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# Synthesis, analgesic, anti-inflammatory, and antimicrobial activity of some novel pyrimido[4,5-*b*]quinolin-4-ones

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**Abstract**—Novel series of pyrimido[4,5-*b*]quinolines (**3a–c**), triazolo[4′,3′:1,2]pyrimido[4,5-*b*]-quinolines (**7a–e**, **9**, and **14**), tetrazolo [4′,3′:1,2]pyrimido[4,5-*b*]quinolin-5-one (**13**), [1,3]-pyrazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolines (**12a** and **12b**), and 2-pyrazolyl-pyrimido[4,5-*b*]-quinolines (**15**, **16a**, **16b**, and **19**) have been synthesized. Some of the new compounds were tested against various bacteria and fungi species. In addition, the analgesic and anti-inflammatory activities are reported. Compounds **8** and **9a** possess high activity toward the fungi as compared with the reference drug Nystatin. The tested compounds **5** and **8** have moderate anti-inflammatory activities. Moreover compounds **5**, **8**, **10**, and **16a**, have activities higher than the reference drug in peripheral analgesic activity testing, Compounds **5**, **7a**, **11a**, and **16a** have potencies as the reference drug in central analgesic activity testing.


## 1. Introduction

Since resistance to antimicrobial drugs is widespread, there is an increasing need for identification of novel structure leads that may be of use in designing new, potent, and less toxic antimicrobial agents. *Imiquimod*, *Cli-oquinol*, and *Enoxacin* are antiviral, antifungal, and antibacterial drugs which contain pyrimidine and quinoline rings. Pyrimidoquinoline nuclei have been a source of great interest to organic, medicinal and materials scientists over many years, which is present in a number of biologically active organic compounds[1–6] which exhibit, antibacterial,[7] anticancer,[8] and medicinal,[9,10] anti-inflammatory activities.[11,12] Moreover, the increasing biological importance of pyrimidoquinoline derivatives particularly in the field of chemotherapy, prompted us to develop and identify the new molecules as triazolo[4′,3′:1,2]pyrimido-[4,5-*b*]quinoline, tetrazolo[4′,3′:1,2] pyrimido[4,5-*b*]quinolin-5-one, and [1,3]pyrazolo-[3′,2′:1,2]pyrimido[4,5-*b*]quinoline derived from pyrimido [4,5-*b*]quinolin-4-ones in order to investigate the effect of such structural variation on the anticipated antimicrobial, anti-inflammatory, and analgesic activities.

## 2. Results and discussion

The present work is an extension of our studies on pyridopyrimidone derivatives and their behavior as functional and bifunctional groups.[12–16] We now wish to report the synthesis of various types of functionalized heterocyclic derivatives and study their biological activities. Thus, heating under reflux of 6-aminothiouracil **2** with α,β-unsaturated ketones **1** gave 5-aryl-2-thioxo-2,3,6,7,8,9-hexahydro-1*H*,4*H*-pyrimido[4,5-*b*]quinolin-4-ones (**3a–c**) and the non-oxidized form 2-thioxo-5,10-dihydro-1*H*,4*H*-pyrimido[4,5-*b*]quinolin-4-ones **3′a–c** were isolated (Method A). The cyclo-condensation process depending on the reaction conditions, the prolonged reaction time in refluxing dimethylformamide furnishes the pure oxidation forms (**3a–c**) in good yield. Also, the latter pyrimidopyrimidines **3a–c** were obtained by the cyclo-condensation reaction of 6-aminothiouracil (**2**) with cyclohexanone and aldehyde (one-pot synthesis) Method B, as shown in Scheme 1.

Beside the correct values in elemental analyses and spectral data of structures **3** and **3′** as shown in Section 3, structure **3** was established chemically by investigating its reactivity toward various reagents. Alkylation of an ethanolic potassium hydroxide solution of **3a** with methyl iodide yielded 5-phenyl-2-methylthio-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**4**). Also, compounds **3a** and **4** gaves 2-hydrazino-5-phenyl-2,3,6,7,8,

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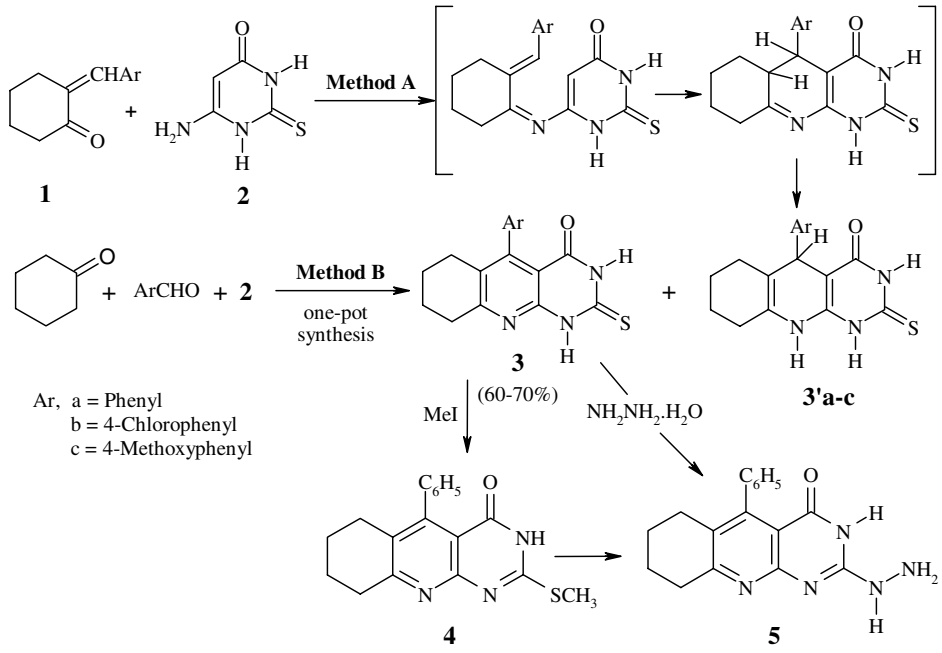



**Scheme 1.**

9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (**5**), with the evolution of hydrogen sulfide or methylmercaptan, upon treatment with hydrazine hydrate as shown in Scheme 1.

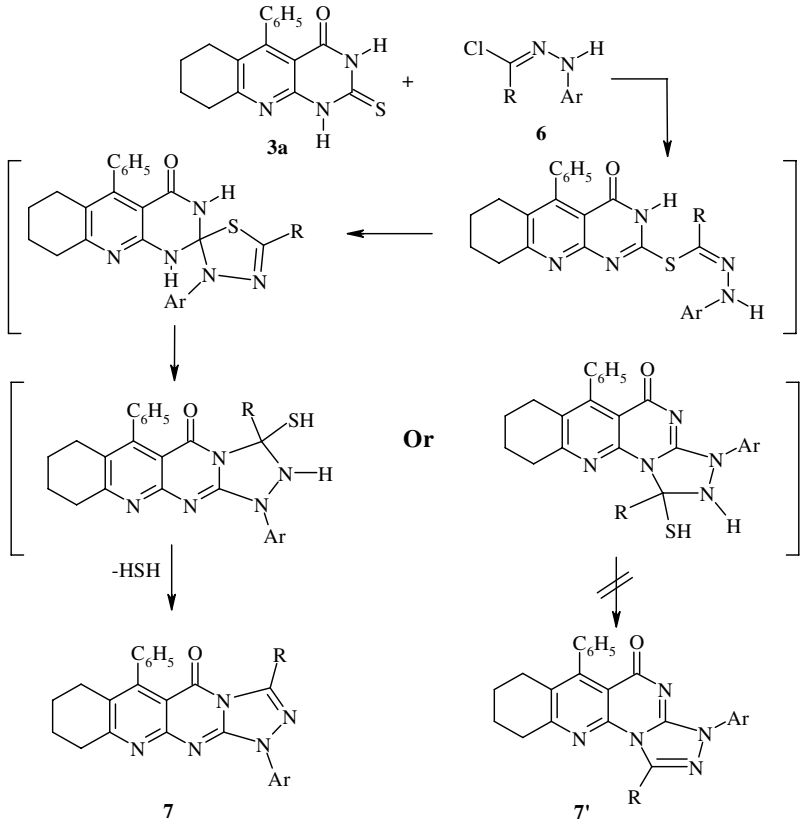
Meanwhile, stirring compound **3a** under reflux with hydrazonoyl chlorides (**6a**–**e**)[17,18] in dry chloroform with a few drops of triethylamine for long time (TLC) afforded 6-phenyl-7,8,9,10-tetrahydro-1,3-substituted-triazolo[4′,3′:1, 2]pyrimido[4,5-*b*]quinolin-5-one derivatives (**7a**–**f**) rather than the isomeric structures **7′** via removal of $H_2S$ as shown in Scheme 2. Structure **7** as shown in Scheme 2 was preferred on the basis of the literature that pyrimidines and fused pyrimidines underwent cyclization at the N-neighboring to the carbonyl group (N-3 not N-1). Moreover, the infrared spectrum indicated that the absorption peak for the carbonyl group (imide) in position 4 shifted by 15–20 $cm^{-1}$ more than that for amide.[19] $^1H$ and $^{13}C$ NMR spectral data. Thus, $^1H$ NMR spectrum for the compound **7a**, as an example, showed signals at $\delta$ 1.72–1.74 (m, 2H, $CH_2$), 1.87–1.89 (m, 2H, $CH_2$), 2.33–2.38 (t, 2H, $CH_2$), 3.13–3.18 (t, 2H, $CH_2$), 7.10–7.25 (m, 2H, phenyl), 7.32–7.54 (m, 9H, phenyl), 7.65–7.68 (m, 2H, phenyl), and 8.38–8.41 (m, 2H, phenyl). The reaction mechanism may proceed as shown in Scheme 2.

Moreover, 2-hydrazino **5** could be considered as a starting material for the synthesis of new polynuclear heterocycles such as azolo-pyrimidoquinolines, pyrimidoquinoline-as-triazines as well as the synthesis of some pyrazolopyrimido-qinolone derivatives. Thus, heating compound **5** with aliphatic acids, namely formic or acetic acid, resulted in the formation of triazolo[4′,3′:1,2]-pyrimido[4,5-*b*]quinoline (**9a** and **9b**), as shown in Scheme 3. On the other hand, heating compound **5** with acetic acid for 3 h yielded 2-acetyl-hydrazino derivative **8**, which on further heating with acetic acid for long time gave **9b**. Also, the 2-hydrazino derivative **5** reacted with potassium thiocyanate in boiling acetic acid to give 3-amino-triazolo[4′, 3′:1,2]pyrimido[4,5-*b*]quinoline **10** (Scheme 3).

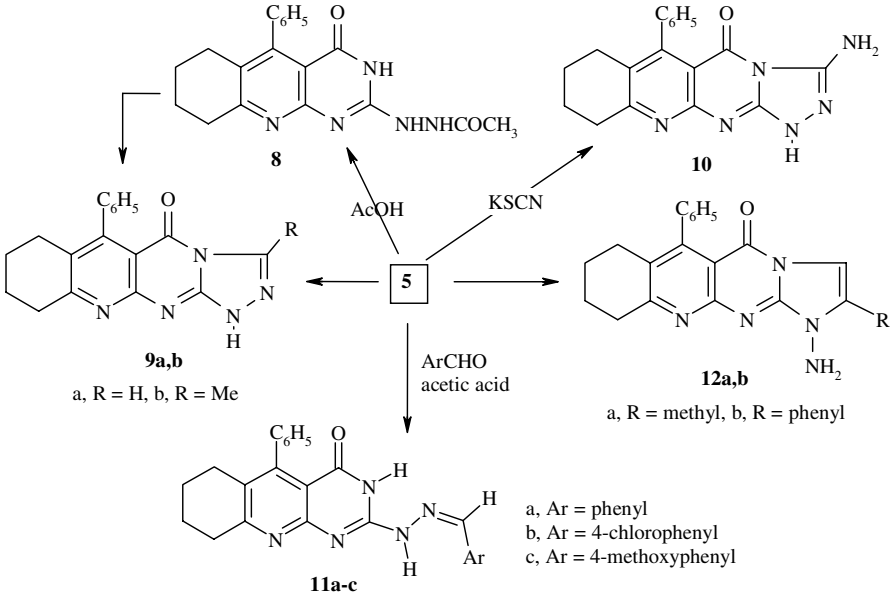
Beside the correct values in elemental analyses, the spectral data of **10** are in agreement with the assigned structure. The latter reaction of 2-hydrazino with potassium thiocyanate may be accomplished via the intermediates, as shown in the considered mechanism (Scheme 4). Also, compound **5** gave the 2-arylmethylene hydrazone derivatives **11a**–**c** when treated with the appropriate aldehyde in boiling glacial acetic acid (Scheme 3).

The reaction of **5** with α-haloketones namely, chloroacetone or phenacyl bromide in dry xylene, yielded the respective [1,3]pyrazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolines (**12a** and **12b**) as shown in Scheme 3. $^1H$ NMR spectrum of **12a**, as an example, showed signals at $\delta$ 1.65–1.66 (m, 2H, $CH_2$), 1.80–1.82 (m, 2H, $CH_2$), 2.22–2.23 (t, 2H, $CH_2$), 2.97–2.99 (t, 2H, $CH_2$), 3.23 (s, 3H, $CH_3$), 5.62 (br s, $NH_2$, $D_2O$ exchangeable), 7.06–7.08 (m, 2H, aromatic protons), 7.35–7.45 [m, 3H aromatic protons), and 8.10 (s, 1H, pyrazolyl proton). Also, its IR spectrum displayed absorption bands at 3420 and 1687 $cm^{-1}$ corresponding to (NH) and (CO) groups, respectively.

Treatment of **5** with nitrous acid at 0 °C led to the formation of 6-phenyl-7,8,9,10-tetrahydrotetrazolo[4′, 3′:1,2]pyrimido[4,5-*b*]quinolin-5-one (**13**) (Scheme 5), which was in equilibrium with the 2-azido-pyrimido[4,5-*b*]pyrimidine (**13′**) tautomer. The IR spectrum of **13** displayed absorption bands at 3243 (NH) and 1700 $cm^{-1}$ (CO) and characteristic absorption band for the azido group[20] at 2320 $cm^{-1}$. Aminopyrimidines are reported in the literature for its biological activities as



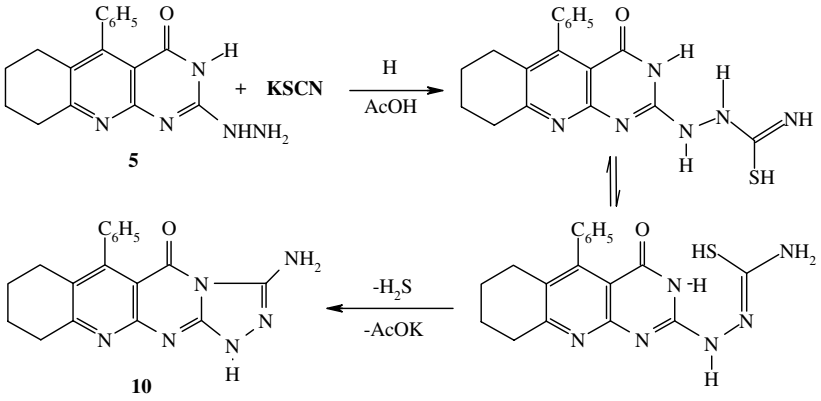

**Scheme 2.**



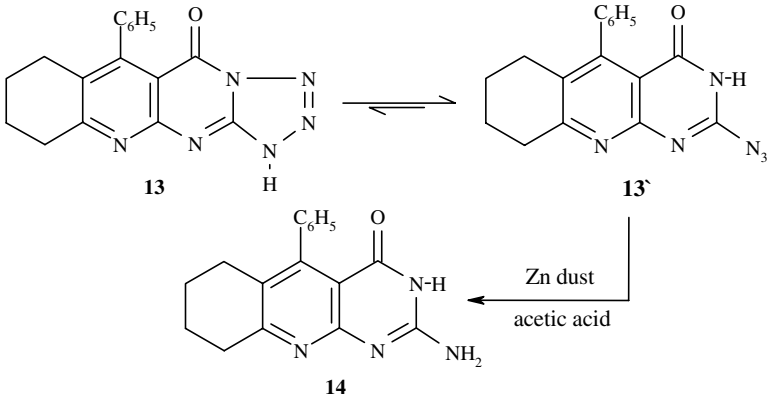

**Scheme 3.**

anticancer, antibacterial, and antimalarial.[19] Therefore, compound **13** was reduced into 2-aminopyrimido[4,5-*b*]-pyrimidine (**14**) by zinc dust and acetic acid.

The 2-hydrazino derivative **5** reacted with β-diketones, β-ketoesters and β-cyano-esters to form 2-(1-pyrazolyl) derivatives. Thus, heating compound **5** with each of eth-




**Scheme 4.**




**Scheme 5.**

ylcyanoacetane, pentane-2,4-dione, and/or 3-chloropentane-2,4-dione yielded the respective 2-(pyrazol-5-one-1-yl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (**15**, **16a**, and **16b**) (Scheme 6).

Trials to synthesize the pyrimidotriazine derivative **17′** by treatment of **5** with chloro acetylchloride in anhydrous dioxane were not successful. Instead the 2-chloroacetyl-hydrazino derivatives **17** were obtained (Scheme 6). Beside the correct values in elemental analyses, the spectral data (IR, $^1$H NMR, Mass spectra) of **17** are in agreement with the assigned structure (see Section 3). Finally, compound **5** condensed with ethyl acetoacetate, upon heating in boiling ethanol, to afford 5-phenyl-2-(ethyl acetoacetatehydrazone)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (**18**), which could be cyclized either by prolonged heating in ethanol or by heating in ethanolic sodium ethoxide solution to give 5-phenyl-2-(3-methyl-4*H*-pyrazol-5-one-1-yl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (**19**).

### 2.1. Antimicrobial activity

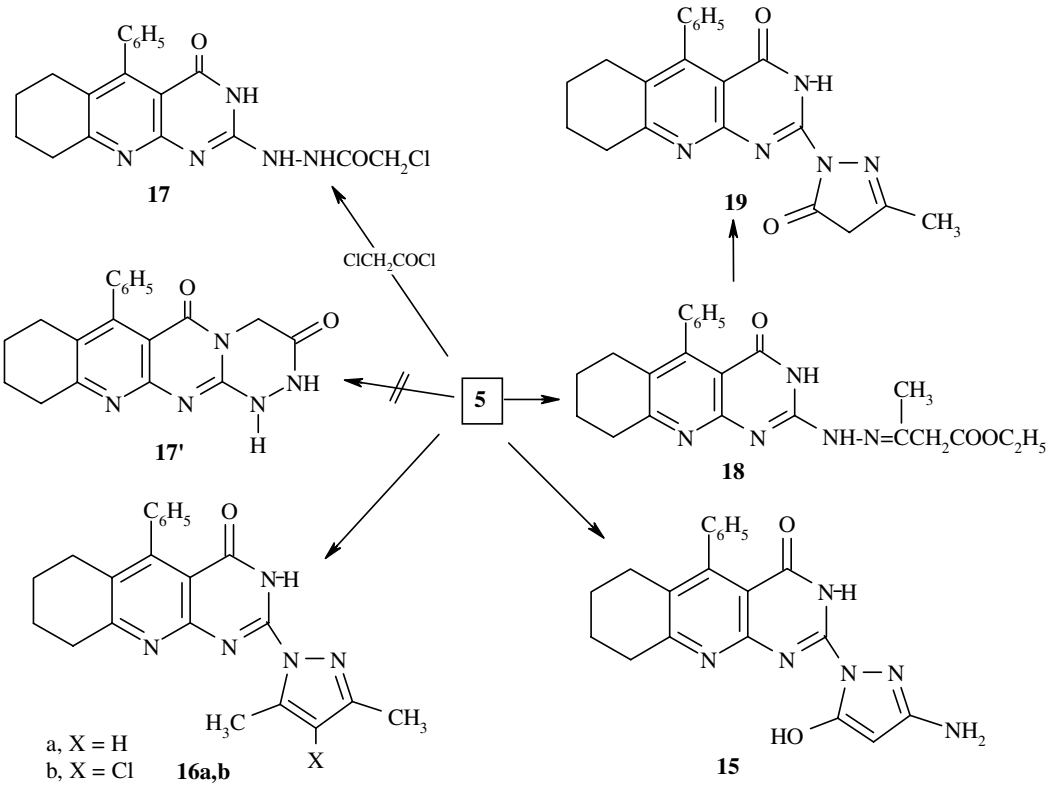
From the data obtained in Table 1, it is clear that all the tested compounds were found to be active against G −ve) and G (+ve) bacteria compared to the reference drug Nalidixic acid. On the other hand, compounds **8** and **9b** possess high activity toward the fungi as compared with the reference drug Nystatin, while the rest of the compounds showed no activity toward the fungi. The MIC (minimum inhibitory concentration) of the most active compounds toward the micro-organisms showed that the MICs ranged between 20 and 30 μg/disk. The activity is tested at concentration of 50 μg/disk. The minimum inhibitory concentrations (MIC) for compounds with high activity **8**, **9b**, **10**, **12b**, **13**, and **16a** are presented in Table 2.

### 2.2. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenin-induced paw edema test in rats. The Anti-inflammatory activity data (Table 3) indicated that, compounds **8** and **5** show significant anti-inflammatory activity in descending order in comparison to the control group, with 50% and 40% potency with respect to indomethacin. Compounds **16a** and **10** have no anti-inflammatory activity.

### 2.3. Analgesic activity

Test for analgesic activity was performed by Turner[21] and Collier[22] technique using Swiss albino mice. The results of analgesic activity (Table 4) indicated that some of the tested compounds show central analgesic activity (Hot-plate test), especially compounds **11a**, **5**, **7a**, **16a**,




**Scheme 6.**

**Table 1.** Preliminary antimicrobial activity test for tested compounds

| Compound | Microorganism inhibition zone diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Gram −ve bacteria | | | Gram +ve bacteria | Fungi | |
| | *Escherichia coli* | *Klebsiella pneumonia* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Candida albicans* | *Candida gabrata* |
| **13** | 18 | 16 | 22 | 17 | — | — |
| **16a** | 19 | 20 | 22 | 21 | 9 | 9 |
| **9b** | 20 | 20 | 21 | 21 | 18 | 13 |
| **8** | 20 | 18 | 22 | 19 | 17 | 16 |
| **3a** | 12 | 14 | 15 | 13 | — | — |
| **5** | 13 | 13 | 15 | 11 | — | — |
| **10** | 20 | 22 | 22 | 21 | — | — |
| **3b** | 13 | 14 | 14 | 13 | — | — |
| **12b** | 21 | 22 | 23 | 19 | — | — |
| **11a** | 15 | 18 | 14 | 16 | 7 | 7 |
| Nystastin | — | — | — | — | 20 | 19 |
| Nalidixic acid | 24 | 25 | 25 | 25 | — | — |

Inhibition zone, 6–10 mm slight activity, 11–15 mm moderate activity, more than 15 mm high activity.

**Table 2.** The minimum inhibitory concentration (MIC)

| Compound | *Escherichia coli* | *Klebsiella pneumonia* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Candida albicans* |
|---|---|---|---|---|---|
| **13** | 30 | 30 | 30 | 30 | — |
| **16a** | 30 | 30 | 30 | 30 | — |
| **9b** | 20 | 20 | 20 | 20 | 50 |
| **8** | 20 | 20 | 20 | 20 | 50 |
| **10** | 30 | 30 | 30 | 30 | — |
| **12b** | 20 | 20 | 20 | 20 | — |

**3a**, **12b**, **9b** and **13** in descending order, where compound **11a** showed activity equal to acetylsalicylic acid. Compounds **10** and **8** have no central analgesic activity. Also, Table 5 shows that, compounds **8**, **16a**, **10**, **5**, **12b**, **3a**, and **11a** were found to have significant peripheral analgesic activity in descending order, where the first four

**Table 3.** Acute inflammation in rat using Plethysmometer

| Group | 1 h | | 2 h | | 3 h | | 4 h | |
|---|---|---|---|---|---|---|---|---|
| | Edema rate (%) | Pot. (%) | Edema rate (%) | Pot. (%) | Edema rate (%) | Pot. (%) | Edema rate (%) | Pot. (%) |
| Control | 34.75 ± 3.12[b] | 0 | 52.96 ± 1.87[b] | 0 | 81.77 ± 3.28[b] | 0 | 91.57 ± 4.53[b] | 0 |
| **16a** | 31.08 ± 1.84[b] (10.5) | 14.5 | 52.49 ± 1.88 [b] (0.9) | 1.5 | 81.73 ± 4.11[b] (0.1) | 0.1 | 90.86 ± 2.96[b] (0.8) | 1.7 |
| **8** | 25.73 ± 1.24[ab] (25.9) | 45.5 | 40.89 ± 3.08[ab] (22.8) | 39.2 | 62.25 ± 4.18[ab] (23.9) | 55.9 | 71.61 ± 3.33[ab] (21.8) | 46.9 |
| **5** | 24.15 ± 0.89[ab] (30.5) | 53.5 | 41.95 ± 2.00 [b] (20.8) | 35.7 | 63.96 ± 1.91[ab] (21.8) | 51.1 | 74.52 ± 3.29[ab] (18.6) | 40.1 |
| **10** | 31.23 ± 2.46[b] (10.1) | 17.7 | 52.31 ± 4.79 [b] (1.2) | 2.1 | 79.47 ± 2.33[b] (2.8) | 6.6 | 82.88 ± 1.87[b] (9.5) | 20.5 |
| Indom-ethacin | 14.95 ± 0.93[a] (56.9) | 100 | 22.15 ± 0.80[a] (58.2) | 100 | 46.91 ± 5.00[a] (42.6) | 100 | 49.07 ± 2.74[a] (46.4) | 100 |

Values represent means ± SE of six animals for each group.
Each value in parentheses indicates the percentage inhibition rate.
The potency (pot.) was calculated compared to the reference drug indomethacin.
[a] $P < 0.05$, statistically significant from control. One-way ANOVA (Dunnett's test).
[b] $P < 0.05$, statistically significant from indomethacin. One-way ANOVA (Dunnett's test).

**Table 4.** Central analgesic activity (hot-plate test)

| Group | Reaction time (s) | | | |
|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min |
| Control | 6.23 ± 0.31 | 7.10 ± 0.36[b] | 7.60 ± 0.41[b] | 8.51 ± 0.40[b] |
| **7a** | 8.16 ± 0.56 | 9.84 ± 0.28[a] | 11.62 ± 0.57[b] | 11.88 ± 0.47[b] |
| **13** | 6.90 ± 0.62 | 9.16 ± 0.57[a] | 10.78 ± 0.45[a] | 10.08 ± 0.24[b] |
| **11a** | 7.24 ± 0.59 | 10.08 ± 0.78[a] | 12.52 ± 0.82[a] | 12.68 ± 0.61[a] |
| **12b** | 7.40 ± 0.37 | 9.42 ± 0.45[a] | 11.41 ± 0.29[a] | 10.67 ± 0.59[ab] |
| **9b** | 6.26 ± 0.41 | 8.68 ± 0.48 | 8.80 ± 0.52 | 10.40 ± 0.18[b] |
| **5** | 7.64 ± 0.20 | 7.54 ± 0.26[b] | 10.61 ± 0.60[a] | 12.00 ± 0.36[a] |
| **10** | 5.38 ± 0.30 | 5.14 ± 0.26[b] | 6.74 ± 0.28[b] | 7.62 ± 0.60[b] |
| **8** | 6.42 ± 0.62 | 8.62 ± 0.34 | 8.06 ± 0.56[b] | 9.22 ± 0.47[b] |
| **16a** | 7.08 ± 0.92 | 8.93 ± 0.74 | 10.15 ± 1.20 | 11.50 ± 0.40[a] |
| **3a** | 7.08 ± 0.12 | 8.65 ± 0.87 | 10.98 ± 0.91[a] | 11.12 ± 0.75[ab] |
| Acetyl salicylic acid | 6.49 ± 0.40 | 10.03 ± 0.12[a] | 11.39 ± 0.53[a] | 13.15 ± 0.38[a] |

Values represent means ± SE of five animals for each group.
[a] $P < 0.05$, statistically significant from control. One-way ANOVA (Dunnett's test).
[b] $P < 0.05$, statistically significant from ASA. One-way ANOVA (Dunnett's test).

**Table 5.** Peripheral analgesic activity (Writhing test)

| Group | No. of writhes/ 20 min | Protection (%) | Potency (%) |
|---|---|---|---|
| Control | 48.0 ± 2.24[b] | 0 | 0 |
| **7a** | 45.8 ± 2.91[b] | 4.6 | 8.89 |
| **13** | 41.3 ± 2.39[b] | 14.0 | 27.07 |
| **11a** | 31.3 ± 2.48[ab] | 34.9 | 67.51 |
| **12b** | 30.2 ± 1.69[a] | 37.1 | 71.76 |
| **9b** | 45.2 ± 1.77[b] | 5.8 | 11.2 |
| **5** | 22.6 ± 1.96[a] | 52.9 | 102.32 |
| **10** | 20.6 ± 1.81[a] | 57.1 | 110.44 |
| **8** | 19.0 ± 1.18[a] | 60.4 | 116.83 |
| **16a** | 19.6 ± 0.51[a] | 59.2 | 114.51 |
| **3a** | 31.0 ± 1.58[ab] | 35.4 | 68.47 |
| Acetylsalicylic acid | 23.2 ± 1.36[a] | 51.7 | 100 |

Values represent means ± SE of six animals for each group.
[a] $P < 0.05$, statistically significant from control. One-way ANOVA (Dunnett's test).
[b] $P < 0.05$, statistically significant from aspirin. One-way ANOVA (Dunnett's test).

compounds showed activity superior to acetylsalicylic acid.

These findings represent an important advantage to compounds **5** and **8** as anti-inflammatory with high analgesic activity equal to that of acetylsalicylic acid.

## 3. Experimental

### 3.1. Chemistry

Melting points were determined on griffin apparatus. The $^1$H NMR and $^{13}$C NMR spectra were recorded on JEOL EX-270 run for HNMR at 270 MHz and run for CNMR at 67.5 MHz: or on JEOL ECA-500 run for HNMR at 500 MHz and run for CNMR at 125 MHz and JEOL JNM-LA-400 FT NMR Spectrometer (Universität Konstanz, Germany) and chemical shifts were expressed as $\delta$ values against $Si(CH_3)_4$ as internal standard. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1430 spectrometer (National Research Centre and Department of Chemistry, Cairo University). Mass Spectra were run at 70 eV on HP-5988A Mass spectrometer, Micro-analytical Centre, Cairo University. The biological activities were screened in Pharmacological Unit, National Research Centre, Egypt.

**3.1.1. 5-Aryl-2-thioxo-2,3,5,6,7,8,9,10-octahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-ones (3′a–c): General procedure.** A mixture of compound **1** (0.01 mol) and 6-aminothiouracil (**2**) (0.01 mol) was refluxed in 50 mL dimethylformamide for 8–10 h (under TLC control).

The reaction mixture was cooled, poured onto water (100 mL). The precipitate was filtered off, dried, and crystallized from the appropriate solvent to produce **3′a–c**.

**3.1.1.1. 5-Phenyl-2-thioxo-2,3,5,6,7,8,9,10-octahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (3′a).** The compound was obtained from **1a** (0.01 mol), as a pale yellow powder, crystallized from ethanol in a 63% yield, mp 225–228 °C (melted); IR, $cm^{-1}$; 3400 (br s, NHs), 3031 (CH aryl), 2923 (CH alkyl), 1685 (CO), 1625 (C=N). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.53–1.67 (m, 2H, $CH_2$), 1.73–1.82 (m, 2H, $CH_2$), 2.22 (t, 2H, $CH_2$), 2.78 (t, 2H, $CH_2$), 5.43 (s, 1H, H-C5), 7.07–7.09 (m, 2H, phenyl), 7.32–7.39 (m, 3H, phenyl) and 8.60, 10.05, 11.20 (3br s, 3NH, $D_2O$ exchangeable); MS *m/z* 311 ($M^+$, 100); Anal. $C_{17}H_{17}N_3OS$ (311.4); requires: C, 65.56; H, 5.50; N, 13.49. Found: C, 65.49; H, 5.51; N, 13.42.

**3.1.1.2. 5-(4-Chlorophenyl)-2-thioxo-2,3,5,6,7,8,9,10-octahydro-1*H*-pyrimido[4,5-*b*]-quinolin-4-one (3′b).** This compound was obtained from **1b** (0.01 mol), as a yellow powder, crystallized from ethanol in a 71% yield, mp 213–215 °C; IR, $cm^{-1}$; 3380 (br s, NHs), 3026 (CH aryl), 2918 (CH alkyl), 1681 (CO), 1610 (C=N). $^1H$ NMR (DMSO-$d_6$, $\delta$, ppm); 1.51–1.60 (m, 2H, $CH_2$), 1.63–1.74 (m, 2H, $CH_2$), 2.19 (t, 2H, $CH_2$), 2.85 (t, 2H, $CH_2$), 5.49 (s, 1H, H-C5), 7.24 (d, 2H, phenyl, $J$ = 8.4 Hz), 7.49 (d, 2H, phenyl, $J$ = 8.6 Hz), and 8.00, 10.80, 11.55 (3br s, 3NH, $D_2O$ exchangeable); MS *m/z* 345 ($M^+$, 100); Anal. $C_{17}H_{16}ClN_3OS$ (345.8); requires: C, 59.03; H, 4.66; N, 12.15. Found: C, 59.05; H, 4.63; N, 12.16.

**3.1.1.3. 5-(4-Methoxyphenyl)-2-thioxo-2,3,5,6,7,8,9,10-octahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (3′c).** This compound was obtained from **1c** (0.01 mol), as a yellow powder, crystallized from ethanol in a 67% yield, mp 184–187 °C (melted); IR, $cm^{-1}$; 3390 (br s, NHs), 3029 (CH aryl), 2934 (CH alkyl), 1682 (CO), 1620 (C=N); 1.48–1.56 (m, 2H, $CH_2$), 1.60–1.74 (m, 2H, $CH_2$), 2.17 (t, 2H, $CH_2$), 2.86 (t, 2H, $CH_2$), 3.86 (s, 3H, $OCH_3$), 5.45 (s, 1H, H-C5), 7.25 (d, 2H, phenyl, $J$ = 8.5 Hz), 7.58 (d, 2H, phenyl, $J$ = 8.4 Hz), and 8.20, 11.30, 12.00 (3br s, 3NH, $D_2O$ exchangeable); MS *m/z* 341 ($M^+$, 100); Anal. $C_{18}H_{19}N_3O_2S$ (341.4); requires: C, 63.32; H, 5.61; N, 12.31. Found: C, 63.29; H, 5.59; N, 12.27.

### 3.1.2. 5-Aryl-2-thioxo-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-ones (3a–c): General procedure

**3.1.2.1. Method A.** A mixture of compound **1** (0.01 mol) and 6-aminothiouracil (**2**) (0.01 mol) was refluxed in 50 mL dimethylformamide for 20–30 h (under TLC control). The reaction mixture was cooled; the deposited precipitate was filtered off, washed with ethanol and dried, and crystallized from the appropriate solvent to produce **3a–c** in good yield.

**3.1.2.2. Method B.** A mixture of benzaldehyde (0.01 mol), cyclohexanone (0.01 mol), and 6-aminothiouracil (**2**) (0.01 mol) was refluxed in 50 mL dimethylformamide, over 50 h (under TLC control). The reaction mixture was cooled; the precipitate was filtered off, washed with ethanol and dried, and crystallized from dimethylformamide.

**3.1.2.3. 5-Phenyl-2-thioxo-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (3a).** The compound was obtained from **1a** (0.01 mol), as a yellow powder, crystallized from ethanol/dioxane (2:1) in a 75% yield, mp 310 °C (melted); IR, $cm^{-1}$; 3370 (br s, NH), 3047 (CH aryl), 2919 (CH alkyl), 1688 (CO), 1615 (C=N). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.59–1.66 (m, 2H, $CH_2$), 1.75–1.81 (m, 2H, $CH_2$), 2.21 (t, 2H, $CH_2$), 2.90 (t, 2H, $CH_2$), 7.07–7.09 (m, 2H, phenyl), 7.32–7.39 (m, 3H, phenyl), and 11.20, 12.12 (2br s, 2NH, $D_2O$ exchangeable); $^{13}C$ NMR (DMSO-$d_6$, $\delta$ ppm); 21.98, 26.38, 33.07, 39.06 (4C, 4$CH_2$), 107.6, 127.0, 127.1, 127.1, 127.9, 137.4, 149.8, 151.5, 158.5 (9C, $sp^2$ carbon atoms with two asymmetric carbon atoms) and 163.4 (CO), 175.5 (CS); MS *m/z* 309 ($M^+$, 100); Anal. $C_{17}H_{15}N_3OS$ (309.4); requires: C, 65.99; H, 4.89; N, 13.58. Found: C, 66.03; H, 4.90; N, 13.62.

**3.1.2.4. 5-(4-Chlorophenyl)-2-thioxo-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (3b).** This compound was obtained from **1b** (0.01 mol), as a yellow powder, crystallized from ethanol in a 75% yield, mp 324 °C; IR, $cm^{-1}$; 3345 (br s, NH), 3037 (CH aryl), 2920 (CH alkyl), 1687 (CO), 1608 (C=N). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.50–1.60 (m, 2H, $CH_2$), 1.61–1.76 (m, 2H, $CH_2$), 2.18 (t, 2H, $CH_2$), 2.88 (t, 2H, $CH_2$), 7.13 (d, 2H, phenyl, $J$ = 8.6 Hz), 7.47 (d, 2H, phenyl, $J$ = 8.6 Hz), and 8.21, 12.22 (2br s, 2NH, $D_2O$ exchangeable); MS *m/z* 345 ($M^+$ + 2,23), 334 ($M^+$ + 1,29), 343 ($M^+$, 100); Anal. $C_{17}H_{14}ClN_3OS$ (343.8); requires: C, 59.38; H, 4.10; N, 12.22. Found: C, 59.41; H, 4.15; N, 12.26.

**3.1.2.5. 5-(4-Methoxyphenyl)-2-thioxo-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (3c).** This compound was obtained from **1c** (0.01 mol), as a pale yellow powder, crystallized from ethanol/dioxane (2:1) in a 75% yield, mp 320 °C (melted); IR, $cm^{-1}$; 3400 (br s, NH), 3019 (CH aryl), 2921 (CH alkyl), 1683 (CO), 1603(C=N); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.52–1.61 (m, 2H, $CH_2$), 1.63–1.79 (m, 2H, $CH_2$), 2.23 (t, 2H, $CH_2$), 2.91 (t, 2H, $CH_2$), 3.89 (s, 3H, $OCH_3$), 7.23 (d, 2H, phenyl, $J$ = 8.4 Hz), 7.50 (d, 2H, phenyl, $J$ = 8.5 Hz), and 8.10, 11.30 (2br s, 2NH, $D_2O$ exchangeable); MS *m/z* 339 ($M^+$, 100); Anal. $C_{18}H_{17}N_3O_2S$ (339.4); requires: C, 63.89; H, 5.05; N, 12.38. Found: C, 63.92; H, 5.10; N, 12.39.

### 3.1.3. 5-Phenyl-2-Methylthio-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (4).

To a warm ethanolic potassium hydroxide solution (prepared by dissolving 0.01 mol of potassium hydroxide in 30 mL absolute ethanol) was added compound **3a** (0.01 mol), the heating was continued for 30 min, the mixture was allowed to cool to room temperature and methyl-iodide (0.12 mol) was added. The mixture was stirred under reflux for 3 h, cooled to room temperature, and poured onto cold water (100 mL). The solid precipitated was filtered off, washed with water and dried. The compound was obtained as yellow crystals, crystallized from ethanol in an 87% yield,

mp 250 °C (melted); IR, $cm^{-1}$; 3354 (br s, NH), 3039 (CH aryl), 2918 (CH alkyl), 1687 (CO), 1629 (C=N); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.50–1.60 (m, 2H, $CH_2$), 1.61–1.76 (m, 2H, $CH_2$), 2.18 (t, 2H, $CH_2$), 2.52 (s, 3H, S-$CH_3$), 2.88 (t, 2H, $CH_2$), 7.11–7.14 (m, 2H, phenyl), 7.39–7.46 (m, 3H, phenyl) and 9.45 (br s, NH, $D_2O$ exchangeable); MS *m/z* 323 ($M^+$, 100); Anal. $C_{18}H_{17}N_3OS$ (323.4); requires: C, 66.85; H, 5.30; N, 12.99. Found: C, 66.89; H, 5.36; N, 13.02.

**3.1.4. 2-Hydrazino-5-phenyl-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (5).** A suspension of compound **3a**/or **4** (0.01 mol) in hydrazine hydrate (99–100%) (25 mL) was stirred under gentle reflux. The insoluble solid went into solution within 10 min with copious evolution of methyl-mercaptan to form a clear solution. After 30 min heating was continued for 8 h and the reaction mixture was allowed to cool to room temperature. The solid which separated was filtered, washed with ethanol, and dried to produce **5**, as a yellow powder, crystallized from DMF in an 80% yield, mp 290 °C (melted); IR, $cm^{-1}$; 3443 (br s, NH), 3034 (CH aryl), 2905 (CH alkyl), 1689 (CO), 1635 (C=N). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.57–1.61 (m, 2H, $CH_2$), 1.64–1.73 (m, 2H, $CH_2$), 2.30 (t, 2H, $CH_2$), 2.90 (t, 2H, $CH_2$), 5.15 (br s, $NH_2$, $D_2O$ exchangeable), 7.07–7.09 (m, 2H, phenyl), 7.32–7.39 (m, 3H, phenyl) and 8.10 (2br s, 2NH, $D_2O$ exchangeable). $^{13}C$ NMR; 21.98, 26.38, 33.07, 39.06 (4C, 4$CH_2$), 107.6, 127.0, 127.2, 127.3, 127.9, 129.8, 137.4, 149.8, 151.5, 158.5 (10C, $sp^2$ carbon atoms with two asymmetric) and 161.7 (CO); MS *m/z* 307 ($M^+$, 100); Anal. $C_{17}H_{17}N_5O$ (307.3); Requires: C, 66.43; H, 5.58; N, 22.79. Found: C, 66.50; H, 5.60; N, 22.65.

**3.1.5. 6-Phenyl-7,8,9,10-tetrahydro-1,3-substituted-triazolo[4′,3′:1,2]pyrimido[4,5-*b*]-quinolin-5-one (7a–e): General procedure.** A mixture of compound **3a** (0.01 mol), the appropriate hydrazonoyl chloride (**6a**–**e**) (0.01 mol) was stirred under reflux in dry chloroform (30 mL) in the presence of four drops of triethylamine for 5 h. The solvent was evaporated under reduced pressure. The solid produced was washed three times by 30 mL methanol and crystallized from the appropriate solvent to produce (**7a**–**e**) in high yields.

**3.1.5.1. 1,3,6-Triphenyl-7,8,9,10-tetrahydro-triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinol-in-5-one (7a).** It was obtained from **3a** (0.01 mol) and *N*-phenylbenzene-carbohydrazonoyl chloride **6a** (0.01 mol), as white needles, crystallized from benzene in a 65% yield, 320 °C (melted); IR, $cm^{-1}$; 3046 (CH aryl), 2920 (CH alkyl), 1687 (CO), 1611 (C=N), 1596 (C=C). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.72–1.74 (m, 2H, $CH_2$), 1.87–1.89 (m, 2H, $CH_2$), 2.33–2.38 (t, 2H, $CH_2$), 3.13–3.18 (t, 2H, $CH_2$), 7.10–7.25 (m, 2H, phenyl), 7.32–7.54 (m, 9H, phenyl), 7.65–7.68 (m, 2H, phenyl), 8.38–8.41 (m, 2H, phenyl); MS *m/z* 469 ($M^+$, 100); Anal. $C_{30}H_{23}N_5O$ (469.5); requires: C, 76.74; H, 4.94; N, 14.92. Found: C, 76.70; H, 4.96; N, 14.88.

**3.1.5.2. 3-Acetyl-1-(4-chlorophenyl)-6-phenyl-7,8,9,10-tetrahydrotriazolo[4′,3′:1,2]-pyrimido[4,5-*b*]quinolin-5-one (7b).** It was obtained from **3a** (0.01 mol) and 2-oxo-*N*-(4-chlorophenyl)-propane hydrazonoyl chloride **6b** (0.01 mol), as a brown powder, crystallized from benzene in a 65% yield, mp 330 °C (melted); IR, $cm^{-1}$; 3032 (CH aryl), 2923 (CH alkyl), 1682 (CO), 1580 (C=N), 1556 (C=C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.69–1.74 (m, 2H, $CH_2$), 1.83–1.88 (m, 2H, $CH_2$), 2.31–2.37 (t, 2H, $CH_2$), 2.63 (s, 3H, $CH_3$), 3.11–3.17 (t, 2H, $CH_2$), 7.10–7.19 (m, 2H, phenyl), 7.26–7.37 (m, 3H, phenyl), 7.46 (d, 2H, phenyl, $J$ = 8.4 Hz), 7.71 (d, 2H, phenyl, $J$ = 8.4 Hz); MS *m/z* 471 ($M^+$ + 2,26), 470 ($M^+$ + 1,27), 469 ($M^+$, 100); Anal. $C_{26}H_{20}ClN_5O_2$ (469.9); requires: C, 66.45; H, 4.29; N, 14.90. Found: C, 66.48; H, 4.31; N, 14.87.

**3.1.5.3. 3-Acetyl-1-(4-nitroxyphenyl)-6-phenyl-7,8,9,10-tetrahydro-triazolo[4′,3′:1,2]-pyrimido[4,5-*b*]quinolin-5-one (7c).** It was obtained from **3a** (0.01 mol) and 2-oxo-*N*-(4-nitrophenyl)-propane hydrazonoyl chloride **6c** (0.01 mol), as a brown powder, crystallized from benzene in a 68% yield, mp 270 °C (melted); IR, $cm^{-1}$; 3040 (CH aryl), 2918 (CH alkyl), 1687 (CO), 1600 (C=N), 1587 (C=C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.70–1.76 (m, 2H, $CH_2$), 1.85–1.91 (m, 2H, $CH_2$), 2.33–2.38 (t, 2H, $CH_2$), 2.66 (s, 3H, $CH_3$), 3.14–3.19 (t, 2H, $CH_2$), 7.08–7.16 (m, 2H, phenyl), 7.22–7.35 (m, 3H, phenyl), 7.43 (d, 2H, phenyl, $J$ = 8.3 Hz), 7.73 (d, 2H, phenyl, $J$ = 8.5 Hz); MS *m/z* 480 ($M^+$, 78); Anal. $C_{26}H_{20}N_6O_4$ (480.5); requires: C, 64.99; H, 4.19; N, 17.49. Found: C, 65.95; H, 4.23; N, 17.52.

**3.1.5.4. 3-Ethyl-(1,6-diphenyl-7,8,9,10-tetrahydro-triazolo[4′,3′:1,2]pyrimido-[4,5-*b*]-quinolin-5-one)acetate (7d).** It was obtained from **3a** (0.01 mol) and chloro-(phenylhydrazono)-ethylacetate **6d** (0.01 mol), as a white powder, crystallized from benzene in a 66% yield, mp 270 °C (melted); IR, $cm^{-1}$; 3039 (CH aryl), 2913 (CH aliphatic), 1689 (CO), 1588 (C=N), 1553 (C=C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.34 (t, 3H, $CH_3$, $J$ = 7.0 Hz), 1.73–1.76 (m, 2H, $CH_2$), 1.85–1.88 (m, 2H, $CH_2$), 2.31–2.36 (t, 2H, $CH_2$), $\delta$ 3.15–3.18 (t, 2H, $CH_2$), 4.02 (q, 2H, $CH_2$, $J$ = 7.0 Hz), 7.15–7.29 (m, 2H, phenyl), 7.34–7.56 (m, 4H, phenyl), 7.67–7.72 (m, 2H, phenyl), 8.21–8.30 (m, 2H, phenyl); Anal. $C_{27}H_{23}N_5O_3$ (465.4); requires: C, 69.67; H, 4.98; N, 15.05. Found: C, 69.80; H, 4.89; N, 15.08.

**3.1.5.5. 3-Ethyl-[1-(4-tolyl)-6-phenyl-7,8,9,10-tetrahydrotriazolo[4′,3′:1,2]pyrimido-[4,5-*b*]quinolin-5-one]acetate (7e).** It was obtained from **3a** (0.01 mol) and chloro-(4-tolylhydrazono)-ethylacetate **6e** (0.01 mol), as a brown powder, crystallized from benzene in a 63% yield, mp 280 °C (melted); IR, $cm^{-1}$; 3029 (CH aryl), 2923 (CH aliphatic), 1685 (CO), 1596 (C=N), 1543 (C=C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.30 (t, 3H, $CH_3$, $J$ = 7.2 Hz), 1.69–1.75 (m, 2H, $CH_2$), 1.83–1.89 (m, 2H, $CH_2$), 2.30–2.38 (t, 2H, $CH_2$), 2.40 (s, 3H, $CH_3$), $\delta$ 3.17–3.20 (t, 2H, $CH_2$), 4.03 (q, 2H, $CH_2$, $J$ = 7.1 Hz), 7.15–7.29 (m, 2H, phenyl), 7.34 (d, 2H, phenyl, $J$ = 8.4 *Hz*), 7.40–7.45 (m, 3H, phenyl), 7.75 (d, 2H, phenyl, $J$ = 8.5 Hz), MS *m/z* 479 ($M^+$, 100); Anal. $C_{28}H_{25}N_5O_3$ (479.5); requires: C, 70.13; H, 5.25; N, 14.60. Found: C, 70.21; H, 5.29; N, 14.54.

**3.1.6. 2-Acetylhydrazido-2,3,6,7,8,9-hexahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (8).** A solution of compound **5** (0.01 mol) in glacial acetic acid was refluxed for 3 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL), the solid soformed was collected by filtration, dried and crystallized from ethanol (dark yellow powder) in a 75% yield, mp 230–232 °C (melted); IR, $cm^{-1}$; 3389, 3100 (br s, 2NH), 2922 (CH alkyl), 1709 (CO), 1686 (CO), 1612 (C=N). The $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.74–1.76 (m, 2H, $CH_2$), 1.90–1.95 (m, 2H, $CH_2$), 2.37–2.39 (t, 2H, $CH_2$), 3.00 (s, 1H, $CH_3$), 3.07–3.08 (t, 2H, $CH_2$), 7.06–7.30 (m, 3H, phenyl), 7.32–7.41 (m, 2H, phenyl), 8.94, 9.05, 10.98 (3br s, 3NH, $D_2O$ exchangeable); MS *m*/*z* 349 ($M^+$, 100), 334 ($M^+$–$CH_3$, 56). Anal. $C_{19}H_{19}N_5O_2$ (349.4); requires: C, 65.30; H, 5.48; N, 20.04. Found: C, 65.26; H, 5.51; N, 20.11.

**3.1.7. 6-Phenyl-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-5-one (9a).** A mixture of **5** (0.01 mol), formic acid (10 mL), and 2 mL of concentrated hydrochloric acid was heated under reflux for 6 h. The reaction mixture was allowed to cool to room temperature and poured onto water (100 mL). The formed solid was collected by filtration, washed with ethanol (20 mL), dried, and crystallized from ethanol, orange powder, in a 67% yield, mp 212–214 °C (melted); IR, $cm^{-1}$; 3350 (br s, NH), 2980 (CH alkyl), 1685 (CO), 1580 (C=N), 1500 (C=C). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.64–1.69 (m, 2H, $CH_2$), 1.82–1.87 (m, 2H, $CH_2$), 2.18–2.24 (t, 2H, $CH_2$), 2.94–2.99 (t, 2H, $CH_2$), 7.09–7.15 (m, 2H, phenyl), 7.33–7.40 (m, 3H, phenyl), 8.10 (s, 1H, azomethine proton), and 9.45 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 317 ($M^+$, 80); Anal. $C_{18}H_{15}N_5O$ (317.3); requires: C, 68.12; H, 4.76; N, 22.07. Found: C, 68.11; H, 4.73; N, 22.03.

**3.1.8. 3-Methyl-6-phenyl-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]-quinolin-5-one (9b).** A mixture of **5** (0.01 mol) and glacial acetic acid (30 mL) was stirred under reflux for 6 h (TLC). The reaction mixture was allowed to cool to room temperature and then poured onto water (100 mL). The solid formed was collected by filtration, washed with ethanol (20 mL), dried, and crystallized from ethanol, orange powder, in a 67% yield, mp 310–313 °C (melted); IR, $cm^{-1}$; 3316 (br s, NH), 2986 (CH alkyl), 1678 (CO), 1583 (C=N), 1507 (C=C). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.63–1.67 (m, 2H, $CH_2$), 1.80–1.86 (m, 2H, $CH_2$), 2.19–2.23 (t, 2H, $CH_2$), 2.95–3.00 (t, 2H, $CH_2$), 3.25 (s, 3H, $CH_3$), 7.05–7.13 (m, 2H, phenyl), 7.35–7.41 (m, 3H, phenyl) and 9.05 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 331 ($M^+$, 100); Anal. $C_{19}H_{17}N_5O$ (331.37); requires: C, 68.86; H, 5.17; N, 21.14. Found: C, 68.83; H, 5.12; N, 21.09.

**3.1.9. 3-Amino-6-phenyl-7,8,9,10-tetrahydro[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*]-quinolin-5-one (10).** A mixture of **5** (0.01 mol) and potassium thiocyanate (0.15 mol) was heated under reflux in acetic acid for 6 h. The reaction mixture was allowed to cool to room temperature and poured onto water. The precipitate formed was collected by filtration, dried and crystallized from ethanol/dioxane (2:1) as a yellow powder in a 68% yield, mp 223–225 °C (melted); IR, $cm^{-1}$; 3412 (NH) and 1678 (CO). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.68–1.73 (m, 2H, $CH_2$), 1.80–1.89 (m, 2H, $CH_2$), 2.37–2.39 (t, 2H, $CH_2$), 2.99–3.06 (t, 2H, $CH_2$), 7.06–7.08 (m, 2H, phenyl), 7.30 (br s, $NH_2$, $D_2O$ exchangeable), 7.44–7.45 (m, 3H, phenyl), 8.45 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 332 ($M^+$, 82); Anal. $C_{18}H_{16}N_6O$ (332.4); requires: C, 65.04; H, 4.85; N, 25.29. Found: C, 65.11; H, 4.90; N, 25.35.

**3.1.10. 5-Aryl-2-arylmethylenehydrazone-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]-quinolin-4-one (11a–c): General procedure.** A mixture of **5** (0.01 mol), the appropriate aromatic aldehyde (0.01 mol), and anhydrous sodium acetate (0.02 mol) was stirred under reflux in glacial acetic acid (30 mL) for 30 min. The reaction mixture was allowed to cool to room temperature, whereby the formed solid was filtered off and crystallized from appropriate solvent to produce **11a**–**c** in high yield.

**3.1.10.1. 5-Phenyl-2-phenylmethylenehydrazone-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]quino-lin-4-one (11a).** It was obtained from compound **5** (0.01 mol) and benzaldehyde (0.01 mol) as pale yellow crystals, crystallized from acetic acid in an 85% yield, mp 308–310 °C (melted); IR, $cm^{-1}$; 3250 (br s, NH), 3040 (CH aryl), 2920 (CH alkyl), 1670 (CO), 1600 (C=N), 1500 (C=C), $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.69–1.72 (m, 2H, $CH_2$), 1.83–1.90 (m, 2H, $CH_2$), 2.30–2.31 (t, 2H, $CH_2$), 2.99–3.01 (t, 2H, $CH_2$), 7.12–7.24 (m, 2H, phenyl), 7.37–7.43 (m, 8H, phenyl) and 7.60, 10.85 (2br s, 2NH, $D_2O$ exchangeable), 8.30 (s, 1H, azomethine proton); MS *m*/*z* 395 ($M^+$, 76); Anal. $C_{24}H_{21}N_5O$ (395.4); requires: C, 72.89.63; H, 5.35; N, 17.71. Found: C, 72.86; H, 5.40; N, 17.75.

**3.1.10.2. 2-(4-Chlorophenylmethylenehydrazone-5-phenyl-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (11b).** It was obtained from compound **5** (0.01 mol) and 4-chlorobenzaldehyde (0.01 mol) as pale yellow crystals, crystallized from acetic acid in an 83% yield, mp 320–322 °C (melted); IR, $cm^{-1}$; 3368 (br s, NH), 3044 (CH aryl), 2916 (CH alkyl), 1676 (CO), 1605 (C=N), 1517 (C=C), $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.61–1.62 (m, 2H, $CH_2$), 1.77–1.90 (m, 2H, $CH_2$), 2.18 (t, 2H, $CH_2$), 2.86–2.90 (t, 2H, $CH_2$), 7.08–7.09 (m, 2H, phenyl), 7.39–7.43 (m, 3H, phenyl, $J = 8.4$ Hz), 7.89–7.92 (d, 2H, phenyl, $J = 8.5$ Hz), 8.03–8.05 (d, 2H, phenyl) 8.12 (s, 1H, azomethine proton) and 10.83, 11.64 (br s, 2NH, $D_2O$ exchangeable); MS *m*/*z* 431 ($M^+ + 2$, 19), 430 ($M^+ + 1$,27), 429 ($M^+$, 89); Anal. $C_{24}H_{20}ClN_5O$ (429.9); requires: C, 67.05; H, 4.69; N, 16.29. Found: C, 67.11; H, 4.72; N, 16.34.

**3.1.10.3. 2-(4-Methoxyphenylmethylenehydrazone-5-phenyl-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (11c).** It was obtained from compound **5** (0.01 mol) and 4-methoxybenzaldehyde (10 m mol) as a yellow powder, crystallized from acetic acid in an 81% yield, mp 306–308 °C (melted); IR, $cm^{-1}$; 3368 (br s, NH), 3044 (CH aryl), 2916 (CH alkyl), 1676 (CO), 1605 (C=N), 1517 (C=C), $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.67–1.68 (m, 2H, $CH_2$), 1.83–1.89 (m, 2H, $CH_2$),

2.28–2.29 (t, 2H, $CH_2$), 2.96–2.98 (t, 2H, $CH_2$), 3.72 (s, 3H, $OCH_3$), 7.09–7.10 (m, 2H, phenyl), 7.10–7.24 (d, 2H, phenyl, $J = 8.6$ Hz), 7.40–7.42 (m, 3H, phenyl), 7.52–7.61 (d, 2H, phenyl, $J = 8.6$ Hz), 7.65 (s, 1H, azomethine proton) and 8.25, 11.28 (br s, 2NH, $D_2O$ exchangeable); Anal. $C_{25}H_{23}N_5O_2$ (425.5); Requires: C, 70.57; H, 5.45; N, 16.46. Found: C, 70.52; H, 5.47; N, 16.50.

**3.1.11. 7-Phenyl-3-(methyl/or phenyl)-8,9,10,11-tetrahydro[1,3]pyrazolo[3′,2′:1,2]-pyrimido[4,5-*b*]quinolin-6-one (12a and 12b): General procedure.** A mixture of compound **5** (0.01 mol) and chloroacetone or phenacylbromide (0.01 mol) was heated under reflux for 5 h in 30 mL of dry xylene. The solid that separated upon cooling was filtered off and crystallized from appropriate solvent to produce **13a** and **13b** in high yield.

**3.1.11.1. 7-Phenyl-3-methyl-8,9,10,11-tetrahydro[1,3]pyrazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolin-6-one (12a).** It was obtained from compound **5** (0.01 mol) and chloroacetone (0.01 mol); as white crystals, crystallized from ethanol in a 58% yield, mp 350–353 °C (dec); IR, $cm^{-1}$; 3420 (br s, NH), 2950 (CH alkyl), 1687 (CO), 1600 (C═N), 1543 (C═C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm); 1.63–1.69 (m, 2H, $CH_2$), 1.80–1.85 (m, 2H, $CH_2$), 2.21–2.26 (t, 2H, $CH_2$), 2.97–2.99 (t, 2H, $CH_2$), 3.23 (s, 3H, $CH_3$), 5.62 (br s, $NH_2$, $D_2O$ exchangeable), 7.06–7.08 (m, 2H, phenyl), 7.35–7.45 (m, 3H, phenyl), 8.10 (s, 1H, pyrazolyl proton); Anal. $C_{20}H_{19}N_5O$ (345.4); requires: C, 69.54; H, 5.54; N, 20.28. Found: C, 69.49; H, 5.58; N, 20.31.

**3.1.11.2. 3,7-Diphenyl-3-phenyl-8,9,10,11-tetrahydro[1,3]pyrazolo[3′,2′:1,2]pyrimi-do[4,5-*b*]quinolin-6-one (12b).** It was obtained from compound **5** (0.01 mol) and phenacylbromide (0.01 mol), as a yellow powder, crystallized from ethanol in a 55% yield, mp 297–300 °C (melted); IR, $cm^{-1}$; 3279 (br s, NH), 3039 (CH aryl), 2916 (CH alkyl), 1686 (CO), 1647 (C═N), 1600 (C═C), $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.65–1.69 (m, 2H, $CH_2$), 1.81–1.87 (m, 2H, $CH_2$), 2.21–2.25 (t, 2H, $CH_2$), 2.96–3.02 (t, 2H, $CH_2$), 7.06–7.08 (m, 2H, phenyl), 7.35–7.56 (m, 8H, phenyl), 8.13 (s, 1H, pyrazolyl proton), 11.30 (br s, $NH_2$, $D_2O$ exchangeable); MS *m*/*z* 407 ($M^+$, 100); Anal. $C_{25}H_{21}N_5O$ (407.5); requires: C, 73.69; H, 5.19; N, 17.19. Found: C, 73.65; H, 5.14; N, 17.15.

**3.1.12. 6-Phenyl-7,8,9,10-tetrahydrotetraazolo[4′,3′:1,2]pyrimido[4,5-*b*]quinolin-5-one (13).** To an ice-cold solution of compound **5** (0.01 mol) in acetic acid (10 mL) was added dropwise a solution of sodium nitrite (0.15 mol) in a least amount of water in an ice bath at −5 °C. The reaction mixture was allowed to stand overnight at room temperature, then poured onto water (100 mL). The solid precipitated was filtered off and crystallized from ethanol to produce **13** as yellow powder in a 50% yield, mp 330–332 °C (dec); IR, $cm^{-1}$; 3243 (br s, NH), 2934 (CH alkyl), 1700 (CO), 1629 (N═N), 1582 (C═N), 1506 (C═C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.73–1.75 (m, 2H, $CH_2$), 1.89–1.95 (m, 2H, $CH_2$), 2.37–2.45 (t, 2H, $CH_2$), 3.11–3.22 (t, 2H, $CH_2$), 7.08–7.14 (m, 2H, phenyl), 7.43–7.48 (m, 3H, phenyl), and 9.45 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 318 ($M^+$, 95); Anal. $C_{17}H_{14}N_6O$ (318.3); requires: C, 64.47; H, 4.43; N, 26.41. Found: C, 64.09; H, 4.41; N, 26.39.

**3.1.13. 2-Amino-5-phenyl-6,7,8,9-tetrahydro-pyrimido [4,5-*b*]quinolin-4-one (14).** To a well-stirred solution of the appropriate tetrazolothienopyrimidine **13** (0.01 mol) in glacial acetic acid (30 mL) was added portionwise activated zinc dust (5.00 g) at room temperature over a period of 30 min. Stirring was continued for additional 3 h. Thereafter the reaction mixture was heated on a water bath (80–90 °C) for 3 h. The progress of reduction was monitored by TLC. After allowing the reaction mixture to cool to room temperature, it was poured onto cold water (100 mL). The insoluble solid which separated was filtered, washed with water, and dried. The crude solid was extracted with hot diethyl ether and the solid obtained after the removal of ether under reduced pressure was crystallized from ethanol in a 45% yield, mp 220–222 °C (melted); IR, $cm^{-1}$; 3310 (br s, $NH_2$), 2910 (CH alkyl), 1687 (CO), 1589 (C═N), 1551 (C═C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.73–1.75 (m, 2H, $CH_2$), 1.89–1.95 (m, 2H, $CH_2$), 2.37–2.45 (t, 2H, $CH_2$), 3.11–3.22 (t, 2H, $CH_2$), 7.08–7.14 (m, 2H, phenyl), 7.43–7.48 (m, 3H, phenyl), and 11.48 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 292 ($M^+$, 74); Anal. $C_{17}H_{16}N_4O$ (292.3); requires: C, 69.84; H, 5.52; N, 19.16. Found: C, 69.80; H, 5.49; N, 19.09.

**3.1.14. 2-(3-Amino-5-hydroxy-4*H*-pyrazol-5-one-1-yl)-5-phenyl-6,7,8,9-tetrahydro-pyrimido[4,5-*b*]quinolin-4-one (15).** To a warm ethanolic sodium ethoxide solution (prepared by dissolving (0.01 mol) sodium metal in absolute ethanol (30 mL)) were added compound **5** (0.01 mol) and ethyl-cyanoacetate (0.01 mol). The mixture was stirred under reflux for 8 h, the reaction mixture was allowed to cool to room temperature, then poured onto cold water (100 mL), neutralized with acetic acid. The solid product precipitated was filtered off, washed with water, ethanol, dried, and crystallized from ethanol, pale yellow powder, in a 72% yield, mp330 °C (dec.); IR, $cm^{-1}$; 3318 (br s, NH), 2921 (CH alkyl), 1687 (CO), 1601 (C═N), 1520 (C═C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.52–1.54 (m, 2H, $CH_2$), 1.66–1.69 (m, 2H, $CH_2$), 2.10 (t, 2H, $CH_2$), 2.78–2.80 (t, 2H, $CH_2$), 3.55 (br s, 1H, OH coupled with $H_2O$ of DMSO), 5.10 (br s, $NH_2$, $D_2O$ exchangeable), 6.02 (s, 1H, pyrazolyl proton), 6.96–6.99 (m, 2H of phenyl), 7.31–7.36 (m, 3H, phenyl) and 9.10 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 374 ($M^+$, 100); Anal. $C_{20}H_{18}N_6O_2$ (374.4); requires: C, 64.16; H, 4.84; N, 22.45. Found: C, 64.19; H, 4.90; N, 22.42.

**3.1.15. 2-(3-Methyl-5-substituted-methyl-4-(un)substituted-pyrazol-1-yl)-5-phenyl-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (16a and 16b): General procedure.** A mixture of compound **5** (0.01 mol) and the β-diketone (0.01 mol) in absolute ethanol (30 mL) was stirred under reflux for 5 h. The reaction mixture was allowed to cool to 0 °C for 3 h, the deposited precipitate was filtered off, dried, and crystallized from an appropriate solvent to produce **16a** and **16b** in high yields.

**3.1.15.1. 2-(3,5-Dimethyl-pyrazol-1-yl)-5-phenyl-6,7,8,9-tetrahydropyrimido[4,5-*b*]-quinolin-4-one (16a).** It was obtained from the reaction of **5** (0.01 mol) with pentan-2,4-dione (0.01 mol), as pale light crystals, crystallized from ethanol in a 75% yield, mp 282–285 °C (melted); IR, $cm^{-1}$; 3300 (br s, NH), 3042 (CH aryl), 2937 (CH alkyl), 1694 (CO), 1629 (C=N), 1548 (C=C). $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): 1.72–1.73 (m, 2H, $CH_2$), 1.85–1.90 (m, 2H, $CH_2$), 2.22 (s, 3H, $CH_3$), 2.38–2.40 (t, 2H, $CH_2$), 2.86 (s, 3H, $CH_3$), 3.16–3.20 (t, 2H, $CH_2$), 6.05 (s, 1H, pyrazolyl proton), 7.11–7.12 (m, 2H, phenyl), 7.44–7.47 (m, 3H, phenyl), 10.23 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 371 ($M^+$, 100); Anal. $C_{22}H_{21}N_5O$ (371.4); requires: C, 71.14; H, 5.70; N, 18.85. Found: C, 71.20; H, 5.67; N, 18.90.

**3.1.15.2. 2-(3,5-Dimethyl-4-chloro-pyrazol-1-yl)-5-phenyl-6,7,8,9-tetrahydropyrimi-do[4,5-*b*]quinolin-4-one (16b).** It was obtained from the reaction of **5** with 3-chloropentan-2,4-dione (0.01 mol), as a light yellow powder and crystallized from ethanol in a 72% yield, 261–263 °C (melted); IR, $cm^{-1}$; 3286 (br s, NH), 3080 (CH aryl), 2939 (CH alkyl), 1678 (CO), 1605 (C=N), 1543 (C=C); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.64–1.70 (m, 2H, $CH_2$), 1.75–1.80 (m, 2H, $CH_2$), 2.25–2.27 (t, 2H, $CH_2$), 2.30 (s, 3H, $CH_3$), 2.48 (s, 3H, $CH_3$), 2.98–3.00 (t, 2H, $CH_2$), 7.10–7.11 (m, 2H, phenyl), 7.35–7.40 (m, 3H, phenyl), 11.82 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 407 ($M^+$ + 2, 33), 406 ($M^+$ + 1,25), 405 ($M^+$, 100); Anal. $C_{22}H_{20}ClN_5O$ (405.9); requires: C, 65.10; H, 4.97; N, 17.26. Found: C, 65.06; H, 4.93; N, 17.31.

**3.1.16. 2-Chloroacetylhydrazido-5-phenyl-6,7,8,9-tetrahydro-1*H*-pyrimido[4,5-*b*]quinolin-4-one (17).** A mixture of compound **5** (0.01 mol) and chloroacetyl chloride (0.01 mol) was gently heated on a water bath (60–80 °C) in dry dioxane (30 mL) for 6 h and the reaction mixture was allowed to cool to room temperature. The solid formed was filtered off, dried, and crystallized from ethanol in a 65% yield, mp 330 °C (dec.); IR, $cm^{-1}$; 3365 (br s, NH), 3043 (CH aryl), 2908 (CH alkyl), 1731, 1687 (2CO), 1630 (C=N); $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm): 1.64–1.65 (m, 2H, $CH_2$), 1.80–1.82 (m, 2H, $CH_2$), 2.23 (t, 2H, $CH_2$), 2.98–3.00 (t, 2H, $CH_2$), 3.25–3.28 (s, 2H, $CH_2$), 7.10–7.14(m, 2H, phenyl), 7.30–7.40 (m, 3H, phenyl), 9.45, 1.45, 11.45 (3br s, 3NH, $D_2O$ exchangeable); MS *m*/*z* 385 ($M^+$ + 2, 28), 384 ($M^+$ + 1,36), 383 ($M^+$, 100); Anal. $C_{19}H_{18}ClN_5O_2$ (383.8); requires: C, 59.45; H, 4.73; N, 18.25. Found: C, 59.43; H, 4.80; N, 18.12.

**3.1.17. 2-(Ethyl-5-phenyl-6,7,8,9-tetrahydropyrimido[4,5-*b*]-quinolin-4-one)acetate-hydrazone (18).** A mixture of compound **5** (0.01 mol) and ethyl acetoacetate (0.01 mol) was refluxed in absolute ethanol (30 mL) for 5 h. The reaction mixture was allowed to cool to room temperature and the solid precipitate produced was filtered off and crystallized from ethanol, a pale brown powder, in a 58% yield, mp 245 °C (melted); IR, $cm^{-1}$; 3250 (br s, NH), 2942 (CH alkyl), 1740, 1680 (2CO), 1580 (C=N), 1500 (C=C), $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.22–1.29 (t, 3H, $CH_3$, $J$ = 7.2 Hz), 1.63–1.68 (m, 2H, $CH_2$), 1.78–1.84 (m, 2H, $CH_2$), 2.03 (s, 3H, $CH_3$), 2.24–2.27 (t, 2H, $CH_2$), 2.96–3.02 (t, 2H, $CH_2$), 3.40 (s, 2H, $CH_2$), 4.09–4.14 (q, 2H, $CH_2$, $J$ = 7.2 Hz), 7.06–7.08 (m, 2H, phenyl), 7.36–7.41 (m, 3H, phenyl), 9.80–10.55 (2br s, 2NH, $D_2O$ exchangeable); MS *m*/*z* 419 ($M^+$, 65); Anal. $C_{23}H_{25}N_5O_3$ (419.5); requires: C, 65.85; H, 6.01; N, 16.70. Found: C, 65.83; H, 6.04; N, 16.87.

**3.1.18. 5-Phenyl-2-(3-methyl-4*H*-pyrazol-5-one-1-yl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (19)**

**3.1.18.1. Method A.** A solution of compound **5** (0.01 mol) and ethyl acetoacetate (0.01 mol) in sodium ethoxide solution (prepared by dissolving 0.01 mol of sodium metal in 30 mL absolute ethanol was heated under reflux with stirring for 6 h. The reaction mixture was allowed to cool and poured onto cold water (100 mL) and neutralized by acetic acid, whereby a solid was precipitated, which was filtered off and crystallized from ethanol to produce **19** as a yellow powder in a 62% yield, mp 330 °C (dec.).

**3.1.18.2. Method B.** A solution of compound **18** (0.01 mol) was heated under reflux with sodium ethoxide solution (prepared by dissolving (0.01 mol) of sodium metal in 30 mL absolute ethanol) for 3 h. The reaction mixture was allowed to cool, poured onto water 100 mL, and neutralized by acetic acid; the precipitate formed was filtered off and crystallized from ethanol in a 68% yield: IR (KBr, $cm^{-1}$); 3400 (br s, NH), 2936 (CH alkyl), 1698, 1684 (2CO), 1550 (C=N), 1500 (C=C), $^1H$ NMR (DMSO-$d_6$, $\delta$ ppm) 1.65–1.68 (m, 2H, $CH_2$), 1.80–1.86 (m, 2H, $CH_2$), 1.99 (s, 3H, $CH_3$), 2.20–2.24 (t, 2H, $CH_2$), 2.97–3.03 (t, 2H, $CH_2$), 4.53 (s, 2H, $CH_2$), 7.03–7.08 (m, 2H, phenyl), 7.33–7.40 (m, 3H, phenyl), 10.3 (br s, NH, $D_2O$ exchangeable); MS *m*/*z* 373 ($M^+$, 100); Anal. $C_{21}H_{19}N_5O_2$ (373.4); requires: C, 67.54; H, 5.13; N, 18.75. Found: C, 67.60; H, 5.25; N, 18.83.

### 3.2. Antimicrobial screening

The bacterial isolates representing Gram (−ve) and Gram (+ve) bacteria were recovered on Nutrient and Macconky agar. The two fungal isolates *Candida albicans* and *Candida glabrate* were isolated on Sabourauds dextrose agar (Oxoid). They are isolated from clinical samples and identified to the species level according to different API systems (Biomerilux). The selected compounds were tested in vitro using the agar disk diffusion method[23,24] taking Nalidixic acid and Nystatin as reference drugs for bacteria and fungi, respectively. The antimicrobial potentialities of the tested compounds were estimated by placing pre-sterilized filter paper disks (5 mm in diameter) impregnated with 50 μg/disk using dimethylsulfoxide (DMSO) as solvent which showed no inhibition zones. The inhibition zones of the tested compounds were measured after 24–28 h incubation at 37 °C for bacteria and at 28 °C after 5 days for fungi. The minimal inhibitory concentration (MIC) determination method of the biologically active compounds (Table 2) was applied using different concentrations per disk against G (−ve) and G (+ve) bacteria, and fungi. Reference Nystatin and Nalidixic acid disks were supplied by

Pasteur laboratory in Egypt of concentration 100 U and 30 μg, respectively.

### 3.3. Animals

Both sex of Swiss mice weighing 25–30 g used in analgesic activity and adulte male of Sprague-Dawley rats weighing between 150 and 180 g were used in anti-inflammatory activity, taking into account international principle and local regulations concerning the care and use of laboratory animals.[25] The animals had free access to standard commercial diet and water ad libitum and were kept in rooms maintained at 22 ± 1 °C with 12 h light dark cycle.

### 3.4. Anti-inflammatory effect

The anti-inflammatory testing was performed according to the method of Winter et al.[26] For this purpose, 36 rats weighing 150–180 g were used. Edema was induced in the left hand paw of all rats by subcutaneous (sc) injection of 0.1 ml of 1% (w/v) carrageenin in distilled water into their footpads. Rats were divided into six groups of six mice each. The 1st group was kept as control and was given the respective volume of the solvent (10% v/v of Tween 80 in distilled water). The 2nd to 5rd groups were orally administered aqueous suspension of synthesized compounds at a dose of 15 mg/kg 1 h before carrageenin injection. The last group was administered indomethacin (Indocid®) in a dose of 10 mg/kg orally as a standard reference.[27] The paw volume of each rat was measured using Plethysmometer; before carrageenin injection and then hourly for 4 h post-administration of aqueous suspension of synthesized compounds. The edema rate and inhibition rate of each group were calculated as follows: Edema rate $(E)\% = V_t - V_c/V_c$, and inhibition rate $(I)\% = E_c - E_t/E_c$, where $V_c$ is the volume before carrageenin injection (ml), $V_t$ is the volume at $t$ h after carrageenin injection (ml), $E_c$ is the edema rate of control group, $E_t$ is the edema rate of treated group).

### 3.5. Analgesic effect

Experimental models used in this study were selected to investigate both centrally and peripherally mediated analgesic effects of the tested compounds. For this purpose; the acetic acid abdominal constriction method was used to elucidate the peripheral effect and the hot-plate test to reveal central activity of the tested compounds.

**3.5.1. Hot-plate test.** The experiment was carried out as described by *Turner*[21] using hot-plate apparatus, maintained at 53 ± 0.5 °C. Seventy-two mice were divided into 12 groups, six animals in each group. The reaction time of the mice to the thermal stimulus was the time interval between placing the animal in the hot plate and when it licked its hind paw or jumped. Reaction time was measured prior to aqueous suspension of synthesized compounds and drug treatment (0 min). Group I was kept as normal control and received the solvent (10% v/v Tween 80 in distilled water). The suspension of synthesized compounds in 10% v/v Tween 80 in distilled water was orally administered to mice of groups II to XI at doses of 15 mg/kg. Mice of group VI (reference) were orally treated with acetyl salicylic acid at a dose of 100 mg/kg. The reaction time was again measured at 15 min and repeated at 30, 60, and 90 min after treatment. To avoid tissue damage to the mice paws, cut-off time for the response to the thermal stimulus was set at 60 s. The reaction time was calculated for each synthesized compound and drug-treated group.

**3.5.2. Writhing test.** An acetic acid-induced abdominal constriction in mice (Writhing effect) was determined by the method described by Collier et al.[22] Seventy-two mice were divided into 12 equal groups and pre-treated as follows: Group I which served as a control orally received 10% v/v Tween 80 in distilled water. Groups II to XI received the aqueous suspension of synthesized compounds in 10% v/v Tween 80 in distilled water orally at dose 15 mg/kg. Group VI was orally received acetyl salicylic acid at a dose of 100 mg/kg. After 30 min, each mouse was administered 0.7% of an aqueous solution of acetic acid (10 ml/kg) intraperitoneally and the mice were then placed in transparent boxes for observation. The number of writhes was counted for 20 min after acetic acid injection. The number of writhes in each treated group was compared to that of a control un-treated group. The number of writhing and stretching was recorded and the percentage protection was calculated using the following ratio: (Control mean − Treated mean/Control mean) × 100.

### 3.6. Statistical analysis

All statistical analyses were done by SPSS version 10 by one-way ANOVA using Dunnett's test.

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## References and notes

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