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Design and Synthesis of Some Substituted Thiazolo[3,2-a]Pyrimidine

Derivatives of Potential Biological Activities

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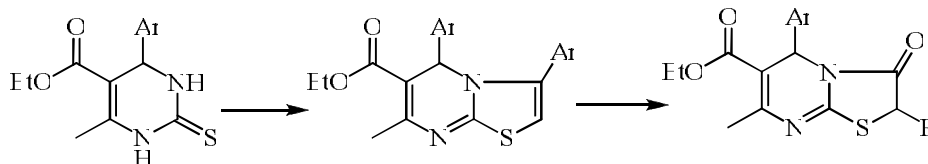
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Graphical abstract: synthesis of some new thiazolo[3,2-a]pyrimidine derivatives for the evaluation of their antimicrobial, anti-inflammatory and analgesic effects, if any.



Abstract

In continuation to our previous work, thiazolopyrimidines **2a-x** were synthesized through intramolecular cyclization of 2-phenacylthio-dihydropyrimidine hydrobromides **1a-x** using polyphosphoric acid. On the other hand, thiazolo[3,2-a]pyrimidine-3-one **3** was coupled with aryldiazonium salts or condensed with isatin to afford compounds **4a-c** or **5**, respectively. Chemical structure of the target compounds was substantiated by IR, FT-IR, ^1H -, ^{13}C and DEPT- ^{13}C -NMR, MS as well as microanalyses. Moreover, the lipophilicity of the target compounds as expressed from Clog P. The antimicrobial screening of the test compounds **2a-x**, **4a-c** and **5** revealed moderate activity in comparison to reference drugs. Compounds **2a-c**, **2e**, **2o** and **2v** showed a gradual increase in their anti-inflammatory activity reaching its maximum at 5 h compared to indomethacin. Furthermore, the analgesic activity of compounds **2a-c**, **2e**, **2o** and **2v** revealed a maximum activity after 5 h of injection compared to aspirin and the LD_{50} of compounds **2e** and **2v** were determined.

Keywords: Thiazolopyrimidines, synthesis, Antimicrobial, anti-inflammatory, analgesic

1. Introduction

Ever since the appearance of bacterial and fungal resistance towards the known chemotherapeutic agent and the amazing discovery of new chemotherapeutic molecule constitutes a real challenge. Consequently, new potent chemotherapeutic agents are needed, and if possible, with new modes of actions (Sahu et al., 2008). In addition, microbial infections often produce pain and inflammation. Chemotherapeutic, analgesic and anti-inflammatory drugs are prescribed simultaneously in normal practice. Consequently, compound possessing all three activities is recommended (Sahu et al., 2008). Hence, the treatment of pain continues to be the subject of considerable pharmaceutical and clinical research (Sahu et al., 2008).

In recent years, thiazolopyrimidines have been of interest due to their vital role in many biological activities (Viveka et al., 2012) such as antimicrobial (Ghorab et al., 2000; Sayed et al., 2006; Sherif et al., 1993; Tozkoparan et al., 1999; Youssef and Amin, 2012), anti-inflammatory (Dinakaran et al., 2012; Jeanneau-Nicolle et al., 1992; Sayed et al., 2006; Sherif et al., 1993; Tozkoparan et al., 1999), analgesic (Tozkoparan et al., 1999), antioxidant (Youssef and Amin, 2012), insecticides (Sherif et al., 1993), antidepressant (Tozkoparan et al., 1999), antiviral (Ravendra et al., 2012; Sayed et al., 2006), antitumor (Sayed et al., 2006; Tozkoparan et al., 1999), and calcium channel blocker activities (Balkan et al., 1992a; Balkan et al., 1996; Jeanneau-Nicolle et al., 1992; Tozkoparan et al., 1999). Additionally, it was reported that such a ring system has the ability to inhibit the enzyme 3', 5'-cyclic AMP phosphodiesterase (Sherif et al., 1993), to have a theophylline-like activity (Sherif et al., 1993) and to be active against virulent Lewis lung tumor in mice (Sherif et al., 1993).

In our previous work, a series of ethyl 6-methyl-4-(substituted)phenyl-2-(substituted)-phenacylthio-1,4-dihydropyrimidine-5-carboxylate hydrobromide **1a-x** have been synthesized and evaluated for their antibacterial and antifungal activities (Hussein et al., 2011).

Enlightened by aforementioned data and in continuation to our previous work, we herein the synthesis of some new thiazolo[3,2-a]pyrimidine derivatives for the evaluation of their antimicrobial, anti-inflammatory and analgesic effects, if any. This work is concerned with study the effect of structural modification on the biological activities of the target compounds.

2. Results and Discussion

2.1. Chemistry.

Compounds **1a-x** were cyclodehydrated into the corresponding thiazolo[3,2-a]pyrimidine compounds **2a-x** by heating with freshly prepared polyphosphoric acid (PPA) for 2 h followed by neutralization with ammonia, **Scheme 1**.

Basically, such products of **2a-x** had been formulated as the 5*H*-thiazolo [3,2-*a*] pyrimidine structure **A** based on comparison of ¹H-NMR spectral data for both compounds **1a-x** and **2a-x** (Balkan et al., 1992b; Sherif et al., 1993); **Figure 1**.

¹H-NMR spectrum of compound **1b** (Hussein et al., 2011), **Figure 2**, show the C6-CH₃ as singlet signal at δ 2.56 ppm and C4-H as singlet signal at δ 5.58 ppm. ¹H-NMR spectrum of compound **2b** as an example, **Figure 2**, showed a singlet signal (3H) at δ 2.50 ppm assigned for the C7-CH₃ protons and singlet signal (1H) at δ 6.13 ppm assigned for the pyrimidine H-5. The appearance of the CH₃ protons signal at the same position on both **1b** and **2b** and the down field shift for the pyrimidine H-5 in **2b**, at δ 6.13 ppm compared to the pyrimidine H-4 in **1b**, which appeared at δ 5.58 ppm, indicates that the environment around carbon-5 in **2b** differs from that around carbon-4 in **1b**. Consequently, we have assigned structure **A**, **Figure 1**, for the reaction products **2a-x** (Balkan et al., 1992b; Sherif et al., 1993).

Structures of the synthesized compounds **2a-x** were verified by IR, ¹H-, ¹³C and DEPT-¹³C-NMR, mass spectra as well as elemental microanalyses. IR spectra of compounds **2a-x** showed strong absorption bands at 1680-1636 cm⁻¹ for the carbonyl groups of the ester and at 1589-1561 cm⁻¹ indicating C=N stretching vibration. ¹H-NMR spectra revealed a triplet (3H) of quartet (2H) at δ 1.12-1.20 and 4.00-4.13 ppm, respectively of ethyl group and multiplet at δ 6.30-8.40 ppm indicating aromatic protons. On the other hand, ¹³C-NMR spectrum of compound **2k** showed a signal at δ 14.26 ppm corresponding to CH₃ of ethyl group, δ 57.16 ppm corresponding to CH₂ of ethyl group, δ 166.43 ppm indicating C-8a and δ 166.61 ppm attributed to carbonyl group. Moreover, DEPT ¹³C-NMR spectrum of compound **2k** showed inversion of CH₂ of the ethyl group and disappearance of all quaternary carbons while the CH₃ and CH groups stay the same. Moreover, mass spectra (EI) of compounds **2b**, **2d**, **2e**, **2i**, **2j**, **2n**, **2o**, **2q** and **2u** revealed the molecular ion peaks (M⁺) corresponding to their molecular weights and M⁺+2 for compounds **2j**, **2n**, **2o**, **2q** and **2u**. Physicochemical and spectral data of compounds **2a-x** are shown in experimental section.

The active methylene carbon atom in compound **3** (Sherif et al., 1993; Shiryaev et al., 2013) was coupled readily with equimolar amount of freshly prepared aryldiazonium salt to give the corresponding 2-arylhydrazono derivatives, compounds **4a-c**. The spectroscopic properties of these products suggest their existence, to a significant extent, in the tautomeric azo forms; hydrazono and diazenyl isomers in ~ 90 : 10% respectively. The hydrazono isomer is the major one due stabilization through formation of intramolecular hydrogen bonding, while the presence of diazenyl isomer was proved by the existence of highly deshielded (C2-H) in accordance with the reported data (Rida et al., 1986; Weisberger & Taylor, 1981), **Scheme 2**.

Structures of the synthesized compounds **4a-c** were verified by IR, ^1H -, ^{13}C and DEPT- ^{13}C -NMR, mass spectra in addition to elemental microanalyses. IR spectra of compounds **4a-c** showed NH at 3145-3220 cm^{-1} , ester carbonyl group at 1711-1717 cm^{-1} and the carbonyl at position 3 at 1693-1695 cm^{-1} . ^1H -NMR spectra for compounds **4a-c** were characterized by appearance of singlet signal at δ 8.13-8.83 ppm corresponding to NH proton (exchangeable with D_2O) for hydrazono isomer. The most characteristic signals indicated the presence of the possible two isomeric forms were the signals for CH_3 and CH_2 of the ethyl group that appeared as two sets of triplet at δ 1.00-1.40 ppm and two sets of quartet at δ 3.50-4.40 ppm, respectively. On the other hand, ^{13}C -NMR spectrum, of compound **4c** revealed the presence of two ethyl CH_3 of the two isomers at δ 13.98 and 18.34 ppm, benzylic carbon at δ 20.69 ppm, 7- CH_3 at δ 22.55 ppm, C-5 at δ 55.25 ppm, two ethyl CH_2 of the two isomers at δ 58.44 and 60.63 ppm, C-6 at δ 109.69 ppm, C-2 of diazenyl isomer at δ 114.50 ppm, aromatic carbons at δ 120.69-139.54 ppm, C-7 at δ 139.71 ppm, C-2 of hydrazono isomer at δ 151.77 ppm (C=N). It also showed signals at δ 153.36, 161.03 and 165.26 ppm corresponding to C-8a, amidic C=O and ester C=O, respectively. Moreover, DEPT ^{13}C -NMR spectrum, of compound **4c** was characterized by the presence of two signals at δ 14.00 ppm and δ 18.32 ppm corresponding to the two ethyl CH_3 groups of the two isomers and inversion of two signals at δ 58.44 ppm and δ 60.63 ppm indicating the presence of two ethyl CH_2 groups for the two isomers. In addition, all

quaternary carbons are disappeared while the signals of CH₃ and CH stay the same. Furthermore, mass spectra (EI) of compounds **4b** and **4c** revealed the molecular ion peaks (M⁺) corresponding to their molecular weights and M⁺+2 for compound **4c**. Physicochemical and spectral data of compounds **4a-c** are shown in experimental section.

Structure of compound **5** was verified by FT-IR, ¹H-NMR, mass spectra as well as elemental microanalyses. FT-IR spectrum of compound **5** showed characteristic bands at 3200 cm⁻¹ indicating the presence of NH group, 1715 cm⁻¹ corresponding to ester C=O and strong absorption band at 1697 cm⁻¹ corresponding to both amidic C=O and indole C=O groups. ¹H-NMR spectrum showed disappearance of the active methylene protons of SCH₂ signal at δ 3.85 ppm indicating condensation of isatin at C2 of compound **3**. It also showed a multiplet signal at δ 6.83-8.96 ppm (9H) indicating aromatic protons (5 protons of phenyl ring + 4 protons of indole ring) as well as singlet signal at δ 11.16 ppm indicating the presence of indole NH group (exchangeable with D₂O). Moreover, mass spectrum (EI) of compound (**5**) revealed the molecular ion peak (M⁺) corresponding to its molecular weight. Physicochemical and spectral data of compound **5** are cited in experimental section.

Lipophilicity. The lipophilicity of the target compounds **2a-x**, **4a-c** and **5** is expressed in the term of Clog P values (experimental section). Most of the target compounds exhibited high values for Clog P are the most active as antimicrobial agents.

2.2. Antimicrobial activities.

2.2.1. Antibacterial activity

Results of antibacterial activity of compounds **2a-x**, **Table 1**, revealed that at a concentration of 100 μmol/mL most of the test compounds were active against most of the used bacterial strains. Compounds (**2f**, **2h**, **2j-l** and **2p-r**; with Clog P from 6.30 to 8.28) were completely inactive against all used organisms while compound (**2v**; with Clog P 6.97) was active only against *Bacillus cereus* and compound (**2x**; with Clog P 6.80) was active only against *Staphylococcus aureus*.

In addition, compounds **2a-x** showed 33.3-66.7% antibacterial activity of that of chloramphenicol against *Staphylococcus aureus*, 40.0-73.33% against *Bacillus cereus*, 40.0-52.0% against *Escherichia coli*, 66.7-120.0% against *Pseudomonas aeruginosa* and 33.3-52.3% against *Serratia marcescens*. Moreover, the variation of the antibacterial activity with concentrations (MICs) was showed in **Table 3**. It was noted that, the most sensitive organisms to the test compounds were *Bacillus cereus* and *Pseudomonas aeruginosa*.

Again, from **Tables 1** and **3**, compounds (**2c**, **2e**, **2g** and **2t**; with Clog P 7.27, 7.05, 7.42 and 7.92 respectively) have a wide spectrum of antibacterial activity as being able to inhibit all test bacterial organisms with MICs ranging from 50 to 3.125 $\mu\text{mol/mL}$. Compounds (**2m** and **2s**; with Clog P 7.27 and 7.05 respectively) were effective against four out of five bacterial strains with MICs ranging from 100 to 25 $\mu\text{mol/mL}$.

In a previous work deals with design, synthesis and antimicrobial activity of the new *S*-phenacylated dihydropyrimidine hydrobromide compounds (**1a-x**) was reported.¹² Thiazolopyrimidines **2a-x** were synthesized and tested for their antibacterial activity. The results indicated that, the cyclized compounds (**2a-e**, **2g**, **2m** and **2s-w**; with Clog P from 6.47 to 7.92) showed an improvement in the antibacterial activity than the open analogues.

Moreover, the results of the antibacterial activity of compounds **4a-c** and **5**, **Table 1** indicated that at a concentration of 100 $\mu\text{mol/mL}$ the test compounds **4a-c** were active against *Staphylococcus aureus* and *Escherichia coli* while compound **5** was active only against *Staphylococcus aureus*. Compounds (**4a-c** and **5**; with Clog P from 4.95 to 5.66) were completely inactive against *Bacillus cereus*, *Pseudomonas aeruginosa* and *Serratia marcescens*.

2.2.2. Antifungal activity

Results of antifungal activity of compounds **2a-x**, **Table 2**, indicated that at a concentration of 100 $\mu\text{mol/mL}$ most of the test compounds were active against most of the used bacterial strains. Compounds (**2b**, **2j**, **2l**, **2p-r** and **2v**; with Clog P from 6.97 to 7.42) were

completely inactive against all used organisms while compounds (**2f**, **2k** and **2x**; with Clog P 6.30, 7.92 and 6.80 respectively) were active only against *Candida albicans*. Compound (**2c**; with Clog P 7.27) was possessed antifungal activity equal to that of the reference drug against *Scopulariopsis brevicaulis* while compound (**2t**; with Clog P 7.92) was exceeded that of the reference drug against *Scopulariopsis brevicaulis*. In addition, the active compounds **2a-x** showed 33.3-62.5% antifungal activity of that of Clotrimazole against *Candida albicans* with MICs ranged from 12.5-100 $\mu\text{mol/mL}$, 33.3-79.2% against *Geotrichum candidum* with MICs ranged from 3.125-100 $\mu\text{mol/mL}$, 40.0-92.0% against *Fusarium oxysporum* with MICs ranged from 3.125-100 $\mu\text{mol/mL}$, 28.6-50.0% against *Aspergillus flavus* with MICs at 100 $\mu\text{mol/mL}$, 41.7-75.0% against *Trichophyton rubrum* with MICs ranged from 3.125-100 $\mu\text{mol/mL}$ and 46.2-123.1 against *Scopulariopsis brevicaulis* with MICs ranged from 25-100 $\mu\text{mol/mL}$

Moreover, the results indicated that compounds (**2a**, **2c**, **2e**, **2g**, **2m** and **2t**; with Clog P 6.55, 7.27, 7.05, 7.42, 7.27 and 7.92 respectively) have a wide spectrum of antifungal activity being able to inhibit all test fungal organisms with MICs ranging from 100 to 3.125 $\mu\text{mol/mL}$. Compounds (**2d**, **2h**, **2s**, **2u** and **2w**; with Clog P 6.47, 8.28, 7.05, 7.77 and 7.55 respectively) were effective against five out of six fungal strains with MICs ranging from 100 to 3.125 $\mu\text{mol/mL}$, **Table 4**.

Also, in continuation to our previous work ([Hussein et al., 2011](#)), thiazolopyrimidines **2a-x** were synthesized and tested for their antifungal activity. The results revealed that, the cyclized compounds (**2a**, **2c-e**, **2g**, **2h**, **2m**, **2o**, **2s-u** and **2w**; with Clog P from 6.47 to 8.28) showed an improvement in the antifungal activity than the open analogues.

On the other hand, the results of the antifungal activity of compounds (**4a-c** and **5**; with Clog P from 4.95 to 5.66), indicated that at a concentration of 100 $\mu\text{mol/mL}$ all the compounds were completely inactive against all the test microorganisms.

It is noteworthy to mention that, within each series, increase in the Clog P values is accompanied by increase in the antimicrobial activity. The most active compounds comprise in

their structures an electron withdrawing group (R_1 = halogen; R_2 = halogen) while the least active compounds comprise in their structures an electron donating group (R_1 = H, CH_3 ; R_2 = OCH_3 , CH_3).

2.3. Pharmacological screening.

2.3.1. Anti-inflammatory activity

The most active compounds **2a-e**, **2o** and **2t-x** as antimicrobial agents were selected for evaluation of their *in vivo* anti-inflammatory effects.

The results of anti-inflammatory activity (**Table 5**), revealed that all the test compounds showed a gradual increase of anti-inflammatory activity up to its maximum at 5 h ranging from 73.72 : 97.58% with compounds **2a-c** and **2v** were the most active giving 92.86 : 97.58% of that of indomethacin.

It is noteworthy to mention that, the result of anti-inflammatory activity indicates that most of the compounds that showed highest anti-inflammatory activity having in their structure $R = p\text{-Cl-C}_6\text{H}_4$ or $p\text{-OCH}_3\text{C}_6\text{H}_4$ moieties which indicate the crucial role of halogen or the OCH_3 in the anti-inflammatory activity. these results comply with the previously reported findings of Kalgutkar *et al* (Kalgutkar et al., 2000).

2.3.2. Analgesic activity

The most active compounds **2a-c**, **2e**, **2o** and **2v** with regard to their anti-inflammatory activity were selected for testing their analgesic activity in mice using the hot plate method (Janssen & Jageneau, 1957) in comparison to aspirin as a reference drug.

Results of analgesic activity (**Table 6**), revealed that compounds **2e** and **2v** showed a gradual increase of analgesic activity up to its maximum after 5 h of injection with 84.04% and 82.26% of that of aspirin respectively, whereas the maximum activity of compound **2o** appears after 4 h of injection with 62.64% of the aspirin activity. On the other hand, compounds **2a** and **2b** revealed the maximum activity at 3 h interval with

35.36 and 37.80% of that of aspirin respectively while compound **2c** was the least active giving 34.39% of aspirin at 5 h interval.

The previous finding of analgesic activity indicates that compounds with the highest analgesic activity having R = *p*-Cl-C₄H₄, *p*-OCH₃C₆H₄ or *p*-CH₃C₆H₄ moieties.

2.3.3. Acute toxicity (LD₅₀)

The most active compounds **2e** and **2v** with regard to their anti-inflammatory and analgesic activity were selected for testing their acute toxicity using aspirin as a reference drug.

The median lethal dose (LD₅₀) of compounds **2e** and **2v** was determined in mice according to a reported method (Sztaricskai et al., 1999) and was found to be nontoxic up to >1 g/kg whereas the LD₅₀ of aspirin equals to 533 mg/Kg (*i.p.*) (Abdel-Alem et al., 1980).

3. Conclusion

This work was devoted for the syntheses of some new thiazolo[3,2-*a*]pyrimidine derivatives **2a-x**, **4a-c** and **5**. The structures of the target compounds were confirmed. The target compounds were tested for their antimicrobial activity and the most active compounds were screened for their MICs. They showed moderate activity against most of the used bacterial and fungal strains. Moreover, the lipophilicity of the target compounds as expressed from Clog P and the most active antimicrobial compounds comprise in their structures an electron withdrawing group (R₁ = halogen; R₂ = halogen) while the least active compounds comprise in their structures an electron donating group (R₁ = H, CH₃; R₂ = OCH₃, CH₃).

In addition, the most active compounds **2a-c**, **2e**, **2o** and **2v** as antimicrobial were tested for their anti-inflammatory effects and they showed a gradual increase in their anti-inflammatory activity reaching its maximum at 5 h in the carrageenan induced paw edema test.

The *p*-Cl-C₄H₄ or *p*-OCH₃C₆H₄ derivatives showed the highest anti-inflammatory activity, which indicate the crucial role of halogen or the OCH₃ groups.

Furthermore, the most active compounds **2a-c**, **2e**, **2o** and **2v** with regard to their anti-inflammatory activity were selected for testing their analgesic activity. The tested compounds (**2e** and **2v**) showed a gradual increase in their analgesic activity up to its maximum after 5 h of injection. In addition, the LD₅₀ of them was determined in mice and was found nontoxic up to >1 g/kg whereas the LD₅₀ of aspirin is 533 mg/Kg (*i.p.*).

4. Experimental

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, Model SMP1, Staffordshire, UK) and were uncorrected. Monitoring of the chemical reactions was carried out by TLC using precoated silica gel plates (kieselgel 0.25 mm, 60 G F254, Merk, Darmstadt, Germany) and CHCl₃/CH₃COCH₃ (9:1 v/v) as the mobile phase. Visualization of the spots was effected by ultraviolet lamp (Spectroline Model CM-10, Seattle, USA), at wavelengths 254 and 365 nm and/or iodine stain.

IR and FT-IR spectra were carried out as KBr discs on a Shimadzu IR-470 spectrometer (Shimadzu, Kyoto, Japan) and Thermo scientific 912A0683 spectrometer (Thermo scientific, Japan) respectively, at the Faculty of Pharmacy, Assiut University, Assiut, Egypt. ¹H-NMR spectra were scanned on a Varian EM-360L NMR spectrophotometer (60 MHz, Varian, CA, USA) at the Faculty of Pharmacy, Assiut University. ¹H, ¹³C and DEPT ¹³C-NMR spectra were scanned on JEOLJNM-LA series FT-NMR system (400 MHz, JEOL, Tokyo, Japan) at Assiut University Unit of Trace Analysis, Assiut University, Assiut, Egypt. Chemical shifts are expressed in δ-values (ppm) relative to tetramethylsilane (TMS) as an internal standard, using CDCl₃ or DMSOd₆ as solvents, and deuterium oxide was used for the detection of the exchangeable protons.

Mass spectra were recorded on a JMS-600H mass spectrometer (JEOL, Tokyo, Japan) at Assiut University Unit of Trace Analysis, Assiut University, Assiut, Egypt

and on Gas chromatography Mass, Quadrable-2010 Plus (Shimadzo, Kyoto, Japan) at the Micro Analytical Center, Faculty of Science, Cairo University, Cairo, Egypt.

Elemental microanalyses were performed on a Vario EL-CHNS Elemental Analyzer (Elementar Analysis GmbH, Germany), at Assiut University Unit of Trace Analysis, Assiut University, Assiut, Egypt and on a Vario elemental analyzer III (Vario, Hanau, Germany) at the Micro Analytical Center, Faculty of Science, Cairo University, Cairo, Egypt.

Calculations of logarithm partition coefficient values (CLog P). The log P values of the target compounds **2a-x**, **4a-c** and **5**, were computed with a routine method called calculated log P (Clog P) contained in a PC-software package (McLogP 2.0, BioByte Corp., CA, USA). A representation of the molecular structure where the hydrogens were omitted or “suppressed” (SMILES notation) was entered into the program, which computes the log P bases on Crippen`s fragmentation (Leo 2001).

Most of chemicals used were of commercial grade: Aniline, anhydrous sodium acetate (El Nasr Pharm. Co. Egypt), *p*-chloroaniline, isatin (Aldrich, Germany), bromoacetic acid and *p*-methyl aniline (Fluka, Germany). All other chemicals and solvents are of the reagent grade.

4.1. General method for synthesis of ethyl 3,5-di(substituted)phenyl-7-methyl-5H-thiazolo[3,2-*a*]pyrimidine-6-carboxylate, **2a-x**.

The appropriate compound, **1a-x**, was heated at 110-120 °C for 2 h with 10-15 times their weight of freshly prepared polyphosphoric acid (Dennler & Frasca, 1966). The reaction mixture was cooled, poured with stirring into ice-water, neutralized with concentrated ammonia and stirred overnight until crystallization was complete. The separated solid product was filtered, washed with water, dried and crystallized from hexane except compounds **2h**, **2i**, **2n**, **2q**, **2w** were crystallized from ethyl acetate-hexane mixture (2:1), **Scheme 1**.

Ethyl 3-(4-bromophenyl)-7-methyl-5-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2b: m.p. 154-155 °C; yield 83 %; R_f. 0.28 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.42;

IR (KBr) $\bar{\nu}$ (cm⁻¹): 1676 (C=O), 1560 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.50 (3H, s, CH₃), 4.06 (2H, q, CH₂CH₃), 6.13 (2H, s, pyr. H-5, thia H-2), 6.53-7.56 (9H, m, Ar-H); EI-MS m/z (%) 455 (M^+ , 26). Anal. Calcd (%) for C₂₂H₁₉BrN₂O₂S: C, 58.03; H, 4.21; N, 6.15. Found, C, 58.00; H, 4.19; N, 6.06.

Ethyl 3-(4-methoxyphenyl)-7-methyl-5-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2d: m.p. 135-137 °C; yield 88 %; R_f. 0.58 (CHCl₃:CH₃COCH₃ 9:1); CLog P 6.47; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1661 (C=O), 1574 (C=N) ; ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.50 (3H, s, CH₃), 4.06 (2H, q, CH₂CH₃), 6.13 (2H, s, pyr. H-5, thia H-2), 6.53-7.56 (9H, m, Ar-H); EI-MS m/z (%) 406 (M^+ , 51.82), 407 (M^+ +1, 50), 408 (M^+ +2, 49). Anal. Calcd (%) for C₂₃H₂₂N₂O₃S: C, 67.96; H, 5.46; N, 6.89. Found, C, 67.83; H, 5.47; N, 6.83.

Ethyl 7-methyl-3-(4-methylphenyl)-5-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2e: m.p. 110-112 °C; yield 89%; R_f. 0.28 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.05; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1679 (C=O), 1564 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.21 (3H, t, CH₂CH₃), 2.47 (3H, s, -C₆H₄-CH₃), 2.58 (3H, s, CH₃), 4.12 (2H, q, CH₂CH₃), 6.18 (1H, s, pyr. H-5), 6.24 (1H, s, thia H-2), 6.69-7.66 (9H, m, Ar-H); EI-MS: m/z (%) 390 (M^+ , 23), 391 (M^+ +1, 6), 392 (M^+ +2, 2). Anal. Calcd (%) for C₂₃H₂₂N₂O₂S: C, 70.74; H, 5.68; N, 7.17. Found, C, 70.63; H, 5.59; N, 7.26.

Ethyl 7-methyl-3-(4-nitrophenyl)-5-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2f: m.p. 142 °C; yield 72 %; R_f. 0.24 (CHCl₃:CH₃COCH₃ 9:1); CLog P 6.30; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1678 (C=O), 1589 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.40 (3H, s, CH₃), 4.06 (2H, q, CH₂CH₃), 6.09 (1H, s, pyr. H-5), 6.24 (1H, s, thia H-2), 6.66-8.33 (9H, m, Ar-H). Anal. Calcd (%) for C₂₂H₁₉N₃O₄S: C, 62.69; H, 4.54; N, 9.97. Found, C, 62.66; H, 4.75; N, 9.92.

Ethyl 5-(4-bromophenyl)-7-methyl-3-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2g: m.p. 111-113 °C; yield 70 %; Rf. 0.36 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.42; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1679 (C=O), 1570 (C=N); ¹H-NMR (CDCl₃): δ 1.15 (3H, t, CH₂CH₃), 2.46 (3H, s, CH₃), 4.06 (2H, q, CH₂CH₃), 6.12 (2H, s, pyr. H-5, thia H-2), 6.40-7.53 (9H, m, Ar-H). Anal. Calcd (%) for C₂₂H₁₉BrN₂O₂S: C, 58.03; H, 4.21; N, 6.15. Found, C, 57.83; H, 4.30; N, 6.06.

Ethyl 3,5-di(4-bromophenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2h: m.p. 192-193 °C; yield 90 %; Rf. 0.36 (CHCl₃:CH₃COCH₃ 9:1); CLog P 8.28; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1678 (C=O), 1562 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.12 (3H, t, CH₂CH₃), 2.46 (3H, s, CH₃), 4.00 (2H, q, CH₂CH₃), 6.00 (1H, s, pyr. H-5), 6.03 (1H, s, thia H-2), 6.39-7.50 (9H, m, Ar-H). Anal. Calcd (%) for C₂₂H₁₈Br₂N₂O₂S: C, 49.46; H, 3.40; N, 5.24. Found, C, 49.63; H, 3.69; N, 5.07.

Ethyl 5-(4-bromophenyl)-3-(4-chlorophenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2i: m.p. 185-187 °C; yield 85 %; Rf. 0.36 (CHCl₃:CH₃COCH₃ 9:1); CLog P 8.13; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1678 (C=O), 1564 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.43 (3H, s, CH₃), 4.03 (2H, q, CH₂CH₃), 6.00 (1H, s, pyr. H-5), 6.15 (1H, s, thia H-2), 6.42-7.50 (8H, m, Ar-H); EI-MS m/z (%) 489 (M^+ , 10), 490 (M^+ +1, 0.28). Anal. Calcd (%) for C₂₂H₁₈BrClN₂O₂S: C, 53.95; H, 3.70; N, 5.72. Found, C, 53.70; H, 3.63; N, 6.02.

Ethyl 5-(4-bromophenyl)-3-(4-methoxyphenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2j: m.p. 185-187 °C; yield 85 %; Rf. 0.32 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.34; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1679 (C=O), 1565 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.40 (3H, s, CH₃), 3.81 (3H, s, OCH₃), 4.03 (2H, q, CH₂CH₃), 6.06 (2H, s, pyr. H-5, thia H-2), 6.50-7.30 (8H, m, Ar-H); EI-MS m/z (%) 485 (M^+ , 4), 486 (M^+ +1, 18), 487 (M^+ +2, 7). Anal. Calcd (%) for C₂₃H₂₁BrN₂O₃S: C, 56.91; H, 4.36; N, 5.77. Found, C, 56.87; H, 4.40; N, 6.10.

Ethyl 5-(4-bromophenyl)-7-methyl-3-(4-methylphenyl)-5H-thiazolo[3,2-a]pyrimidine-

6-carboxylate, 2k: m.p. 183-185 °C; yield 88 %; R_f. 0.36 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.92; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1678 (C=O), 1564 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.22 (3H, t, CH₂CH₃), 2.46 (3H, s, -C₆H₄-CH₃), 2.48 (3H, s, CH₃), 4.11 (2H, q, CH₂CH₃), 6.18 (2H, s, pyr. H-5, thia H-2), 6.68-7.31 (8H, m, Ar-H); ¹³C-NMR (CDCl₃): (ppm) 14.26 (CH₂CH₃); 21.37 (-C₆H₄-CH₃), 23.59 (7-CH₃), 57.16 (CH₂CH₃), 59.77 (C-5), 100.66 (C-6), 102.50 (C-2), 122.02-139.89 (Ar-C + Ar-CH), 141.17 (C-3), 156.05 (C-7), 166.43 (C-8a), 166.61 (CO); DEPT ¹³C-NMR (CDCl₃): (ppm) 14.24(CH₂CH₃), 21.38 (7-CH₃), 23.62 (-C₆H₄-CH₃), 57.16 (inverted CH₂CH₃), 59.77 (C-5), 102.55 (C-2), 128.25-131.25 (Ar-CH). Anal. Calcd (%) for C₂₃H₂₁BrN₂O₂S: C, 58.85; H, 4.51; N, 5.97. Found, C, 58.82; H, 4.57; N, 6.33.

Ethyl 5-(4-bromophenyl)-7-methyl-3-(4-nitrophenyl)-5H-thiazolo[3,2-a]pyrimidine-6-

carboxylate, 2l: m.p. 194-195 °C; yield 87 %; R_f. 0.22 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.16; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1679 (C=O), 1564 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.50 (3H, s, CH₃), 4.10 (2H, q, CH₂CH₃), 6.06 (1H, s, pyr. H-5), 6.27 (1H, s, thia H-2), 6.50-8.40 (8H, m, Ar-H). Anal. Calcd (%) for C₂₂H₁₈BrN₃O₄S: C, 52.81; H, 3.63; N, 8.40. Found, C, 52.99; H, 3.87; N, 8.02.

Ethyl 5-(4-chlorophenyl)-7-methyl-3-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-

carboxylate, 2m: m.p. 155-156 °C; yield 85 %; R_f. 0.38 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.27; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1680 (C=O), 1563 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.16 (3H, t, CH₂CH₃), 2.50 (3H, s, CH₃), 4.13 (2H, q, CH₂CH₃), 6.15 (2H, s, pyr. H-5, thia H-2), 6.63-7.63 (9H, m, Ar-H). Anal. Calcd (%) for C₂₂H₁₉ClN₂O₂S: C, 64.30; H, 4.66; N, 6.82. Found, C, 64.47; H, 4.71; N, 6.60.

Ethyl 3-(4-bromophenyl)-5-(4-chlorophenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-

6-carboxylate, 2n: m.p. 157-158 °C; yield 76 %; R_f. 0.42 (CHCl₃:CH₃COCH₃ 9:1); CLog P 8.13; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1636 (C=O), 1562 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.20 (3H, t,

CH₂CH₃), 2.48 (3H, s, CH₃), 4.12 (2H, q, CH₂CH₃), 6.12 (1H, s, pyr. H-5), 6.22 (1H, s, thia H-2), 6.66-7.72 (8H, m, Ar-H). EI-MS m/z (%) 489 (M^+ , 51), 490 (M^++1 , 25), 491 (M^++2 , 29). Anal. Calcd (%) for C₂₂H₁₈BrClN₂O₂S: C, 53.95; H, 3.70; N, 5.72. Found, C, 53.83; H, 3.89; N, 5.59.

Ethyl 3,5-di(4-chlorophenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2o: m.p. 154-155 °C; yield 91 %; Rf. 0.39 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.98; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1677 (C=O), 1564 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.46 (3H, s, CH₃), 4.06 (2H, q, CH₂CH₃), 6.03 (1H, s, pyr. H-5), 6.12 (1H, s, thia H-2), 6.60-7.48 (8H, m, Ar-H). EI-MS: m/z (%) 445 (M^+ , 12), 446 (M^++1 , 20), 447 (M^++2 , 5). Anal. Calcd (%) for C₂₂H₁₈Cl₂N₂O₂S: C, 59.33; H, 4.07; N, 6.29. Found, C, 59.48; H, 4.00; N, 6.30.

Ethyl 5-(4-chlorophenyl)-3-(4-methoxyphenyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2p: m.p. 154-156 °C; yield 75 %; Rf. 0.36 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.19; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1680 (C=O), 1561 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.46 (3H, s, CH₃), 3.78 (3H, s, OCH₃), 4.06 (2H, q, CH₂CH₃), 6.06 (2H, s, pyr. H-5, thia H-2), 6.50-7.13 (8H, m, Ar-H). Anal. Calcd (%) for C₂₃H₂₁ClN₂O₃S: C, 62.65; H, 4.80; N, 6.35. Found, C, 62.42; H, 4.75; N, 6.10.

Ethyl 5-(4-chlorophenyl)-7-methyl-3-(4-methylphenyl)-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2q: m.p. 100-102 °C; yield 80 %; Rf. 0.44 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.77; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1679 (C=O), 1567 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.39 (6H, s, 2CH₃), 4.06 (2H, q, CH₂CH₃), 6.15 (1H, s, pyr. H-5), 6.18 (1H, s, thia H-2), 6.48-7.39 (8H, m, Ar-H). Anal. Calcd (%) for C₂₃H₂₁ClN₂O₂S: C, 65.01; H, 4.98; N, 6.59. Found, C, 65.83; H, 4.91; N, 6.23.

Ethyl 5-(4-chlorophenyl)-7-methyl-3-(4-nitrophenyl)-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2r: m.p. 120-121 °C; yield 92 %; Rf. 0.32 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.01; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1679 (C=O), 1565 (C=N); ¹H-NMR (CDCl₃): δ 1.20 (3H, t, CH₂CH₃), 2.46

(3H, s, CH₃), 4.13 (2H, q, CH₂CH₃), 6.13 (1H, s, pyr. H-5), 6.50 (1H, s, thia H-2), 6.60-8.36 (8H, m, Ar-H). Anal. Calcd (%) for C₂₂H₁₈ClN₃O₄S: C, 57.96; H, 3.98; N, 9.22. Found, C, 57.79; H, 4.00; N, 8.79.

Ethyl 7-methyl-5-(4-methylphenyl)-3-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2s: m.p. 150-151 °C; yield 88 %; R_f. 0.40 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.05; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1679 (C=O), 1567 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.15 (3H, t, CH₂CH₃), 2.16 (3H, s, C₆H₅-CH₃), 2.46 (3H, s, CH₃), 4.00 (2H, q, CH₂CH₃), 6.03 (2H, s, pyr. H-5, thia H-2), 6.39-7.47 (9H, m, Ar-H). Anal. Calcd (%) for C₂₃H₂₂N₂O₂S: C, 70.74; H, 5.68; N, 7.17. Found, C, 70.51; H, 6.38; N, 7.08.

Ethyl 3-(4-bromophenyl)-7-methyl-5-(4-methylphenyl)-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2t: m.p. 174-176 °C; yield 79 %; R_f. 0.42 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.92; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1678 (C=O), 1563 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.20 (3H, t, CH₂CH₃), 2.20 (3H, s, -C₆H₄-CH₃), 2.50 (3H, s, CH₃), 4.06 (2H, q, CH₂CH₃), 6.00 (1H, s, pyr. H-5), 6.11 (1H, s, thia H-2), 6.50-7.66 (8H, m, Ar-H). Anal. Calcd (%) for C₂₃H₂₁BrN₂O₂S: C, 58.85; H, 4.51; N, 5.97. Found, C, 58.84; H, 4.57; N, 6.15.

Ethyl 3-(4-chlorophenyl)-7-methyl-5-(4-methylphenyl)-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2u: m.p. 173-175 °C; yield 85 %; R_f. 0.39 (CHCl₃:CH₃COCH₃ 9:1); CLog P 7.77; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1677 (C=O), 1566 (C=N); ¹H-NMR (CDCl₃): δ 1.16 (3H, t, CH₂CH₃), 2.20 (3H, s, -C₆H₄-CH₃), 2.42 (3H, s, CH₃), 4.06 (2H, q, CH₂CH₃), 6.03 (1H, s, pyr. H-5), 6.10 (1H, s, thia H-2), 6.50-7.50 (8H, m, Ar-H). EI-MS *m/z* (%) 424 (*M*⁺, 53), 425 (*M*⁺+1, 20), 426 (*M*⁺+2, 36). Anal. Calcd (%) for C₂₃H₂₁ClN₂O₂S: C, 65.01; H, 4.98; N, 6.59. Found, C, 65.06; H, 5.10; N, 6.61%.

Ethyl 3-(4-methoxyphenyl)-7-methyl-5-(4-methylphenyl)-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2v: m.p. 162-164 °C; yield 89 %; R_f. 0.33 (CHCl₃:CH₃COCH₃ 9:1); CLog P 6.97; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1668 (C=O), 1567 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.13

(3H, t, CH_2CH_3), 2.17 (3H, s, $-\text{C}_6\text{H}_4-\text{CH}_3$), 2.46 (3H, s, CH_3), 3.75 (3H, s, OCH_3), 4.00 (2H, q, CH_2CH_3), 5.94 (1H, s, pyr. H-5), 6.00 (1H, s, thia H-2), 6.42-7.00 (8H, m, Ar-H). Anal. Calcd (%) for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$: C, 68.55; H, 5.75; N, 6.66. Found, C, 67.93; H, 6.79; N, 6.49.

Ethyl 7-methyl-3,5-di(4-methylphenyl)-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2w: m.p. 195-196 °C; yield 88 %; R_f. 0.39 (CHCl_3 : CH_3COCH_3 9:1); CLog P 7.55; IR (KBr) $\bar{\nu}$ (cm^{-1}): 1680 (C=O), 1570 (C=N); $^1\text{H-NMR}$ (CDCl_3): (ppm) 1.16 (3H, t, CH_2CH_3), 2.20 (3H, s, $-\text{C}_6\text{H}_4-\text{CH}_3$), 2.50 (6H, s, $-\text{C}_6\text{H}_4-\text{CH}_3$, CH_3), 4.06 (2H, q, CH_2CH_3), 6.03 (1H, s, pyr. H-5), 6.10 (1H, s, thia H-2), 6.50-8.00 (8H, m, Ar-H). Anal. Calcd (%) for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_2\text{S}$: C, 71.26; H, 5.98; N, 6.93. Found, C, 71.13; H, 5.95; N, 6.64.

Ethyl 7-methyl-5-(4-methylphenyl)-3-(4-nitrophenyl)-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 2x: m.p. 134-135 °C; yield 91 %; R_f. 0.33 (CHCl_3 : CH_3COCH_3 9:1); CLog P 6.80; IR (KBr) $\bar{\nu}$ (cm^{-1}): 1652 (C=O), 1560 (C=N); $^1\text{H-NMR}$ (CDCl_3): δ 1.16 (3H, t, CH_2CH_3), 2.20 (3H, s, $-\text{C}_6\text{H}_4-\text{CH}_3$), 2.42 (3H, s, CH_3), 4.03 (2H, q, CH_2CH_3), 6.00 (1H, s, pyr. H-5), 6.23 (1H, s, thia H-2), 6.42-8.31 (8H, m, Ar-H). Anal. Calcd (%) for $\text{C}_{23}\text{H}_{21}\text{N}_3\text{O}_4\text{S}$: C, 63.43; H, 4.86; N, 9.65. Found, C, 62.88; H, 5.38; N, 9.51.

4.2. General method for synthesis of ethyl 5-phenyl-2,3-dioxo-2-(un)substituted phenyl hydrazono-7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 4a-c. The appropriate aryldiazonium chloride (10 mmol) prepared by adding concentrated hydrochloric acid (3 mL) to the appropriate aromatic amine (10 mmol) at 0-5 °C and treating the resulting hydrochloride solution with a cold solution of sodium nitrite (0.69 g, 10 mmol; in 5 mL water). Then, it was added drop wise with constant stirring at 0-5 °C to a cold solution of compound **3** (3.16 gm, 10 mmol,) in an absolute ethanol (80 mL), containing sodium acetate (3.02 gm, 37 mmol). The reaction mixture was stirred at room temperature for 2 h then diluted with cold water (30 mL) whereby the resultant crude product which precipitated was filtered, washed with water, dried and crystallized from absolute ethanol, **Scheme 2**.

Ethyl 7-methyl-3-oxo-5-phenyl-2-(2-phenylhydrazono)-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 4a: m.p. 130-131 °C; yield 85 %; R_f. 0.41 (CHCl₃:CH₃COCH₃ 9:1); CLog P 4.95; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1717 (ester C=O), 1603 (C=N); ¹H-NMR (CDCl₃): (ppm) 1.23 (two sets, 6H, t, CH₂CH₃), 2.60 (3H, s, CH₃), 4.03 (two sets, 4H, q, CH₂CH₃), 6.33 (1H, s, pyr. H-5), 7.10-7.76 (11H, m, Ar-H, C2-H), 8.83 (1H, s, NH; exchangeable with D₂O). Anal. Calcd (%) for C₂₂H₂₀N₄O₃S: C, 62.84; H, 4.79; N, 13.32. Found, C, 62.55; H, 4.70; N, 13.39.

Ethyl 2-[2-(4-chlorophenyl)hydrazono]-7-methyl-3-oxo-5-phenyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 4b: m.p. 125-126 °C; yield 62 %; R_f. 0.39 (CHCl₃:CH₃COCH₃ 9:1); CLog P 5.66; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1710 (ester C=O), 1598 (C=N); ¹H-NMR (CDCl₃, 400 MHz): (ppm) 1.23 (two sets, 6H, t, CH₂CH₃), 2.60 (3H, s, CH₃), 4.03 (two sets, 4H, q, CH₂CH₃), 6.30 (1H, s, pyr. H-5), 7.00-7.70 (11H, m, Ar-H, C2-H), 8.13 (1H, s, NH; exchangeable with D₂O). EI-MS *m/z* (%) 454.95 (*M*⁺, 27), 455.95 (*M*⁺+1, 38), 456.90 (*M*⁺+2, 10). Anal. Calcd (%) for C₂₂H₁₉ClN₄O₃S: C, 58.08; H, 4.21; N, 12.32. Found, C, 57.19; H, 4.62; N, 12.00.

Ethyl 7-methyl-2-[2-(4-methylphenyl)hydrazono]-3-oxo-5-phenyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, 4c: m.p. 136-137 °C; yield 88 %; R_f. 0.45 (CHCl₃:CH₃COCH₃ 9:1); CLog P 5.45; IR (KBr) $\bar{\nu}$ (cm⁻¹): 1711 (ester C=O), 1604 (C=N); ¹H-NMR (CDCl₃, 400 MHz): (ppm) 1.15 (two sets, 6H, t, CH₂CH₃), 2.19 (3H, s, -C₆H₄-CH₃), 2.45 (3H, s, CH₃), 3.66 (2H, q, CH₂CH₃), 4.04 (2H, q, CH₂CH₃), 6.12 (1H, s, pyr. H-5), 6.95-7.35 (11H, m, Ar-H, C2-H), 8.62 (1H, s, NH; exchangeable with D₂O); ¹³C-NMR (CDCl₃): (ppm) 13.98 (CH₂CH₃), 18.34 (CH₂CH₃), 20.69 (-C₆H₄-CH₃), 22.55 (7-CH₃), 55.25 (C-5), 58.44 (CH₂CH₃), 60.63 (CH₂CH₃), 109.69 (C-6), 114.50 (C2-H), 120.69-139.71 (Ar-C + Ar-CH), 151.77 (C-7), 153.36 (C-8a), 161.03 (C-3), 165.26 (ester C=O); DEPT ¹³C-NMR (CDCl₃): δ 14.00 (CH₂CH₃), 18.32 (CH₂CH₃), 20.73 (-C₆H₄-CH₃), 22.59 (7-CH₃), 55.25 (C-5), 58.44

(inverted $\underline{\text{CH}_2\text{CH}_3}$), 60.63 (inverted $\underline{\text{CH}_2\text{CH}_3}$), 114.52 (C-2), 128.02-132.76 (Ar-CH). Anal. Calcd (%) for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_3\text{S}$: C, 63.58; H, 5.10; N, 12.89. Found, C, 63.53; H, 5.09; N, 12.63.

4.3. Synthesis of ethyl 5-phenyl-3-oxo-2-isatinyldene-7-methyl-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate, compound, 5. To a solution of compound **3** (0.63 g, 2 mmol) in glacial acetic acid (20 ml); isatin (0.35 g, 2.4 mmol) and fused sodium acetate (0.57 g, 7 mmol) were added. The reaction mixture was heated under reflux for 4 h, during which the product was partly crystallized out. After cooling, the reddish compound was filtered, washed with ethanol, dried and crystallized from ethanol, **Scheme 2. 5**; m.p. 298-299 °C; yield 62 %; R_f. 0.32 (CHCl_3 : CH_3COCH_3 9:1); CLog P 4.95; IR (KBr) $\bar{\nu}$ (cm^{-1}): 1684 (ester C=O), 1599 (C=N), 3170 (NH); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz): (ppm) 1.26 (3 H, t, $\underline{\text{CH}_2\text{CH}_3}$); 2.50 (3H, s, CH_3); 4.20 (2H, q, $\underline{\text{CH}_2\text{CH}_3}$); 6.33 (1H, s, pyr. H-5); 6.83-8.96 (9 H, m, Ar-H); 11.16 (1 H, s, NH; exchangeable with D_2O). EI-MS: m/z (%) 445 (M^+ , 44), 446 (M^++1 , 12), 447 (M^++2 , 5). Anal. Calcd (%) for $\text{C}_{22}\text{H}_{19}\text{ClN}_4\text{O}_3\text{S}$: C, 64.71; H, 4.30; N, 9.43. Found, C, 64.81; H, 4.45; N, 9.33.

5. Biological testing

5.1. Antimicrobial activity

5.1.1. Antibacterial activity

Compounds **2a-x**, **4a-c** and **5** were tested for their antibacterial activity *in vitro*, in comparison with chloramphenicol as a reference drug using the standard agar cup diffusion method (Janssen & Jageneau, 1957; Sherif et al., 1993) at the Assiut University Mycological Center (AUMC), Faculty of Science, Assiut University, Assiut, Egypt. Five bacterial species representing both Gram-positive and Gram-negative strains which are common contaminants of the environments in Egypt and some of which are involved in human and animal diseases were selected. The used bacterial strains are *Staphylococcus aureus* (AUMC No. B-54)) and *Bacillus cereus* (AUMC NoB-5) as representatives of Gram-positive strains, and *Escherichia*

coli (AUMC No.B-53), *Pseudomonas aeruginosa* (AUMC No. B-73), as well as *Serratia marcescens* (AUMC No. B-55) as representatives of Gram-negative strains (Janssen & Jageneau, 1957).

To prepare inocula for bioassay, bacterial strains were individually cultured for 24 h in 100 ml conical flasks containing 30 ml nutrient broth medium. Bioassay was done in 10 cm sterile plastic Petri plates in which bacterial suspension (1ml/plate) and 15 ml Nutrient agar medium (15 ml/plate) were poured. After solidification of the media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. Tested compounds dissolved in dimethyl sulfoxide (DMSO) at 100 μ mol/ml were pipetted in the cavities (20 μ l/cavity). Cultures were then incubated at 28 ± 2 °C for 48 h. Results were taken as the diameter (in mm) of inhibition zone around cavities and the results are cited in **Table 1**.

5.1.2. Antifungal activity

The synthesized compounds **2a-x**, **4a-c** and **5** were tested for their antifungal activity *in vitro*, in comparison with clotrimazole as a reference drug using the standard agar cup diffusion method (Janssen & Jageneau, 1957; Sherif et al., 1993) at the Assiut University Mycological Center (AUMC), Faculty of Science, Assiut University, Assiut, Egypt. Six pathogenic, phytogenic, or food-poisoning fungal species were used in the present study: *Candida albicans* (AUMC No. 418), *Geotrichum candidum* (AUMC No. 226), *Aspergillus flavus* (AUMC No. 1276), *Trichophyton rubrum* (AUMC No. 1804), *Scopulariopsis brevicaulis* (AUMC No. 729), *Fusarium oxysporum* (AUMC No. 5119) (Janssen & Jageneau, 1957).

To prepare inocula for bioassay, Fungi were grown for 7 days in 100 ml conical flasks containing 30 ml sabouraud's dextrose broth. Bioassay was done in 10 cm sterile plastic Petri plates in which fungal suspension (1ml/plate) and 15 ml sabouraud's dextrose agar medium (15 ml/plate) were poured. After solidification of the media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. Tested compounds dissolved in dimethyl sulfoxide (DMSO) at 100 μ mol/ml were pipetted in the cavities (20 μ l/cavity).

Cultures were then incubated at 28 ± 2 °C up to 7 days. Results were read as the diameter (in mm) of inhibition zone around cavities and the results are cited in **Table 2**.

5.1.3. The Minimum Inhibitory Concentrations (MICs).

The test compounds giving positive results were diluted with DMSO to prepare a series of descending concentrations and were similarly assayed as mentioned before and the least concentration (below which no activity) was recorded as the MIC. The results are cited in **Tables 3 and 4**.

5.2. Pharmacological screening

5.2.1. Anti-inflammatory activity

Male adult albino rats (130-150 g) were obtained from the animal house, Faculty of Medicine, Assiut University, Assiut, Egypt. Animals were housed in separate cages, 6 animals each, in temperature-controlled rooms at 25 °C. Animals were allowed free access to rodent chow and water and maintained at a 12 h light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use, care as found in the European Community Guidelines (Nargund et al., 1994).

Indomethacin (Liometacin[®] vial, Nile Company), Carrageenan (Sigma, USA), sodium carboxymethylcellulose (NaCMC) and normal saline were obtained from local market.

For determination of anti-inflammatory activity, all tested compounds and the reference drug were suspended in (1% sodium carboxymethylcellulose (NaCMC) solution in normal saline). Suspensions of the test compounds, reference drug and 1% (NaCMC)-saline solution (negative control) were injected *i.p.* (1 mL each).

Compounds **2a-e**, **2o** and **2t-x** were evaluated for their anti-inflammatory activity according to carrageenan induced paw edema method (Nargund et al., 1994) in comparison to indomethacin as a reference drug. The test is based on the pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 mL of 1% solution in normal

saline) into the right hind of the rats. Male adult albino rats were divided into groups, each of three animals. The thickness of rat paw was measured by a Vernier caliper (SMIEC, China) before and 1 h after carrageenan injection to detect the carrageenan induced inflammation. Each tested compound, at a dose of 54 $\mu\text{mol/Kg}$, was injected *i. p.* to a thirteen different groups of rats 1 h after carrageenan injection. Control group received a vehicle (1% NaCMC solution in normal saline), while the reference group received indomethacin *i.p.* at 54 $\mu\text{mol/Kg}$.

The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.5, 1, 2, 3, 4, and 5 h after injection of the test compounds, reference drug, and control. The results are listed in **Table 5**.

5.2.2. Analgesic activity

The analgesic activity of the most active anti-inflammatory compounds **2a-c**, **2e** and **2v** was evaluated in mice using the hot plate method (Janssen & Jageneau, 1957) in comparison to aspirin as a reference drug. In this method, the time taken by the mouse to lick its feet or to jump within a plexiglass cylinder placed on a hot plate surface (55°C) was determined. The reaction time was taken as the end point and the increase in hot plate latency was taken as a measure of the analgesic activity.

All tested compounds and the reference drug were suspended in 1% sodium carboxymethylcellulose (NaCMC) saline solution. Suspensions of the tested compounds, reference drug and 1% sodium carboxymethylcellulose (NaCMC) saline solution (negative control) were injected *i.p.* (1 mL each).

Male adult albino mice (25-30 g) were divided into eight groups, each of three animals. Solutions or suspensions of the test compounds and the reference drug in 1% sodium carboxymethylcellulose (NaCMC) solution were injected *i.p.* in a dose level of 300 $\mu\text{mol/kg}$ into mice. Control group of animals were similarly treated with 1% sodium carboxymethylcellulose (NaCMC) saline solution. The reaction time was evaluated directly 0.5, 1, 2, 3, 4 and 5 hours of first injection. The results are cited in **Table 6**.

5.2.3. Acute toxicity (LD₅₀)

The median lethal dose (LD₅₀) of the most active anti-inflammatory and analgesic compounds **2e** and **2v** was determined in mice.

Groups of male adult albino mice, each of three animals (25-30 g), were injected *i.p.* with graded doses of the test compounds. The percentage mortality in each group of animals was determined 24 h latter to injection (Sztaricskai et al., 1999).

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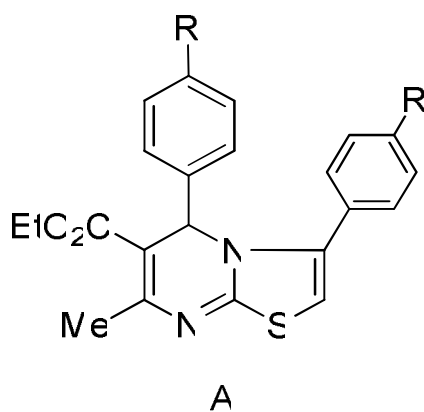


Figure 1 — Structure of compound A

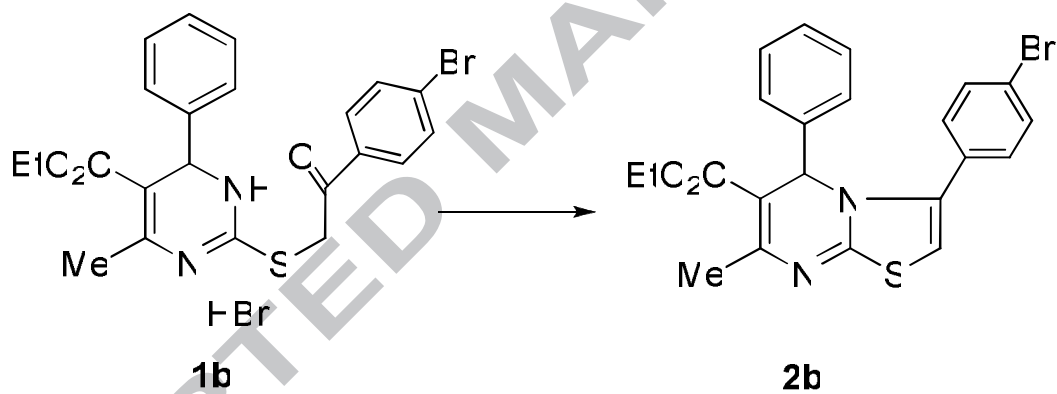
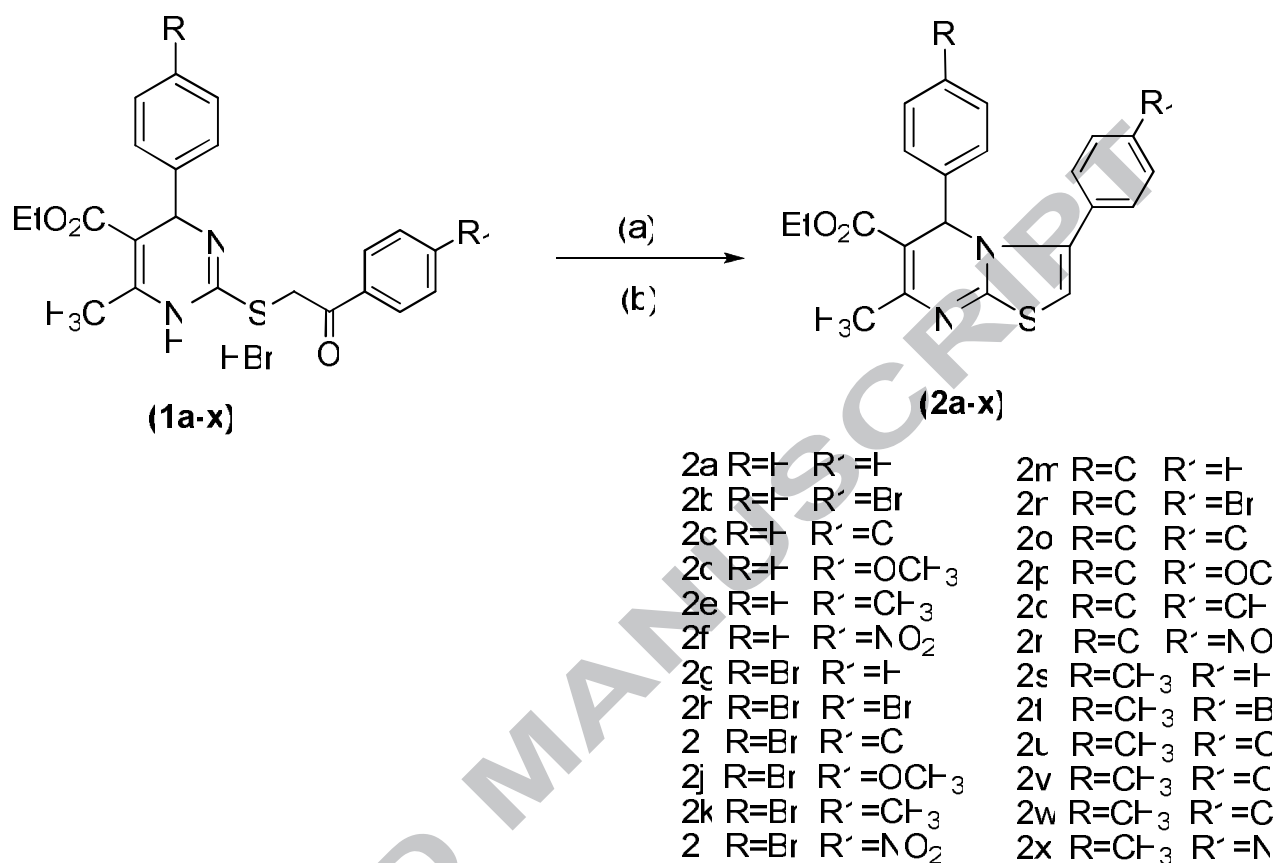
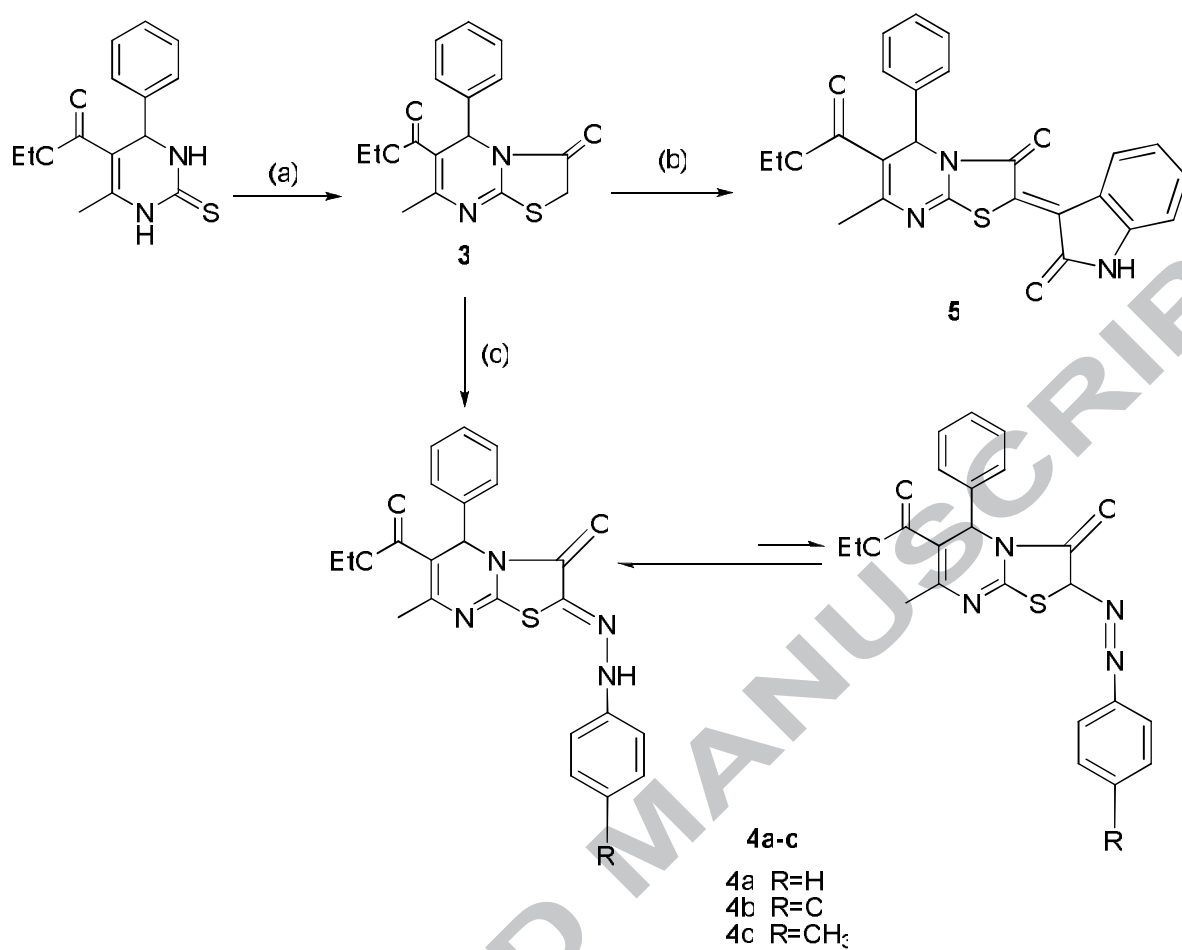


Figure 2 — Structure of compounds 1b & 2b



Scheme 1 — Synthetic route for compounds **2a-x**. (a) 1) polyphosphoric acid, 110 °C, (b) NH₄OH



Scheme 2 — Synthetic route for compounds **4a-c** and **5**. (a) bromoacetic acid, (b) isatin, sod. acetate, (c) *p*-(un)substituted-aniline, sod. nitrite, conc. HCl, 0-5 °C

Table 1 Antibacterial activity of compounds (**2a-x**, **4a-c** and **5**) and Chloramphenicol (inhibition zones in mm at 100 µmol/mL)

Comp No.	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. marcescens</i>
2a	8	18	-	-	-
2b	8	18	-	-	-
2c	11	17	10	13	12
2d	8	18	-	-	-
2e	13	22	13	13	16
2f	-	-	-	-	-
2g	16	21	13	18	18
2h	-	-	-	-	-
2i	10	12	13	-	-
2j	-	-	-	-	-
2k	-	-	-	-	-
2l	-	-	-	-	-
2m	12	12	12	-	16 pi
2n	8	-	12	13	-
2o	11	-	11	-	-
2p	-	-	-	-	-
2q	-	-	-	-	-
2r	-	-	-	-	-
2s	11	17	10	14	-
2t	11	12	10	10	12
2u	10	-	10	10	-
2v	-	12	-	-	-
2w	11	15	10	-	-
2x	11	-	-	-	-
4a	8	-	8	-	-
4b	8	-	8	-	-
4c	10	-	8	-	-
5	10	-	-	-	-
CHL	24	30	25	15	34
Control (DMSO)	-	-	-	-	-

Pi = partial inhibition. - = no inhibition. CHL= Chloramphenicol as antibacterial standard. AUMC = Assiut University

Mycological Center

Table 2 Antifungal activity of compounds (**2a-x**, **4a-c** and **5**) and clotrimazole (inhibition zones in mm at 100 μ mol/mL)

Comp No.	<i>C. albicans</i>	<i>G. candidum</i>	<i>F. oxysporum</i>	<i>A. flavus</i>	<i>T. rubrum</i>	<i>S. brevicaulis</i>
2a	13	14	13	8	18	12
2b	-	-	-	-	-	-
2c	15	15	22	12	22	26
2d	13	16	13	-	22	15
2e	14	19	22	13	27	16
2f	11	-	-	-	-	-
2g	14	15	18	14	25	22
2h	12	8	15	-	16	18
2i	-	10	10	-	16 p. i	22
2j	-	-	-	-	-	-
2k	8	-	-	-	-	-
2l	-	-	-	-	-	-
2m	14	14	16	12	16	20
2n	-	13	12	-	-	14
2o	13	14 pi	10	-	-	15
2p	-	-	-	-	-	-
2q	-	-	-	-	-	-
2r	-	-	-	-	-	-
2s	10	16	13	-	18	16
2t	14	17	23	13	22	32
2u	8	14	18	-	16 pi	16
2v	-	-	-	-	-	-
2w	13	14	14	-	15	16
2x	8	-	-	-	-	-
4a	-	-	-	-	-	-
4b	-	-	-	-	-	-
4c	-	-	-	-	-	-
5	-	-	-	-	-	-
CLO	24	24	25	28	36	26
Control (DMSO)	-	-	-	-	-	-

Pi = partial inhibition. - = no inhibition. CLO = Clotrimazole antifungal standard. AUMC = Assiut University Mycological Center

Table 3 Antibacterial activity of compounds (**2a-x**) and chloramphenicol (inhibition zone in mm and MICs given in brackets at 100 μ mol/mL)

Comp No.	<i>S.</i> <i>aureus</i>	<i>B.</i> <i>cereus</i>	<i>E.</i> <i>coli</i>	<i>P.</i> <i>aeruginosa</i>	<i>S.</i> <i>marcescens</i>
2a	8(50)	10(3.125)	-	-	-
2b	8(25)	12(100)	-	13(100)	-
2c	8(12.5)	9(6.25)	8(6.25)	8(6.25)	10(50)
2d	8(100)	10(0.78)	-	-	-
2e	8(3.125)	8(12.5)	10(50)	10(50)	11(50)
2g	10(25)	11(25)	8(25)	10(50)	10(50)
2i	10(50)	12(100)	8(50)	-	-
2m	8(50)	10(50)	8(50)	-	16(100) p.i
2n	8(100)	-	12(100)	13(100)	-
2o	10(25)	-	11(100)	-	-
2s	8(25)	12(50)	8(25)	14(100)	-
2t	8(50)	12(50)	9(25)	8(50)	9(50)
2u	10(100)	-	10(100)	10(100)	-
2v	10(50)	-	-	-	-
2w	11(100)	8(6.25)	8(50)	-	-
2x	10(12.5)	-	-	-	-
CHL	10(0.39)	12(0.19)	11(1.56)	10(6.25)	14(3.125)

Pi = partial inhibition. - = no inhibition. CHL=Chloramphenicol as antibacterial standard. AUMC = Assiut University Mycology Center

Table 4 Antifungal activity of compounds (**2a-x**) and Clotrimazole (inhibition zone in mm and MICs given in brackets at 100 μ mol/mL)

Comp No.	<i>C. albicans</i>	<i>G. candidum</i>	<i>F. oxysporum</i>	<i>A. flavus</i>	<i>T. rubrum</i>	<i>S. brevicaulis</i>
2a	8(50)	8(25)	8(12.5)	8(100)	8(12.5)	12(100)
2c	8(25)	8(3.125)	10(6.25)	12(100)	8(3.125)	8(25)
2d	8(12.5)	8(3.125)	10(6.25)	-	8(3.125)	16(50) p.i
2e	10(12.5)	10(6.25)	10(3.125)	13(100)	10(3.125)	8(25)
2f	10(25)	-	-	-	-	-
2g	8(12.5)	8(12.5)	10(25)	14(100)	10(25)	10(25)
2h	12(100)	8(50)	8(25)	-	10(50)	8(25)
2i	-	10(100)	10(100)	-	16(100) p.i	8(50)
2k	8(100)	-	-	-	-	-
2m	14(100)	10(50)	10(50)	12(100)	10(50)	20(100)
2n	-	10(12.5)	8(50)	-	-	14(50)
2o	13(100)	8(25)	10(100)	-	-	13(50)
2s	10(100)	12(12.5)	13(100)	-	13(50)	16(100)
2t	10(50)	12(25)	12(25)	13(100)	8(25)	11(25)
2u	8(100)	10(12.5)	10(50)	-	8(50)	23(50) p.i
2w	8(50)	8(6.25)	8(25)	-	12(50)	8(50)
2x	8(25)	-	-	-	-	-
CLO	12(1.56)	12(0.39)	15(0.39)	18(0.05)	10(0.05)	24(1.56) p.i

Pi = partial inhibition. - = no inhibition. CLO = Clotrimazole antifungal standard. AUMC = Assiut University Mycological Center

Table 5 Percentage of edema inhibition of compounds (**2a-e**, **2o**, **2t-x**) and indomethacin on carrageenan induced paw edema in rats

Compd. No.	Edema inhibition (%) \pm SE					
	0.5 h	1 h	2 h	3 h	4 h	5 h
Negative control	--	--	--	--	--	--
Indomethacin	16.07 \pm 0.29 [*]	35.76 \pm 0.00 ^{**}	58.03 \pm 0.17 ^{**}	75.69 \pm 0.29 ^{**}	88.26 \pm 0.17 ^{**}	89.39 \pm 0.17 ^{**}
2a	8.15 \pm 0.17 ^{***}	28.69 \pm 0.17 ^{**}	41.97 \pm 0.29 ^{**}	75.69 \pm 0.29 ^{**}	74.04 \pm 0.17 ^{**}	83.01 \pm 0.17 ^{**}
2b	8.15 \pm 0.17 ^{***}	28.69 \pm 0.17 ^{**}	41.97 \pm 0.17 ^{**}	67.59 \pm 0.00 ^{**}	81.13 \pm 0.17 ^{**}	83.01 \pm 0.17 ^{**}
2c	11.99 \pm 0.33 [*]	32.12 \pm 0.44 ^{**}	51.64 \pm 0.29 ^{**}	64.83 \pm 0.17 ^{**}	83.40 \pm 0.17 ^{**}	85.06 \pm 0.17 ^{**}
2d	0.00 \pm 0.17 ^{****}	14.35 \pm 0.00 [*]	25.91 \pm 0.17 ^{**}	46.03 \pm 0.17 ^{**}	57.44 \pm 0.29 ^{**}	65.90 \pm 0.33 ^{**}
2e	4.08 \pm 0.00 ^{****}	17.99 \pm 0.17 ^{**}	32.30 \pm 0.29 ^{**}	51.38 \pm 0.00 ^{**}	64.54 \pm 0.29 ^{**}	72.28 \pm 0.17 ^{**}
2o	8.15 \pm 0.02 ^{**}	21.41 \pm 0.02 ^{**}	45.26 \pm 0.02 ^{**}	59.48 \pm 0.03 ^{**}	71.63 \pm 0.03 ^{**}	78.67 \pm 0.02 ^{**}
2t	4.08 \pm 0.00 ^{****}	14.35 \pm 0.00 [*]	25.91 \pm 0.17 ^{**}	54.13 \pm 0.17 ^{**}	64.53 \pm 0.29 ^{**}	68.07 \pm 0.29 ^{**}
2u	4.08 \pm 0.00 ^{****}	17.99 \pm 0.17 ^{**}	32.30 \pm 0.29 ^{**}	48.62 \pm 0.20 ^{**}	59.86 \pm 0.17 ^{**}	68.07 \pm 0.29 ^{**}
2v	16.07 \pm 0.00 [*]	35.76 \pm 0.00 ^{**}	58.03 \pm 0.50 ^{**}	67.59 \pm 0.29 ^{**}	78.72 \pm 0.29 ^{**}	87.23 \pm 0.29 ^{**}
2w	4.08 \pm 0.00 ^{****}	17.99 \pm 0.17 ^{**}	32.30 \pm 0.29 ^{**}	48.62 \pm 0.17 ^{**}	64.53 \pm 0.29 ^{**}	65.90 \pm 0.33 ^{**}
2x	4.08 \pm 0.00 ^{****}	17.99 \pm 0.17 ^{**}	32.30 \pm 0.29 ^{**}	46.03 \pm 0.17 ^{**}	59.86 \pm 0.17 ^{**}	68.07 \pm 0.29 ⁸⁸

Significant difference at ^{*} $p < 0.01$ versus control value; ^{**} $p < 0.001$ versus control value; ^{***} $p < 0.05$ versus control value; non significantly different at ^{****} $p > 0.05$ versus control value (student's test)

Table 6 Analgesic activity of compounds (**2a-c**, **2e**, **2o** and **2v**) and aspirin using the hot-plate test

Comp no.	The average Reaction time (s) at different time intervals after compound administration \pm SE					
	0.5 h	1h	2 h	3 h	4 h	5 h
Control	24.00 \pm 2.08	25.00 \pm 1.73	22.67 \pm 1.20	22.67 \pm 1.20	23.33 \pm 1.20	23.00 \pm 1.15
Aspirin	26.33 \pm 3.18 [*]	40.00 \pm 2.89 ^{**}	68.00 \pm 4.73 ^{**}	82.67 \pm 2.73 ^{**}	91.67 \pm 4.06 ^{**}	94.00 \pm 3.22 ^{**}
2a	24.33 \pm 2.40 [*]	25.33 \pm 2.40 [*]	28.00 \pm 2.08 [*]	29.00 \pm 1.53 [*]	26.33 \pm 1.76 [*]	27.33 \pm 1.45 [*]
2b	24.00 \pm 1.53 [*]	28.00 \pm 1.53 [*]	29.33 \pm 1.45 [*]	31.00 \pm 2.30 [*]	31.33 \pm 1.86 [*]	31.33 \pm 1.86 [*]
2c	25.00 \pm 2.08 [*]	26.33 \pm 1.86 [*]	30.33 \pm 2.91 [*]	25.33 \pm 2.96 [*]	29.00 \pm 2.08 [*]	32.33 \pm 1.76 [*]
2e	38.00 \pm 3.79 ^{***}	45.33 \pm 3.18 ^{**}	60.67 \pm 5.61 ^{**}	57.00 \pm 4.73 ^{**}	69.00 \pm 3.51 ^{**}	79.00 \pm 0.58 ^{**}
2o	17.00 \pm 1.16 [*]	21.00 \pm 0.58 [*]	25.33 \pm 2.60 [*]	38.33 \pm 1.67 ^{**}	57.00 \pm 2.00 ^{**}	55.33 \pm 3.38 ^{**}
2v	25.33 \pm 2.60 [*]	40.00 \pm 2.89 ^{**}	68.00 \pm 2.08 ^{**}	75.67 \pm 2.40 ^{**}	71.67 \pm 4.80 ^{**}	77.33 \pm 3.28 ^{**}

Significantly different at ^{**} $p < 0.001$ versus control value; ^{***} $p < 0.01$ versus control value; Non significantly different at ^{*} $p > 0.05$ versus control value (student's test)