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Original article

# Synthesis, biological and medicinal significance of *S*-glycosido-thieno[2,3-*d*]-pyrimidines as new anti-inflammatory and analgesic agents

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ABSTRACT

Several 2-thioglycosides were prepared. Glycosylation of 2-thioxo-thieno[2,3-*d*]-pyrimidines **5a**,**b** with 1-bromo-2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyle **7**, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl and galacto-pyranosyl bromide **8a**,**b** gave the protected β-D-nuclosides **10a**,**b** and **13a**–**d** in high yields, which were transformed to deacetylated derivatives **14a**,**b** and **15a**–**d**. The structures of the compounds were elucidated by spectral and elemental analysis. Anti-inflammatory and Analgesic activities screening of the new compounds (at a dose of 100 mg/kg body weight) utilizing in vivo acute carrageenan-induced paw oedema standard method exhibited that the deacetylated derivatives **14a**,**b** and **15a**–**d** possess highly promising activities.



## 1. Introduction

Rheumatic diseases are the most prevalent causes of disability in Western countries, and non-steroidal anti-inflammatory drugs (NSAIDs) are still the most commonly used remedies. NSAIDs cause several serious adverse effects; the most important one is gastric injury that might later cause gastric ulceration and renal injury [1]. Attempts to develop non-steroidal anti-inflammatory drugs that are devoid of classical NSAID toxicity, especially gastrointestinal injury, follow several strategies. One of which is selective cyclo-oxygenase-2 inhibition (COX-2) [2,3]. Although agents that inhibit COX-2 while sparing COX-1 represented a new attractive therapeutic development, they also gave rise to some of the side effects seen with traditional dual COX inhibitors (NSAIDs), namely, effects on the kidney that might manifest as an increased incidence of hypertension, edema and associated clinical states [4–6]. Therefore, selective COX-2 inhibitors may not be the proper strategy to overcome the damaging effects of conventional NSAIDs which are used for the chronic inflammatory diseases in a long term basis. The other strategy is the inhibition of inducible nitric oxide synthase (iNOS), which contributes to acute and chronic inflammation [7–9].

In addition, it is known that bacterial infections often produce pain and inflammation. In normal practice, two groups of agents (chemotherapeutic, analgesic and anti-inflammatory) are prescribed simultaneously. Unfortunately, none of the drugs possesses these three activities in a single component. Therefore, our aim is to find a compound having dual effect analgesic, anti-inflammatory and anti-microbial activities. While searching for such a compound, we have found that 2-thioxo-thieno[2,3-*d*]-pyrimidine ring is one of the moieties on which studies have been concentrated. In our laboratory, we have designed and synthesized some 2-thioxo-thieno[2,3-*d*]pyrimidine and pyridopyrimidine derivatives in the search for new non-steroidal anti-inflammatory agents [10–23]. A considerable number of the prepared compounds have been found to have analgesic-anti-inflammatory activity comparable to or higher than that of indomethacin. 2-thioxopyrimidines is also a isostere of purines and promizole whose anti-microbial activities have been extensively investigated and performed [24,25]. All of these have made us think that pyrimidines are promising compounds for finding a drug which has analgesic, anti-inflammatory and anti-microbialaction.

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of

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nucleic acids to their current use in the chemotherapy of AIDS. Recently, pyrimidines derivatives have generated wide-spread interest due to their antiviral properties (Fig. 1); 5-Iododeoxyuridine (**A**) is an antiviral agent of high selectivity. 5-Trifluromethyl-2′-deoxyuridine (**B**) has been found useful against infections resistant to IDU therapy. Ara-A 9-$\beta$-D-arabinofuranosyl adenine (**C**), a relatively new antiviral drug, is effective against herpes infections of eye, brain and skin. It is especially effective against IDU-resistant herpes virus [26]. Also, Retrovir (AZT-16, **D**) is a potent inhibitor of the in vivo replication and cytopathic effects of HIV and has been recently approved for use against AIDS and severe ARC [27].

There are a large number of pyrimidine-based antimetabolites. They are usually structurally related to the endogenous substrates that they antagonize. The structural modification may be on the pyrimidine ring or on the pendant sugar groups. One of the early metabolites prepared was 5-fluorouracil [28]. Other active pyrimidines and thieno[2,3-*d*]pyrimidines nucleosides have recently been synthesized as antiviral against HIV-1 and Herpes simplex Virus (HSV-1) [29–31]. However, thioglycosides of thieno[2,3-*d*]-pyrimidine are not known in the literature.

## 2. Results and discussion

### 2.1. Chemistry

On the basis of the above considerations, original nucleoside analogs directed upon reverse transcriptase still aroused considerable interest [32]. In the hope of elucidating and/or finding better therapeutic agents, S-glycosido-thieno[2,3-*d*]pyrimidine analogs seem to be one of the recommended targets and extension of our work on pyrimidines [33], *C*-nucleosides [13,22] and thieno[2,3-*d*]pyrimidines [10–12]. Also, due to the data of S-glycosides are relatively few, all this promoted us to research and developed in the synthesis of these types of nucleosides.

With respect to the previous studies carried out in our laboratory, 5-acetyl and/or 5-ethylcarboxylate-3-ethyl-(2-amino-4-methylthiophene)carboxylate precursors [34] have been regarded as promising intermediates to produce 2-thioxo-thieno[2,3-*d*]pyrimidine with some structural analogies with natural nucleobases. The interaction of acetylacetone with ethyl cyanoacetate and sulfur metal in absolute ethanol in the presence of diethylamine led to ethyl thiophene-3-carboxylate **1b**. The hydrazide **3b** obtained by refluxing of ethylcarboxylate **1b** with hydrazine hydrate in ethanol. 2-Thioxo-thieno[2,3-*d*]pyrimidin-4-ones (**5a**,**b**) were produced by two way. The first: action of carbon disulfide on hydrazide **3a**,**b** and the second: action of dimethylsulfate and carbon disulfide on 5-acetyl and/or 5-ethylcarboxylate-3-ethyl(2-amino-4-methylthiophene)carboxylate in dimethylsulfoxide followed by hydrazine hydrate (99%) as shown in Scheme 1.

Compounds **5a**,**b** was found to be useful for the syntheses of the interesting S-glycosides. As a model experiment the alkylation of **5a** was carried out by the reaction of one equivalent of methyliodide with the potassium salt **6a** generated in situ by the reaction of **5a** with alcoholic potassium hydroxide. The structure of the new 2-methylthio-quinazoline **7** was confirmed by all spectroscopic data.

The $^{13}$C NMR spectrum as an example, reveled that the corresponding signal of the C-2 (C–$SCH_3$) appeared at $\delta$ 159 ppm. The chemical shifts in the $^{13}$C NMR spectrum of the 2-thioxo- (**5a**) and 2-methylthio-thieno[2,3-*d*]pyrimidine (**7**) indicated that the site of the alkylation is the sulfur atom rather than the nitrogen atom (Scheme 2, Table 1).

In order to compare $^{13}$C NMR data, $^{13}$C chemical shifts of new compounds **5a**, **7** and **13a** together with those previously reported for compounds **5c** and **5d** are shown in Scheme 2. The NMR study of compound **5a** was carried out in DMSO-$d_6$ solution because this compound is very insoluble in $CDCl_3$. $^{13}$C chemical shifts of compound **5a** were assigned by comparing the chemical shifts of the corresponding 2-thioxo, 2-oxo-derivatives **5c** and **5d**. On the other hand, the correct assignment for C-2 in compound **7** and **13a** was checked by comparison with the corresponding chemical shifts of related derivatives **A** and **B** previously reported [35,36], (see Scheme 2).

As can be seen in Table 1 and Scheme 2, the chemical shift of C-2 is shifted downfield about 25 ppm by changing an oxo group (compounds **5c**) by a thioxo group (compounds **5d** and **5a** about 174 ppm). Comparing $^{13}$C NMR spectra of unsubstituted derivative **5a** and S-derivative **7** (recorded both in DMSO-$d_6$), it is interesting to point out whilst the C-2 signal appears shifted 25 ppm to up-field in **7**, as expected for the change of an N–C=S to N=C–S-group. NMR spectra of soluble compounds in $CDCl_3$ **13a**–**d** were recorded in this solvent. In order to compare $^{13}$C chemical shifts of compounds **7** and **13a**, the $^{13}$C NMR spectrum of **13a** was also recorded in DMSO-$d_6$ showing both spectra only slight or no chemical shift differences. One possible explanation, of this anomalous fact, could be the existence of compound **13a** mainly in the form S-glycosides (see Scheme 3).

The synthetic route we used for the preparation of 2-*S*-($\beta$-D-glycopyranosyl/or furanosyl)-thieno[2,3-*d*]pyrimidine is outlined in Scheme 3. The heterocycle thieno[2,3-*d*]pyrimidines **5a**,**b** was converted into its potassium salt **6a**,**b** with used of KOH in acetone and was stirred at room temperature for long time with 2,3,4,6-tetra-*O*-acetyl-$\alpha$-D-glucopyranosyl bromide (**8b**) or 2,3,4,6-tetra-*O*-acetyl-$\alpha$-D-galacto-pyranosyl bromide (**8c**), afforded the *S*-glycosylated nucleosides **13a**–**d** in good yields (68–78%). Thin layer chromatography (chloroform:methanol, 8:2) indicated the formation of the pure compounds. The structures of the S-glycosides were confirmed by elemental analysis and spectral data (IR, $^1$H NMR, $^{13}$C NMR). The $^1$H NMR spectrum of compound **13a** as an example, showed the

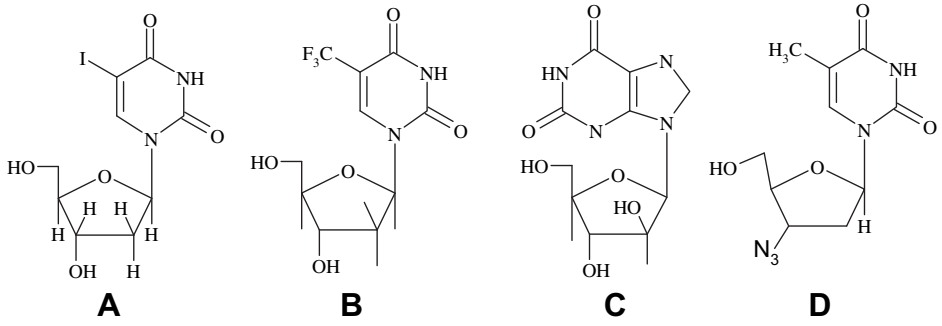
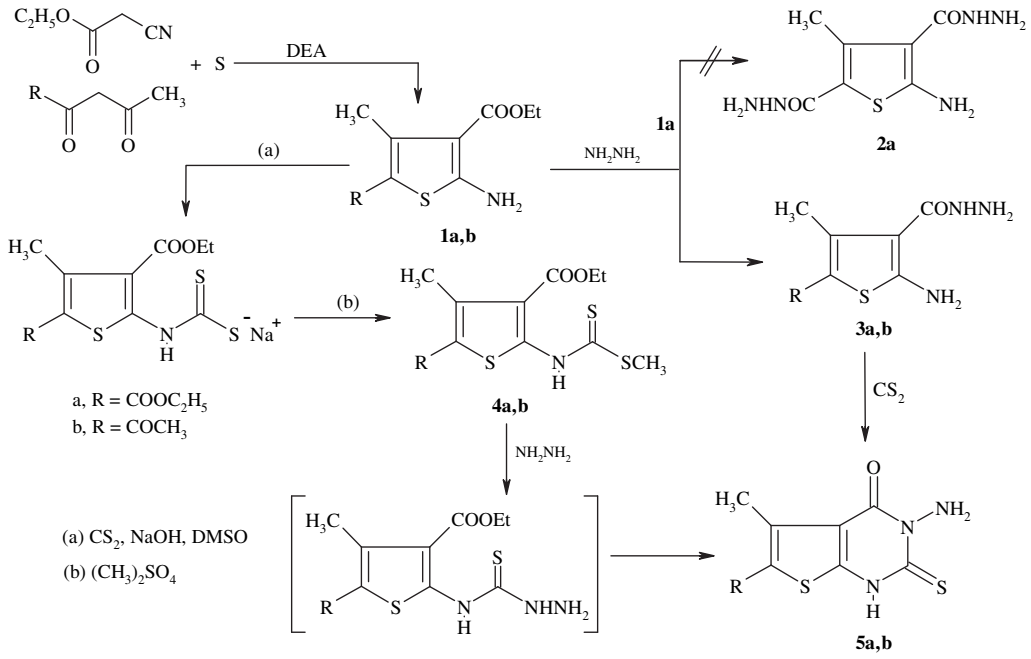
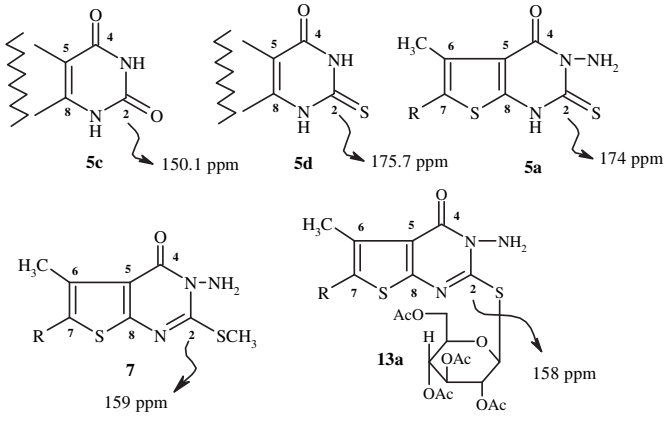



**Fig. 1.**




**Scheme 1.** Synthetic route of thioxo-thieno[2,3-d]pyrimidines

anomeric proton of the glucose moiety as a doublet at $\delta$ 5.69 ppm with a coupling constant $J = 10.68$ Hz indicating $\beta$-configuration of the anomeric center. The other protons of the gluco-pyranose ring resonated at $\delta$ 3.95–5.10 ppm, while the four acetoxy groups appeared as four singles at $\delta$ 1.90, 2.00, 2.05 and 2.10 ppm. The $^{13}$C NMR revealed the absence of the thione carbon atom at about 174 ppm and a resonance of –N=C–N– carbon atom (C-2) at $\delta$ 158 ppm was indicated to the chemical shift of the corresponding carbon atom (Scheme 3). The signals at $\delta$ 166.5, 169.2, 169.3, 169.5 ppm are due to the four acetoxy carbonyl atoms (4C=O), and the four signals around $\delta$ 20.29–20.36 ppm are assigned to the acetate methyl carbon atoms. Also, the six signals at $\delta$ 67.78, 69.82, 72.54, 75.01, 81.94 and 98.68 ppm were assigned to C-5′, C-6′, C-4′, C-2′, C-3′ and C-1′, respectively. Moreover, the IR spectra of compounds **13** revealed the absence of the stretching signal of a thione group. Similarly, the reaction of heterocycle base **5a**,**b** with 1-bromo-2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyle (**8a**) furnished the *S*-glycosated product **10a**,**b**. The structures assignment of this product is based on their elemental analysis and the spectral data (see Experimental).




**Scheme 2.** C-2 Chemical shifts of 2-thioxopyrimidines **5a**, **7** and **13a**.

In addition to the above synthetic methods a so called "one-pot" protocol of the silyl-Hilbert-Johnson reaction was described [37]. This 'one-pot' protocol was used by Wolfe for the synthesis of β-D-ribo-nucleosides [38]. In this procedure, the nucleobase was silyated with HDMS in presence of $(NH_4)_2SO_4$ in MeCN and glycosylated with 1,2,3,4,6-penta-*O*-acetyl-α-D-glyco-pyranose in presence of $SnCl_4$. Here, MeCN was used as a solvent and HMDS (Hexamethyldisilane) as glycosylation catalyst (Vorbrüggen conditions). The nucleobases were silyated and directly glycosylated in one step. Townsend applied this procedure for the synthesis of toyocamycin [39]. We used the 'one-pot' method for the synthesisd of the 2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glycopyranosylthio)-thieno[2,3-*d*]pyrimidin-4-one analogs **13a**,**b**. The nucleobases **5a**,**b** were silylated with HMDS in anhydrous MeCN at room temperature in presence of ammonium sulfate and then reacted with 1,2,3,4,6-penta-*O*-acetyl-α-D-glyco-pyranose (Scheme 3). This afforded the glycosylated compounds **13a**,**b** (in 54% yields) after working up.

Deacetylation of *S*-nucleosides **10a**,**b** and **13a**–**d** proceeded smoothly via methanolic ammonia treatment to afford the free nucleosides **14a**,**b** and **15a**–**d** in good to excellent yields (Scheme 3). The $^1$H NMR data of the compounds **14** and **15** revealed the absence of the acetyl protons and appearance of the $D_2O$ exchangeable OH protons at $\delta$ 3.10–3.50. The IR data of the compound **14a** as a typical example showed also the absence of the acetyl function and the appearance of the characteristic OH's band at 3360 (br) cm$^{-1}$.

## 2.2. Pharmacology

Analgesic activities of the resulting compounds were investigated by *p*-benzoquinone-induced writhing test [40], which is a well established method of testing the analgesic activity of compounds and sufficiently sensitive to detect the effect of analgesics which are less active than aspirin. Anti-inflammatory activities of the compounds were assessed by utilizing carrageenan-induced hind paw edema model [41]. Since the carrageenan edema has been used in the development of indomethacin, many researchers adapted this procedure for screening potential anti-inflammatory compounds.

**Table 1**
$^{13}$C NMR chemical shifts of the bases and S-Glycosides.

| Compd. | $CH_3$ | $OCH_2$ | CO(Es/Ac) | CO(4) | C-2 | C-5 | C-6 | C-7 | C-8 | C(1′) | C(2′) | C(3′) | C(4′) | C(5′) | C(6′) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **5a**[a] | 14.83, 17.07 | 59.83 | 165.6 | 162.2 | 174.5 | 113.4 | 120.4 | 139.3 | 153.5 | – | – | – | – | – | – |
| **5b**[a] | 19.89, 20.78 | – | 190.2 | 165.7 | 175.3 | 118.9 | 121.7 | 141.3 | 149.8 | – | – | – | – | – | – |
| **7**[a] | 13.98, 20.07 | 60.26 | 189.6 | 167.2 | 159.0 | 123.6 | 128.5 | 146.7 | 152.6 | – | – | – | – | – | – |
| **10a**[a] | 14.69, 14.86, 16.62, 16.79, 20.56 | 60.01 | 167.7, 168.2, 169.4, 169.9 | 164.2 | 158.9 | 105.5 | 124.1 | 142.9 | 156.3 | 83.68 | 68.40 | 68.09 | 67.62 | 67.48 | – |
| **10b**[b] | 17.67, 18.94, 21.02, 25.40 | – | 168.1, 168.4, 169.3, 169.9 | 163.7 | 158.2 | 116.5 | 133.2 | 140.6 | 155.9 | 88.45 | 77.14 | 81.45 | 76.88 | 67.35 | – |
| **13a**[a] | 14.31, 16.26, 20.29, 20.32, 20.35, 20.36 | 59.62 | 166.5, 169.2, 169.3, 169.5, 169.9 | 164.5 | 160.1 | 105.8 | 122.4 | 142.8 | 156.9 | 98.68 | 75.01 | 81.94 | 72.54 | 67.78 | 69.82 |
| **13b**[a] | 14.62, 16.76, 20.26, 20.28, 20.30, 20.33 | 59.68 | 166.7,168.9, 169.2, 169.7, 170.3 | 163.2 | 159.3 | 109.8 | 124.8 | 141.5 | 156.5 | 92.50 | 73.45 | 80.78 | 70.89 | 66.57 | 69.36 |
| **13c**[b] | 17.61, 18.87, 20.66, 2075, 20.84, 25.36 | – | 166.3, 168.5, 169.6, 169.8, 170.7 | 163.8 | 159.6 | 117.6 | 125.4 | 143.6 | 157.9 | 87.66 | 77.11 | 81.38 | 74.25 | 68.33 | 68.79 |
| **13d**[b] | 17.62, 18.68, 20.64, 20.75, 20.85 | – | 165.7, 166.5, 168.4, 169.2, 170.3 | 162.5 | 159.3 | 115.7 | 123.6 | 142.9 | 156.5 | 90.06 | 77.38 | 81.95 | 74.90 | 66.34 | 67.87 |
| **14a**[a] | 13.90, 19.32 | 60.73 | 167.5 | 165.3 | 157.4 | 118.7 | 126.3 | 140.8 | 154.8 | 85.79 | 69.48 | 67.29 | 66.55 | 68.21 | – |
| **14b**[a] | 20.21, 21.51 | – | 185.9 | 164.8 | 158.6 | 120.4 | 126.9 | 141.6 | 156.2 | 84.41 | 68.37 | 68.43 | 67.08 | 67.79 | – |
| **15a**[c] | 14.00, 20.73 | 61.02 | 166.8 | 162.5 | 159.8 | 117.9 | 125.9 | 143.0 | 153.8 | 85.64 | 73.44 | 77.27 | 70.50 | 77.63 | 61.53 |
| **15b**[c] | 14.08, 19.68 | 60.91 | 168.3 | 167.5 | 158.4 | 118.6 | 128.2 | 142.6 | 155.0 | 87.36 | 75.54 | 76.93 | 71.38 | 77.87 | 62.08 |
| **15c**[c] | 20.34, 21.96 | – | 188.4 | 163.3 | 157.9 | 119.0 | 127.5 | 141.9 | 153.9 | 86.76 | 74.29 | 77.35 | 70.89 | 78.20 | 61.75 |
| **15d**[c] | 20.27, 22.06 | – | 186.0 | 162.9 | 156.9 | 116.9 | 126.8 | 145.3 | 156.1 | 89.78 | 74.12 | 78.03 | 70.58 | 77.47 | 60.59 |

Es, CO-Ester; Ac, CO-Acetyl.
[a] Measured in DMSO-$d_6$.
[b] Measured in $CDCl_3$.
[c] Measured in DMSO-$d_6$+$D_2O$.

Carrageenan-induced edema is a non-specific inflammation maintained by the release of histamine, 5-hydroxytryptamine, kinins and later by prostaglandins [42]. The inhibitory effect of acid NSAIDs, such as indomethacin, is usually weak in the first phase (1–2 h), in contrast with their strong inhibition in the second phase (3–4 h) [43]. Good inhibition of the second phase of carrageenan-induced edema was observed for the compounds tested, suggesting that they interfere with prostaglandin synthesis (Table 2).

The obtained pharmacological results indicate that among the compounds, the derivatives **5a**,**b**, which did not carry the S-glycosides moiety on the second position, was found weaker than that bearing S-glycosides residue on this position and some of the title compounds possess a good analgesic activity coupled with notable anti-inflammatory properties. Moreover, all compounds showed a remarkable gastric tolerance except **13d**. Some preliminary conclusions can be drawn as follows: As shown in Table 2, most compounds showed potent inhibitory activities between 31.2 and 53.3% on 100 mg/kg dose. When substituents were taken into consideration within them, it was determined that substituted 3-aminothieno[2,3-*d*]pyrimidines bearing S-glycosides (**14a**,**b** and **15a**–**d**) had a stronger inhibitory effect on analgesic, anti-inflammatory activity than that of the acetylated-S-glycosides derivatives (**10a**,**b** and **13a**–**d**). According to this, we suggested the S-glycoside with free hydroxyl groups was an important structural characteristic of the analgesic-anti-inflammatory action of these derivatives. Then the effect of the substitutes on the 3-aminothieno[2,3-*d*]pyrimidines was investigated. It was clear that compounds bearing 2-(β-D-ribofuranosylthio) residues (**14a**, **14b**) displayed the best activity. By contrast, 2-(β-D-glucopyranosyl-thio)-/or 2-(β-D-galactopyranosyl-thio)- (**15a**–**d**) analogs were less active, which can be hypothesized that the larger subsistent causes decreasing of the activity for this series may be detrimental to the overall activity. Among the compounds It appeared that presence of an ethyl-carboxylate substituent in position-6 of the 3-aminothieno[2,3-*d*]-pyrimidine nucleus led to more active compounds (**14a**, **15a**,**b**) compared with acetyl substituted derivatives in the same position of 3-aminothieno[2,3-*d*]pyrimidine (**14b**, **15c**,**d**). A quiet similar pattern of anti-inflammatory activity was observed with that of analgesic activity. Although not significant, inhibitory ratios for all compounds were above 30% for the last two measurements. However, the inhibitory effects of **14a** and **14b** reached to significant values after 360 min. Among the compounds examined in this study, the compounds **14a**,**b**, **15a**–**c** possessed the most prominent and consistent activity. Compounds **14a**,**b**, **15a**,**c** deserve attention and may be considered for further evaluation.

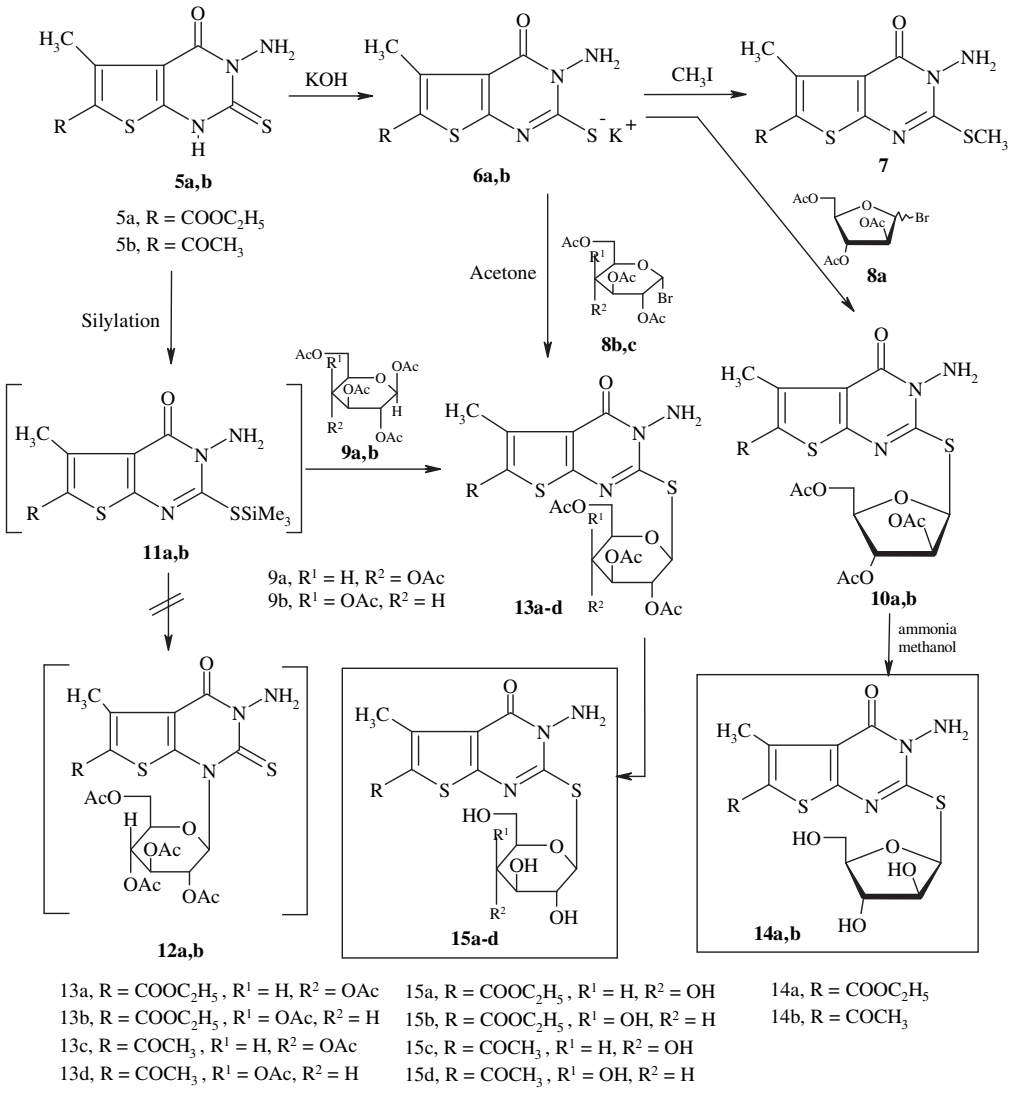
## 3. Exprimental

### 3.1. Chemistry

Melting points were determined on the Electrothermal 9100 melting point apparatus (Electrothermal, UK) and are uncorrected. The IR spectra (KBr) were recorded on an FT-IR NEXCES spectrophotometer (Shimadzu, Japan). The $^1$H NMR spectra were measured with a Jeol ECA 500 MHz (Japan) in DMSO-$d_6$ or $CDCl_3$ and chemical shifts were recorded in δ ppm relative to TMS. Mass spectra (EI) were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA). The purity of the compounds was checked on Aluminium plates coated with silica gel (Merck). The Pharmacological evaluations of the products were carried out in Pharmacological Unit Pharmacology Department (NRC, Cairo, Egypt). The starting compounds **1a**, **3a**, **5a** were prepared according to previously reported procedures [34,44].

#### 3.1.1. 5-Acetyl-3-ethyl(2-amino-4-methylthiophene) carboxylate (*1b*)

According to Gewald et al. [44] and the developed method, a mixture of acetylacetone (10 mmol), ethylcyano-acetate (10 mmol), sulfur (10 mmol) and diethylamine (10 mmol) was heated (70 °C) under stirring in absolute ethanol for 4 h, then leave the mixture for 24 h at 0 °C. The formed solid was collected by filtration, washed with ethanol (20 mL), dried and crystallized from absolute ethanol, as yellow crystals in a 88% yield, m.p. 160–162 °C; IR ($cm^{-1}$, ν); 3424 (br, $NH_2$), 2937 (CH alkyl), 1745, 1718 (2CO); $^1$H NMR (DMSO-$d_6$, δ, ppm): 1.37 (t, 3H, $J$ = 7.0 Hz, $CH_3$), 2.42 (s, 3H, $CH_3$), 2.69 (s, 3H, $CH_3$), 4.32 (q, 2H, $J$ = 7.0 Hz, $OCH_2$) 6.79 (br, 2H, $NH_2$, $D_2O$ exchangeable); $^{13}$C NMR: δ 14.35 ($CH_3$), 16.88 ($CH_3$), 30.19 ($CH_3$), 60.25 ($CH_2$), 109.15, 120.78, 146.58, 166.87 (4*C* of thiophene ring), 190.23 (*C*O), 203.41 (*C*O); Its MS (*m*/*z*), 227 ($M^+$, 100);




**Scheme 3.** Synthetic route of acetylated-S-glycoside and S-glycosides of thieno[2,3-d]pyrimidines.

**Table 2**
Percent analgesic activity and inhibition of carrageenan paw edema (CPE) of the compounds **5a**,**b** and **10–15**.

| Anti-inflammatory Activity | | | | | | |
|---|---|---|---|---|---|---|
| Comp. No | Swelling in thickness ($\times 10^{-2}$ mm) ± SEM (% inhibition) | | | | Analgesic Activity | Ratio of ulceration |
| | 90 min | 180 min | 270 min | 360 min | Number of writhin ± SEM (% Inhibition) | |
| Control | 41.7 ± 4.13 | 47.7 ± 4.36 | 54.2 ± 4.95 | 59.0 ± 5.31 | 44.3 ± 3.84 | 0/6 |
| **5a** | 41.2 ± 4.03 (1.2) | 44.8 ± 4.31 (6.1) | 49.7 ± 4.84 (8.3) | 54.0 ± 4.80 (8.5) | 33.7 ± 1.94 (23.9**) | 0/6 |
| **5b** | 44.8 ± 4.31 (6.1) | 44.0 ± 4.39 (7.8) | 49.7 ± 4.84 (8.3) | 54.0 ± 4.80 (8.5) | 28.5 ± 2.41 (35.7**) | 0/6 |
| **10a** | 28.3 ± 2.06 (32.1) | 30.7 ± 2.51 (35.6)* | 34.7 ± 2.14 (35.9)** | 36.0 ± 2.98 (38.9)** | 20.7 ± 1.33 (53.3***) | 0/6 |
| **10b** | 29.8 ± 2.14 (28.5) | 33.5 ± 1.98 (29.8) | 38.7 ± 1.89 (28.6)* | 38.0 ± 1.79 (35.6)** | 22.7 ± 2.52 (48.8***) | 0/6 |
| **13a** | 36.2 ± 2.69 (13.2) | 40.7 ± 2.64 (14.7) | 43.2 ± 2.77 (20.3) | 45.8 ± 2.09 (22.3) | 27.5 ± 2.75 (37.9*) | 1/6 |
| **13b** | 30.5 ± 2.64 (26.9) | 35.0 ± 2.81 (26.6) | 37.7 ± 2.63 (30.4)* | 40.5 ± 2.80 (31.4)* | 25.2 ± 2.10 (43.1***) | 0/6 |
| **13c** | 31.0 ± 3.13 (25.7) | 36.3 ± 3.15 (23.9) | 38.0 ± 2.5 (29.9)* | 38.8 ± 1.70 (34.2)** | 24.2 ± 1.56 (45.4***) | 0/6 |
| **13d** | 41.2 ± 3.54 (14.9) | 44.0 ± 4.39 (7.8) | 48.5 ± 4.29 (10.5) | 51.3 ± 4.14 (13.1) | 38.2 ± 2.95 (13.8) | 2/6 |
| **14a–3a** | 28.3 ± 2.06 (32.1) | 30.7 ± 2.51 (35.6)* | 34.7 ± 2.14 (35.9)** | 36.0 ± 2.98 (38.9)** | 20.7 ± 1.33 (53.3***) | 0/6 |
| **14b–3b** | 29.8 ± 2.14 (28.5) | 33.5 ± 1.98 (29.8) | 38.7 ± 1.89 (28.6)* | 38.0 ± 1.79 (35.6)** | 22.7 ± 2.52 (48.8***) | 0/6 |
| **15a–3c** | 29.3 ± 2.91 (29.7) | 32.8 ± 3.06 (31.2) | 35.8 ± 2.04 (33.9)* | 38.7 ± 2.42 (34.4)** | 21.7 ± 2.03 (51.0***) | 1/6 |
| **15b–3f** | 30.5 ± 2.64 (26.9) | 35.0 ± 2.81 (26.6) | 37.7 ± 2.63 (30.4)* | 40.5 ± 2.80 (31.4)* | 25.2 ± 2.10 (43.1***) | 0/6 |
| **15c–3d** | 31.0 ± 3.13 (25.7) | 36.3 ± 3.15 (23.9) | 38.0 ± 2.5 (29.9)* | 38.8 ± 1.70 (34.2)** | 24.2 ± 1.56 (45.4***) | 0/6 |
| **15d–3g** | 35.7 ± 2.80 (14.4) | 39.8 ± 2.70 (16.6) | 44.7 ± 2.94 (17.5) | 46.8 ± 3.46 (20.7) | 28.5 ± 2.41 (35.7**) | 0/6 |
| ASA | – | – | – | – | 21.3 ± 1.80 (51.9***) | 2/6 |
| INDO | 30.7 ± 4.18 (29.5)* | 33.0 ± 3.38 (30.6)* | 35.3 ± 3.31 (41.9)** | 34.2 ± 3.11 (42.7)*** | – | – |

Aspirin (ASA); Indomethacin (INDO); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

$C_{10}H_{13}NO_3S$ (227.2); Requires (Found): C, 52.84 (52.81); H, 5.76 (5.75); N, 6.16 (6.14).

*3.1.2. 5-Acetyl-2-amino-3-carbohydrazide-4-methylthiophene (**3b**)*

A suspension of dry compound **1b** (10 mmol) in hydrazine hydrate (80%) (5 mL) was stirred under gentle reflux. The insoluble solid dissolved within 10 min with copious evolution of hydrogen sulfide to form a clear solution. After 30 min when the solid product started separating out, heating was continued for 4 h. The reaction mixture was then allowed to cool to room temperature. The solid was filtered, washed with ethanol, dried and crystallized from ethanol; as yellow crystals in a 78% yield, m.p. 112–114 °C; IR ($cm^{-1}$, ν); 3426–3156 (br, NH, $NH_2$), 2966 (CH alkyl), 1693, 1637 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 2.40 (s, 3H, C$H_3$), 2.66 (s, 3H, C$H_3$), 3.55 (br, 2H, N$H_2$, $D_2O$ exchangeable), 6.79 (br, 2H, N$H_2$, $D_2O$ exchangeable), 8.05 (br, 1H, N*H*, $D_2O$ exchangeable); Its MS (*m*/*z*), 213 ($M^+$); $C_8H_{11}N_3O_2S$ (213.2); Requires (Found): C, 45.05 (45.07); H, 5.20 (5.17); N, 19.70 1 (19.69).

*3.1.3. Methyl-N-(4-methyl-5-substituted-3-carboxy-thiophene)dithiocarbamate (**4a**,**b**)*

*General procedure*; To a vigorously stirred solution of 2-amino-3-carboxy-thiophene (**1a**,**b**) (20 mmol) in dimethylsulfoxide (10 mL) at room temperature, carbon disulfide (1.98 g, 26 mmol) and aqueous sodium hydroxide (1.2 mL, 20 mol solution) were added simultaneously over 30 min, the stirring was continued for further 30 min. Dimethylsulfate (2.5 g, 20 mmol) was added drop wise to the reaction mixture with stirring at 5–10 °C, it was further stirred for 2 h and poured into ice-water, the solid obtained was filtered, dried and crystallized from ethanol.

*3.1.3.1. Methyl N-(4-methyl-3,5-dicarboxyethyl-thiophene)dithiocarbamate (**4a**).* It was obtained from **1a**, yield 87%, m.p. 116–118 °C, IR ($cm^{-1}$, ν); 3426 (br, NH), 2985 (CH alkyl), 1708, 1663 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.36 (t, 3H, $J$ = 7.00 Hz, C$H_3$), 1.38 (t, 3H, $J$ = 7.02 Hz, C$H_3$), 1.44 (s, 1H, S*H*), 2.72 (s, 3H, SC$H_3$), 2.77 (s, 3H, C$H_3$), 4.31 (q, 2H, $J$ = 7.00 Hz, C$H_2$), 4.41 (q, 2H, $J$ = 7.02 Hz, C$H_2$), 7.25 (br, 2H, N$H_2$), 9.30 (br, 1H, N*H*) (NH, N$H_2$, $D_2O$ exchangeable); Its MS (*m*/*z*), 347 ($M^+$, 100%); $C_{13}H_{17}NO_4S_3$ (347.4); Requires (Found): C, 44.93 (44.87); H, 4.93 (4.88); N, 4.03 (4.06).

*3.1.3.2. Methyl N-(4-methyl-5-acetyl-3-carboxyethyl-thiophene)dithiocarbamate (**4b**).* It was obtained from **1b**, yellow crystals, yield 91%, m.p. 134–137 °C, IR ($cm^{-1}$, ν); 3407 (br, NH), 2985 (CH alkyl), 1678, 1665 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.36 (t, 3H, $J$ = 7.01 Hz, C$H_3$), 2.42 (s, 3H, C$H_3$), 2.68 (s, 3H, C$H_3$), 2.70 (s, 3H, SC$H_3$), 4.30 (q, 2H, $J$ = 7.01 Hz, C$H_2$), 6.60 (br, 1H, N*H*, $D_2O$ exchangeable); Its MS (*m*/*z*), 317 ($M^+$, 100%); $C_{12}H_{15}NO_3S_3$ (317.4); Requires (Found): C, 45.40 (45.37); H, 4.76 (4.73); N, 4.41 (4.39).

*3.1.4. 3-Amino-6-ethyl(5-methyl-2-thioxo-thieno[2,3-d]pyrimidin-4-one)carboxylate or 6-acetyl-5-methyl-2-thioxo-thieno[2,3-d]-pyrimidin-4-one (**5a**,**b**)*

*General procedure*; To a solution of each of **4a**,**b** (10 mmol) in ethanol 30 mL was treated with hydrazine hydrate (10 mmol, 99%) and refluxed on a water bath until the methylmercaptan evolution ceases (8 h). After cooling, the solid obtained was filtered, dried and recrystallized from ethanol/acetone mixture.

*3.1.4.1. 3-Amino-6-ethyl(5-methyl-2-thioxo-thieno[2,3-d]pyrimidin-4-one)carboxylate (**5a**).* It was obtained from **4a**, brown crystals, yield 75%, m.p. 192–194 °C, IR ($cm^{-1}$, ν); 3425 (br, NH), 2927 (CH alkyl), 1695, 1676 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.28 (t, 3H, $J$ = 7.0 Hz, C$H_3$), 2.44 (s, 3H, C$H_3$), 4.19 (q, 2H, $J$ = 7.04 Hz, C$H_2$), 7.54 (br, 2H, N$H_2$), 9.30 (br, 1H, N*H*), (NH, N$H_2$, $D_2O$ exchangeable); Its MS (*m*/*z*), 285 ($M^+$, 100%); $C_{10}H_{11}N_3O_3S_2$ (285.3); Requires (Found): C, 42.09 (42.07); H, 3.89 (3.85); N, 14.73 (14.71).

*3.1.4.2. 3-Amino-6-acetyl-5-methyl-2-thioxo-thieno[2,3-d]pyrimidin-4-one (**5b**).* It was obtained from **4b**, orange crystals, yield 85%, m.p. 254–257 °C, IR ($cm^{-1}$, ν); 3423–3273 (br, NH, $NH_2$), 2944 (CH alkyl), 1669, 1609 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 2.28 (s, 3H, C$H_3$), 2.67 (s, 3H, C$H_3$), 7.19 (br, 2H, N$H_2$), 7.54 (br, 1H, N*H*), 9.30 (br, 1H, N*H*); Its MS (*m*/*z*), 255 ($M^+$, 100%); $C_9H_9N_3O_2S_2$ (255.3); Requires (Found): C, 42.34 (42.31); H, 3.55 (3.58); N, 16.46 (16.44).

*Method B*. To a warmed ethanolic sodium hydroxide solution (0.40 g in 50 mL ethanol), compound **3b** (10 mmol) and carbon disulfide (excess 5 mL) were added. The mixture was heated under reflux for 15 h. The reaction mixture was allowed to cool to 0 °C, the deposited precipitate was filtered off, washed by water (20 mL), dried and crystallized from dioxane as dark yellow crystals.

*3.1.5. 3-Amino-5-methyl-6-ethylcarboxylate-2-methyllthio-thieno[2,3-d]pyrimidin-4-one (**7**)*

To a warmed ethanolic KOH solution prepared by dissolving (10 mmol) of KOH in 50 mL (ethanol) was added each of compound **5a** (10 mmol), the heating was continued for 30 min and the mixture was allowed to cool to room temperature, and the proper methyliodide (12 mmol) was added. The mixture was stirred under reflux for 5 h, then cool to room temperature, poured into cold water (100 mL). The solid product precipitated was filtered off washed with 100 mL water. The product was dried and crystallized from dioxane as a yellow powder, yield 85%, m.p. 219–221 °C, IR ($cm^{-1}$, ν); 3385 (br, $NH_2$), 2936 (CH alkyl), 1680, 1665 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.28 (t, 3H, C$H_3$), 2.20 (s, 3H, C$H_3$), 2.38 (s, 3H, SC$H_3$), 3.67 (q, 2H, OC$H_2$), 8.10 (br, 2H, N$H_2$); Its MS (*m*/*z*), 299 ($M^+$, 100%); $C_{11}H_{13}N_3O_3S_2$ (299.3); Requires (Found): C, 44.13 (44.09); H, 4.37 (4.40); N, 14.03 (13.98).

*3.1.6. Preparation of the acetylated-S-nucleosides (**10a**,**b**) and (**13a**–**d**)*

*Method A*: To a solution of **5a**,**b** (0.01 mol) in aqueous potassium hydroxide (0.01 mol) in distilled water (5 mL) was added a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-gluco-/or galacto-pyranosyl bromide (**8b**,**c**) or 1-bromo-2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyle (**8a**) (15 mmol) in acetone (40 mL). The reaction mixture was stirred at room temperature for 24 h (under TLC control). The solvent was evaporated under reduced pressure at 40 °C, and the crude product was filtered off and washed with distilled water to remove KBr formed. The product was dried, and crystallized from the proper solvent.

*Method B*: Compound **5a**,**b** (10 mmol) was stirred under reflux, and dry conditions in 50 mL hexamethyldisilane (HMDS) in the presence of ammonium sulfate (10 mmol) for 50–60 h. The clear solution formed was cooled and the solvent was evaporated in vacuo to give the silylated compound **7** as yellow oil. The letter oil was dissolved in acetonitrile (10 mL) and was added to a solution of 1,2,3,4,6-penta-*O*-acetyl-α-D-gluco-pyranose (**9**) in acetonitrile (5 mL) followed by addition of $SnCl_4$ (1.8 mL). The reaction mixture was stirred at room temperature for 16–20 h (under TLC control). The mixture was poured into saturated sodium bicarbonate solution and extracted the thioglycosides by diethylether + ethylacetate (1:1, 100 mL). Evaporate the solvent under reduced pressure to furnish crude nucleosides which were purified by column chromatography (30% ethylacetate in ether) to afford the pure thioglycosides.

*3.1.6.1. 3-Amino-5-methyl-6-ethyl[2-(2′,3′,5′-tri-O-acetyl-β-D-arabino-furanosyl-thio)-thieno[2,3-d]pyrimidine-4-one]carboxylate (**10a**).* It was obtained from compound **5a** (10 mmol) and 1-bromo-2,3,5-tri-

*O*-acetyl-α-D-arabinofuranosyle (**8a**) (10 mmol); as yellow powder, crystallized from *n*-hexane; yield 67%, m.p. 140–142 °C, IR ($cm^{-1}$, ν); 3431 (br, $NH_2$), 2978 (CH alkyl), 1752 (4CO), 1671 (CO imide); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.24 (t, 3H, $J = 7.46$ Hz, C*H*$_3$), 1.96–2.04 (3s, 9H, 3C*H*$_3$CO), 2.46 (s, 3H, C*H3*), 3.78 (q, 2H, $J = 7.46$ Hz, OC*H*$_2$), 3.95 (m, 1H, *H*-4′), 4.20 (m, 2H, *H*-5′, *H*-5″), 5.25 (m, 1H, *H*-3′), 5.51 (m, 1H, *H*-2′), 5.55 (d, 1H, $J = 3.65$ Hz, *H*-1′), 8.10 (br, 2H, N*H*$_2$); Its MS (*m*/*z*), 543 ($M^+$, 28%); $C_{21}H_{25}N_3O_{10}S_2$ (543.5); Requires (Found): C, 46.40 (46.41); H, 4.63 (4.58); N, 7.73 (7.69).

*3.1.6.2. 3-Amino-6-acetyl-5-methyl-2-(2′,3′,5′-tri-O-acetyl-β-D-arabinofuranosyl-thio)-thieno[2,3-d]pyrimidin-4-one (**10b**).* It was obtained from compound **5b** (10 mmol) and 1-bromo-2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyle (**8a**) (10 mmol) as yellow powder, crystallized from pet-ether 40–60, yield 85%, m.p. 199–201 °C, IR ($cm^{-1}$, ν); 3426 (br, $NH_2$), 2929 (CH alkyl), 1747 (3CO acetyl), 1680 CO acetyl, 1625 (CO imide); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 2.00 (s, 3H, C*H*$_3$), 2.05, 2.06, 2.09 (3s, 9H, 3C*H*$_3$CO), 2.33 (s, 3H, C*H3*), 3.84 (m, 1H, *H*-4′), 4.79 (m, 2H, *H*-5′, *H*-5″), 5.34 (m, 1H, *H*-3′), 5.38 (m, 1H, *H*-2′), 5.80 (d, 1H, $J = 3.72$ Hz, *H*-1′), 8.00 (br, 2H, N*H*$_2$); Its MS (*m*/*z*), 513 ($M^+$, 23%); $C_{20}H_{23}N_3O_9S_2$ (513.5); Requires (Found): C, 46.78 (46.75); H, 4.51 (4.48); N, 8.18 (8.19).

*3.1.6.3. 3-Amino-5-methyl-6-ethyl[2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl-thio)-thieno[2,3-d]pyrimidin-4-one]carboxlate (**13a**).* It was obtained from compound **5a** (10 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**8b**) (10 mmol); as a pale yellow powder, yield 70%, m.p. 186–188 °C, IR ($cm^{-1}$, ν); 3435 (br s, NH), 2938 (CH alkyl), 1742 (CO), 1686 (CO), 1667 CO; $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.30 (t, 3H, $J = 7.52$ Hz, C*H*$_3$), 1.90, 2.00, 2.05, 2.10 (4s, 12H, 4C*H*$_3$CO), 2.63 (s, 3H, C*H*$_3$), 3.85 (q, 2H, $J = 7.52$ Hz, C*H*$_2$), 3.95 (m, 1H, *H*-5′), 4.15 (m, 2H, *H*-6′, *H*-6″), 4.30 (m, 1H, *H*-4′), 4.95 (t, 1H, *H*-2′), 5.10 (t, 1H, $J = 9.59$ Hz, *H*-3′), 5.69 (d, 1H, $J = 10.68$ Hz, *H*-1′), 8.00 (br,2H, N*H*$_2$); Its MS (*m*/*z*), 615 ($M^+$, 13%); 331 ($M^+ - C_{10}H_{11}N_3O_3S_2$, 36%), 285 ($M^+ + 1 - C_{14}H_{19}O_9$, 30%); $C_{24}H_{29}N_3O_{12}S_2$ (615.6); Requires (Found): C, 46.82 (46.79); H, 4.75 (4.72); N, 6.82 (6.79).

*3.1.6.4. 3-Amino-5-methyl-6-ethyl[2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl-thio)-thieno[2,3-d]pyrimidin-4-one]carboxlate (**13b**).* It was obtained from compound **5a** (10 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galacto-pyranosyl bromide (**8c**) (10 mmol) as yellow powder, crystallized from *n*-hexane, yield 68%, m.p. 109–111 °C, IR ($cm^{-1}$, ν); 3420 (br s, NH), 2951 (CH alkyl), 1739 (CO), 1689 (CO), 1664 CO; $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.26 (t, 3H, $J = 7.49$ Hz, C*H*$_3$), 1.89, 2.03, 2.05, 2.11 (4s, 12H, 4C*H*$_3$CO), 2.59 (s, 3H, C*H*$_3$), 3.68 (q, 2H, $J = 7.49$ Hz, C*H*$_2$), 3.98 (m, 1H, *H*-5′), 4.20 (m, 2H, *H*-6′, *H*-6″), 4.22 (m, 1H, *H*-4′), 5.11 (t, 1H, *H*-2′), 5.31 (t, 1H, $J = 9.59$ Hz, H-3′), 5.54 (d, 1H, $J = 10.68$ Hz, *H*-1′), 7.98 (br, 2H, N*H*$_2$); Its MS (*m*/*z*), 615 ($M^+$, 23%); 331 ($M^+ - C_{10}H_{11}N_3O_3S_2$, 48%), 285 ($M^+ + 1 - C_{14}H_{19}O_9$, 28%); $C_{24}H_{29}N_3O_{12}S_2$ (615.6); Requires (Found): C, 46.82 (46.76); H, 4.75 (4.71); N, 6.82 (6.84).

*3.1.6.5. 3-Amino-6-acetyl-5-methyl-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl-thio)-thieno[2,3-d]pyrimidin-4-one (**13c**).* It was obtained from compound **5b** (10 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**8b**) (10 mmol); as a pale yellow powder, crystallized from *n*-hexane; yield 78%, m.p. 186–189 °C, IR ($cm^{-1}$, ν); 3450 (br, $NH_2$), 2946 (CH alkyl), 1755 (4CO acetyl), 1694 (CO acetyl), 1626 (CO imide); $^1H$ NMR ($CDCl_3$, δ, ppm): 1.97, 2.00, 2.01, 2.03 (4s, 12H, 4C*H*$_3$CO), 2.11 (s, 3H, C*H*$_3$), 2.74 (s, 3H, C*H*$_3$), 3.91 (m, 1H, *H*-5′), 4.13 (m, 2H, *H*-6′, *H*-6″), 5.12 (t, 1H, *H*-4′), 5.34 (m, 1H, *H*-2′), 5.36 (t, 1H, $J = 9.60$ Hz, *H*-3′), 5.65 (d,1H, $J = 11.0$, *H*-1′), 7.29 (br, 2H, N*H*$_2$); Its MS (*m*/*z*), 585 ($M^+$, 29%); $C_{23}H_{27}N_3O_{11}S_2$ (585.5); Requires (Found): C, 47.17 (47.15); H, 4.65 (4.68); N, 7.17 (7.19).

*3.1.6.6. 3-Amino-6-acetyl-5-methyl-2-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl-thio)-thieno[2,3-d]pyrimidin-4-one (**13d**).* It was obtained from compound **5b** (10 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galacto-pyranosyl bromide (**8c**) (10 mmol); as a pale yellow powder, crystallized from *n*-hexane, yield 71%, m.p. 130–132 °C, IR ($cm^{-1}$, ν); 3433 (br, $NH_2$), 2963 (CH alkyl), 1750 (4CO acetyl), 1688 (CO acetyl), 1630 (CO imide); $^1H$ NMR ($CDCl_3$, δ, ppm): 1.97, 2.01, 2.04, 2.09 (4s, 12H, 4C*H*$_3$CO), 2.16 (s, 3H, C*H*$_3$), 2.34 (s, 3H, C*H*$_3$), 3.93 (m, 1H, *H*-5′), 4.10 (m, 2H, *H*-6′, *H*-6″), 4.76 (m, 1H, *H*-4′), 5.31 (m, 1H, *H*-2′), 5.44 (t, 1H, $J = 9.58$ Hz, *H*-3′), 5.63 (d,1H, $J = 10.72$ Hz, *H*-1′), 7.40 (br, 2H, N*H*$_2$); Its MS (*m*/*z*), 585 ($M^+$, 21%); $C_{23}H_{27}N_3O_{11}S_2$ (585.5); Requires (Found): C, 47.17 (47.18); H, 4.65 (4.63); N, 7.17 (7.14).

### *3.1.7. General procedure for deacetylation of compounds **10a**,**b** and **14a**–**d***

To a stirred solution of compounds **10a**,**b** and **13a**–**d** (1 mmol) in methanol (10 mL) was added portion wise NaOMe 0.054 g (1 mmol) in anhydrous methanol (10 mL) by saturated ammonia gas at room temperature and the solution was stirred overnight. After evaporation of solvent in vacuo, $H_2O$ (10 mL) was added and the mixture was extracted several times with $CH_2Cl_2$ to remove the ester formed during the deprotection. To the resulting aqueous solution was added an ion exchange resin (Dowex 50W × 2, $H^+$ form), previously washed with methanol. After stirring for tin minutes, the solution was filtered, evaporated in vacuo and the residue was flashed chromatographed on silica gel with the gradient 0–10% methanol in chloroform to give compounds **14a**,**b** and **15a**–**d**. Also, purification by heating the crude in *n*-hexane (100 mL, three times) and crystallization from methanol gave a pale yellow powder.

*3.1.7.1. 3-Amino-6-ethyl[5-methyl-2-(β-D-arabinofuranosyl-thio)-thieno[2,3-d]-pyrimidin-4-one]carboxylate (**14a**).* It obtained from **10a**. yield 44%, m.p. 183–185 °C, IR ($cm^{-1}$, ν); 3465 (br, NH), 3360 (br s, OH's), 2961 (CH alkyl), 1685, 1662 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 1.22 (t, 3H, $J = 7.50$ Hz, C*H*$_3$), 2.40 (s, 3H, C*H*$_3$), 3.56 m, H-C(5′), 3.80 (q, 2H, $J = 7.50$ Hz, OC*H*$_2$), 3.88 (m, H-C(4′), 4.05 (m, H-C(3′), 4.23 (m, H-C(2′)), 5.04 (t, $J = 5.30$ Hz, $J = 4.92$ Hz, O*H*-C(5′), 5.15 (d, $J = 4.31$ Hz, O*H*-C(3′), 5.36 (d, $J = 5.91$ Hz, O*H*-C(2′), 6.04 (d, $J = 5.63$ Hz, H-C(1′), 9.20 (br, 2H, N*H*$_2$); Its MS (*m*/*z*), 417 ($M^+$, 19%); $C_{15}H_{19}N_3O_7S_2$ (417.4); Requires (Found): C, 43.15 (43.12); H, 4.59 (4.55); N, 10.07 (10.09).

*3.1.7.2. 3-Amino-6-acetyl-5-methyl-2-(β-D-arabinofuranosyl-thio)-thieno[2,3-d]-pyrimidin-4-one (**14b**).* It obtained from **10b**. yield 39%, m.p. 231–233 °C, IR ($cm^{-1}$, ν); 3420 (br, NH), 3350 (br s, OH's), 2928 (CH alkyl), 1700, 1672 (2CO); $^1H$ NMR (DMSO-$d_6$, δ, ppm): 2.04 (t, 3H, C*H*$_3$), 2.29 (s, 3H, C*H3*), 3.50 m, H-C(5′), 3.81 m, H-C(4′), 4.02 m, H-C(3′), 4.26 (m, H-C(2′)), 5.01 (t, $J = 5.32$ Hz, $J = 4.88$ Hz, O*H*-C(5′), 5.12 (d, $J = 4.35$ Hz, O*H*-C(3′), 5.33 (d, $J = 5.94$ Hz, O*H*-C(2′), 6.07 (d, $J = 5.60$ Hz, H-C(1′), 8.40 (br, 2H, N*H*$_2$); Its MS (*m*/*z*), 387 ($M^+$, 31%); $C_{14}H_{17}N_3O_6S_2$ (387.4); Requires (Found): C, 43.40 (43.37); H, 4.42 (4.39); N, 10.85 (10.78).

*3.1.7.3. 3-Amino-6-ethyl[5-methyl-2-(β-D-glucopyranosyl-thio)-thieno-[2,3-d]pyrimidin-4-one]carboxylate (**15a**).* It obtained from **13a**. yield 49%, m.p. 213–215 °C, IR ($cm^{-1}$, ν); 3425 (br, NH), 3345 (br s, OH's), 2932 (CH alkyl), 1672, 1668 (2CO); $^1H$ NMR (DMSO-$d_6$, $D_2O$ δ, ppm): 1.23 (t, 3H, $J = 7.3$ Hz, C*H*$_3$), 2.36 (s, 3H, C*H*$_3$), 3.25 (dd, 2H, *H*-6′, *H*-6″), 3.35–3.42 (m, 2H, *H*-2′, *H*-3′), 5.52 (dd, 1H, *H*-5′), 3.71 (dd, 1H, *H*-4′), 4.05 (q, 2H, $J = 7.04$ Hz, OC*H*$_2$), 4.59 (d, 1H, $J = 8.7$ Hz, *H*-1′); Its MS (*m*/*z*), 447 ($M^+$, 26%); $C_{16}H_{21}N_3O_8S_2$ (447.4); Requires (Found): C, 42.94 (42.87); H, 4.73 (4.69); N, 9.39 (9.36).

*3.1.7.4. 3-Amino-6-ethyl[5-methyl-2-(β-D-galactopyranosyl-thio)-thieno[2,3-d]pyrimidin-4-one]carboxylate (**15b**).* It obtained from

**13b**. yield 51%, m.p. 151–153 °C, IR ($cm^{-1}$, ν); 3455 (br, NH), 3386 (br s, OH's), 2926 (CH alkyl), 1695, 1665 (2CO); $^1$H NMR (DMSO-$d_6$, $D_2O$, δ, ppm): 1.30 (t, 3H, $J = 7.00$ Hz, $CH_3$), 2.36 (s, 3H, $CH_3$), 3.31 (dd, 2H, *H*-6′, *H*-6″), 3.32–3.46 (m, 2H, *H*-2′, *H*-3′), 3.61 (dd, 1H, *H*-5′), 3.76 (dd, 1H, *H*-4′), 4.11 (q, 2H, $J = 7.02$ Hz, $CH_2$), 4.92 (d, 1H, $J = 9.01$ Hz, *H*-1′); Its MS (*m*/*z*), 447 ($M^+$, 22%); $C_{16}H_{21}N_3O_8S_2$ (447.4); Requires (Found): C, 42.94 (42.89); H, 4.73 (4.70); N, 9.39 (9.40).

*3.1.7.5. 3-Amino-6-acetyl-5-methyl-2-(β-D-glucopyranosyl-thio)-thieno[2,3-d]pyrimidin-4-one (**15c**).* It obtained from **13c**. yield 42%, m.p. 201–203 °C, IR ($cm^{-1}$, ν); 3410 (br, NH), 3380 (br s, OH's), 2940 (CH alkyl), 1710, 1664 (2CO); $^1$H NMR (DMSO-$d_6$, $D_2O$, δ, ppm): 2.19 (s, 3H, $CH_3$), 2.28 (s, 3H, $CH_3$), 3.50 (t, $J = 9.2$ Hz, 1H, *H*-4′), 3.46–3.54 (m, 2H, *H*-2′, *H*-3′), 3.75 (dd, $J = 6.1, 12.4$ Hz, *H*-6′), 3.93 (dd, $J = 1.70$, 12.41 Hz, *H*-6″), 5.19 (d, $J = 7.10$ Hz, 1H, *H*-1′); Its MS (*m*/*z*), 417 ($M^+$, 18%); $C_{15}H_{19}N_3O_7S_2$ (417.4); Requires (Found): C, 43.15 (43.11); H, 4.59 (4.55); N, 10.06 (10.08).

*3.1.7.6. 3-Amino-6-acetyl-5-methyl-2-(β-D-galactopyranosyl-thio)-thieno[2,3-d]pyrimidin-4-one (**15d**).* It obtained from **13d**. yield 46%, m.p. 165–167 °C, IR ($cm^{-1}$, ν); 3436 (br, NH), 3390 (br s, OH's), 2961 (CH alkyl), 1705, 1668 (2CO); $^1$H NMR (DMSO-$d_6$, $D_2O$, δ, ppm): 2.16 (s, 3H, $CH_3$), 2.30 (s, 3H, $CH_3$), 3.38 (dd, 2H, *H*-6′, *H*-6″), 3.41–3.58 (m, 2H, *H*-2′, *H*-3′), 3.68 (dd, 1H, *H*-5′), 3.87 (dd, 1H, *H*-4′), 5.09 (d, $J = 8.96$ Hz, 1H, *H*-1′); Its MS (*m*/*z*), 417 ($M^+$, 14%); $C_{15}H_{19}N_3O_7S_2$ (417.4); Requires (Found): C, 43.15 (43.13); H, 4.59 (4.53); N, 10.06 (10.03).

### 3.2. Pharmacological screening

#### 3.2.1. Animals

Male Swiss albino mice (20–25 g) were obtained from the animal house colony of the National Research Center, Cairo, Egypt. The animals left for two days for acclimatization to animal room conditions and were maintained on standard pellet diet and water ad libitum [45]. The food was withdrawn on the day before the experiment, but allowed free access of water. All procedures involving animals were carried out in accordance with the guide for the care and use of laboratory animals (National Academy of Science of Egypt) and were approved by the Animals Studies Committee at Washington University. Test samples and reference compounds were suspended in 0.5% carboxymethyl cellulose and administered to each mouse by using gastric gavages needle. The control group animals, however, received same volume of dosing vehicle. In the pharmacological studies, the animals were first administered in 100 mg/kg (body weight) dose of the test drugs.

#### 3.2.2. p-Benzoquinone-induced abdominal constriction test in mice

60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ; Merck) solution in distilled water. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movement) were counted for the next 15 min, starting on the 5th min after the *p*-Benzoquinone injection. The data represent average of the total number of writhes observed [40]. The anti-nociceptive activity was expressed as percentage change from writhing controls. Aspirin (ASA) was used as reference.

#### 3.2.3. Carrageenan-induced paw edema model

For the determination of the effects on carrageenan-induced paw oedema the modified method of Kasahara et al. [46] was employed. 60 min after the oral administration of either test sample or dosing vehicle, each mice was injected with freshly prepared (0.5 mg/25 μL) suspension of carrageenan (Sigma, St. Louis, Missouri, U.S.A.) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw. As the control, 25 μL saline solutions were injected into that of the left hind paw. Paw oedema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods [41]. Indometacin (INDO) was used as a reference compound.

#### 3.2.4. Gastric ulceration study

All the animals were sacrificed immediately after the last measurement under ether anesthesia and stomachs were removed. Then the stomachs were examined for lesions under a dissecting microscope. Stomachs exhibiting one or more ulcers were considered positive.

#### 3.2.5. Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were tested by two tailed Student's *t*-test. $p < 0.05$ was considered to be significant.

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