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Original article

Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as anti-oxidant, anti-inflammatory and analgesic activities

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Abstract

The 5,10-dihydro-2-thioxo-pyrimido[4,5-*b*]quinolines (**2a–c**) and its oxidized form **3** were prepared and used as key intermediates for the synthesis of thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]-quinolines (**5a–c**), isoxazolo[5″,4″:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolines (**6a–c**), 4-chloro-2-methylthio-pyrimido[4,5-*b*]quinoline, its amino derivatives (**19–21**) and 10,11,12,13-tetrahydro-5*H*-quino[2′,3′:4,5]pyrimido[6,1-*b*]quinazoline (**22**). The newly synthesized compounds were characterized by IR, NMR (^{1}H, ^{13}C) and mass spectral studies. Representative of the synthesized compounds was tested and evaluated for anti-oxidant, anti-inflammatory and analgesic activities. Compounds **2a–c** showed the highest inhibitory anti-oxidant activity either using erythrocyte hemolysis or ABTS methods. Compounds **2a**, **10b**, **16**, and **17a** manifested the best protective effect against DNA damage induced by bleomycin. Compounds **2c**, **5a**, **20a**, **2a**, and **2b** exhibited a potent anti-inflammatory activity using carrageenan-induced paw edema test in rats.

Keywords: Thiazolo[3′,2′:1,2]- and 2,3-dihydroisoxazolo[4″,5″:5′,4′]thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolones; Pyrimido[4,5-*b*]quinolone; 4-Amino derivatives; Anti-oxidant; Anti-inflammatory; Analgesic activities

1. Introduction

Pyrimido[4,5-*b*]quinolones and their derivatives have attracted interest over the years because of their varied biological activities [1,2], recently found application in drug development for the treatment of allergies [3], hypertension [4], inflammation [5], central depressant agent [6], bacterial and [7] HIV infections [8], antimalarial [9], anti-histaminic agents [10], antitumor [11] and more recently for the treatment of pain [12], antithrombotic activity [13] and as new inhibitions of bacterial DNA gyrase B [14]. In view of the above mentioned findings and as continuation of our effort [15–17] to identify new candidates that may be value in designing new, potent, selective and less toxic antimicrobial agents, we report in the present work the synthesis of some pyrimido[4,5-*b*]quinoline, 2,10-diarylidene-7,8,9,10-tetrahydro-thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinoline-3,5-diones, 4-chloro-pyrimido[4,5-*b*]quinoline, and its 4-amino derivatives starting from 6-amino-thiouracil and α,β-unsaturated ketones [18] in order to investigate their anti-oxidant, analgesic and anti-inflammatory activities.

2. Chemistry

Our group has actively been working on the development of synthetic strategies for the preparation of 2-thioxo-pyrimido[4,5-*b*]quinolin-4-ones (**3**) from α,β-unsaturated ketones **2** (Scheme 1). Thus, in a so-called cyclic strategy 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (**3**) was obtained by the reaction of an α,β-unsaturated ketone (**1**) and 6-aminothiouracil in refluxing dimethylformamide for a long time. The structure

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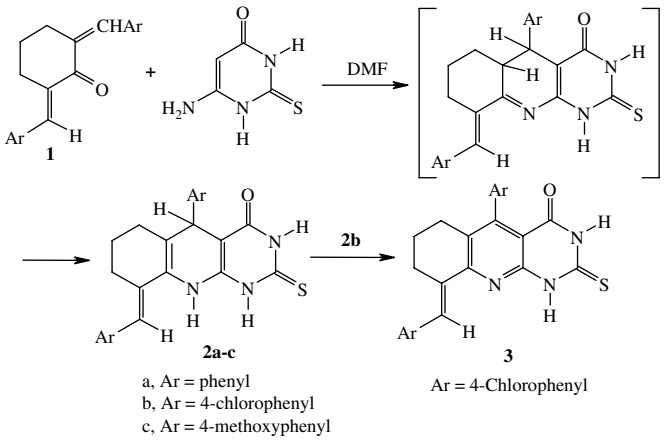
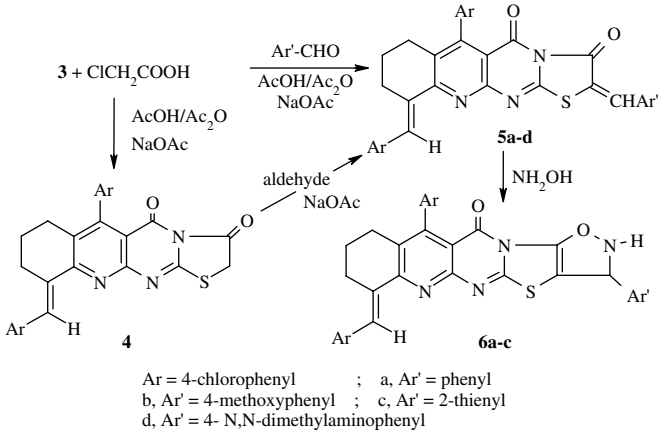


Scheme 1.

of **3** was established by analytical and spectral data [17], and also the 5,10-dihydro derivatives **2a–c** were obtained after refluxing for 3–4 h.

In addition, we report here simple and convenient methods for the syntheses of thiazolopyridopyrimidines, isoxazolothiazolopyridopyrimidines and pyridotriazolo-pyrimidines. Thus, when a ternary mixture of 9-(4-chlorophenylmethylene)-5-(4-chlorophenyl)-2-thioxo-2,3,6,7,8,9-hexahydropyrimido[4,5-*b*]-quinolin-4-one (**3**), chloroacetic acid and a proper aldehyde was heated under reflux in a mixture of acetic acid, acetic anhydride and anhydrous sodium acetate, 6-aryl-2,10-diarylmethylene-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinoline-3,5-diones (**5a–c**) were obtained in high yields (Scheme 2). The structure assignments were based on an independent preparation of **5a** by condensation of 6-aryl-10-arylmethylene-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-*b*]-quinoline-3,5-dione **4** with aldehyde (Scheme 2) and the correct values in elemental analysis and compatible spectral data. The ^{13}C NMR spectrum for compound **5c** showed the signals at 24.90, 30.72, 30.95 ppm for three sp^3 carbons, 115.6–155.6 ppm for 22 sp^2 carbons with four symmetric carbon atoms, and 166.3 and 168.6 (2CO) ppm. Moreover, compound



Scheme 2.

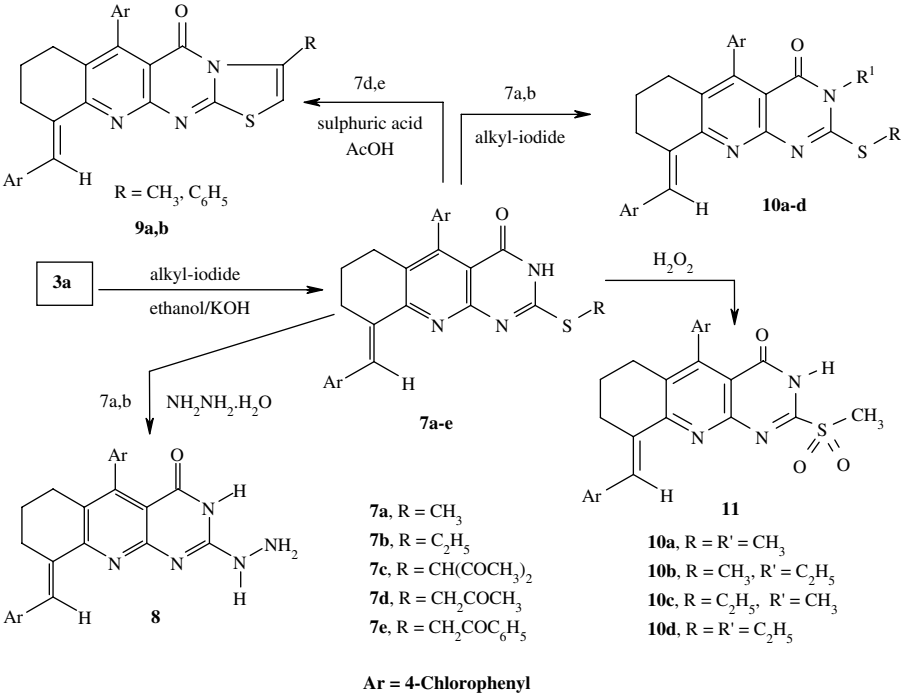
4 was prepared by the reaction of compound **3** with chloroacetic acid in the presence of a mixture from acetic acid, acetic anhydride and sodium acetate. Its IR spectrum displayed absorption bands around 1696 and 1680 cm^{-1} for two carbonyl groups.

Compounds **5a–c** underwent cycloaddition with hydroxylamine hydrochloride, by heating in boiling acetic acid in the presence of sodium acetate, to give 7-arylidene-3,11-diaryl-2,3,7,8,9,10-hexahydroisoxazolo[5″,4″:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolin-12-ones (**6a–c**), with a new ring system. The ^{1}H NMR spectrum of **6** showed a doublet signal around 5.89–6.02 ppm which supported the oxazole proton, furthermore the formation of **6** from **5** proceeded by first 1,4-addition of hydroxylamine on the ethylenic double bond, followed by loss of water [19].

Alkylation of an ethanolic KOH solution of **3** with an alkyl halide yielded the 2-alkylthio derivatives **7a–e**. Assignment of structures **7** is based on the fact that each **7a**,**b** gave the same 5-aryl-2-hydrazino-9-arylmethylene-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (**8**) with the evolution of methyl or ethyl mercaptan on treatment with hydrazine hydrate (Scheme 3). The ^{13}C NMR (DMSO-d_6) spectrum for **7c** showed the signals at 16.41, 23.06, 24.21, 27.99, 28.25, 40.72 ppm for six sp^3 carbons, and around 115.2–154.4 ppm for 16 sp^2 carbons with four symmetric carbon atoms and at 167.57, 185.68 and 192.30 ppm assigned for three carbonyl groups.

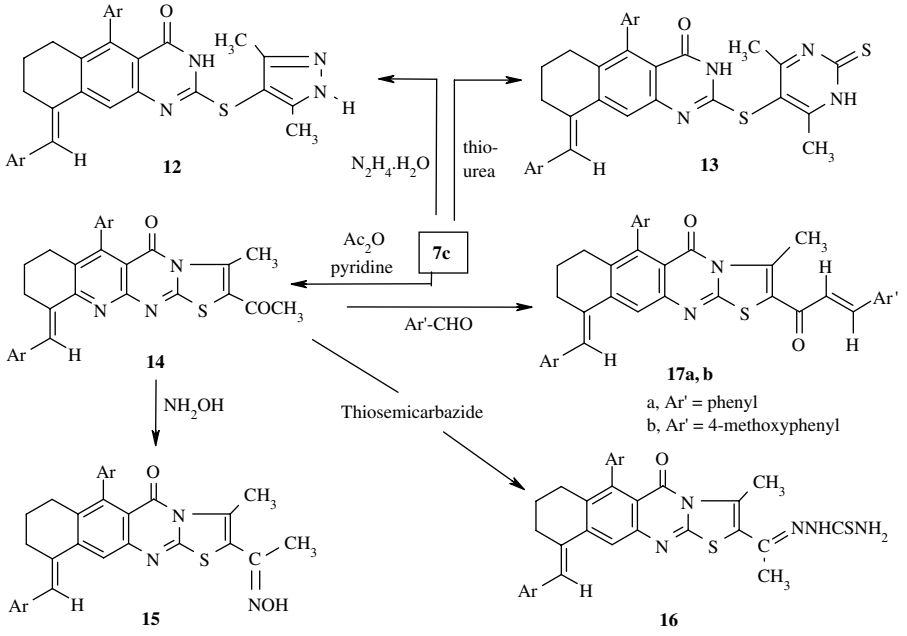
On the other hand, trials to add hydrazine hydrate to **5** failed and yielded instead the 2-hydrazino-**8**, which was reported according to Shishoo [20], upon heating the 2-methylthio derivative with hydrazine hydrate. Assignments of structures **8** are based on correct elemental analyses and IR and NMR spectroscopies.

The reaction of **3** in an ethanolic potassium hydroxide solution with α-haloketones, such as chloroacetone and/or phenacylbromide, yielded 2-(*S*-acetone or *S*-phenacyl)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydr opyrimido[4,5-*b*]quinolin-4-ones (**7d**,**e**) (Scheme 3). Assignments of structures **7d**,**e** are based on correct elemental analyses. The IR spectra are in agreement with the structure and revealed the presence of a free keto-group around 1720 cm^{-1}. The latter compounds **7d**,**e** were cyclized under reflux in a mixture of glacial acetic acid and sulfuric acid to give 3-(methyl or phenyl)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrim ido[4,5-*b*]quinoline-3,5-diones (**9a**,**b**). Structures **9a**,**b** were preferred on the basis of ^{1}H and ^{13}C NMR spectral data. Also the IR spectra are in agreement with the structure and revealed the absence of the NH group at position 3. The 2-alkylthio derivatives **7a**,**b** underwent further alkylation at the N-3 nitrogen atom on treatment with alkyl iodides, in aqueous ethanolic sodium ethoxide solution, to afford 2-alkylthio-3-alkyl-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetra-hydropyrimido[4,5-*b*]quinolin-4-ones (**10a–d**). Also, oxidation of **7a** with hydrogen peroxide in acetic acid yielded the 2-methylsulfone (**11**) (Scheme 3). Structures **11** were preferred from analytical data and spectroscopic analysis and also on the shifting of the 2-methylthio group at position 2 from 2.84 to 3.30 ppm.



Scheme 3.

On the other hand, compound **7c**, as typical 1,3-diketones, reacted with hydrazine hydrate and thiourea to afford the corresponding 2-(3,5-dimethyl-1*H*-pyrazol-4-yl-thio) and 2-(4,6-dimethyl-2-thioxo-pyrimidin-5-yl-thio) derivatives **12** and **13**, respectively (Scheme 4). Heating **7c** under reflux on acetic anhydride/pyridine mixture led to cyclization and formation of 2-acetyl-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrahydro-thiazolo[3′,2′:1,2]pyrimido[4,5-*b*] quionolin-5-one (**14**) in good yield. Assignments of structures **14** were based on spectral data besides our previous report on a related compound [19]. Thus the N-3 nitrogen atom and not the N-1 nitrogen atom was involved in the cyclization. The ^{13}C NMR spectrum of **14** showed the signals at 12.70, 24.81 (2C, $2CH_3$) ppm, 25.72, 29.91, 30.13 (3C, $3CH_2$) ppm, 18 sharp lines around 104.2–155.1 ppm supported the presence of 22 sp^2 carbon atoms (included four symmetric carbons of the



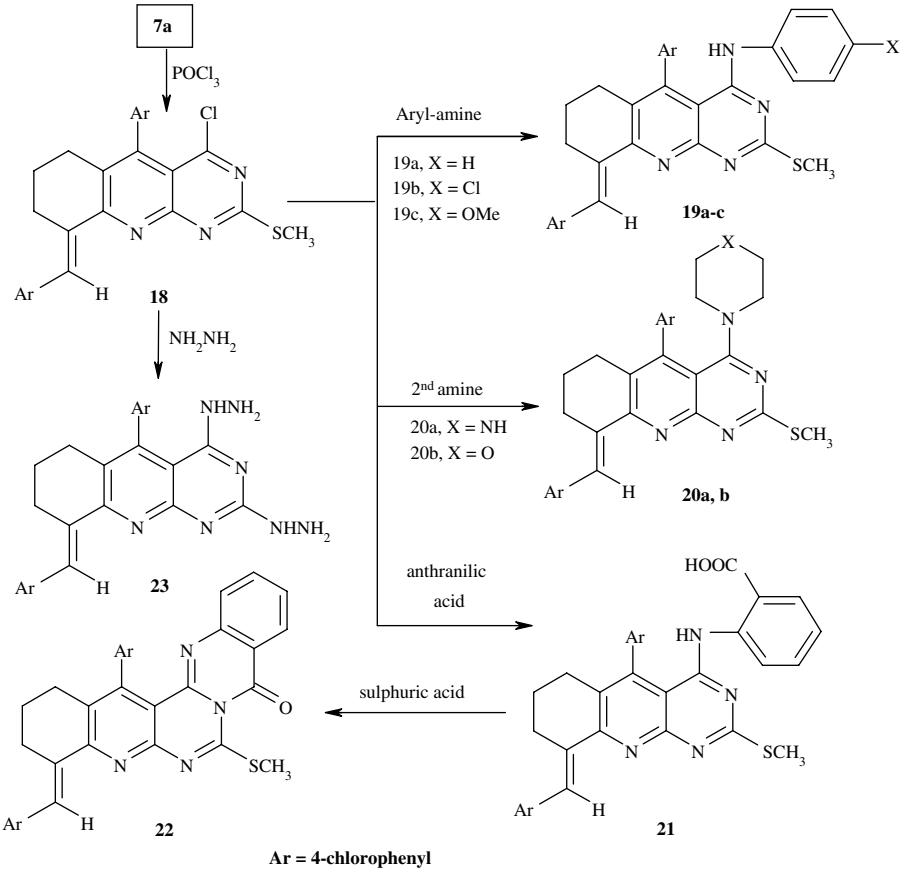
Scheme 4.

p-substituted phenyl) and two signals at 165.3, 193.1 due to the two carbonyl groups.

In support of structure **14**, characteristic reactions for the 2-acetyl group were observed. Thus, its reaction with each of hydroxylamine hydrochloride and thiosemicarbazide gave the corresponding oxime and thiosemicarbazone derivatives **15** and **16**, respectively (Scheme 4). Compounds **15** and **16** gave correct values in elemental analyses and compatible data in IR and ^{1}H NMR spectra. The mass spectrum of **14** showed a molecular ion peak at *m*/*z* 545 for [M^+] (100%). Moreover, compound **14** yielded the 6-aryl-10-aryl-methylene-2-cinnamoyl-3-methyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolin-5-one derivatives **17a**,**b** on heating with the proper aldehyde in the presence of a catalytic amount of piperidine (Scheme 5). The IR spectra of **17** displayed two carbonyl absorption bands around 1700–1685 cm^{-1}. Also the ^{1}H NMR of **17** revealed the presence two doublet signals supporting the AB system hydrogen atoms in the *trans*-form for the ethylenic protons around 5.28–5.53 with *J* coupling around 10.9, 11.5 Hz.

Finally, it is well known in pyrimidine chemistry that position 4 in pyrimidines and fused pyrimidines shows distinct activities towards nucleophiles. Therefore, 4-chloro-2-methylthio-pyrimido[4,5-*b*]quinoline (**18**) was prepared from the reaction of **7a** with phosphorus oxychloride in dry dioxane [20] and its activity towards nucleophiles such as primary aromatic amines, secondary amines and hydrazine hydrates was investigated. Thus heating compound **18** under reflux with aryl-amine, namely aniline, 4-chloroaniline and *p*-anisidine in acetic acid, produced the 4-arylamino-2-methylthio-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolines (**19a–c**) (Scheme 5).

The IR spectrum of **18** revealed the absence of any absorption bands in the NH region. The IR spectra of **19** displayed an absorption band at 3395–3420 cm^{-1} (NH). Moreover, the reaction of 4-chloropyridopyrimidine (**18**) with anthranilic acid afforded 4-(*o*-carboxyphenyl)-amino-pyrimidoquinoline (**21**) (Scheme 5). The IR spectra of **21** displayed an absorption band at 3510 cm^{-1} (OH), 3368 cm^{-1} (NH), and 1716 cm^{-1} for carbonyl group. The latter compound **21** underwent cyclization when boiled with glacial acetic acid in the presence of catalytic amount of sulfuric acid to give 14-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7-methylthio-10,11,12,13-tetrahydro-5*H*-quino[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-5-one (**22**). Structure **22** was preferred on the basis of ^{1}H NMR and ^{13}C NMR spectral data. Similarly, the 2,4-dihydrazinopyridopyrimidine derivative **23** was synthesized from **18** and hydrazine hydrate by heating in dry dioxane. Beside the correct values in elemental analyses spectral data of **23** are in agreement with the assigned structure.



Scheme 5.

3. Results and discussion

3.1. Anti-oxidant activity screening

A variety of quinoline compound derivatives were tested for anti-oxidant activity as reflected in the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rate erythrocyte hemolysis. The pro-oxidant activities of the aforementioned compounds were assessed by their effects on bleomycin-induced DNA damage. The pyrimido[4,5-*b*]quinoline derivatives manifested potent anti-oxidative activity in the lipid peroxidation assay but showed no inhibitory activity in the hemolysis assay.

All compounds have been tested to bleomycin-dependant DNA damage. The results indicated that they may have some protective activity to DNA by certain mechanism. A series of compounds (**2a**, **2c**, **2b**) exhibited a high anti-oxidant activity. On the other hand, compounds **2a**, **10b**, **16**, **17a** protect the DNA from the induced damage by bleomycin (Tables 1–4).

3.2. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats. The anti-inflammatory activity data (Table 5) indicated that all the test compounds protected rats from carrageenan-induced inflammation and some of the tested compounds are more potent than our earlier reported compounds [21]. Compounds **2a–c**, **5a**, **6a**, **7a**, **20a** showed similar and more anti-inflammatory activity than diclofenac sodium.

Table 1
Anti-oxidant assays by erythrocyte hemolysis ($A/B \times 100$)

Compounds	Absorbance of samples (*A*)	Hemolysis (%)
Complete hemolysis with distilled H_2O (*B*)	0.660	
Ascorbic acid	0.026	3.93
2a	0.236	35.75
2b	0.084	12.72
2c	0.054	8.18
3	0.055	8.33
5a	0.038	5.75
5b	0.030	4.54
5c	0.042	6.36
5d	0.048	7.27
6a	0.062	9.39
6b	0.048	7.27
6c	0.031	4.64
7a	0.028	4.24
7b	0.038	5.60
7c	0.052	7.87
7d	0.055	8.33
7e	0.043	6.51
10a	0.027	4.09
10b	0.026	3.93
10c	0.035	5.30
10d	0.203	30.75
14	0.040	6.06
15	0.048	7.22
16	0.041	6.21
17a	0.030	4.54
17b	0.039	5.90
18	0.029	4.39

Table 2
Anti-oxidant assays by ABTS method [Abs (control) − Abs (test)/Abs (control) × 100]

Compounds	Absorbance of samples	Inhibition (%)
Control of ABTS	0.54	0
Ascorbic acid	0.06	88.9
2a	0.10	81.5
2b	0.22	59.3
2c	0.18	66.7
3	0.30	44.4
5a	0.32	40.7
5b	3.36	33.3
5c	0.40	25.9
5d	0.34	37.0
6a	0.36	33.3
6b	0.41	24.1
6c	0.38	29.6
7a	0.36	33.3
7b	0.34	37.0
7c	0.31	42.6
7d	0.35	35.2
7e	0.36	33.3
10a	0.37	31.5
10b	0.34	37.0
10c	0.37	31.5
10d	0.35	35.2
14	0.32	40.7
15	0.28	48.1
16	0.20	63.0
17a	0.33	38.9
17b	0.42	22.2
18	0.34	37.0

3.3. Analgesic activity

Test for analgesic activity was performed by Turner [28] and Collier [29] technique using Swiss albino mice. The results of analgesic activity indicated that all test compounds exhibited significant activity. Compounds **2a–c**, **5a**, **6a**, **20a**,**b** have nearly the same activity of reference drug, and the remaining tested compounds have good central analgesic activity (Table 6). Also dihydropyrimido[4,5-*b*]quinolines (**2a–c**) and thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]-quinoline-3,5-dione (**5a**) have good activity higher than the reference drug in peripheral analgesic activity testing. Isoxazolothiazolo (**6a**), 2-alkylthio- (**7a**,**c**), 4-piperazino (**20a**) and 4-morpholino-pyrimido[4,5-*b*]quinolines (**20a**,**b**) have the same potency as reference drug. Furthermore, the

Table 3
Assay for bleomycin-dependent DNA damage (DNA)

Compounds	Absorbance of samples
Ascorbic acid	0.020
2a	0.025
10b	0.021
16	0.065
17a	0.035

Table 4
Comparison between dihydrocompounds **2a–c** in anti-oxidant assays by ABTS method

2a		**2b**		**2c**	
H		Cl		OCH_3	
ABTS (%)	H (%)	ABTS (%)	H (%)	ABTS (%)	H (%)
81.50	35.75	59.30	12.72	66.70	8.18

remaining compounds have moderate activity in peripheral analgesic activity testing (Table 7).

4. Conclusions

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 polycondensed new heterocyclic ring systems. Compounds **2a–c** showed the highest inhibitory anti-oxidant activity either using erythrocyte hemolysis or ABTS methods. These quinolones are either phenyl, or 4-chlorophenyl or 4-methoxyphenyl. This means the presence of the phenyl group will potentiate the activity which may increase by introducing an electron-donating group e.g. OCH_3, or electron-withdrawing group e.g. Cl in *p*-position. Compounds **2a**, **10b**, **16** and **17a** manifested the best protective effect against DNA damage induced by bleomycin. The phenyl derivative of **2** proved to be more active than that of methoxy or chloro derivatives. Compounds **2c**, **5a**, **20a**, **2a** and **2b** exhibited a potent anti-inflammatory activity using carrageenan-induced paw edema test in rats. The *p*-methoxy derivative of **2** retained the best activity in all tested assays. This may suggest that the mechanism of anti-inflammatory and analgesic activities of compounds **2a–c**, especially **2c**, may be due to the potent anti-oxidant activity to maintain the integrity of both the cell membrane and DNA of the cells.

Table 5
Percent inflammatory activity of the tested compounds (carrageenan-induced paw edema in rats)

Compounds	Percent protection		
	1 h	2 h	3 h
2a	49.0 ± 1.27*	53.2 ± 1.32**	41.0 ± 1.31*
2b	56.3 ± 1.37**	62.0 ± 1.76**	40.2 ± 1.05*
2c	59.5 ± 1.06**	62.4 ± 2.03**	46.5 ± 1.26*
5a	59.8 ± 1.41**	59.3 ± 1.32*	42.3 ± 1.26*
6a	49.6 ± 2.41*	57.1 ± 1.63*	34.8 ± 1.33*
7a	50.1 ± 1.51**	56.2 ± 1.64**	32.4 ± 1.21*
7c	46.3 ± 1.53*	48.6 ± 139*	38.6 ± 1.39*
14	46.7 ± 2.28*	44.2 ± 1.83*	39.2 ± 1.04*
18	41.2 ± 1.38*	42.5 ± 1.46*	31.1 ± 1.33*
19a	42.3 ± 1.63*	45.8 ± 1.47*	32.9 ± 1.26*
19b	44.1 ± 1.83*	49.0 ± 1.14	30.5 ± 1.83*
19c	39.8 ± 1.42*	42.3 ± 1.42*	25.3 ± 1.32*
20a	43.1 ± 162*	52.0 ± 1.95**	42.0 ± 1.92*
20b	39.2 ± 1.39*	47.1 ± 1.45*	37.3 ± 1.21*
23	34.0 ± 1.36*	39.6 ± 1.46*	36.7 ± 1.54*
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 ± SE ($n = 6$). Significance levels *$p < 0.5$, **$p < 0.001$ as compared with respective control. Dose is 20 mg/kg for the selected tested compound.

5. Experimental

5.1. Chemistry

All melting points were measured using an Electrothermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were recorded by Vario, Elementar apparatus (Shimadzu) (Table 8). The IR spectra (KBr) were recorded on a Perkin–Elmer 1650 spectrometer (USA). ^{1}H NMR spectra were determined on a JEOL EX-270 run for H NMR at 270 MHz and run for C NMR at 67.5 MHz: or on JEOL ECA-500 run for H NMR at 500 MHz and run for C NMR at 125 MHz, and chemical shifts were expressed in parts per million relative to $SiMe_4$ as internal standards. Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan). The starting materials are prepared according to El-Gazzar [18].

5.1.1. Synthesis of 5-aryl-9-(arylmethylene)-5,10-dihydro-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**2a–c**): general procedure

A mixture of **1a–c** (0.01 mol) and 6-aminothiouracil (0.01 mol) was refluxed in dimethylformamide (50 mL) for 3–4 h. The reaction mixture was cooled, the deposited precipitate was filtered off, washed with ethanol and dried, and crystallized from dimethylformamide to obtain **2a–c**, as yellow powder products.

*5.1.1.1. Synthesis of 5,10-dihydro-5-phenyl-9-(phenylmethylene)-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**2a**).* It was obtained from **1a**. IR (KBr, cm^{-1}): 3385 (br, NH's), 3056 (CH aryl), 2924 (CH alkyl), 1686 (CO), 1635 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65–1.67 (m, 2H, CH_2), 2.29 (t, 2H, $J = 5.83$ Hz, CH_2), 2.80 (t, 2H, $J = 5.85$ Hz, CH_2), 5.43 (s, 1H, C5–H), 7.00–7.13 (m, 2H, phenyl), 7.16–7.19 (m, 5H, phenyl), 7.25–7.28 (m, 3H, phenyl), 8.21 (s, 1H, CH), 9.50, 10.40, 12.00 (3 br s, 3NH, D_2O exchangeable); its MS, [M^+], *m/z* 399 (100%).

*5.1.1.2. Synthesis of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-5,10-dihydro-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**2b**).* It was obtained from **1b**. IR (KBr, cm^{-1}): 3390 (br, NH's), 3036 (CH aryl), 2941 (CH alkyl), 1690 (CO), 1615 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm); 1.63–1.66 (m, 2H, CH_2), 2.28 (t, 2H, $J = 5.78$ Hz, CH_2), 2.78 (t, 2H, $J = 5.83$ Hz, CH_2), 5.41 (s, 1H, C5–H), 7.12 (d, 2H, $J = 8.35$ Hz, phenyl), 7.17 (d, 2H, $J = 8.37$ Hz, phenyl), 7.22–7.28 (m, 4H, phenyl), 8.21 (s, 1H, CH), 10.20, 11.00, 12.30 (3 br s, 3NH, D_2O exchangeable); its MS, [M^+], *m/z* 467 (100%), [$M^+ + 2$], *m/z* 469 (52%), [$M^+ + 4$], *m/z* 471 (13%).

*5.1.1.3. Synthesis of 5-(4-methoxyphenyl)-9-(4-methoxyphenylmethylene)-5,10-dihydro-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**2c**).* It was obtained from **1c**. IR (KBr, cm^{-1}): 3410 (br, NH's), 3047 (CH aryl), 2916 (CH alkyl), 1686 (CO), 1630 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.61–1.65 (m, 2H, CH_2), 2.24 (t, 2H, $J = 5.84$ Hz, CH_2),

Table 6
Central analgesic activity (hot-plate test)

Compounds	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]
2a	9.40 ± 0.35	9.42 ± 0.45[a]	10.41 ± 0.29[a]	11.67 ± 0.59[a,b]
2b	7.40 ± 0.38	9.42 ± 0.45[a]	10.41 ± 0.29[a]	11.67 ± 0.59[a,b]
2c	8.16 ± 0.57	9.84 ± 0.28[a]	11.62 ± 0.57[a]	11.88 ± 0.47[a]
5a	8.90 ± 0.65	9.16 ± 0.57[a]	10.78 ± 0.45[a]	11.08 ± 0.24[b]
6a	6.24 ± 0.57	7.08 ± 0.78[a]	9.52 ± 0.82[a]	12.68 ± 0.61[a]
7a	7.40 ± 0.36	8.42 ± 0.45[a]	10.41 ± 0.29[a]	10.67 ± 0.59[a,b]
7c	8.26 ± 0.40	8.68 ± 0.48	9.80 ± 0.52	10.40 ± 0.18[b]
14	6.64 ± 0.20	7.54 ± 0.26[b]	8.61 ± 0.60[a]	12.00 ± 0.36[a]
18	8.26 ± 0.40	9.68 ± 0.48	9.80 ± 0.52	10.40 ± 0.18[b]
19a	7.26 ± 0.40	8.50 ± 0.48	9.86 ± 0.52	10.47 ± 0.18[b]
19b	9.38 ± 0.30	10.14 ± 0.26[b]	10.74 ± 0.28[b]	7.62 ± 0.60[b]
19c	8.42 ± 0.61	8.62 ± 0.34	9.06 ± 0.56[b]	9.22 ± 0.47[b]
20a	6.08 ± 0.90	7.93 ± 0.74	10.15 ± 1.20	11.50 ± 0.40[a]
20b	7.08 ± 0.13	8.65 ± 0.87	10.98 ± 0.91[a]	11.12 ± 0.75[a,b]
23	8.09 ± 0.34	8.82 ± 0.35[a]	9.86 ± 0.58[a]	10.46 ± 0.48[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 ± SE of six animals for each group.
[a] $p < 0.05$: statistically significant from control (Dunnett's test).
[b] $p < 0.05$: statistically significant from ASA (Dunnett's test).

2.73 (t, 2H, $J = 5.79$ Hz, CH_2), 3.87, 3.91 (2s, 6H, $2CH_3$), 5.45 (s, 1H, C5–H), 7.14 (d, 2H, $J = 8.42$ Hz, phenyl), 7.20 (d, 2H, $J = 8.43$ Hz, phenyl), 7.26–7.29 (m, 4H, phenyl), 8.30 (s, 1H, CH), 9.20, 10.20, 11.50 (3 br s, 3NH, D_2O exchangeable); its MS, [M^+], *m/z* 459 (100%).

*5.1.2. Synthesis of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**3**)*

A mixture of **1b** (0.01 mol) and 6-aminothiouracil (0.01 mol) was refluxed in dimethylformamide (50 mL) for 20–30 h. The reaction mixture was cooled, the deposited precipitate was filtered off, washed with ethanol and dried, and crystallized from dimethylformamide. IR (KBr, cm^{-1}): 3380 (br, NH's), 3025 (CH aryl), 2921 (CH alkyl), 1688 (CO), 1631 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.64–1.67 (m, 2H, CH_2), 2.30 (t, 2H, $J = 6.00$ Hz, CH_2), 2.75–2.97 (t, 2H, $J = 5.93$ Hz, CH_2), 7.11 (d, 2H, $J = 8.40$ Hz, phenyl), 7.15 (d, 2H, $J = 8.39$ Hz, phenyl), 7.17–7.19 (m, 4H, phenyl), 8.19 (s, 1H, CH), 10.30, 11.50 (2 br s, 2NH); ^{13}C NMR (DMSO-d_6, δ, ppm): 22.27, 26.73, 27.18 (3C, $3CH_2$), 108.3, 127.9, 128.2, 128.5, 128.6, 129.4, 130.1, 131.1, 132.3,

Table 7
Percent analgesic activity (peripheral, writhing test)

Compounds	Percent protection			
	30 min	1 h	2 h	3 h
2a	61.2 ± 1.15*	65.1 ± 1.31**	69.2 ± 1.03**	50.1 ± 1.73*
2b	60.4 ± 1.51**	69 ± 1.56**	74.3 ± 1.84**	47.3 ± 172*
2c	69.0 ± 1.05**	74 ± 1.93**	75.4 ± 1.51**	58.2 ± 1.17*
5a	73.2 ± 1.05**	76 ± 1.39**	77.2 ± 1.31	60.3 ± 1.39**
6a	46.7 ± 1.51*	53 ± 1.49**	59.3 ± 1.69	36.5 ± 1.09*
7a	50.4 ± 1.34*	52 ± 1.02*	55.5 ± 1.37	37.6 ± 1.49*
7c	46.0 ± 1.93*	54 ± 1.41*	56.6 ± 1.39	37.3 ± 1.23*
14	43.5 ± 1.92*	49 ± 1.37*	53.1 ± 1.16	38.4 ± 1.81*
18	41.4 ± 1.16*	47 ± 1.32*	48.8 ± 1.39	36.2 ± 1.74*
19a	43.4 ± 1.21*	48 ± 1.42*	51.3 ± 1.60	39.6 ± 1.83*
19b	43.2 ± 1.32*	45 ± 1.03*	46.1 ± 1.39	34.7 ± 1.15*
19c	41.5 ± 1.43*	52 ± 1.26*	49.3 ± 1.92	37.3 ± 1.39*
20a	49.5 ± 1.05*	50 ± 1.63*	49.4 ± 1.37	30.5 ± 1.61*
20b	45.2 ± 1.46*	47 ± 1.52*	45.2 ± 1.47	27.8 ± 1.43*
23	41.0 ± 1.63*	45 ± 1.32*	49.7 ± 1.92	33.1 ± 1.32*
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 ± SE ($n = 6$). Significance levels *$p < 0.5$, **$p < 0.001$ as compared with respective control. Dose is 20 mg/kg for the selected tested compound.

Table 8
Physical and chemical properties of synthesized compounds

Compounds	Yield (%)	M.p. (°C)	Mol. formula (M_r)	Found (calculated) (%)		
				C	H	N
2a	66	330–332	$C_{24}H_{21}N_3OS$ (399.5)	72.15 (72.17)	5.30 (5.27)	10.52 (10.49)
2b	64	215–217	$C_{24}H_{19}Cl_2N_3OS$ (468.4)	61.54 (61.56)	4.09 (4.11)	8.97 (8.85)
2c	67	228–230	$C_{26}H_{25}N_3O_3S$ (459.5)	67.95 (67.94)	5.48 (5.46)	9.14 (9.16)
3	85	295–297	$C_{24}H_{17}Cl_2N_3OS$ (466.3)	61.81 (61.78)	3.67 (3.65)	9.01 (9.06)
4	73	302–304	$C_{26}H_{17}Cl_2N_3O_2S$ (506.4)	61.66 (61.64)	3.38 (3.37)	8.29 (8.30)
5a	85	354–356	$C_{33}H_{21}Cl_2N_3O_2S$ (594.5)	66.66 (66.70)	3.56 (3.59)	7.09 (7.12)
5b	80	330–332	$C_{34}H_{23}Cl_2N_3O_3S$ (624.5)	65.38 (65.40)	3.71 (3.68)	6.73 (6.75)
5c	80	316–218	$C_{31}H_{19}Cl_2N_3O_2S_2$ (600.5)	62.00 (62.04)	3.19 (3.22)	6.99 (7.01)
5d	80	195–197	$C_{35}H_{26}Cl_2N_4O_2S$ (637.5)	65.93 (65.95)	4.11 (4.13)	8.79 (8.83)
6a	81	238–240	$C_{33}H_{22}Cl_2N_4O_2S$ (609.5)	65.02 (65.00)	3.64 (3.67)	9.19 (9.20)
6b	82	188–190	$C_{34}H_{24}Cl_2N_4O_3S$ (639.5)	63.85 (63.88)	3.78 (3.80)	8.76 (8.75)
6c	80	290–292	$C_{31}H_{20}Cl_2N_4O_2S_2$ (615.5)	60.48 (60.51)	3.27 (3.29)	9.10 (9.12)
7a	80	327–330	$C_{25}H_{19}Cl_2N_3OS$ (480.4)	62.50 (62.47)	3.98 (3.94)	8.75 (8.79)
7b	74	310–312	$C_{26}H_{21}Cl_2N_3OS$ (494.4)	63.15 (63.17)	4.28 (4.30)	8.50 (8.46)
7c	85	239–241	$C_{29}H_{23}Cl_2N_3O_3S$ (564.5)	61.70 (61.68)	4.10 (4.08)	7.44 (7.41)
7d	88	205–207	$C_{27}H_{21}Cl_2N_3O_2S$ (522.4)	62.07 (62.05)	4.05 (4.03)	8.04 (8.01)
7e	90	228–230	$C_{32}H_{23}Cl_2N_3O_2S$ (584.5)	65.76 (65.73)	3.96 (3.98)	7.19 (7.20)
8	80	278–280	$C_{24}H_{19}Cl_2N_5O$ (464.4)	62.08 (62.11)	4.12 (4.09)	15.08 (15.06)
9a	82	290–292	$C_{27}H_{19}Cl_2N_3OS$ (504.4)	64.26 (64.27)	3.79 (3.80)	8.33 (8.35)
9b	80	272–274	$C_{32}H_{21}Cl_2N_3OS$ (566.5)	67.84 (67.82)	3.74 (3.71)	7.42 (7.45)
10a	75	218–220	$C_{26}H_{21}Cl_2N_3OS$ (494.4)	63.15 (63.12)	4.28 (4.31)	8.50 (8.48)
10b	77	245–247	$C_{27}H_{23}Cl_2N_3OS$ (508.4)	63.78 (63.76)	4.56 (4.54)	8.26 (8.30)
10c	78	146–148	$C_{27}H_{23}Cl_2N_3OS$ (508.4)	63.78 (63.80)	4.56 (4.60)	8.26 (8.29)
10d	76	168–170	$C_{28}H_{25}Cl_2N_3OS$ (522.5)	64.36 (64.40)	4.82 (4.85)	8.04 (8.02)
11	71	243–246	$C_{25}H_{19}Cl_2N_3O_3S$ (512.4)	58.60 (58.61)	3.74 (3.72)	8.20 (8.17)
12	58	270–273	$C_{29}H_{23}Cl_2N_5OS$ (560.5)	62.14 (62.09)	4.13 (4.15)	12.49 (12.47)
13	60	261–264	$C_{30}H_{23}Cl_2N_5OS_2$ (604.5)	59.60 (59.58)	3.83 (3.85)	11.58 (11.61)
14	80	221–223	$C_{29}H_{21}Cl_2N_3O_2S$ (546.4)	63.74 (63.75)	3.87 (3.90)	7.69 (7.71)
15	70	269–271	$C_{29}H_{22}Cl_2N_4O_2S$ (561.5)	62.03 (62.02)	3.95 (3.97)	9.98 (10.01)
16	80	181–183	$C_{30}H_{24}Cl_2N_6OS_2$ (619.6)	58.16 (58.18)	3.90 (3.87)	13.57 (13.49)
17a	61	289–291	$C_{36}H_{25}Cl_2N_3O_2S$ (634.5)	68.14 (68.12)	3.97 (3.95)	6.62 (6.58)
17b	65	256–259	$C_{37}H_{27}Cl_2N_3O_3S$ (664.6)	66.86 (66.84)	4.10 (4.12)	7.22 (7.19)
18	80	360–362	$C_{25}H_{18}Cl_3N_3S$ (498.8)	60.19 (60.17)	3.63 (3.65)	8.42 (8.43)
19a	80	245–247	$C_{31}H_{24}Cl_2N_4S$ (555.5)	67.03 (67.01)	4.35 (4.29)	10.09 (10.07)
19b	74	282–285	$C_{31}H_{23}Cl_3N_4S$ (589.9)	63.11 (63.09)	3.93 (3.90)	9.50 (9.47)
19c	76	257–260	$C_{32}H_{26}Cl_2N_4OS$ (585.5)	65.64 (65.62)	4.47 (4.45)	9.57 (9.59)
20a	68	227–230	$C_{29}H_{23}Cl_2N_5S$ (544.5)	63.97 (64.01)	4.26 (4.23)	12.86 (12.84)
20b	63	241–243	$C_{29}H_{22}Cl_2N_4OS$ (545.5)	63.85 (63.82)	4.07 (4.05)	10.27 (10.30)
21	77	223–225	$C_{32}H_{24}Cl_2N_4O_2S$ (599.5)	64.10 (64.09)	4.03 (4.01)	9.35 (9.37)
22	75	184–186	$C_{32}H_{22}Cl_2N_4OS$ (581.5)	66.09 (66.11)	3.81 (3.79)	9.63 (9.65)
23	90	298–300	$C_{24}H_{21}Cl_2N_7$ (478.4)	60.25 (60.23)	4.43 (4.45)	20.50 (20.52)

132.4, 135.5, 135.7, 136.2, 149.7, 158.4 (15 line for 19 sp^2 carbon atoms), 162.2 (CO), 176.4 (CS); its MS, [M^+], *m/z* 465 (100%), [$M^+ + 2$], *m/z* 467 (45%), [$M^+ + 4$], *m/z* 469 (11%).

*5.1.3. Synthesis of 6-(chlorophenyl)-10-(4-chlorophenyl methylene)-7,8,9,10-tetrahydrothiazolo [3′,2′:1,2]pyrimido[4,5-b] quinoline-3,5-dione (**4**)*

A mixture of **3** (0.01 mol), chloroacetic acid (0.01 mol) and anhydrous sodium acetate (0.02 mol) was heated gently with stirring on a water bath (60 °C) for 2 h. The reaction mixture was allowed to cool to room temperature and poured into water (100 mL). The deposited precipitate was filtered off and crystallized from dioxane. The compound was produced as a yellow powder. IR (KBr, cm^{-1}): 3035 (CH aryl), 2917 (CH alkyl), 1696, 1680 (2CO), 1629 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.63–1.67 (m, 2H, CH_2), 2.34 (t, 2H, J = 5.86 Hz, CH_2), 2.66 (t, 2H, J = 5.89 Hz, CH_2), 3.00 (s, 2H, CH_2), 7.03 (d, 2H, J = 8.42 Hz, phenyl), 7.21 (d, 2H, J = 8.40 Hz, phenyl), 7.36 (d, 2H, J = 8.37 Hz, phenyl), 7.57 (d, 2H, J = 8.40 Hz, phenyl), 8.18 (s, 1H, CH).

*5.1.4. Synthesis of 2-arylmethylene-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7,8,9,10-tetrahydro thiazolo[3′,2′:1,2]pyrimido[4,5-b]quinoline-3,5-diones (**5a–c**): general procedure*

Method A. A mixture of **3** (0.01 mol), chloroacetic acid (0.01 mol), the appropriate aromatic aldehyde (0.01 mol) and (0.02 mol) anhydrous sodium acetate was stirred under reflux in 30 mL of glacial acetic acid and 15 mL of acetic

anhydride for 15 h. The reaction mixture was cooled and poured into cold water (100 mL). The deposited precipitate was filtered off and crystallized from appropriate solvent to produce **5a–c**.

Method B. A mixture of **4** (0.01 mol), the appropriate aromatic aldehyde (0.01 mol) and anhydrous sodium acetate (0.02 mol) was stirred under reflux in 30 mL of glacial acetic acid and 15 mL of acetic anhydride for 5 h. The reaction mixture was allowed to cool to room temperature and poured into cold water (100 mL). The deposited precipitate was filtered off and crystallized from appropriate solvent to produce **5a**.

*5.1.4.1. Synthesis of 6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-2-phenylmethylene-7,8,9,10-tetrahydrothiazolo-[3′,2′:1,2]pyrimido[4,5-b]quinoline-3,5-dione (**5a**).* It was obtained from benzaldehyde as yellow powder and crystallized from dioxane. IR (KBr, cm^{-1}): 3055 (CH aryl), 2937 (CH alkyl), 1687, 1675 (2CO), 1626 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.67 (t, 2H, $J = 6.01$ Hz, CH_2), 2.35 (t, 2H, $J = 5.95$ Hz, CH_2), 2.65–2.82 (m, 2H, CH_2), 6.91–7.10 (m, 3H, phenyl), 7.15–7.26 (m, 2H, phenyl), 7.30–7.41 (m, 2H, phenyl), 7.42–7.72 (m, 4H, phenyl), 8.10 (s, 1H, CH), 8.20 (s, 1H, CH).

*5.1.4.2. Synthesis of 6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-2-(4-methoxyphenylmethylene)-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinoline-3,5-dione (**5b**).* It was obtained from 4-anisaldehyde as yellow powder and crystallized from dimethylformamide. IR (KBr, cm^{-1}): 3051 (CH aryl), 2927 (CH alkyl), 1690, 1676 (2CO), 1632 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65 (t, 2H, $J =$ 5.86 Hz, CH_2), 2.34–2.49 (m, 2H, CH_2), 2.81 (t, 2H, $J =$ 5.94 Hz, CH_2), 3.81 (s, 3H, OCH_3), 7.10 (d, 2H, $J = 8.42$ Hz, phenyl), 7.19 (d, 2H, $J = 8.40$ Hz, phenyl), 7.47 (d, 2H, $J = 8.37$ Hz, phenyl), 7.53 (d, 2H, $J = 8.39$ Hz, phenyl), 7.58–7.71 (m, 4H, phenyl), 7.86 (s, 1H, CH), 8.07 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, δ, ppm): 24.9, 30.7, 30.9 (3C, $3CH_2$), 55.9 (1C, OCH_3), 114.2, 115.9, 119.7, 127.4, 127.5, 127.8, 127.9, 128.8, 129.4, 130.2, 133.4, 133.5, 133.9, 134.8, 136.1, 142.3, 143.2, 152.6, 152.9, 155.9, 159.9, 163.7 (22 line for 28 sp^2 carbon atoms) and 166.3, 168.6 (2CO).

*5.1.4.3. Synthesis of 6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-2-(2-thienyl-methylene)-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinoline-3,5-dione (**5c**).* It was obtained from 2-thiophene carboxaldehyde as brown crystals and crystallized from dioxane. IR (KBr, cm^{-1}): 3023 (CH aryl), 2902 (CH alkyl), 1696, 1672 (2CO), 1651 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65 (t, 2H, $J = 6.00$ Hz, CH_2), 2.32 (t, 2H, $J = 5.99$ Hz, CH_2), 2.72–2.73 (m, 2H, CH_2), 7.21 (d, 2H, $J = 8.37$ Hz, phenyl), 7.26 (d, 2H, $J = 8.40$ Hz, phenyl), 7.31 (t, 1H, $J = 4.32$ Hz, thiophene), 7.41 (d, 2H, $J = 8.42$ Hz, phenyl), 7.55 (d, 2H, $J = 8.44$ Hz, phenyl), 7.63 (d, 1H, $J = 4.92$ Hz, thiophene), 7.67 (d, 1H, $J = 4.83$ Hz, thiophene), 8.10 (s, 1H, CH), 8.30 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, δ, ppm): 24.90, 30.72, 30.95 (3C, $3CH_2$), 115.6, 119.7, 121.9, 127.1, 127.8, 128.2, 128.8, 129.4, 130.2, 130.5, 133.4, 133.5, 134.8, 136.1, 137.8, 142.0, 143.0, 148.3, 148.7, 152.6, 152.9, 155.6 (22 line for 26 sp^2 carbon atoms) and 166.3, 168.6 (2C=O); its MS, [M^+], *m/z* 599 (100%), [$M^+ + 2$], *m/z* 601 (26%), [$M^+ + 4$], *m/z* 603 (6%).

*5.1.4.4. Synthesis of 6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-2-(4-N,N-dimethyl-aminophenylmethylene)-7,8,-9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinoline-3,5-dione (**5d**).* It was obtained from *N*,*N*-dimethylaminobenzaldehyde as brown crystals and crystallized from dioxane. IR (KBr, cm^{-1}): 3079 (CH aryl), 2924 (CH alkyl), 1700, 1678 (2CO), 1600 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.64 (t, 2H, $J = 5.79$ Hz, CH_2), 2.33 (t, 2H, $J = 5.82$ Hz, CH_2), 2.77–2.79 (m, 2H, CH_2), 2.88 (s, 6H, $2CH_3$), 7.17 (d, 2H, $J = 8.42$ Hz, phenyl), 7.28 (d, 2H, $J = 8.40$ Hz, phenyl), 7.51–7.72 (m, 4H, phenyl), 7.95 (s, 1H, CH), 8.22 (s, 1H, CH); its MS, [M^+], *m/z* 636 (100%), [$M^+ + 2$], *m/z* 638 (56%), [$M^+ + 4$], *m/z* 640 (12%).

*5.1.5. Synthesis of 3-aryl-11-(4-chlorophenyl)-7-(4-chlorophenylmethylene)-2,3,7,8,9,10-hexahydroisoxazolo [5″,4″:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-12-ones (**6a–c**): general procedure*

A mixture of **5a–c** (0.01 mol), hydroxylamine hydrochloride (0.01 mol), and anhydrous sodium acetate (0.01 mol) was stirred under reflux in 30 mL glacial acetic acid for 5 h. The reaction mixture was allowed to cool to room temperature and poured into cold water (100 mL). The deposited precipitate was filtered off, dried and crystallized from appropriate solvent to produce **6a–c**.

*5.1.5.1. Synthesis of 11-(4-chlorophenyl)-7-(4-chlorophenylmethylene)-3-phenyl-2,3,7,8,9,10-hexahydroisoxazolo[5″,-4″:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-12-one (**6a**).* It was obtained from **5a** as green crystals and crystallized from benzene. IR (KBr, cm^{-1}): 3410 (br, NH), 3056 (CH aryl), 2928 (CH alkyl), 1692 (CO), 1640 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65 (t, 2H, $J = 5.89$ Hz, CH_2) 2.34 (t, 2H, $J = 6.00$ Hz, CH_2), 2.72–2.83 (m, 2H, CH_2), 6.00 (d, 1H, $J = 6.43$ Hz, isooxazole proton), 7.02–7.15 (m, 3H, phenyl), 7.18–7.28 (m, 2H, phenyl), 7.30–7.43 (m, 2H, phenyl), 7.52–7.70 (m, 4H, phenyl), 8.24 (s, 1H, CH), 10.30 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 608 (100%), [$M^+ + 2$], *m/z* 610 (49%), [$M^+ + 4$], *m/z* 614 (10%).

*5.1.5.2. Synthesis of 11-(4-chlorophenyl)-7-(4-chlorophenylmethylene)-3-(4-methoxy-phenyl)-2,3,7,8,9,10-hexahydroisoxazolo[5″,4″:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-b]-quinolin-12-one (**6b**).* It was obtained from **5b** as green powder and crystallized from ethanol. IR (KBr, cm^{-1}): 3400 (br, NH), 3067 (CH aryl), 2923 (CH alkyl), 1685 (CO), 1630 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.67 (t, 2H, $J = 6.02$ Hz, CH_2), 2.30–2.39 (m, 2H, CH_2), 2.76 (t, 2H, $J = 6.00$ Hz, CH_2), 3.87 (s, 3H, OCH_3), 5.89 (d, 1H, $J = 6.54$ Hz,

isooxazole proton), 7.10 (d, 2H, $J = 8.43$ Hz, phenyl), 7.18 (d, 2H, $J = 8.39$ Hz, phenyl), 7.45 (d, 2H, $J = 8.37$ Hz, phenyl), 7.53 (d, 2H, $J = 8.39$ Hz, phenyl), 7.58–7.76 (m, 4H, phenyl), 8.27 (s, 1H, CH), 10.00 (br s, NH, D_2O exchangeable); its MS, [M^+], *m/z* 638 (100%), [$M^+ + 2$], *m/z* 640 (53%), [$M^+ + 4$], *m/z* 642 (15%).

*5.1.5.3. Synthesis of 11-(4-chlorophenyl)-7-(4-chlorophenylmethylene)-3-(2-thienyl)-2,3,7,8,9,10-hexahydroisoxazolo-[5″,4″:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-12-one (**6c**).* It was obtained from **5c** as a yellow powder and crystallized from ethanol. IR (KBr, cm^{-1}): 3340 (br, NH), 3049 (CH aryl), 2929 (CH alkyl), 1688 (CO), 1625 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.67 (t, 2H, $J = 5.97$ Hz, CH_2), 2.33 (t, 2H, $J = 5.95$ Hz, CH_2), 2.71–2.76 (m, 2H, CH_2), 6.02 (d, 1H, $J = 6.38$ Hz, isooxazole proton), 7.21 (d, 2H, $J = 8.41$ Hz, phenyl), 7.26 (t, 1H, $J = 4.40$ Hz, thiophene), 7.32 (d, 2H, $J = 8.37$ Hz, phenyl), 7.46 (d, 2H, $J = 8.43$ Hz, phenyl), 7.57 (d, 2H, $J = 8.42$ Hz, phenyl), 7.67 (d, 1H, $J = 4.86$ Hz, thiophene), 7.89 (d, 1H, $J = 4.76$ Hz, thiophene), 8.30 (s, 1H, CH), 10.80 (br s, NH, D_2O exchangeable); its MS, [M^+], *m/z* 614 (100%), [$M^+ + 2$], *m/z* 616 (46%), [$M^+ + 4$], *m/z* 618 (11%).

*5.1.6. Synthesis of 2-alkylthio-9-(4-chlorophenylmethylene)-5-(4-chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-ones (**7a–e**): general procedure*

To a warmed ethanolic KOH solution (prepared by dissolving 0.01 mol of KOH in 50 mL ethanol) was added each of **3** (0.01 mol), the heating was continued for 30 min and the mixture was allowed to cool to room temperature, and the proper halo-compound (0.012 mol) was added. The mixture was stirred under reflux for 5 h, then cooled to room temperature and poured into cold water (100 mL). The solid product precipitated was filtered off, washed with 100 mL water, the product was dried and crystallized to produce **7a–e**.

*5.1.6.1. Synthesis of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]-quinolin-4-one (**7a**).* It was obtained from **3** and methyl iodide (0.012 mol) as pale yellow crystals and crystallized from dioxane. IR (KBr, cm^{-1}): 3403 (br, NH), 3036 (CH aryl), 2925 (CH alkyl), 1687 (CO), 1652 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.67 (t, 2H, $J = 5.96$ Hz, CH_2), 2.33 (t, 2H, $J = 6.00$ Hz, CH_2), 2.73–2.77 (m, 2H, CH_2), 2.84 (s, 3H, SCH_3), 7.20 (d, 2H, $J = 8.36$ Hz, phenyl), 7.33 (d, 2H, $J = 8.45$ Hz, phenyl), 7.45 (d, 2H, $J = 8.43$ Hz, phenyl), 7.59 (d, 2H, $J = 8.39$ Hz, phenyl), 8.34 (s, 1H, CH), 9.20 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 479 (100%), [$M^+ + 2$], *m/z* 481 (56%), [$M^+ + 4$], *m/z* 483 (16%) [$M^+ - SCH_3$], *m/z* 432 (44%).

*5.1.6.2. Synthesis of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-ethylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**7b**).* It was obtained from **3** and ethyl iodide (0.012 mol) as orange crystals and crystallized from dioxane. IR (KBr, cm^{-1}): 3385 (br, NH), 3061 (CH aryl), 2908 (CH alkyl), 1679 (CO), 1635 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.36 (t, 3H, $J = 7.40$ Hz, CH_3), 1.68 (t, 2H, $J = 5.69$ Hz, CH_2), 2.30 (t, 2H, $J = 5.78$ Hz, CH_2), 2.70–2.76 (m, 2H, CH_2), 3.48 (q, 2H, $J = 7.38$ Hz, SCH_2), 7.21 (d, 2H, $J = 8.37$ Hz, phenyl), 7.34 (d, 2H, $J = 8.38$ Hz, phenyl), 7.47 (d, 2H, $J = 8.41$ Hz, phenyl), 7.57 (d, 2H, $J = 8.40$ Hz, phenyl), 8.22 (s, 1H, CH), 10.00 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 493 (100%), [$M^+ + 2$], *m/z* 495 (31%), [$M^+ - CH_3$], *m/z* 478 (36%), [$M^+ - SC_2H_5$], *m/z* 432 (85%).

*5.1.6.3. Synthesis of 2-(acetylacetonethio)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**7c**).* It was obtained from **3** and chloroacetylacetone as yellow crystals and crystallized from dioxane. IR (KBr, cm^{-1}): 3410 (br, NH), 3056 (CH aryl), 2918 (CH alkyl), 1720, 1715, 1686, (3CO), 1658 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.74 (t, 2H, $J = 5.87$ Hz, CH_2), 2.29 (t, 2H, $J = 5.91$ Hz, CH_2), 2.69–2.73 (m, 2H, CH_2), 3.11 and 3.16 (2s, 6H, $2COCH_3$), 4.04 (s, 1H, CH), 6.94 (d, 2H, $J = 8.37$ Hz, phenyl), 7.17 (d, 2H, $J = 8.40$ Hz, phenyl), 7.27 (d, 2H, $J = 8.39$ Hz, phenyl), 7.52 (d, 2H, $J = 8.41$ Hz, phenyl), 8.32 (s, 1H, CH), 9.80 (br, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 16.41, 23.06 ($2CH_3$), 24.21, 27.99, 28.25 (3C, $3CH_2$), 40.72 (1C, CH), 115.2, 126.1, 126.9, 129.2, 129.3, 129.5, 129.9, 130.0, 133.7, 135.0, 135.4, 139.1, 139.3, 140.7, 152.3, 154.4 (16 line for 20 sp^2 carbon atoms), 167.57, 185.68, 192.30 (3CO); its MS, [M^+], *m/z* 563 (100%), [$M^+ + 2$], *m/z* 565 (46%), [$M^+ + 4$], *m/z* 567 (14%), [$M^+ - COCH_3$], *m/z* 520 (45%), [$M^+ - CH(COCH_3)_2$], *m/z* 454 (85%), [$M^+ - SCH(COCH_3)_2$], *m/z* 422 (19%).

*5.1.6.4. Synthesis of 2-(S-acetone)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**7d**).* It was obtained from **3** and chloroacetone as yellow crystals and crystallized from ethanol. IR (KBr, cm^{-1}): 3430 (br, NH), 3059 (CH aryl), 2909 (CH alkyl), 1718, 1683 (2CO), 1651 (C=N); ^1H NMR (DMSO-d_6): 1.63–1.66 (m, 2H, CH_2), 1.84 (s, 3H, CH_3), 2.30 (t, 2H, $J = 5.98$ Hz, CH_2), 2.80 (t, 2H, $J = 6.00$ Hz, CH_2), 3.98 (s, 2H, CH_2), 7.11 (d, 2H, $J = 8.40$ Hz, phenyl), 7.20 (d, 2H, $J = 8.38$ Hz, phenyl), 7.27–7.34 (m, 4H, phenyl), 8.26 (s, 1H, CH), 10.30 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 521 (100%), [$M^+ + 2$], *m/z* 523 (52%), [$M^+ + 4$], *m/z* 525 (7%), [$M^+ - COCH_3$], *m/z* 480 (75%), [$M^+ - CH_2COCH_3$], *m/z* 466 (66%), [$M^+ - SCH_2CO-CH_3$], *m/z* 434 (28%).

*5.1.6.5. 2-(S-Phenacyl)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**7e**).* It was obtained from **3** and phenacylbromide as pale yellow crystals and crystallized from ethanol/dioxane (1:1). IR (KBr, cm^{-1}): 3437 (br, NH), 3078 (CH aryl), 2923 (CH alkyl), 1721, 1689 (2CO), 1647 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.67 (t, 2H, $J = 5.96$ Hz, CH_2), 2.35 (t, 2H, $J = 5.89$ Hz, CH_2), 2.65–2.82 (m, 2H, CH_2), 3.97 (s, 2H, CH_2), 6.98–7.10 (m, 3H, phenyl), 7.17–7.26 (m, 2H, phenyl), 7.33–7.41 (m, 2H, phenyl), 7.50–7.72 (m, 4H, phenyl), 8.22

(s, 1H, CH), 10.30 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 583 (100%), [$M^+ + 2$], *m/z* 585 (53%), [$M^+ + 4$], *m/z* 587 (10%), [$M^+ - C_6H_5$], *m/z* 506 (100%), [$M^+ - COC_6H_5$], *m/z* 478 (15%).

*5.1.7. Synthesis of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-hydrazino-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**8**)*

A suspension of **7a**,**b** (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 methylmercaptan to form a clear solution. After 30 min when the solid product started separating out, 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, and obtained as yellow powder crystals and crystallized from dimethylformamide. IR (KBr, cm^{-1}): 3198 (br s, NH), 3036 (CH aryl), 2934 (CH alkyl), 1676 (CO), 1676 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.64–1.69 (m, 2H, CH_2), 2.31 (t, 2H, $J = 5.98$ Hz, CH_2), 2.77 (t, 2H, $J = 6.01$ Hz, CH_2), 7.09 (d, 2H, $J = 8.34$ Hz, phenyl), 7.40 (d, 2H, $J = 8.37$ Hz, phenyl), 7.43–7.47 (m, 4H, phenyl), 8.02 (s, 1H, CH), 8.75 (br s, NH_2, D_2O exchangeable), 9.50, 11.00 (2 br s, 2NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ, ppm): 22.27, 22.73, 27.19 (3C, $3CH_2$), 109.1, 127.7, 127.8, 128.3, 129.3, 129.4, 131.1, 131.6, 133.4, 136.1, 136.3, 137.9, 141.5, 148.2 150.0, 155.1 (16 lines for 20 sp^2 carbon atoms), 160.2 (CO); its MS, [M^+], *m/z* 463 (100%), [$M^+ + 2$], *m/z* 465 (56%), [$M^+ + 4$], *m/z* 467 (14%).

*5.1.8. Synthesis of 6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-(methyl or phenyl)-7,8,9-hexahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-5-ones (**9a**,**b**): general procedure*

A solution of **7d**,**e** (0.01 mol) in glacial acetic acid (40 mL) and catalytic amount of sulfuric acid (1 mL) was stirred under reflux for 8 h. The reaction mixture was allowed to cool, poured into cold water (100 mL), neutralized by ammonia solution, and the solid precipitate was filtered off, washed with water, dried and crystallized to produce **9a**,**b**.

*5.1.8.1. Synthesis of 6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]-pyrimido[4,5-b]quinolin-5-one (**9a**).* The compound was obtained from **7d** as yellow crystals and crystallized from dimethylformamide. IR (KBr, cm^{-1}): 3066 (CH aryl), 2908 (CH alkyl), 1696 (CO), 1650 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65–1.69 (m, 2H, CH_2), 2.13 (s, 3H, CH_3), 2.30 (t, 2H, $J = 5.85$ Hz, CH_2), 2.76 (t, 2H, $J = 5.81$ Hz, CH_2), 7.13 (d, 2H, $J = 8.37$ Hz, phenyl), 7.39 (d, 2H, $J = 8.36$ Hz, phenyl), 7.42–7.57 (m, 4H, phenyl), 7.83 (s, 1H, thiazole), 8.30 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, δ, ppm): 21.56 (CH_3), 25.71, 29.83, 30.09 (3C, $3CH_2$), 106.7, 118.9, 127.4, 126.5, 128.3, 128.7, 129.2, 131.4, 133.6, 134.2, 135.4, 136.7, 142.5, 145.5, 148.4, 152.1, 152.8, 154.2 (18 line for 22 sp^2 carbon atoms), 166.7 (CO); its MS, [M^+], *m/z* 503 (100%), [$M^+ + 2$], *m/z* 505 (55%), [$M^+ + 4$], *m/z* 507 (13%), [$M^+ - CH_3$], *m/z* 488 (53%).

*5.1.8.2. Synthesis of 6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-phenyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]-pyrimido[4,5-b]quinolin-5-one (**9b**).* It was obtained from **7e** as yellow crystals and crystallized from dimethylformamide. IR (KBr, cm^{-1}): 3054 (CH aryl), 2918 (CH alkyl), 1695 (CO), 1647 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.66–1.70 (m, 2H, CH_2), 2.28 (t, 2H, $J = 5.93$ Hz, CH_2), 2.75 (t, 2H, $J = 5.99$ Hz, CH_2), 6.97–7.05 (m, 4H, phenyl), 7.22 (d, 2H, $J = 8.39$ Hz, phenyl), 7.46–7.80 [m, 6H (5H, phenyl + s, 1H, thiazole)], 8.08 (d, 2H, $J = 8.43$ Hz, phenyl), 8.28 (s, 1H, CH); its MS, [M^+], *m/z* 565 (100%), [$M^+ + 2$], *m/z* 567 (57%), [$M^+ + 4$], *m/z* 569 (12%), [$M^+ - C_6H_5$], *m/z* 488 (41%).

*5.1.9. Synthesis of 3-alkyl-2-alkylthio-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-ones (**10a–d**): general procedure*

To a warmed ethanolic sodium ethoxide solution (prepared by dissolving 0.01 mol of sodium metal in 30 mL absolute ethanol) was added each of **7a** or **7b** (0.01 mol), the heating was continued for 30 min, and the mixture was allowed to cool to room temperature and the proper alkyliodide (0.012 mol) was added. The mixture was stirred under reflux for 3 h, cooled to room temperature, and poured into cold water (100 mL). The solid so-precipitated was filtered off, washed with water and dried, to produce **10a–c** in high yields.

*5.1.9.1. Synthesis of 5-(4-chlorophenyl)-2-methylthio-3-methyl-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**10a**).* It was obtained from **7a** and methyl iodode as red crystals and crystallized from benzene. IR (KBr, cm^{-1}): 3056 (CH aryl), 2940 (CH alkyl), 1696 (CO), 1618 (C=N); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.64–1.68 (m, 2H, CH_2), 2.24 (t, 2H, $J = 5.90$ Hz, CH_2), 2.65 (s, 3H, SCH_3), 2.76 (t, 2H, $J = 5.93$ Hz, CH_2), 4.20 (s, 3H, N–CH_3), 6.97 (d, 2H, $J = 8.37$ Hz, phenyl), 7.08 (d, 2H, $J = 8.40$ Hz, phenyl), 7.21 (d, 2H, $J = 8.43$ Hz, phenyl), 7.51 (d, 2H, $J = 8.38$ Hz, phenyl), 8.24 (s, 1H, CH); its MS, [M^+], *m/z* 493 (100%), [$M^+ + 2$], *m/z* 495 (61%), [$M^+ - CH_3$], *m/z* 478 (12%), [$M^+ - SCH_3$], *m/z* 446 (5%).

*5.1.9.2. Synthesis of 5-(4-chlorophenyl)-3-ethyl-2-methylthio-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**10b**).* It was obtained from **7a** and ethyl iodode as yellow powder and crystallized from dioxane. IR (KBr, cm^{-1}): 3075 (CH aryl), 2920 (CH alkyl), 1705 (CO), 1625 (C=N); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.30 (t, 3H, $J = 7.38$ Hz, CH_3), 1.66–1.69 (m, 2H, CH_2), 2.27 (t, 2H, $J = 5.86$ Hz, CH_2), 2.68 (s, 3H, SCH_3), 2.78 (t, 2H, $J = 5.90$ Hz, CH_2), 4.38 (q, 2H, $J = 7.49$ Hz, N–CH_2), 6.98 (d, 2H, $J = 8.35$ Hz, phenyl), 7.10 (d, 2H, $J = 8.37$ Hz, phenyl), 7.25 (d, 2H, $J = 8.39$ Hz, phenyl), 7.55 (d, 2H, $J = 8.38$ Hz,

phenyl), 8.18 (s, 1H, CH); its MS, [M^+], *m/z* 507 (63%), [$M^+ - C_2H_5$], *m/z* 478 (18%), [$M^+ - SC_2H_5$], *m/z* 446 (10%).

*5.1.9.3. Synthesis of 5-(4-chlorophenyl)-2-ethylthio-3-methyl-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**10c**).* It was obtained from **7b** and methyl iodode as red crystals and crystallized from benzene. IR (KBr, cm^{-1}): 3065 (CH aryl), 2930 (CH alkyl), 1698 (CO), 1620 (C=N); ^1H NMR ($CDCl_3$, δ, ppm): 1.21 (t, 3H, $J = 7.36$ Hz, CH_3), 1.68–1.71 (m, 2H, CH_2), 2.29 (t, 2H, $J = 5.91$ Hz, CH_2), 2.73 (q, 2H, $J = 7.35$ Hz, SCH_2), 2.79 (t, 2H, $J = 5.87$ Hz, CH_2), 4.32–4.40 (s, 3H, N–CH_3), 6.97 (d, 2H, $J = 8.34$ Hz, phenyl), 7.11 (d, 2H, $J = 8.38$ Hz, phenyl), 7.28 (d, 2H, $J = 8.38$ Hz, phenyl), 7.58 (d, 2H, $J = 8.39$ Hz, phenyl), 8.27 (s, 1H, CH); its MS, [M^+], *m/z* 507 (88%), [$M^+ + 2$], *m/z* 509 (51%), [$M^+ + 4$], *m/z* 511 (10%).

*5.1.9.4. Synthesis of 5-(4-chlorophenyl)-3-ethyl-2-ethylthio-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**10d**).* It was obtained from **7b** and ethyl iodode as yellow powder and crystallized from dioxane. IR (KBr, cm^{-1}): 3029 (CH aryl), 2921 (CH alkyl), 1700 (CO), 1650 (C=N); ^1H NMR ($CDCl_3$, δ, ppm): 1.15–1.20 (t, 3H, CH_3), 1.29 (t, 3H, $J = 7.43$ Hz, CH_3), 1.64–1.70 (m, 2H, CH_2), 2.30 (t, 2H, $J = 5.89$ Hz, CH_2), 2.70 (q, 2H, $J = 7.56$ Hz, SCH_2), 2.76–2.81 (t, 2H, CH_2), 4.26–4.30 (q, 2H, N–CH_2), 7.05 (d, 2H, $J = 8.37$ Hz, phenyl), 7.15 (d, 2H, $J = 8.38$ Hz, phenyl), 7.30 (d, 2H, $J = 8.36$ Hz, phenyl), 7.65 (d, 2H, $J = 8.41$ Hz, phenyl), 8.22 (s, 1H, CH); its MS, [M^+], *m/z* 521 (100%), [$M^+ + 2$], *m/z* 523 (60%), [$M^+ + 4$], *m/z* 525 (19%).

*5.1.10. Synthesis of 5-(4-chlorophenyl)-2-methylsulfone-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**11**)*

A mixture of **7a** (0.01 mol) and excess amount of hydrogen peroxide (5 mL) in acetic acid (30 mL) was heated gently with stirring for 10 h. The reaction mixture was allowed to cool to 0 °C. The deposited precipitate was filtered off and crystallized from dioxane. IR (KBr, cm^{-1}): 3385 (br, NH), 3031 (CH aryl), 2909 (CH alkyl), 1635 (C=N), 1165, 1345 (SO_2); ^1H NMR (DMSO-d_6, δ, ppm): 1.69 (t, 2H, $J = 5.86$ Hz, CH_2), 2.29 (t, 2H, $J = 5.69$ Hz, CH_2), 2.71–2.76 (m, 2H, CH_2), 3.30 (s, 3H, SO_2CH_3), 7.18 (d, 2H, $J = 8.40$ Hz, phenyl), 7.35 (d, 2H, $J = 8.39$ Hz, phenyl), 7.55 (d, 2H, $J = 8.38$ Hz, phenyl), 7.68 (d, 2H, $J = 8.37$ Hz, phenyl), 8.30 (s, 1H, CH), 9.80 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 511 (100%), [$M^+ + 2$], *m/z* 513 (63%), [$M^+ + 4$], *m/z* 515 (16%).

*5.1.11. Synthesis of 2-[3,5-dimethyl-1,2-dihydropyrazol-4-ylthio]-9-(4-chlorophenyl)-5-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**12**)*

A mixture of **7c** (0.01 mol) and hydrazine hydrate (99–100%) in dioxane (20 mL) and ethanol (10 mL) was stirred under reflux for 12 h. The reaction mixture was allowed to cool to room temperature and poured into cold water (100 mL). The deposited precipitate was filtered off, dried, and obtained as pale yellow powder and crystallized from ethanol/dioxane (1:1). IR (KBr, cm^{-1}): 3400 (br s, NH's), 3023 (CH aryl), 2929 (CH alkyl), 1687 (CO), 1653 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.68 (t, 2H, $J = 5.98$ Hz, CH_2), 2.27 (t, 2H, $J = 5.92$ Hz, CH_2), 2.41 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.70–2.75 (m, 2H, CH_2), 6.95–6.99 (m, 4H, phenyl), 7.10 (d, 2H, $J = 8.39$ Hz, phenyl), 7.34 (d, 2H, $J = 8.40$ Hz, phenyl), 8.25 (s, 1H, CH), 9.34, 10.10 (2 br s, 2NH, D_2O exchangeable); its MS, [M^+], *m/z* 559 (100%), [$M^+ + 2$], *m/z* 561 (54%), [$M^+ + 4$], *m/z* 563 (21%).

*5.1.12. Synthesis of 2-(4,6-dimethyl-2-thioxo-1,2-dihydropyrimidin-5-ylthio)-9-(4-chloro-phenylmethylene)-5-(4-chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (**13**)*

A mixture of **7c** (0.01 mol) and thiourea (0.01 mol) was stirred under reflux in dioxane (30 mL) in the presence of catalytic amount of piperidine for 15 h. The reaction mixture was allowed to cool to room temperature, poured into water (100 mL), the deposited precipitate was filtered off, washed with ethanol (30 mL), dried and crystallized from dimethylformamide as yellow powder. IR (KBr, cm^{-1}): 3420 (br s, NH's), 3043 (CH aryl), 2928 (CH alkyl), 1686, (CO), 1630 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.67 (t, 2H, $J = 5.69$ Hz, CH_2), 2.27 (t, 2H, $J = 5.74$ Hz, CH_2), 2.45 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.72–2.77 (m, 2H, CH_2), 7.05 (d, 2H, $J = 8.34$ Hz, phenyl), 7.15 (d, 2H, $J = 8.37$ Hz, phenyl), 7.34 (d, 2H, $J = 8.43$ Hz, phenyl), 7.54 (d, 2H, $J = 8.43$ Hz, phenyl), 8.30 (s, 1H, CH), 9.20, 10.50 (2 br s, 2NH, D_2O exchangeable); its MS, [M^+], *m/z* 603 (53%), [$M^+ + 2$], *m/z* 605 (34%), [$M^+ + 4$], *m/z* 607 (18%).

*5.1.13. Synthesis of 2-acetyl-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-5-one (**14**)*

A solution of **7c** (0.01 mol) in a mixture of acetic anhydride/pyridine (20:10) was stirred under reflux for 4 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL), the deposited precipitate was filtered off, dried and crystallized from benzene. The compound was obtained as yellow crystals. IR (KBr, cm^{-1}): 3066 (CH aryl), 2927 (CH alkyl), 1720, 1687, (2CO), 1630 (C=N); ^1H NMR (DMSO-d_6, δ, ppm): 1.68 (t, 2H, $J = 6.01$ Hz, CH_2), 2.28 (t, 2H, $J = 6.03$ Hz, CH_2), 2.45 (s, 3H, CH_3), 2.70–2.75 (m, 2H, CH_2), 2.83 (s, 3H, CH_3), 6.97–7.12 (m, 4H, phenyl), 7.24 (d, 2H, $J = 8.41$ Hz, phenyl), 7.54 (d, 2H, $J = 8.43$ Hz, phenyl), 8.25 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, δ, ppm): 12.70, 24.81 (2C, $2CH_3$), 25.72, 29.91, 30.13 (3C, $3CH_2$), 104.2, 119.8, 127.8, 127.9, 128.8, 128.9, 129.4, 130.3, 133.5, 133.6, 134.9, 136.1, 143.1, 145.2, 148.6, 152.7, 152.9, 155.1 (18 line for 22 sp^2 carbon atoms), 165.3, 193.1 (2CO); its MS, [M^+], *m/z* 545 (100%), [$M^+ + 2$], *m/z* 547 (56%), [$M^+ + 4$], *m/z* 549 (11%), [$M^+ - CH_3$], *m/z* 530 (15%), [$M^+ - COCH_3$], *m/z* 502 (34%).

*5.1.14. Synthesis of 2-(acetoxime)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-5-one (**15**)*

A mixture of **14** (0.01 mol), hydroxylamine hydrochloride (0.01 mol) in dioxane (30 mL) and catalytic amount of piperidine was added. The reaction mixture was stirred under reflux for 15 h, allowed to cool to room temperature, and poured into water (100 mL). The deposited precipitate was filtered off, dried and crystallized from ethanol/dioxane (1:1). IR (KBr, cm^{-1}): 3540 (br s, OH), 3045 (CH aryl), 2923 (CH alkyl), 1690 (CO), 1650 (C═N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.70 (t, 2H, $J = 5.97$ Hz, CH_2), 2.30 (t, 2H, $J = 5.95$ Hz, CH_2), 2.41 (s, 3H, CH_3), 2.73–2.78 (m, 2H, CH_2), 3.02 (s, 3H, CH_3), 3.47 (br, OH overlapped with H_2O of DMSO, D_2O exchangeable), 7.04 (d, 2H, $J = 8.36$ Hz, phenyl), 7.20 (d, 2H, $J = 8.40$ Hz, phenyl), 7.35 (d, 2H, $J = 8.40$ Hz, phenyl), 7.54 (d, 2H, $J = 8.37$ Hz, phenyl), 8.25 (s, 1H, CH); its MS, [M^+], *m/z* 560 (47%), [$M^+ + 2$], *m/z* 562 (26%), [$M^+ + 4$], *m/z* 564 (6%), [$M^+ - OH$], *m/z* 543 (100%).

*5.1.15. Synthesis of 2-(acetothiosemicarbazone)-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-5-one (**16**)*

A mixture of **14** (0.01 mol), thiosemicarbazide (0.01 mol) in dioxane (30 mL) and catalytic amount of piperidine was added. The reaction mixture was stirred under reflux for 12 h, allowed to cool to room temperature, and poured into water (100 mL). The deposited precipitate was filtered off, dried and crystallized from dioxane. The compound was obtained as green powder. IR (KBr, cm^{-1}): 3400 (br s, NH), 3071 (CH aryl), 2918 (CH alkyl), 1689, (CO), 1625 (C═N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.66 (t, 2H, $J = 6.00$ Hz, CH_2), 2.31 (t, 2H, $J = 6.02$ Hz, CH_2), 2.40 (s, 3H, CH_3), 2.74–2.79 (m, 2H, CH_2), 2.96 (s, 3H, CH_3), 7.05 (d, 2H, $J = 8.36$ Hz, phenyl), 7.22 (d, 2H, $J = 8.38$ Hz, phenyl), 7.2 (d, 2H, $J = 8.39$ Hz, phenyl), 7.55 (d, 2H, $J = 8.36$ Hz, phenyl), 8.22 (s, 1H, CH), 8.67 (br, NH_2, D_2O exchangeable), 9.80 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 618 (47%), [$M^+ + 2$], *m/z* 620 (21%), [$M^+ + 4$], *m/z* 622 (5%), [$M^+ - CH_3$], *m/z* 603 (53%).

*5.1.16. Synthesis of 2-E-cinnamoyl-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrarhydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-5-one (**17a**,**b**): general procedure*

A mixture of **14** (0.01 mol), aldehyde (0.01 mol) and a catalytic amount of piperidine was heated at 170–180 °C in test tube for 3 h. The product was solidified by cooling and addition of methanol (50 mL). The precipitate formed was collected by filtration and crystallized from an appropriate solvent.

*5.1.16.1. Synthesis of 2-E-cinnamoyl-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-5-one (**17a**).* It was obtained from benzaldehyde as brown powder and crystallized from dioxane. IR (KBr, cm^{-1}): 3054 (CH aryl), 2917 (CH alkyl), 1703, 1685, (2CO), 1615 (C═N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65–1.71 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.27–2.33 (m, 2H, CH_2), 2.68–2.73 (m, 2H, CH_2), 5.28, 5.50 (2d, 2H, CH═CH, $J = 11.5$ Hz), 7.01 (d, 2H, $J = 8.40$ Hz, phenyl), 7.10–7.26 (m, 4H, phenyl), 7.27–7.34 (m, 5H, phenyl), 7.58 (d, 2H, $J = 8.42$ Hz, phenyl), 8.34 (s, 1H, CH); its MS, [M^+], *m/z* 633 (100%), [$M^+ + 2$], *m/z* 635 (52%), [$M^+ + 4$], *m/z* 637 (26%).

*5.1.16.2. Synthesis of 6-(4-chlorophenyl)-2-E-(4-methoxy-cinnamoyl)-10-(4-chlorophenylmethylene)-3-methyl-7,8,9,10-tetrahydrothiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-5-one (**17b**).* It was obtained from 4-anisaldehyde as brown powder and crystallized from dioxane. IR (KBr, cm^{-1}): 3045 (CH aryl), 2931 (CH alkyl), 1700, 1685 (2CO), 1620 (C═N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.66–1.72 (m, 2H, CH_2), 2.21 (s, 3H, CH_3), 2.26–2.31 (m, 2H, CH_2), 2.71–2.78 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3), 5.30, 5.53 (2d, 2H, CH═CH, $J = 10.9$ Hz), 6.98 (d, 2H, $J = 8.38$ Hz, phenyl), 7.13–7.29 (m, 4H, phenyl), 7.23–7.44 (m, 4H, phenyl), 7.59 (d, 2H, $J = 8.37$ Hz, phenyl), 8.50 (s, 1H, CH); its MS, [M^+], *m/z* 663 (78%), [$M^+ + 2$], *m/z* 665 (29%).

*5.1.17. Synthesis of 4-chloro-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**18**)*

A solution of **7a** (0.01 mol) in dry dioxane (30 mL) was treated with 7 mL of phosphorus oxychloride and the mixture was stirred under reflux for 3 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL) whereby a solid was separated, filtered off and crystallized from dioxane (yellow powder) in 56% yield, m.p. 313–315 °C (melted); IR (KBr, cm^{-1}): 3049 (CH aryl), 2919 (CH alkyl), 1600 (C═N), 1160 (C–Cl); ^{1}H NMR ($CDCl_3$, δ, ppm): 1.67 (t, 2H, $J = 6.00$ Hz, CH_2), 2.30 (t, 2H, $J = 5.96$ Hz, CH_2), 2.65–2.72 (m, 2H, CH_2), 2.84 (s, 3H, SCH_3), 6.96 (d, 2H, $J = 8.39$ Hz, phenyl), 7.20 (d, 2H, $J = 8.38$ Hz, phenyl), 7.37 (d, 2H, $J = 8.40$ Hz, phenyl), 7.47 (d, 2H, $J = 8.41$ Hz, phenyl), 8.25 (s, 1H, CH); its MS, [M^+], *m/z* 497 (100%), [$M^+ + 2$], *m/z* 499 (59%), [$M^+ + 4$], *m/z* 501 (17%).

*5.1.18. Synthesis of 4-arylamino-5-(4-chlorophenyl)-9-(chlorophenylmethylene)-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**19a–c** or **20a**,**b**): general procedure*

In a warm solution of **18** (0.01 mol) in glacial acetic acid (40 mL) was added the freshly distilled aryl-amine or 2nd amine (0.01 mol). The reaction mixture was stirred under reflux for 3 h, then allowed to cool to 0 °C for 4 h, and the solid obtained was filtered, washed with water (100 mL) dried and recrystallized from appropriate solvent to produce **19a–c** and **20a**,**b**.

*5.1.18.1. Synthesis of 5-(4-chlorophenyl)-9-(chlorophenylmethylene)-4-phenylamino-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**19a**).* It was obtained from aniline as pale yellow crystals and crystallized from dioxane. IR (KBr, cm^{-1}): 3410 (br s, NH), 3032 (CH aryl), 2919 (CH alkyl),

1609 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.66 (t, 2H, $J = 5.97$ Hz, CH_2), 2.31 (t, 2H, $J = 5.89$ Hz, CH_2), 2.67–2.72 (m, 2H, CH_2), 2.86 (s, 3H, SCH_3), 6.93–7.12 (m, 3H, phenyl), 7.22 (d, 2H, $J = 8.43$ Hz, phenyl), 7.36–7.42 (m, 2H, phenyl), 7.45–7.53 (m, 4H, phenyl), 7.65 (d, 2H, $J = 8.41$ Hz, phenyl), 8.35 (s, 1H, CH), 9.20 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 554 (73%), [$M^+ + 2$], *m/z* 556 (29%), [$M^+ + 4$], *m/z* 558 (7%).

*5.1.18.2. Synthesis of 4-(chlorophenylamino)-5-(4-chlorophenyl)-9-(chlorophenylmethylene)-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**19b**).* It was obtained from 4-chloroaniline as yellow crystals and crystallized from dimethylformamide. IR (KBr, cm^{-1}): 3395 (br s, NH), 3049 (CH aryl), 2926 (CH alkyl), 1617 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.67 (t, 2H, $J = 5.86$ Hz, CH_2), 2.30 (t, 2H, $J = 5.88$ Hz, CH_2), 2.68–2.74 (m, 2H, CH_2), 2.88 (s, 3H, SCH_3), 6.98–7.18 (m, 4H, phenyl), 7.23 (d, 2H, $J = 8.44$ Hz, phenyl), 7.34–7.43 (m, 4H, phenyl), 7.65 (d, 2H, $J = 8.42$ Hz, phenyl), 8.32 (s, 1H, CH), 9.50 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 588 (100%), [$M^+ + 2$], *m/z* 590 (57%), [$M^+ + 4$], *m/z* 592 (10%).

*5.1.18.3. Synthesis of 5-(4-chlorophenyl)-9-(chlorophenylmethylene)-4-(4-methoxyphenyl-amino)-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**19c**).* It was obtained from 4-anisidene as yellow crystals and crystallized from dimethylformamide in 76% yield, m.p. 270–272 °C (melted); IR (KBr, cm^{-1}): 3420 (br s, NH), 3054 (CH aryl), 2926 (CH alkyl), 1610 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.65 (t, 2H, $J = 5.96$ Hz, CH_2), 2.30 (t, 2H, $J = 5.99$ Hz, CH_2), 2.67–2.72 (m, 2H, CH_2), 2.83 (s, 3H, SCH_3), 3.89 (s, 3H, OCH_3), 6.92–7.16 (m, 4H, phenyl), 7.25 (d, 2H, $J = 8.38$ Hz, phenyl), 7.33 (d, 2H, $J = 8.39$ Hz, phenyl), 7.45 (d, 2H, $J = 8.37$ Hz, phenyl), 7.65 (d, 2H, $J = 8.40$ Hz, phenyl), 8.40 (s, 1H, CH), 10.00 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 584 (100%), [$M^+ + 2$], *m/z* 586 (59%), [$M^+ + 4$], *m/z* 588 (22%).

*5.1.18.4. Synthesis of 5-(4-chlorophenyl)-9-(chlorophenyl methylene)-4-piprazino-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**20a**).* It was obtained from piperazine as pale yellow crystals and crystallized from dioxane. IR (KBr, cm^{-1}): 3365 (br, NH), 3061 (CH aryl), 2931 (CH alkyl), 1600 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.66 (t, 2H, $J = 6.02$ Hz, CH_2), 2.29 (t, 2H, $J = 6.00$ Hz, CH_2), 2.65–2.70 (m, 2H, CH_2), 2.83 (s, 3H, SCH_3), 3.29–3.33 (m, 2H, CH_2), 3.35–3.38 (m, 4H, CH_2), 3.45–3.49 (m, 2H, CH_2), 7.05 (d, 2H, $J = 8.37$ Hz, phenyl), 7.28 (d, 2H, $J = 8.39$ Hz, phenyl), 7.36 (d, 2H, $J = 8.40$ Hz, phenyl), 7.53 (d, 2H, $J = 8.41$ Hz, phenyl), 8.27 (s, 1H, CH), 9.60 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 543 (100%), [$M^+ + 2$], *m/z* 545 (22%), [$M^+ + 4$], *m/z* 547 (9%).

*5.1.18.5. Synthesis of 5-(4-chlorophenyl)-9-(chlorophenylmethylene)-4-morpholino-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**20b**).* It was obtained from morpholine as yellow crystals and crystallized from dimethylformamide. IR (KBr, cm^{-1}): 3415 (br s, NH), 3067 (CH aryl), 2951 (CH alkyl), 1630 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.71 (t, 2H, $J = 6.02$ Hz, CH_2), 2.28 (t, 2H, $J = 6.00$ Hz, CH_2), 2.67–2.72 (m, 2H, CH_2), 2.77 (s, 3H, SCH_3), 3.26–3.31 (m, 2H, CH_2), 3.39–3.43 (m, 2H, CH_2), 3.82–3.90 (m, 4H, $2CH_2$), 7.04 (d, 2H, $J = 8.38$ Hz, phenyl), 7.26 (d, 2H, $J = 8.37$ Hz, phenyl), 7.39 (d, 2H, $J = 8.40$ Hz, phenyl), 7.57 (d, 2H, $J = 8.41$ Hz, phenyl), 8.33 (s, 1H, CH); its MS, [M^+], *m/z* 544 (65%), [$M^+ + 2$], *m/z* 546 (31%), [$M^+ + 4$], *m/z* 548 (8%).

*5.1.19. Synthesis of 4-(o-carboxyphenyl)amino-5-(4-chlorophenyl)-9-(chlorophenylmethylene)-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**21**)*

It was obtained from anthranilic acid as yellow powder and crystallized from dioxane. IR (KBr, cm^{-1}): 3510 (br, OH), 3368 (br, NH), 3041 (CH aryl), 2927 (CH alkyl), 1716 (CO), 1605 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.68 (t, 2H, $J = 5.86$ Hz, CH_2), 2.27 (t, 2H, $J = 5.90$ Hz, CH_2), 2.69–2.76 (m, 2H, CH_2), 2.85 (s, 3H, SCH_3), 6.92–7.15 (m, 4H, phenyl), 7.22 (d, 2H, $J = 8.34$ Hz, phenyl), 7.34–7.40 (m, 2H, phenyl), 7.49 (d, 1H, $J = 9.63$ Hz, phenyl), 7.57 (d, 2H, $J = 8.32$ Hz, phenyl), 7.70 (d, 1H, $J = 9.46$ Hz, phenyl), 8.25 (s, 1H, CH), 9.10 (br, NH, D_2O exchangeable), 12.30 (br s, OH, D_2O exchangeable); its MS, [M^+], *m/z* 598 (100%), [$M^+ + 2$], *m/z* 600 (67%), [$M^+ + 4$], *m/z* 602 (17%).

*5.1.20. Synthesis of 14-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-7-methylthio-10,11,12,13-tetrahydro-5H-quino[2′,3′:4,5]pyrimido[6,1-b]quinazolin-5-one (**22**)*

A solution of **21** (0.01 mol) in glacial acetic acid (40 mL) and catalytic amount of sulfuric acid (1 mL) was stirred under reflux for 8 h. The reaction mixture was allowed to cool, poured into cold water (100 mL), neutralized by ammonia solution, the solid so-precipitated was filtered off, washed with water, dried and crystallized from dimethylformamide as yellow crystals. IR (KBr, cm^{-1}): 3023 (CH aryl), 2917 (CH alkyl), 1708 (CO), 1630 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 1.69 (t, 2H, $J = 6.01$ Hz, CH_2), 2.28 (t, 2H, $J = 6.03$ Hz, CH_2), 2.68–2.75 (m, 2H, CH_2), 2.83 (s, 3H, SCH_3), 6.94–7.15 (m, 4H, phenyl), 7.21 (d, 2H, $J = 8.41$ Hz, phenyl), 7.32–7.38 (m, 2H, phenyl), 7.52 (d, 1H, $J = 9.07$ Hz, phenyl), 7.62 (d, 2H, $J = 8.38$ Hz, phenyl), 7.73 (d, 1H, $J = 9.23$ Hz, phenyl), 8.45 (s, 1H, CH), 9.50 (br, NH, D_2O exchangeable); its MS, [M^+], *m/z* 580 (100%), [$M^+ + 2$], *m/z* 582 (38%), [$M^+ + 4$], *m/z* 584 (15%).

*5.1.21. Synthesis of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2,4-dihydrazino-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**23**)*

A mixture of **18** (0.01 mol) and hydrazine hydrate (99–100%, 10 mL) was stirred under reflux in dioxane (30 mL) and ethanol (5 mL) for 12 h. The reaction mixture was allowed to cool to 0 °C for 5 h, and the solid was collected by filtration and crystallized from dioxane as a pale yellow powder. IR (KBr,

cm^{-1}): 3438–3350 (br s, NH's), 3035 (CH aryl), 2923 (CH alkyl), 1625 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm); 1.71 (t, 2H, $J = 5.97$ Hz, CH_2), 2.26–2.34 (m, 2H, CH_2), 2.45 (br, NH_2, D_2O exchangeable), 2.75–2.78 (m, 2H, CH_2), 7.07 (d, 2H, $J = 8.45$ Hz, phenyl), 7.16 (d, 2H, $J = 8.42$ Hz, phenyl), 7.30 (d, 2H, $J = 8.40$ Hz, phenyl), 7.43 (d, 2H, $J = 8.39$ Hz, phenyl), 8.32 (s, 1H, CH), 8.54–8.80 (br, NH_2, D_2O exchangeable), 10.70, 11.60 (two, br s, 2NH, D_2O exchangeable).

5.2. Pharmacological screening

5.2.1. Animals

Male Sprague–Dawley rats (150–200 g) were used in the study of anti-oxidant activity and the adult females were used in anti-inflammatory activity study. Both sex of Swiss mice weighing 25–30 g were used in analgesic activity taking into account international principle and local regulations concerning the care and use of laboratory animals [22]. 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.

5.2.2. Reagents

DNA (Type 1, calf thymus), bleomycin sulfate, butylated hydroxyanisole (BHA) and L-ascorbic acid were obtained from Sigma. 2,2′-Azo-bis-(2-amidinopropane) dihydrochloride (AAPH) and 2,2′-azino-bis(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS) were purchased from Wak. All other chemicals were of the highest quality available.

5.2.3. Anti-oxidant screening

5.2.3.1. Assay for erythrocyte hemolysis. Blood was obtained from rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffy coat and washed three times with 10 volumes of 0.15 M NaCl. During the last washing, the erythrocytes were centrifuged at 2500 rpm for 10 min to obtain a constantly packed cell preparation. Erythrocyte hemolysis was mediated by peroxyl radicals in this assay system [23]. A 10% suspension of erythrocytes in pH 7.4 phosphate-buffered saline (PBS) was added to the same volume of 200 mM 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) solution (in PBS) containing samples to be tested at different concentrations. The reaction mixture was shaken gently while being incubated at 37 °C for ~h. The reaction mixture was then removed, diluted with eight volumes of PBS and centrifuged at 2500 rpm for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with eight volumes of distilled water to achieve complete hemolysis, and the absorbance B of the supernatant obtained after centrifugation was measured at 540 nm. The percentage hemolysis was calculated by equation $(1 - A/B) \times 100\%$. The data were expressed as mean standard deviation. L-Ascrobic was used as a positive control.

5.2.3.2. Anti-oxidant activity screening assay – ABTS method. For each of the investigated compounds 2 mL of ABTS solution (60 μM) was added to 3 M MnO_2 solution (25 mg/mL) all prepared in phosphate buffer (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was adjusted at ca. 0.5 at λ 734 nm. Then, 50 μl of (2 mM) solution of the test compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance (A_{test}) was measured and the reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the following equation [24]:

$$\%\ \text{Inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Ascorbic acid (vitamin C) was used as standard anti-oxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of sample. Negative control sample was run with MeOH/phosphate buffer (1:1) instead of tested compound.

5.2.3.3. Bleomycin-dependent DNA damage. The assay was done according to Aeschlach et al. [25] with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 μM) and samples to be tested at different concentrations. L-Ascorbic acid was used as a positive control. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding 0.5 mL thiobarbituric acid (TBA) (1%, w/v) and 0.5 mL HCl (25%, v/v) followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by increase in absorbance at 532 nm.

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

The method adopted resembles essentially that described by Winter et al. [26]; distilled water was selected as vehicle to suspend the standard drugs and the test compounds. The albino rats weighing between 150 and 180 g was starved for 18 h prior to the experiment. The animals were weighed, marked for identification and divided into 17 groups each group containing six 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 diclofenac sodium in a dose of 20 mg/kg orally as aqueous suspension [27]. 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 $(E)\% = V_t - V_0/V_0$, inhibition rate $(I)\% = E_c - E_t/E_c$, where V_0 is the volume before carrageenan injection (mL), V_t is the volume at t hours after carrageenan

injection (mL), E_c, E_t are the edema rate of control group and treated group, respectively.

5.2.5. Analgesic activity using hot-plate test

The experiment was carried out as described by Turner [28] using hot-plate apparatus maintained at 53 ± 0.5 °C. The mice were divided into 17 groups of six 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. 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 weight. 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.

5.2.6. 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. [29]. One hundred and two mice were divided into 17 groups (six in each group), starved for 16 h and pretreated as follows: the 1st group which served as control positive was orally received distilled water in appropriate volumes. The 2nd to 16th groups were received the aqueous suspension of synthesized compounds orally in a dose of 20 mg/kg. The last group was orally received diclofenac sodium in a dose of 20 mg/kg. After 30 min, each mouse was administered 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.

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