formalin (2%, 0.1 mL) was injected into the subplanter region of the left hind paw under light ether anesthesia 1 h after oral administration (po) of the test compound. A second injection of formalin (2%, 0.1 mL) was given on the third day. The changes in the volume of paw were measured plethymographically at the first and eighth days.

#### 3.2.3. Turpentine oil-induced granuloma pouch bioassay (sub-acute inflammatory model)

Male albino rats weighing 120–150 g were used throughout this assay. They were kept in the animal house under standard condition of light and temperature with free access to food and water. One group of five rats was kept as a control and another group received the standard drug diclofenac Na (at a dose of 10 mg/kg body weight, po). Subcutaneous dorsal granuloma pouch was made in ether-anesthetized rats by injecting 2 mL of air, followed by injection of 0.5 mL of turpentine oil into it. All of the test compounds were administered orally (at a dose level of 10 mg/kg body weight) 1 h prior to turpentine oil injection and continued for seven consecutive days. On the eighth day, the paw was opened under anesthesia and the exudates were taken out with a syringe. The volume (mL) of the exudates was measured and the percentage inhibition of inflammation relative to the reference drug (diclofenac Na) was determined as follows:

$$\%\ \text{Inhibition} = V\ \text{control} - V\ \text{treated}/V\ \text{control} \times 100$$

#### 3.2.4. Ulcerogenic activity

Male albino rats (180–200 g) were divided into groups each of five animals and were fasted for 12 h prior to the administration of the test compounds. Water was given ad libitum. Control group received 1% gum acacia orally.

Other groups received diclofenac Na or the test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 300 mg/kg per day. Animals were sacrificed by diethyl ether 6 h after the last dose and their stomachs were removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cold saline and inspected with a 3X magnifying lens for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosion or ulcer.

#### 3.2.5. Acute toxicity

Twelve groups of rats (180–200 g) each consists of five animals, were used in this test. The animals were fasted for 24 h prior to administration of the test compounds. The compounds were given orally in graded doses of 0.1–0.3 g/kg body weight, po. The compounds were screened at graded doses for their acute lethal doses ($ALD_{50}$) and the mortalities were recorded at each dose level after 24 h.

#### 3.2.6. Determination of effective dose 50 ($ED_{50}$)

The selected compounds were further tested at 5, 10, 20, 40, and 50 mg/kg body weight and the $ED_{50}$ was determined by measuring the inhibition of the edema volume 2 h after formalin injection.

#### 3.2.7. Statistical analysis

The data obtained are presented as means ± SE of the mean. The concentration-dependent effects of various drugs in vitro were evaluated statistically by the randomized block design analysis of Variance (ANOVA) followed by 'Student–Newman–Keuls Multiple Comparison Test. The difference in results was considered significant when $P < 0.05$.

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