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Original article

Synthesis of some new pyrimido[2′,1′:2,3]thiazolo[4,5-*b*]quinoxaline derivatives as anti-inflammatory and analgesic agents

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ABSTRACT

Treatment of 6-aminothiouracil (**1**) with 2,3-dichloroquinoxaline (**2**) in ethanol/TEA afforded 6-amino-2-(3-chloroquinoxalin-2-ylthio)pyrimidin-4(3*H*)-one (**3**), which was refluxed in DMF to give 2-aminopyrimido[2′,1′:2,3]thiazolo[4,5-*b*]quinoxaline-4-one (**4**). Compound **4** was utilized as a key intermediate for the synthesis of a new pyrimido[2′,1′:2,3]thiazolo[4,5-*b*]quinoxaline derivatives **5**–**14** *via* the reaction with 2-chlorobenzaldehyde, 2-chlorocyclohex-1-enecarbaldehyde, 2-chlorobenzoic acid, 2,4-dichlorobenzoic acid, 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde, 2-chloro-4,6-dimethylnicotinonitrile, α,β-unsaturated ketones and isonicotinaldehyde, respectively. The chemical structures of the newly synthesized compounds were characterized by IR, NMR and mass spectral analysis. These compounds were also screened for their analgesic and anti-inflammatory activities. Some of these compounds (**3**, **4**, **9**, **10** and **12**–**14**) exhibited promising activities.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation in arthritis for decades. However, long-term clinical usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding, and nephrotoxicity. Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area [1–4]. It is evident from the literature that quinazolines and condensed quinazolines exhibit potent central nervous system (CNS) activities, *e.g.* analgesic, anti-inflammatory [5]. In our ongoing medicinal chemistry research program we have demonstrated that azolopyrimidoquinolines, pyrimidoquinazolines, exhibited good anti-oxidant, anti-inflammatory and analgesic activities [6]. Literature reports show that thienopyrimidines (bioisostere of quinazoline and condensed quinazoline) possess CNS and antibacterial activities [7–9]. On the other hand, thiazoles and their derivatives are found to be associated with various biological activities [10–12], such as antibacterial, antifungal, anti-inflammatory activities. Furthermore, organic compounds bearing thiazolopyrimidine and pyrimidothiazoloquinoxaline nuclei were found to possess potent anti-cancer activity [13]. This biological importance prompted us to synthesize some new heterocyclic derivatives having thiazolopyrimidine (bioisostere of quinazoline) and quinaxoline (bioisostere of quinazoline) moieties starting from 2,3-dichloro-quinoxaline [14,15], in order to investigate their anti-inflammatory and analgesic activities.

2. Chemistry

The target compounds were synthesized as outlined in Schemes 1 and 2. The starting material 2,3-dichloroquinoxaline (**2**) was prepared according to a reported procedure [14,15], and allowed to react with 6-aminouracil in ethanol in the presence of a catalytic amount of TEA to produce 6-amino-2-(3-chloroquinoxalin-2-ylthio)pyrimidin-4(3*H*)-one (**3**), which was refluxed in DMF to give the corresponding thiazolo[4,5-*b*]quinoxaline-4-one derivative **4**. In another route compound **4** was obtained in high yield *via* refluxing a mixture of **1** and **2** in DMF.

The final compounds **5**–**9** were synthesized by refluxing **4** with 2-chlorobenzaldehyde, 2-chlorocyclohex-1-enecarbaldehyde, 2-chlorobenzoic acid, 2,4-dichlorobenzoic acid and 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde. Further, compound **4** was condensed with 2-chloro-4,6-dimethylnicotinonitrile and

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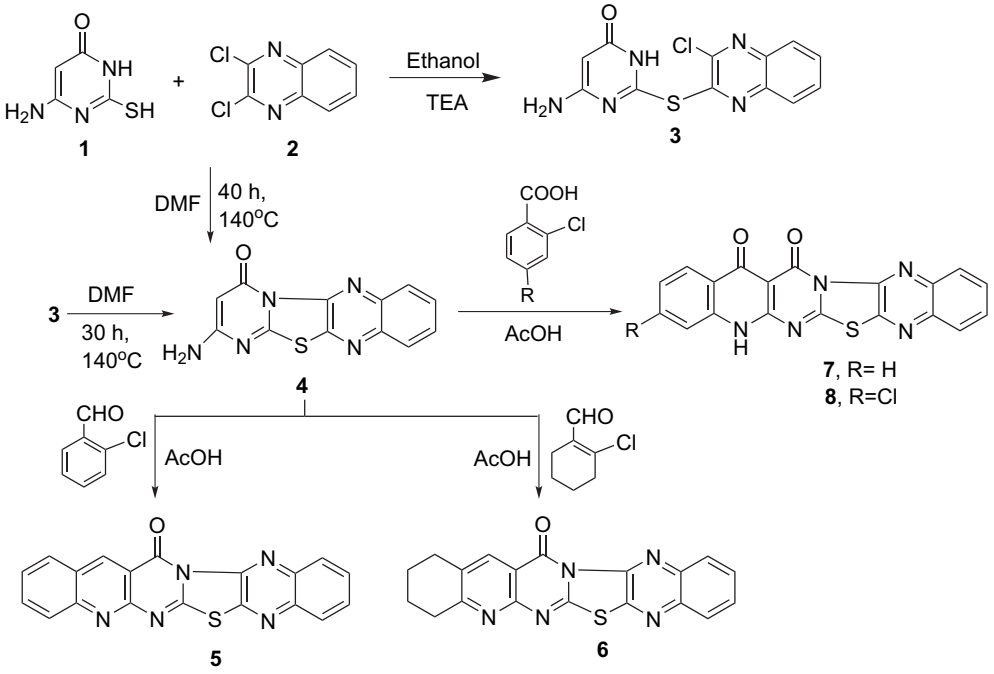
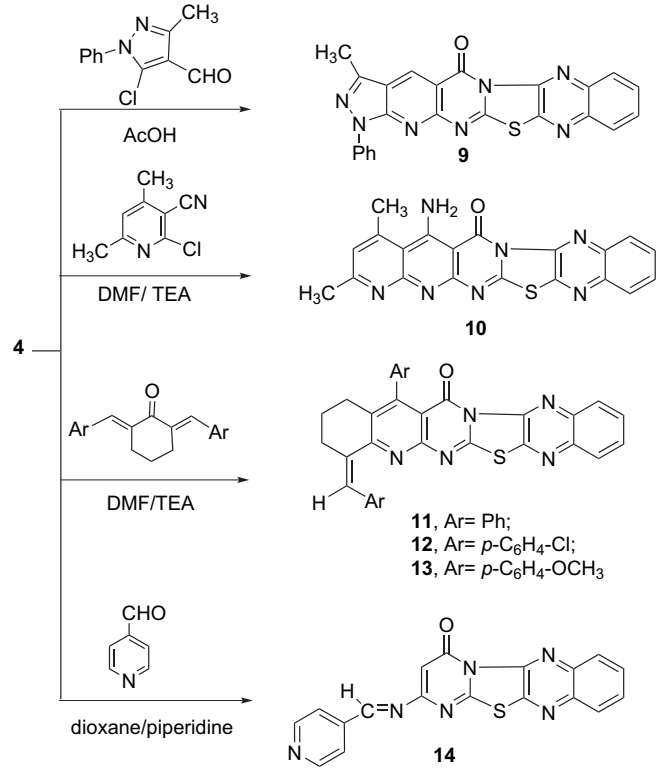


Scheme 1.

α,β-unsaturated ketones in DMF/TEA to afford the corresponding thiazolo[4,5-*b*]quinoxaline-8-one derivatives **10**–**13**. Finally, the Schiff base **14** was obtained by condensation of **4** with iso-nicotinaldehyde in dioxane/piperidine. The structure assignments to new compounds were based on their elemental analysis and spectral (IR, ^{1}H, ^{13}C NMR and mass) data.

IR spectrum of compound **3** showed the broad amide and amino NH peaks at 3423–3349 cm^{-1}. The carbonyl stretching of amide appears at 1654 cm^{-1}, carbonyl group C=O. Further, its ^{1}H NMR spectrum showed a singlet at δ 5.20 ppm corresponding to C_5-H of pyrimidine nucleus, whereas the amino and the amide NH groups resonated at δ 6.36 and 11.61 ppm. The IR spectrum of **4** showed the absorption bands at 3420 and 1661 cm^{-1} due to NH stretching of amino and amidic carbonyl groups, respectively. Its ^{1}H NMR showed a sharp singlet peak at δ 5.30 ppm due to the presence of C_4 of the pyrimidine and the NH_2 group resonated as a broad singlet at δ 6.90 ppm. In ^{13}C NMR spectrum, it showed signals at δ 165.0 and 93.5 ppm due to C_4 and C_5 of pyrimidine nucleus, respectively.

The IR spectrum of **5**–**14** showed characteristic absorption bands within the $\nu = 1697$–1656 cm^{-1} region corresponding to the stretching vibration of amidic group and the absorption bands within the $\nu = 1625$–1604 cm^{-1} region are due to symmetric vibration of C=N group. The absence of the absorption band



Scheme 2.

Table 1
Percent anti-inflammatory activity of the tested compounds (carrageenan-induced paw odema test in rats).

Compd. no.	Percent protection		
	1 h	2 h	3 h
3	58.8 ± 1.40**	58.3 ± 1.31*	41.3 ± 1.25*
4	55.3 ± 1.36**	61.0 ± 1.75**	41.2 ± 1.05*
5	45.3 ± 1.52*	47.6 ± 137*	37.6 ± 1.38*
6	38.8 ± 1.41*	41.3 ± 1.41*	24.3 ± 1.31*
7	40.2 ± 1.37*	41.5 ± 1.45*	30.1 ± 1.32*
8	43.1 ± 1.81*	48.0 ± 1.12	31.5 ± 1.82*
9	48.6 ± 2.40*	56.1 ± 1.62*	33.8 ± 1.32*
10	58.5 ± 1.05**	60.4 ± 2.01**	45.5 ± 1.25*
11	41.3 ± 1.62*	43.8 ± 1.46*	31.9 ± 1.25*
12	45.7 ± 2.27*	43.2 ± 1.80*	38.2 ± 1.04*
13	48.0 ± 1.26*	52.2 ± 1.30**	40.1 ± 1.30*
14	51.1 ± 1.50**	54.2 ± 1.62**	33.4 ± 1.22*
Control	6.1 ± 0.27	5.7 ± 0.32	3.2 ± 0.93
Diclofenac Sodium	52.4 ± 0.92*	60.3 ± 1.52**	42.0 ± 1.36*

Each value represents the mean ± S.E ($n = 6$).
Significance levels *$p < 0.5$, **$p < 0.001$ as compared with respective control.
Dose (20 mg/kg), for the selected tested compound.

corresponding to amino stretching frequency of the parent 2-aminopyrimido[2′,1′:2,3]thiazolo[4,5-*b*]quinoxaline-4-one (**4**) clearly confirmed the formation of compounds **5**–**14**.

The ^{1}H NMR spectrum of compounds **5**, **6** and **9** showed a singlet signal within the δ 8.5–8.65 ppm regions corresponding to C_4 proton of pyridine nucleus. Also, compound **9** revealed a singlet signal at δ 3.28 ppm due to methyl protons. Further, compound **12** displayed characteristic signals at δ 1.63–1.69 (m, 2H, CH_2), 2.08 (t, 2H, CH_2), 2.76 (t, 2H, CH_2), 7.95 (s, 1H, CH methine).

The ^{13}C NMR spectrum of compounds **5** and **6** displayed characteristic signals within δ 165.0–167.0 and 140.1–141.0 ppm due to C_4 of pyrimidine and C_4 of pyridine nucleus, respectively. The methylene carbons of compound **6** appeared at δ 31.2, 31.6, and 34.7, (2C) ppm. Also, compound **7** showed signals at δ 187.4 and 168.2 ppm which were due to ketonic and amidic groups, respectively. Moreover, the carbon of carbonyl and methyl group of compound **9** resonated at δ 169.2 and 14.9, respectively. Furthermore, compound **10** displayed signals at δ 168.0 and (20.1, 22.1) due to CO and two methyl groups, respectively. Finally, compounds **12** and **14** showed signals within δ 167.0–165.0 ppm indicating the presence of amidic group, whereas, the methylene carbons of compound **12** appear at δ 25.1, 32.8 and 36.1 ppm.

3. Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in rats. The anti-inflammatory activity data (Table 1) indicated that all the tested compounds protected rats from carrageenan-induced inflammation, and some of the tested compounds (**3** and **10**) are more potent than our earlier reported ones [16,17]. Compounds **3**, **4**, **10**, and **14** showed similar and higher anti-inflammatory activity than diclofenac sodium.

4. Analgesic activity

The analgesic activity was determined by the hot-plate test (central analgesic activity) and acetic acid induced writhing assay. The results (Tables 2 and 3) revealed that all tested compounds exhibited significant activity. Most of the tested compounds have nearly the same activity as the reference drug, and the remaining tested compounds have good activities in central analgesic activity. Also, compound **10** exhibited activities higher than the reference drug in peripheral analgesic activity testing. The remaining compounds have the same activity in peripheral analgesic activity testing.

Table 2
Central analgesic activity (hot-plate test).

Group	Reaction time (min)			
	0 min	30 min	60 min	90 min
Control	8.23 ± 0.33	8.10 ± 0.36[b]	8.60 ± 0.41[b]	9.51 ± 0.40[b]
3	8.40 ± 0.35	8.42 ± 0.45[a]	9.41 ± 0.29[a]	10.67 ± 0.59[a,b]
4	7.90 ± 0.65	8.42 ± 0.45[a]	9.41 ± 0.29[a]	10.67 ± 0.59[a,b]
5	7.16 ± 0.57	8.84 ± 0.28[a]	10.62 ± 0.57[a]	10.88 ± 0.47[a]
6	7.40 ± 0.35	8.40 ± 0.45[a]	10.40 ± 0.29[a]	10.65 ± 0.58[a,b]
7	7.40 ± 0.38	8.16 ± 0.57[a]	9.78 ± 0.45[a]	10.08 ± 0.24[b]
8	6.23 ± 0.57	7.07 ± 0.78[a]	9.50 ± 0.82[a]	12.66 ± 0.61[a]
9	7.65 ± 0.20	7.55 ± 0.26[b]	8.60 ± 0.60[a]	12.00 ± 0.36[a]
10	8.38 ± 0.30	10.14 ± 0.26[b]	10.74 ± 0.28[b]	7.62 ± 0.60[b]
11	7.25 ± 0.40	7.51 ± 0.48	8.86 ± 0.52	10.45 ± 0.18[b]
12	8.42 ± 0.61	8.61 ± 0.34	9.06 ± 0.55[b]	9.22 ± 0.46[b]
13	8.25 ± 0.40	8.67 ± 0.48	9.82 ± 0.52	10.41 ± 0.18[b]
14	8.25 ± 0.40	9.66 ± 0.48	9.82 ± 0.52	10.41 ± 0.18[b]
Diclofenac sodium	6.49 ± 0.40	10.03 ± 0.12[a]	11.39 ± 0.53[a]	13.15 ± 0.38[a]

Values represent the mean ± S.E. of six animals for each groups.
*Significant at $P < 0.05$.
[a] $P < 0.05$: Statistically significant from control. (Dunnett's test).
[b] $P < 0.05$: Statistically significant from ASA (Dunnett's test).

Table 3
Percent analgesic activity (peripheral, writhing test).

Compd. No.	Percent protection			
	30 min	1 h	2 h	3 h
3	68.0 ± 1.05**	73 ± 1.93**	75.4 ± 1.51**	57.2 ± 1.17*
4	60.4 ± 1.51**	68 ± 1.56**	73.3 ± 1.84**	46.3 ± 172*
5	40.4 ± 1.16*	46 ± 1.32*	47.8 ± 1.39	35.2 ± 1,73*
6	42.2 ± 1.32*	45 ± 1.03*	45.1 ± 1.39	34.7 ± 1.15*
7	42.5 ± 1.92*	49 ± 1.37*	53.1 ± 1.16	38.4 ± 1.81*
8	40.5 ± 1.43*	51 ± 1.26*	47.3 ± 1.92	38.3 ± 1.39*
9	45.7 ± 1.51*	52 ± 1.49**	58.3 ± 1.69	35.5 ± 1.09*
10	72.2 ± 1.05**	75 ± 1.39**	76.2 ± 1.31	60.3 ± 1.39**
11	42.4 ± 1.21*	47 ± 1.42*	50.3 ± 1.60	38.6 ± 1.83*
12	45.0 ± 1.93*	53 ± 1.41*	55.6 ± 1.39	36.3 ± 1.22*
13	51.4 ± 1.34*	53 ± 1.02*	56.5 ± 1.37	38.6 ± 1.48*
14	60.2 ± 1.15*	64.1 ± 1.31**	68.2 ± 1.03**	51.1 ± 1.73*
Control	02.0 ± 0.35	06.0 ± 0.50	04.0 ± 0.59	04.0 ± 0.90
Diclofenac sodium	46.0 ± 095*	55.2 ± 1.16*	62 ± 1.49*	39 ± 1.13*

Each value represents the mean ± S.E ($n = 6$).
Significance levels *$p < 0.5$, **$p < 0.001$ as compared with respective control.
Dose (20 mg/kg), for the selected tested compound.

5. Conclusion

The prepared new ring systems seem to be interesting for biological activity studies. Furthermore, the present investigation offers rapid and effective new procedures for the synthesis of the poly-condensed new heterocyclic ring systems. Compounds **3**, **4**, **9**, **10**, **12**–**14** exhibited potent anti-inflammatory and analgesic activities. It is worth mentioning that the incorporation of pyrimidine, thiazolopyrimidine, pyrazolopyridine, pyridopyridine, *p*-chlorophenyl, *p*-methoxyphenyl or pyridine nucleus to quinoxaline moiety caused significant anti-inflammatory and analgesic activities. In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocyclics based on quinoxaline for anti-inflammatory and analgesic evaluation.

6. Materials and methods

6.1. Animals

Female Sprague–Dawley rats (150–200 g) were used in the study of anti-inflammatory activity. Both sexes of Swiss mice weighing 25–30 g were used in analgesic activity and international principle and local regulations concerning the care and use of laboratory animals were taken into account [18]. 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.

6.2. Anti-inflammatory activity (carrageenan-induced rat hind paw edema model)

The method adopted resembles essentially that described by *Winter et al.* [19], distilled water was selected as vehicle to suspend the standard drugs and the test compounds. The albino rats weighing between 150 and 180 g were starved for 18 h prior to the experiment. The animals were weighed, marked for identification and divided into 17 groups each group containing 6 animals. Edema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 mL of 1% (W/V) carrageenan in distilled water into their footpads. The 1st group was kept as control and was given the respective volume of the solvent (0.5 mL distilled water). The 2nd

to 16th groups were orally administered aqueous suspension of the synthesized compounds in dose of 20 mg/kg 1 h before carrageenan injection. The last group (standard) was administered diclofenc sodium in a dose of 20 mg/kg, orally as aqueous suspension [20]. The paw volume of each rat was measured immediately by mercury plethysmometer, before carrageenan injection and then hourly for 4 h post administration of aqueous suspension of the synthesized compounds. The edema rate and inhibition rate of each group were calculated as follows: Edema rate $(E)\% = V_t - V_o/V_o$, Inhibition rate $(I)\% = E_c - E_t/E_c$ where V_o is the volume before carrageenan injection (mL), V_t is the volume at t h after carrageenan injection (mL), E_c, E_t the edema rates of control and treated groups, respectively.

6.3. Analgesic activity using hot-plate test

The experiment was carried out as described by *Turner* [21], using hot-plate apparatus, maintained at 53 ± 0.5 °C. The mice were divided into 17 groups of 6 animals each. 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. The reaction time was measured prior to aqueous suspension of synthesized compounds and drug treatment (0 min). Group 1 was kept as normal control. The aqueous suspension of synthesized compounds was orally administered to mice of groups 2–16 at doses of 20 mg/kg. Mice of group 17 (reference) were orally treated with diclofenac sodium in a dose of 20 mg/kg body wt. 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.

6.4. Analgesic activity (acetic acid induced writhing response model)

The compounds were selected for investigating their analgesic activity in acetic acid induced writhing response in Swiss albino mice, following the method of *Collier et al.* [22]. One hundred and two mice were divided into 17 groups (six in each group) starved for 16 h pretreated as follows, the 1st group which served as control positive orally received distilled water in appropriate volumes. The 2nd to 16th groups received the aqueous suspension of synthesized compounds orally at a dose of 20 mg/kg. The last group orally received diclofenac sodium in a dose of 20 mg/kg. After 30 min, each mouse was administrated 0.7% of an aqueous solution of acetic acid (10 mL/kg) 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 group. The number of writhing was recorded and the percentage protection was calculated using the following ratio: % protection = (control mean − - treated mean/control mean) × 100.

7. Experimental

All melting points are in degree centigrade and were determined on Gallenkamp electric melting point apparatus. The IR spectra were recorded (KBr) on a Perkin–Elmer 1430 spectrometer (National Research Center and Chemistry Department, Cairo University). The ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL-ECA500 (National Research Center, Egypt) and JEOL JNM-LA-400 FT NMR Spectrometer (Chemistry Department, Cairo University) and chemical shifts were expressed as δ values against TMS as internal standard. Mass spectra were recorded on GCMS-QP 1000 EX Shimadzu Japan (Gas Chromatography–Mass spectrometer). Microanalytical data were obtained at the Micro-analytical Center at Cairo University and National Research Center, Egypt.

7.1. Synthesis of 6-amino-2-(3-chloroquinoxalin-2-ylthio)pyrimidin-4(3H)-one (**3**)

A mixture of 6-aminothiouracil (**1**) (1.43 g, 10 mmol) and 2,3-dichloroquinoxaline (**2**) (1.99 g, 10 mmol) in ethanol containing 0.2 mL of TEA as a catalyst was refluxed for 20 h. The reaction mixture was cooled and the deposited solid was filtered off, dried and recrystallized from DMF/ethanol (1:4) to afford compound **3**.

Pale yellow powder, yield, 85%, mp: 263–265 °C, IR (KBr): ν_{max}, cm^{-1}: 3423–3349 (br, NH, NH$_2$), 1654 (CO), 1619 (C=N). ^{1}H NMR (DMSO-d_6): δ 5.20 (s, 1H, C$_5$-H, pyrimidine), 6.36 (br, 2H, NH$_2$, D$_2$O exchangeable), 7.25–7.87 (m, 4H, Ar-H), 11.61 (s, H, NH, D$_2$O exchangeable). Anal. Calcd for $C_{12}H_8ClN_5OS$ (305.74): C, 47.14; H, 2.64; N, 22.91. Found: C, 47.21; H, 2.68; N, 22.96.

7.2. Synthesis of 2-aminopyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline-4-one (**4**)

Method A: To a solution of 6-aminothiouracil (**1**) (1.43 g, 10 mmol) in DMF (10 mL), 2,3-dichloroquinoxaline (**2**) (1.99 g, 10 mmol) and TEA (0.2 mL) were added. The reaction mixture was refluxed for 40 h, and cooled. The deposited solid was filtered and recrystallized from benzene–DMF to afford compound **4** as a reddish brown powder.

Method B: A mixture of **3** (3.06 g, 10 mmol) in DMF (20 mL) containing TEA (0.5 mL) was refluxed for 30 h. The reaction mixture was cooled, the deposited solid was filtered, dried and recrystallized from benzene–DMF to furnish **4**.

Yield: A, 80; B, 62%, mp: 335–337 °C, IR (KBr): ν_{max}, cm^{-1}: 3420 (br, NH$_2$), 1661 (CO), 1605 (C=N); ^{1}H NMR (DMSO-d_6): δ 5.30 (s, 1H, CH, pyrimidine), 6.90 (br, s, 2H, NH$_2$, D$_2$O exchangeable), 7.25–7.68 (m, 4H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 93.5 (C$_6$-pyrimidine), 125.4, 127.2, 128.2, 129.1, 135.0, 136.4, 141.0, 160.0 (C$_4$-thiazole), 161.0 (C$_2$-thiazole), 164.0, 165.0; $m/z = 270$ ($M^+ + 1$, 16.2), 209 (30.7), 180 (33.3), 132 (30.7), 105 (33.12), 77.0 (100.0), 74 (37.1). Anal. Calcd for $C_{12}H_7N_5OS$ (269.29): C, 53.52; H, 2.62; N, 26.01. Found: C, 53.58; H, 2.67; N, 26.06.

7.3. Synthesis of pyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline derivatives **5**–**9**

7.3.1. General procedure

A suspension of compound **4** (1.99 g, 10 mmol) and 2-chlorobenzaldehyde (1.41 g, 10 mmol), 2-chlorocyclohex-1-enecarbaldehyde (1.45 g, 10 mmol), 2-chlorobenzoic acid (1.57 g, 10 mmol), 2,4-dichlorobenzoic acid (1.91 g, 10 mmol) or 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (2.21 g, 10 mmol), in glacial acetic acid (45 mL) was stirred and heated under reflux for 17–22 h (TLC control). The reaction mixture was cooled, the formed precipitate filtered off, dried and recrystallized from the proper solvent to afford compounds **5**–**9**.

7.3.2. Quinolino[2″,3″:4′,5′]pyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline-8-one (**5**)

Reaction time 22 h, recrystallized from DMF, brown powder, yield, 79%, mp: >390 °C, IR (KBr): ν_{max}, cm^{-1}: 1671 (CO), 1613 (C=N). ^{1}H NMR (DMSO-d_6): δ 7.47–7.69 (m, 8H, Ar-H), 8.65 (s, 1H, C$_4$-H, pyridine). ^{13}C NMR (DMSO-d_6): δ 122.6, 125.1, 127.0, 127.1, 128.0, 128.3, 128.8, 129.1, 129.1, 131.5, 135.0, 136.0, 138.9, 141.0, 148.1, 160.0, 161.0, 168.3, 165.0. Anal. Calcd for $C_{19}H_9N_5OS$ (355.38): C, 64.22; H, 2.55; N, 19.71. Found: C, 64.18; H, 2.51; N, 19.68.

*7.3.3. 3,4,5,6-Tetrahydroquinolino[2″,3″:4′,5′]pyrimido[2′,1′:2,3] thiazolo[4,5-b]quinoxaline-8-one (**6**)*

Reaction time 18 h, recrystallization from DMF, brown powder, yield, 77%, mp: 386–388 °C, IR (KBr): ν_{max}, cm^{-1}: 2955 (br, CH, aliph.) 1675 (CO), 1608 (C═N). ^1H NMR (DMSO-d_6): δ 1.65–1.80 (m, 4H, $2CH_2$), 2.60 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 7.55–7.90 (m, 4H, Ar-H), 8.60 (s, 1H, C_4-H, pyridine). ^{13}C NMR (DMSO-d_6): δ 31.2, 31.6, 34.7 (2C), 122.0, 125.0, 127.0, 128.0, 129.0, 136.0, 136.1, 137.2, 140.1, 143.1, 162.0, 162.1, 163.0, 164.1, 167.0. Anal. Calcd for $C_{19}H_{13}N_5OS$ (359.41): C, 63.50; H, 3.64; N, 19.48. Found: C, 63.57; H, 3.70; N, 19.53.

*7.3.4. Quinolino[2″,3″:4′,5′]pyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline-7(2H),8-dione (**7**)*

Reaction time 17 h, recrystallization from dioxane, brown powder, yield, 80%, mp: 360–362 °C, IR (KBr): ν_{max}, cm^{-1}: 3250 (br, NH), 1710, 1666 (2CO), 1613 (C═N). ^1H NMR (DMSO-d_6): δ 7.08–8.07 (m, 8H, Ar-H), 11.70 (br, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 102.4, 115.7, 119.1, 123.4, 125.0, 127.0, 128.1, 129.0, 131.4, 135.1, 136.3, 136.3, 143.0, 148.3, 162.1, 163.0, 164.0, 168.2, 187.4. Anal. Calcd for $C_{19}H_9N_5O_2S$ (381.46): C, 59.83; H, 5.02; N, 18.36. Found: C, 59.88; H, 5.11; N, 18.43.

*7.3.5. 4-Chloro-quinolino[2″,3″:4′,5′]pyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline-7(2H),8-dione (**8**)*

Reaction time 30 h, recrystallized from benzene–ethanol, reddish brown powder, yield, 78%, mp: 375–377 °C, IR (KBr): ν_{max}, cm^{-1}: 3300 (br s, NH), 1718, 1677 (2CO), 1617 (C═N). ^1H NMR (DMSO-d_6): δ 7.30–8.20 (m, 7H, Ar-H), 10.20 (br, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 101.5, 115.5, 118.5, 124.0, 124.5, 127.0, 128.0, 128.3, 129.0, 132.0, 137.0, 137.8, 141.0, 144.0, 154.0, 163.0, 165.0, 168.0, 170.0, 183.0. Anal. Calcd for $C_{19}H_8ClN_5O_2S$ (405.82): C, 56.23; H, 1.99; N, 17.25. Found: C, 56.26; H, 2.04; N, 17.35.

*7.3.6. 5-Methyl-3-phenyl-3H-pyrazolo[3′,4′:2″,3″]pyrido[5″,6″:4′,5′]pyrimido [2′,1′:2,3]thiazolo[4,5-b]quinoxaline-7-one (**9**)*

Reaction time 20 h, recrystallization from dioxane–benzene, brown powder, yield, 76%, mp: >390 °C, IR (KBr): ν_{max}, cm^{-1}: 2967, 2900 (CH, aliphatic), 1673 (CO), 1621 (C═N). ^1H NMR (DMSO-d_6): δ 3.28 (s, 3H, CH_3), 7.40–8.10 (m, 4H, Ar-H), 8.5 (s, 1H, C_4-H, pyridine). ^{13}C NMR (DMSO-d_6): δ 14.9, 106.5, 118.9, 123.2, 125.3, 126.1, 127.0, 128.0, 129.2, 129.2, 130.1, 135.3, 136.0, 138.4, 139.8, 143.2, 151.90, 162.2, 163.1, 169.2, 172.9. Anal. Calcd for $C_{23}H_{13}N_7OS$ (435.47): C, 63.44; H, 3.01; N, 22.52. Found: C, 63.50; H, 3.06; N, 22.63.

*7.4. Synthesis of pyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline-8-one derivatives **10**–**13***

7.4.1. General procedure

A suspension of compound **4** (2.69 g, 10 mmol) and 2-chloro-4,6-dimethylnicotinonitrile (1.67 g, 10 mmol), 2,6-dibenzylidenecyclohexanone (2.74 g, 10 mmol), 2,6-bis(4-chlorobenzylidene) cyclohexanone (3.43 g, 10 mmol) or 2,6-bis(4-methoxybenzylidene) cyclohexanone (3.34 g, 10 mmol) in DMF (30 mL) containing a catalytic amount of TEA (0.5 mL) was stirred and heated under reflux for 30–42 h (TLC control). The reaction mixture was cooled, the formed precipitate filtered off, dried and recrystallized from the proper solvent to afford compounds **10**–**13**, respectively.

*7.4.2. 7-Amino-4,6-dimethyl-1,8-naphthyridino-[2″,3″:4′,5′] pyrimido[2′,1′:2,3] thiazolo[4,5-b]quinoxaline-8-one (**10**)*

Reaction time 30 h, recrystallized from benzene–DMF, brown powder, yield, 75%, mp: >390 °C, IR (KBr): ν_{max}, cm^{-1}: 3420 (br, NH_2), 1697 (CO), 1625 (C═N). ^{13}C NMR (DMSO-d_6): δ 20.1, 22.1, 109.3, 109.6, 123.8, 125.0, 127.0, 128.1, 129.3, 135.0, 136.4, 143.1, 147.1, 150.6, 158.0, 159.2, 162.3, 163.1, 164.5, 168.0; $m/z = 399$ (M^+, 6.7%), 341 (79.1), 280 (46.3), 255 (89.3), 291 (39.5), 191 (40.7), 152.8 (79.1), 151 (100), 149 (50.8), 126 (52.5), 111 (58.2), 96.9 (84.7), 85 (97.7), 57 (71.7). Anal. Calcd for $C_{20}H_{13}N_7OS$ (399.44): C, 60.14; H, 3.28; N, 24.55. Found: C, 60.07; H, 3.21; N, 24.51.

*7.4.3. 3-Benzylidene-7-phenyl-3,4,5,6-tetrahydroquinolino[2″,3″:4′,5′]pyrimido [2′,1′:2,3]thiazolo[4,5-b]quinoxaline-8-one (**11**)*

Reaction time 40 h, brown powder, yield, 82%, mp: 345–347 °C, IR (KBr): ν_{max}, cm^{-1}: 2995 (br, CH, aliph.), 1656 (CO), 1607 (C═N); ^1H NMR (DMSO-d_6): δ 1.60–1.69 (m, 2H, CH_2), 2.28 (t, 2H, CH_2), 2.73 (t, 2H, CH_2), 7.12–8.03 (m, 14H, Ar-H), 8.20 (s, 1H, CH, methine). $m/z = 523$ (M^+, 7.33%), 474 (12.0), 369 (5.3), 339 (7.3), 17.7 (8.6), 125 (50.6), 90 (100). Anal. Calcd for $C_{32}H_{21}N_5OS$ (523.62): C, 73.40; H, 4.04; N, 13.37. Found: C, 73.47; H, 4.09; N, 13.42.

*7.4.4. 3-(4-Chlorobenzylidene)-7-(4-chlorophenyl)-3,4,5,6-tetrahydroquinolino [2″,3″:4′,5′]pyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline-8-one (**12**)*

Reaction time 42 h, brown powder, yield, 80%, mp: 355–357 °C, IR (KBr): ν_{max}, cm^{-1}: 2929 (br, CH, aliph.), 1663 (CO), 1612 (C═N). ^1H NMR (DMSO-d_6): δ 1.63–1.69 (m, 2H, CH_2), 2.08 (t, 2H, CH_2), 2.76 (t, 2H, CH_2), 7.30–7.68 (m, 12H, Ar-H), 7.95 (s, 1H, CH, methine). ^{13}C NMR (DMSO-d_6): δ 25.1, 32.8, 36.1, 119.8, 121.8, 125.2, 126.1, 127.1 (2C), 127.5 (2C), 127.7 (2C), 128.0, 128.4 (2C), 128.8, 129.1, 129.4, 133.1, 133.40, 134.4, 136.1, 136.2, 143.1, 143.2, 153.1, 155.9, 161.5, 162.2, 163.1, 164.8, 167.0; $m/z = 592$ (M^+, 3.4%), 513 (2.70), 363 (3.3), 289 (4.7), 193 (6.7), 123 (8.6), 57 (100). Anal. Calcd for $C_{32}H_{19}Cl_2N_5OS$ (592.51): C, 64.87; H, 3.23; N, 11.82. Found: C, 64.95; H, 3.28; N, 11.86.

*7.4.5. 3-(4-Methoxybenzylidene)-7-(4-methoxyphenyl)-3,4,5,6-tetrahydro-quinolino[2″,3″:4′,5′]pyrimido[2′,1′:2,3]thiazolo[4,5-b]quinoxaline-8-one (**13**)*

Reaction time 35 h, brown powder, yield, 78%, mp: 377–379 °C, IR (KBr): ν_{max}, cm^{-1}: 2950 (br, CH, aliph.), 1660 (CO), 1614 (C═N); ^{13}C NMR (DMSO-d_6): δ 24.5, 32.1, 37.3, 55.5 (2C), 114.5 (2C), 114.7 (2C), 119.5, 122.1, 124.1, 127.0 (2C), 127.5, 127.9 (2C), 128.1, 129.1, 130.5, 133.1, 137.0, 139.5, 150.5, 155.4, 161.4, 162.2, 162.4, 162.7, 163.0, 163.8, 165.0, 172.0. $m/z = 583.5$ (M^+, 5.5%), 447 (8.0), 413 (4.7), 325 (4.6), 291 (4.7), 205 (3.3), 125 (58.6), 96 (100). Anal. Calcd for $C_{34}H_{25}N_5O_3S$ (583.67): C, 69.97; H, 4.32; N, 11.99. Found: C, 70.03; H, 4.40; N, 12.03.

*7.4.6. Synthesis of 2-[4-(pyridinylmethylene)imino]-2-aminopyrimido[2′,1′:2,3] thiazolo[4,5-b]quinoxaline-4-one (**14**)*

A suspension of compound **4** (2.69 g, 10 mmol) and isonicotinaldehyde (1.07 g, 10 mmol) in dioxane (30 mL) containing piperidine (0.5 mL) as a catalyst, was stirred and heated under reflux for 5 h. The reaction mixture was cooled, the formed precipitate filtered off, dried and recrystallized from methanol–DMF to afford **14**.

Brown powder, yield, 80%, mp: 292–294 °C, IR (KBr): ν_{max}, cm^{-1}: 2990 (br, CH, aliph.) 1697 (CO), 1604 (C═N). ^1H NMR (DMSO-d_6): δ 5.78 (s, 1H, C_3-H), 6.90–7.97 (m, 8H, Ar-H), 8.03 (s, 1H, CH, methine). ^{13}C NMR (DMSO-d_6): δ 112.3, 124.2 (2C), 125.1, 126.2, 128.1, 129.1, 135.1, 136.1, 138.5, 143.1, 149.9 (2C), 158.6, 162.1, 163.2, 164.1, 165.0. Anal. Calcd for $C_{18}H_{10}N_6OS$ (358.39): C, 60.33; H, 2.81; N, 23.45. Found: C, 60.37; H, 2.79; N, 23.43.

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