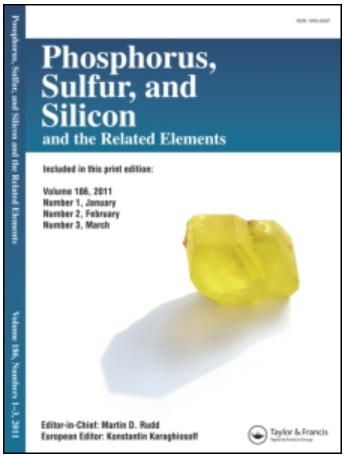

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Synthesis and Antioxidant, Anti-Inflammatory, and Analgesic Activity of Novel Polycyclic Pyrimido[4,5-*b*]quinolines

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Synthesis and Antioxidant, Anti-Inflammatory, and Analgesic Activity of Novel Polycyclic Pyrimido[4,5-*b*]quinolines

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*The behavior of the 2-methylthio-pyrimido[4,5-*b*]quinolin-4-one towards differently substituted amines is reported. Also, the reactivity of 3-aminothiazolo[3′,2′:1,2]-pyrimido[4,5-*b*]quinoline-2-carbonitrile towards formic acid, urea, thiourea, formamide, and carbon disulfide is discussed. Some of the synthesized derivatives possess biological activities as anti-inflammatory and analgesic agents. Some of these selective biologically active compounds were screened for antioxidant properties.*

Keywords 3-Aminothiazolo-[3′,2′:1,2]pyrimido[4,5-b]quinoline-2-carbonitrile; analgesic activities; anti-inflammatory; antioxidant; 2-arylaminopyrimido[4,5-*b*]quinoline; 2-methylthio

INTRODUCTION

Various polysubstituted 2-aminopyrimidines exhibit important pharmacological properties. For example, derivative **A** was reported as a $5HT_2$ receptor antagonist,[1] whereas the bicyclic compound **B** shows sorbitol dehydrogenase inhibition properties[2] (Figure 1). In addition, many bicyclic- and tricyclic compounds that are either natural (phytoestrogens) or obtained by synthesis and that exhibit a quinoline ring, have been studied for their biological activities. They are especially used as radical scavengers such as quercetol or coumestrol[3] or the copper- or iron-chelating molecules such as clioquinol.[4] Also, pyrimidine amide derivatives are novel anti-allergic agents.[5] *S*-Alkylated derivatives are potent antiviral agents.[6] 6-Alkylamino derivatives are inhibitors of *Bacillus subtilis* DNA polymerase III.[7] Aziridino derivatives are

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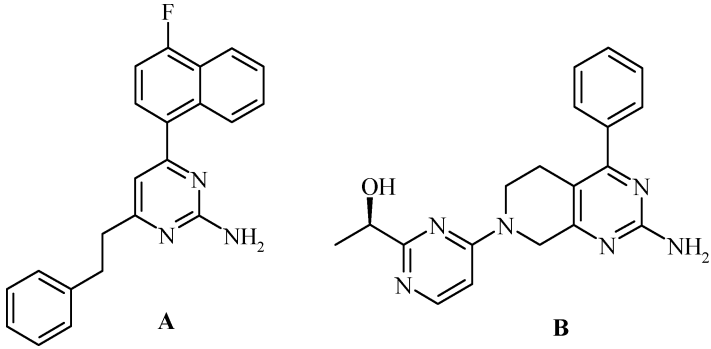


FIGURE 1 Some polysubstituted pyrimidines exhibited pharmacological properties.

new cytotoxic agents with tumor-inhibitory activity.[8] Also, arylamino derivatives of pyrimidines are potential anti-cytomegalovirus agents.[9]

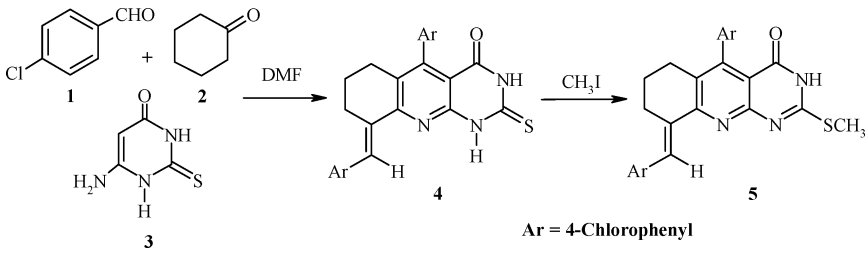
We recently reported the identification of pyrimido[4,5-b]quinoline as a selective antioxidant,[10] with anti-inflammatory and highly analgesic properties. We report here a one-pot synthesis of new tetrahydropyrimido[4,5-*b*]quinoline, 2-arylamino-, 2-piperazino-, morpholino-, and 3-amino-thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolines. Their antioxidant properties were also evaluated by the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) method and the erythrocyte hemolysis method. Also, the anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats, and the analgesic activity was performed by the Turner and Collier technique.

RESULTS AND DISCUSSION

A one-pot synthesis of 5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-thioxo-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (**4**) is described below. 4-Chlorobenz-aldehyde **1** was condensed with cyclohexanone **2** and 6-aminothiouracil **3** in dimethylformamide (DMF) solution. Stirring for a long time under thin layer chromatography (TLC) control afforded **4** in good yield (Scheme 1).

First, a condensation reaction of 2 mol of 4-chlorobenzaldehyde with cyclohexanone with loss of 2 mol of water under formation of an α,β-unsaturated ketone occurs. This ketone reacts with **3** under formation of a Schiff base, which undergoes an interamolecular [4+2]-cycloaddition with subsequent spontaneous dehydrogenation to form the aromatic pyrimido[4,5-*b*]qinoline **4**.

It is well known that positions 2 and 4 in pyrimidine and fused pyrimidines show distinct activities towards nucleophiles. Therefore,

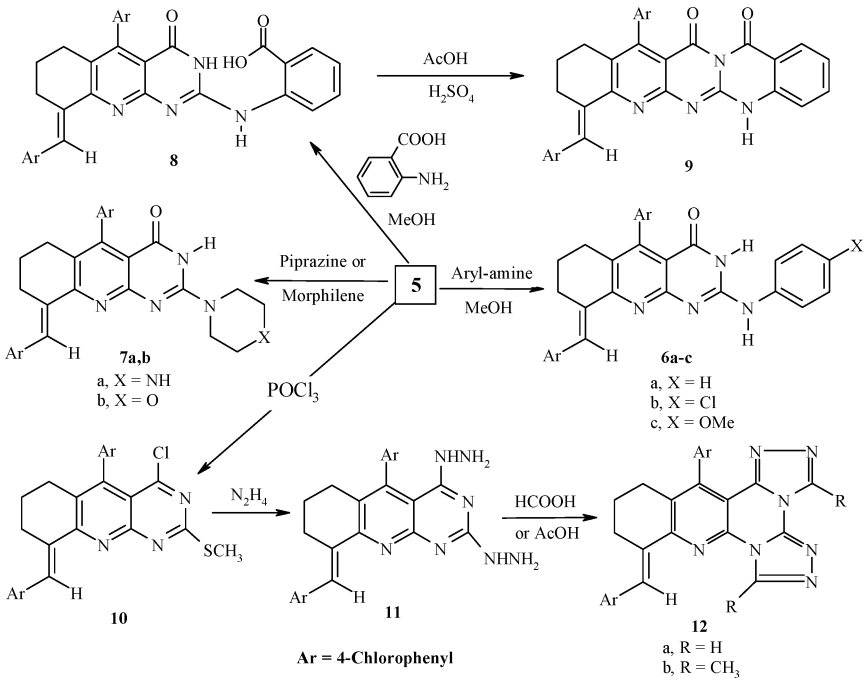


SCHEME 1

2-methylthio-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7, 8, 9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (**5**) was prepared, and its activity towards nucleophiles such as primary aromatic amines and secondary aliphatic amines (piperazine, morpholine) was investigated. Thus heating under reflux of 2-methylthio-pyrimido[4,5-*b*]quinolin-4-one (**5**) with aniline, 4-chloroaniline, and *p*-anisidine in methanol produced 2-arylamino-5-(4-chloro-phenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one derivatives (**6a–c**) with evolution of methanethiol (Scheme 2). The IR spectra of **6a–c** displayed absorption bands around 3365–3420 cm^{-1} (NH) and 1680–1685 cm^{-1} (C=O). Moreover, the ^{1}H-NMR spectra of **6a–c**,**7** revealed the absence of a CH_3S signal. For example, the ^{1}H-NMR spectrum of **6c** showed signals at 1.63–1.67, 2.27–2.33, and 2.67–2.72 for the three CH_2 groups, a signal at 3.89 (OCH_3), signals due to the aromatic protons (6.92–7.69), and the signal for the vinyl proton at 8.40. The broad band at 10.00 belongs to NH (exchangeable by D_2O). Its mass spectrum showed the molecular ion peak [M^+], m/z 555, (100%).

Reaction of **5** with piperazine and morpholine in methanol produced the 2-piperazino- and 2-morpholino-2-methylthio-5-(4-chlorophenyl)-9-(4-chlorophenyl-methylene)-6, 7, 8, 9-tetrahydropyrimido[4, 5-*b*]-quinolin-4-one derivatives (**7a**,**b**).The ^{1}H-NMR spectrum of **7a** showed a triplet at 3.29–3.33, a multiplet at 3.35–3.38, and a triplet at 3.45–3.49 corresponding to the protons of four CH_2 groups of morpholine, in addition to the remaining protons, which supported the proposed structure of compound **7a**.

Moreover, the reaction of **5** with anthranilic acid afforded 2-(*o*-carboxyphenylamino)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-*b*]-quinolin-4-one (**8**). The IR spectrum of **8** displayed bands at 3510 cm^{-1} (OH), 3368 (NH), and 1716 (C=O). Its ^{1}H-NMR spectrum showed signals at δ 1.66–1.70, 2.24–2.30, and 2.69–2.76 for the three methylene groups; a multiplet for the aromatic protons around 6.92–7.72; and a singlet for CH at 8.25 (s, 1H, CH),



SCHEME 2

in addition to two broad bands at 9.10, 12.30 corresponding to the NH and hydroxyl groups. Compound **8** was cyclized by boiling with glacial acetic acid in the presence of a few drops of sulfuric acid to give 12-(4-chlorophenyl)-8-(4-chlorophenylmethylene)-8,9,10,11-terahydroquino[2′,3′:4,5]pyrimido[2,1-*b*]quinazoline-13(*H*),15(*H*)-dione (**9**) (Scheme 2). Structure **9** is preferred on the basis of elemental analysis and spectral data. Its IR spectrum revealed the absence of any absorption bands in the OH region, and a shift of the carbonyl frequency from 1716 to 1683 indicates the formation of the quinazoline ring. Also, its ^{1}H-NMR spectrum supported the proposed structure (see Experimental).

4-Chloro-2-methylthio-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinoline (**10**) was prepared by gentle heating of **5** with phosphorus oxychloride in dry dioxane,[12,13] and its reactivity towards hydrazine hydrate was investigated. The IR spectrum of **10** revealed the absence of any absorption bands in the NH and CO region. Its ^{1}H-NMR spectrum supported the proposed structure. Also, its mass spectrum showed the molecular ion peak [M$^+$], m/z 498, (78%).

Refluxing a mixture of **10** and hydrazine hydrate afforded 5-(4-chloro-phenyl)-9-(4-chlorophenylmethylene)-2,4-dihydrazino-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinoline (**11**). The IR spectrum of **11** displayed absorption bands around 3438–3350 cm^{-1} (NH) groups. Its ^{1}H-NMR spectrum showed broad singles at 2.45, 8.54 indicating the presence of two amino groups. Compound **11** reacted with formic and acetic acid to yield the 6-(4-chlorophenylmethylene)-10-(4-chlorophenyl)-6,7,8,9-tetrahydro-*s*-tria-zolo[3′,4′:6, 1]-*s*-triazolo[3″,4″,:2, 3]pyrimido-[4,5-*b*]quinolines (**12a**,**b**), with a new ring system. Besides the correct values of the elemental analyses, the spectral data of **12** are in agreement with the assigned structure. The IR spectrum of **12** revealed the absence of NH groups. Also, the ^{1}H-NMR spectrum revealed the absence of NH_2 and NH groups, while the two signals at 9.23 and 9.39 for **12a** correspond to the triazole protons. They are replaced in **12b** by two singlets at 2.86, 2.89 due to the methyl groups. In agreement with the structure, the ^{13}C-NMR spectrum of **12a** showed signals at δ 22.25, 26.67, 27.20 due to three sp^3 carbon atoms and 23 sp^2 carbon atoms around 105.71–153.27 ppm, and its mass spectrum showed the molecular ion peak [M^+] at m/z 498 (100%).

The reaction of **4** with bromo-malononitrile in an ethanolic potassium carbonate solution gave 3-amino-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-5-oxo-7,8,-9,10-tetrahydrothiazolo[3′,2′:1,2] pyrimido[4,5-*b*]quinoline-2-carbonitrile (**13**). The IR spectrum of **13** displayed absorption bands corresponding to amino, nitrile, and carbonyl groups, and the ^{1}H-NMR spectrum showed signals at 1.63–1.66, 2.30–2.32, 2.73–2.78 due to the three methylene groups, the aromatic protons around 7.10–7.43, and a singlet due to CH at 8.19, in addition to the broad peak at 8.90 corresponding to the amino group. Its ^{13}C-NMR spectrum showed signals 24.30, 26.97, 28.06 for three sp^3 carbon atoms, one signal at 68.5 (CN), 18 signals corresponding to sp^2 carbon atoms around 108.5–158.7, and the signal of (C=O) at 164.9. Its mass spectrum showed the absorption molecular ion peak [M^+], m/z 530, (100%).

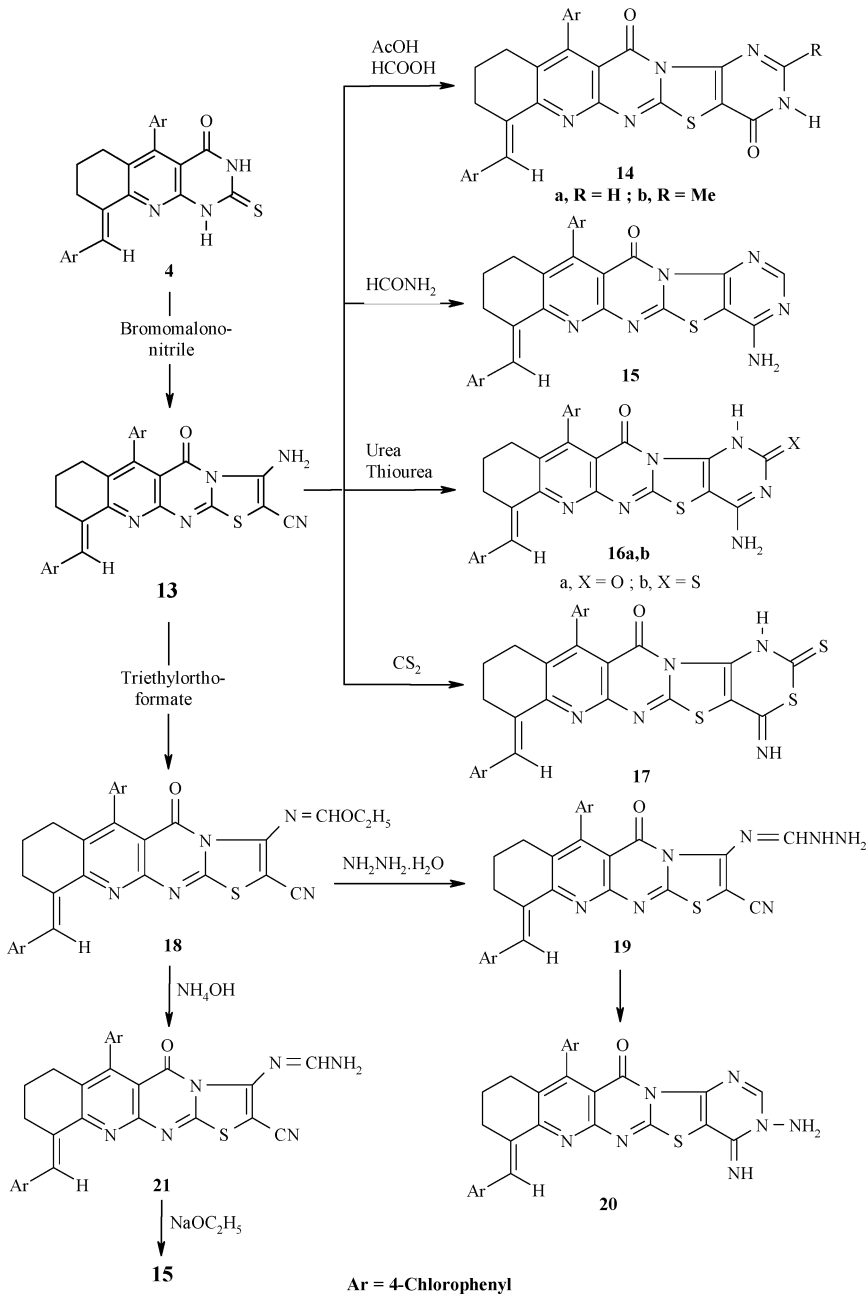
Compound **13**, as a typical β-enaminonitrile derivative, reacted with aliphatic acids to afford 8-(4-chlorophenyl methylene)-12-(4-chlorophenyl)-8,9,10,11-tetrahydropyr-imido[4″, 5″,:4′, 5′]thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinoline-4,13-diones (**14a**,**b**). The IR spectrum of **14** revealed the absence of amino and nitrile groups. The ^{1}H-NMR spectrum of **14b** showed a signal at 2.32 for the methyl group and a broad signal at 10.00 for the NH group. Similarly, compound **13** reacted with formamide, in the presence of DMF, to yield 4-amino-8-(4-chlorophenylmethylene)-12-(4-chloro-phenyl)-8,9,10,

11 - tetrahydropyrimido[4″, 5″, :4′, 5′]thiazolo[3′, 2′:1, 2]pyrimido[4,5-*b*]-quinolin-13-one (**15**). The ^{1}H-NMR exhibited a singlet at 8.15 corresponding to the pyrimidine proton and a broad band at 8.35 for the amino group. When **13** was heated with urea or thiourea, 4-amino-8-(4-chlorophenylmethylene)- 12-(4-chlorophenyl)-8,9,10,11-tetrahydropyrimido[4″, 5″, : 4′, 5′]thiazolo[3′, 2′:1, 2]pyrimido[4,5-*b*]quinoline-2,13-dione (**16a**) and 4-amino-8-(4-chlorophenylmethylene)-12 -(4-chlorophenyl)-8,9,10,11-tetrahydropyrimido[4″,5″,:4′,5′]thiazolo [3′, 2′:1,2]pyrimido[4,5-*b*]quinolin-2-thioxo-13-one (**16b**), respectively, were formed (Scheme 3). The ^{1}H-NMR spectrum of **16b** showed signals around δ 1.64–2.78 due to the three methylene groups, at 7.06–7.47 for the arene protons, a singlet signal at 8.02 for CH, and broad bands at 8.75, 9.50 due to the NH_2 and NH groups.

Alternatively, heating of **13** with carbon disulfide in pyridine led to 8-(4-chlorophenylmethylene)-12-(4-chlorophenyl)-4-imino-8,9, 10, 11-tetrahydroquino[2″, -3″, : 4′, 5′]pyrimido[2′, 1′:2, 3]thiazolo[4, 5-*d*] [1,3]thiazine-13-one (**17**). The ^{1}H-NMR spectrum of **17** showed signals around δ 1.63–2.81, three methylene groups around 7.05–7.51 (Ar-H), and a singlet signal at 8.12 for CH, in addition to broad bands at 9.75, 10.50 due to two NH groups. The ^{13}C-NMR showed signals at δ 22.23, 26.65, 27.33 due to three sp^3 carbon atoms; 23 sp^2 carbon atoms around 108.6–155.3; and two bands at 163.5 and 176.8 corresponding to the carbonyl and thioxo group, respectively.

Condensation of **13** with triethyl orthoformate yielded the corresponding 3-ethoxy-methyleneamino derivative **18**, with expected IR and ^{1}H-NMR data. Compound **18** reacted with hydrazine hydrate in boiling dioxane to give 3-hydrazino-methylene-amino-6-(4-chlorophenyl) - 10 - (4 - chlorophenylmethylene) - 5 - oxo - 7, 8, 9, 10 - tetrahydro - thiazolo[3′, 2′ : 1, 2]pyrimido[4, 5-*b*]quinoline-2-carbonitrile (**19**), which on boiling in an ethanolic sodium ethoxide solution underwent cyclization to give 3-amino-8-(4-chlorophenylmethylene)-12-(4-chlorophenyl)-4 -imino - 8, 9, 10, 11-tetrahydropyrimido-[4″, 5″,:4′, 5′] thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolin-13-one (**20**) (Scheme 3). The IR spectrum of **18** displayed absorption bands at 2215 cm^{-1}(CN), while that of **20** revealed the absence of a nitrile group. The ^{1}H-NMR spectrum of **20** showed the standard signals for the methylene and aromatic protons, the broad signals corresponding to the NH_2 and NH at 8.76 and 10.82, and a singlet at 8.29 for the pyrimidine proton.

Finally, compound **18** was heated with aqueous ammonia to give 3-aminomethyleneamino-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-5-oxo-7, 8, -9, 10-tetrahydrothiazolo[3′, 2′ :1, 2] pyrimido[4,5-*b*]quinoline-2-carbonitrile (**21**). The IR spectrum of



SCHEME 3

21 displayed absorption bands at 3400 cm^{-1} (NH_2), 2216 cm^{-1} (CN), and 1694 cm^{-1} (CO), and the ^{1}H-NMR revealed that the absence of an ethyl group and showed the broad signal at 8.90, characteristic of the NH_2 group. Compound **21** was cyclized to 4-amino-8-(4-chlorophenylmethylene)-12-(4-chlorophenyl) - 8, 9, 10, 11-tetrahydropyrimido[4″, 5″,:4′, 5′]thiazolo[3′,2′:1, 2]pyrimido[4,5-*b*]quinolin-13-one (**15**) upon heating in an ethanolic sodium ethoxide solution.

Antioxidant Activity Screening

The antioxidant activity of pyrimido[4,5-*b*]quinoline and their derivatives was determined with three standard assays. The antioxidant activity was measured as the ability of pyrimido[4,5-*b*]quinoline and its derivatives to react with the preformed radical monocation of $ABTS^+$. This assay is also known as the total radical-trapping antioxidant parameter assay (TRAP assay). The antioxidant activity was also measured by erythrocyte hemolysis (Table I) and by bleomycin-dependent DNA damage.

In all three assays, ascorbic acid was used as a control. The results of the ABTS assay of the antioxidant activity are shown in Table II. This indicates that compounds **7a–c**, **8a**, **8b**, and **15** exhibit high activity due to the presence of arylamino, piperazino, or morpholino groups. While the other synthesized compounds showed moderate activity in comparison to the control, the compounds that exhibit high activity were chosen to assay for bleomycin-dependent DNA damage. The results indicate that they may have some protective activity to DNA by a certain mechanism. The series of compounds **7a–c**, **8a**, **8b**, **13**, and **15** exhibited a high antioxidant activity. On the other hand, these compounds protect the DNA from the induced damage by bleomycin (Table III).

Anti-Inflammatory Activity

The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in rats. The anti-inflammatory activity data (Table IV) 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 ones. Compounds **6a–c**, **7a**,**b**, **13**, and **15** showed similar or higher anti-inflammatory activity than diclofenac sodium.

TABLE I Antioxidant Assays by Erythrocyte Hemolysis

Methods	Erythrocyte hemolysis 1-A/B × 100	
Compounds	Absorbance of samples (A)	% Hemolysis
Complete hemolysis with Dist-H_2O (B)	0.660	
Ascorbic acid	0.026	3.93
4	0.042	6.36
5	0.048	7.27
10	0.062	9.39
6a	0.236	35.75
6b	0.284	36.72
6c	0.154	19.18
7a	0.232	35.70
7b	0.281	36.68
8	0.062	9.39
9	0.048	7.27
11	0.031	4.64
12	0.028	4.24
13	0.218	30.60
14a	0.052	7.87
14b	0.055	8.33
15	0.236	35.75
16a	0.027	4.09
16b	0.055	8.19
17	0.056	8.34
18	0.038	5.76
20	0.032	4.55

Analgesic Activity

The analgesic activity was determined by the hot plate test (central analgesic activity) and acetic-acid–induced writhing assay. The results (Tables V and VI) reveal that all test 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 2-arylaminopyrimido[4,5-*b*]-quinoline (**6a–c**), 2-piperazino-, 2-morphlinopyrimidoquinoline (**7a–c**), and 3-amino-thiazolo[3′,2′,1,2]pyrimido[4,5-*b*]quinoline-2-carbonitrile (**13)** exhibit activities higher than the reference drug in peripheral analgesic activity testing. The remaining compounds have the same activity in peripheral analgesic activity testing.

TABLE II Antioxidant Assays by ABTS Method

Methods	ABTS Abs (control)-Abs (Test)/Abs (control) × 100	
Compounds	Absorbance of samples	% Inhibition
Control of ABTS	0.54	0
Ascorbic acid	0.06	88.9
4	3.37	33.5
5	0.42	26.8
10	0.36	37.8
6a	0.09	82.5
6b	0.20	62.3
6c	0.16	68.7
7a	0.26	50.4
7b	0.31	42.7
8	0.36	33.3
9	0.34	37.0
11	0.31	42.6
12	0.35	35.2
13	0.27	48.3
14a	0.37	31.5
14b	0.34	37.0
15	0.14	62.5
16a	0.35	35.2
16b	0.32	40.7
17	0.28	48.1
18	0.34	37.0
20	0.37	31.5

TABLE III Assay for Bleomycin–Dependent DNA Damage (DNA)

Methods	Bleomycin-dependent DNA damage
Compounds	Absorbance of samples
Ascorbic acid	0.020
6a	0.024
6b	0.022
6c	0.034
7a	0.032
7b	0.030
13	0.025
15	0.028

TABLE IV Percent Inflammatory Activity of the Tested Compounds (Carrageenan-Induced Paw Edema Test in Rats)

Compd. No.	Percent protection		
	1 hour	2 hours	3 hours
6a	$49.0 \pm 1.27^{*}$	$53.2 \pm 1.32^{**}$	$41.0 \pm 1.31^{*}$
6b	$56.3 \pm 1.37^{**}$	$62.0 \pm 1.76^{**}$	$40.2 \pm 1.05^{*}$
6c	$59.5 \pm 1.06^{**}$	$62.4 \pm 2.03^{**}$	$46.5 \pm 1.26^{*}$
7a	$59.8 \pm 1.41^{**}$	$59.3 \pm 1.32^{*}$	$42.3 \pm 1.26^{*}$
7b	$49.6 \pm 2.41^{*}$	$57.1 \pm 1.63^{*}$	$44.8 \pm 1.83^{*}$
9	$46.3 \pm 1.53^{*}$	$48.6 \pm 139^{*}$	$38.6 \pm 1.39^{*}$
11	$46.7 \pm 2.28^{*}$	$44.2 \pm 1.83^{*}$	$39.2 \pm 1.04^{*}$
12a	$41.2 \pm 1.38^{*}$	$42.5 \pm 1.46^{*}$	$31.1 \pm 1.33^{*}$
13	$42.3 \pm 1.63^{*}$	$45.8 \pm 1.47^{*}$	$42.9 \pm 1.63^{*}$
14a	$44.1 \pm 1.83^{*}$	49.0 ± 1.14	$30.5 \pm 1.83^{*}$
14b	$39.8 \pm 1.42^{*}$	$42.3 \pm 1.42^{*}$	$25.3 \pm 1.32^{*}$
15	$43.1 \pm 162^{*}$	$52.0 \pm 1.95^{**}$	$42.0 \pm 1.92^{*}$
Control	6.1 ± 0.27	5.7 ± 0.32	3.2 ± 0.93
Diclofenac Sodium	$52.4 \pm 0.92^{*}$	$60.3 \pm 1.52^{**}$	$42.0 \pm 1.36^{*}$

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.

TABLE V Central Analgesic Activity (Hot Plate Test)

Group	Reaction time (min.)			
	0 min	30 min	60 min	90 min
Control	8.24 ± 0.33	8.11 ± 0.36^{b}	8.62 ± 0.41^{b}	9.52 ± 0.40^{b}
6a	6.08 ± 0.90	7.93 ± 0.74	10.15 ± 1.20	11.51 ± 0.40^{a}
6b	7.08 ± 0.13	8.65 ± 0.87	10.98 ± 0.91^{a}	$11.12 \pm 0.75^{a,b}$
6c	8.09 ± 0.34	8.83 ± 0.35^{a}	9.87 ± 0.58^{a}	10.46 ± 0.48^{b}
7a	7.27 ± 0.40	8.50 ± 0.48	9.86 ± 0.52	10.47 ± 0.18^{b}
7b	9.38 ± 0.30	10.14 ± 0.26^{b}	10.74 ± 0.28^{b}	7.63 ± 0.60^{b}
9	8.43 ± 0.61	8.62 ± 0.34	9.06 ± 0.56^{b}	9.22 ± 0.47^{b}
11	8.26 ± 0.40	8.68 ± 0.48	9.80 ± 0.52	10.40 ± 0.18^{b}
12a	6.64 ± 0.20	7.54 ± 0.26^{b}	8.61 ± 0.60^{a}	12.00 ± 0.36^{a}
13	8.26 ± 0.40	9.68 ± 0.48	9.82 ± 0.52	10.43 ± 0.18^{b}
14a	8.91 ± 0.65	9.19 ± 0.57^{a}	10.78 ± 0.45^{a}	11.08 ± 0.24^{b}
14b	6.24 ± 0.57	7.08 ± 0.78^{a}	9.52 ± 0.82^{a}	12.68 ± 0.61^{a}
15	7.42 ± 0.36	8.43 ± 0.45^{a}	10.43 ± 0.29^{a}	$10.68 \pm 0.59^{a,b}$
Diclofanc sodium	6.51 ± 0.40	10.05 ± 0.12^{a}	11.40 ± 0.53^{a}	13.20 ± 0.38^{a}

Values represent the mean ± S.E. of 6 animals for each groups.

^{a}P< 0.05: Statistically significant from control (Dunnett's test).

^{b}P< 0.05: Statistically significant from ASA (Dunnett's test).

*Significant at $p < 0.05$.

TABLE VI Percent Analgesic Activity (Peripheral, Writhing Test)

Compd. No.	Percent protection			
	30 min	1 hour	2 hours	3 hours
6a	$62.2 \pm 1.15^{*}$	$65.3 \pm 1.31^{**}$	$69.4 \pm 1.03^{**}$	$50.4 \pm 1.73^{*}$
6b	$61.4 \pm 1.51^{**}$	$69 \pm 1.56^{**}$	$74.3 \pm 1.84^{**}$	$48.3 \pm 172^{*}$
6c	$70.0 \pm 1.05^{**}$	$74 \pm 1.93^{**}$	$75.4 \pm 1.51^{**}$	$58.2 \pm 1.17^{*}$
7a	$73.4 \pm 1.05^{**}$	$76.3 \pm 1.39^{**}$	77.6 ± 1.31	$63.3 \pm 1.39^{**}$
7b	$46.7 \pm 1.51^{*}$	$53 \pm 1.49^{**}$	59.3 ± 1.69	$65.3 \pm 1.31^{*}$
9	$50.4 \pm 1.34^{*}$	$52 \pm 1.02^{*}$	55.5 ± 1.37	$37.6 \pm 1.49^{*}$
11	$46.0 \pm 1.93^{*}$	$54 \pm 1.41^{*}$	56.6 ± 1.39	$37.3 \pm 1.23^{*}$
12a	$43.5 \pm 1.92^{*}$	$49 \pm 1.37^{*}$	53.1 ± 1.16	$38.4 \pm 1.81^{*}$
13	$41.4 \pm 1.16^{*}$	$47 \pm 1.32^{*}$	48.8 ± 1.39	$67.3 \pm 1.53^{*}$
14a	$43.4 \pm 1.21^{*}$	$48 \pm 1.42^{*}$	51.3 ± 1.60	$39.6 \pm 1.83^{*}$
14b	$43.2 \pm 1.32^{*}$	$45 \pm 1.03^{*}$	46.1 ± 1.39	$34.7 \pm 1.15^{*}$
15	$41.5 \pm 1.43^{*}$	$52 \pm 1.26^{*}$	49.3 ± 1.92	$37.3 \pm 1.39^{*}$
Control	02.0 ± 0.35	06.0 ± 0.50	04.0 ± 0.59	04.0 ± 0.90
Diclfenac sodium	$46.0 \pm 095^{*}$	$55.2 \pm 1.16^{*}$	$62 \pm 1.49^{*}$	$39 \pm 1.13^{*}$

Each value represents the mean $\pm$ 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.

CONCLUSIONS

The 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 (**6a–c**) showed the highest inhibitory antioxidant activity either using erythrocytes hemolysis or ABTS methods. These quinolones are the phenylamino, 4-chlorophenylamino, or 4-methoxyphenylamino derivatives. This means that the presence of the phenyl group will potentiate the activity, which may increase by introducing an electron-donating group, e.g. OCH_3, or an electron-withdrawing group, e.g., Cl in the *p*-position. Compounds **6a–c**, **7b**,**13**, and **15** manifested the best protective effect against DNA damage induced by bleomycin. Compounds **6a–c**, **7a**,**b**, and **13** exhibited a potent anti-inflammatory activity according to the carrageenan-induced paw edema test in rats. The *p*-methoxy derivative of **6** exhibited the best activity in all tested assays. This suggests the mechanism of anti-inflammatory and analgesic activities of compounds **6a–c**, especially **6c**, to be due to the potent antioxidant activity to maintain the integrity of both the cell membrane and DNA of the cells.

EXPERIMENTAL

All melting points are uncorrected and measured using an Electrothermal IA 9100 apparatus (Shimadzu, Japan). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL JNM-LA-400 FT NMR Spectrometer (University of Konstanz, Germany) and chemical shifts were expressed as δ values against $SiMe_4$ as internal standards. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1430 spectrometer (National Research Center). Mass spectra were recorded on GCMS-Q P1000 EX, Shimadzu, Japan (cas chromatography-mass spectrometer). Microanalytical data were performed by the Microanalytical Center at Cairo University (Egypt). The starting materials are prepared according to El-Gazzar et al.[14,15] The biological evaluation of the products was carried out at the Fermentation Biotechnology & Applied Microbiology (Ferm-BAM) Center at Al-Azhar University, Cairo, Egypt.

5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2-thioxo-6,7,8,9-tetrahydro-pyrimido[4,5-*b*]quinolin-4-one (4)

A mixture of 4-chlorobenzaldehyde (1.40 g, 10 mmol), cyclohexanone (1.12 g, 10 mmol), and 6-aminothiouracil **3** (1.43 g, 0.01 mol) was refluxed in 50 mL DMF for 50 h (under TLC control). The reaction mixture was cooled; the precipitate was filtered off, washed with ethanol, dried, and crystallized from DMF, as a yellow powder, in 80 % yield, m.p. 297–299°C; IR, cm^{-1}: 3361 (brs, NH), 3025 (CH aryl), 2911 (CH alkyl), 1688 (CO), 1631 (C=N). ^{1}H-NMR (DMSO-d_6) ppm: δ 1.64–1.67 (m, 2H, CH_2), 2.29–2.32 (t, 2H, CH_2) 2.75–2.97 (t, 2H, CH_2), 7.10–7.12 (d, 2H, Ar-H), 7.14–7.15 (d, 2H, Ar-H), 7.16–7.17 (dd, 4H, Ar-H), 8.19 (s, 1H, CH), 11.50 (brs, NH). ^{13}C-NMR (DMSO-d_6) ppm: δ 22.27, 26.73, 27.18 (3C, 3CH_2), 108.35, 127.95, 128.29, 128.52, 128.58, 129.44, 130.14, 131.17, 132.34, 132.43, 135.53, 135.70, 136.22, 149.71, 158.47 (15C, SP^2 carbon atoms), 162.25 (CO), 175.42 (CS); MS, [M^+], m/z 465 (100%). $C_{24}H_{17}Cl_2N_3OS$ (465.5); Requires (Found): C, 61.92 (62.01); H, 3.68 (3.64); N, 9.03 (9.11).

5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2-methylthio-6,7,8,9-tetra-hydropyrimido[4,5-*b*]quinolin-4-one (5)

To a warmed ethanolic KOH solution (prepared by dissolving 0.01 mol of KOH in 50 mL ethanol), **4** (4.65 g, 10 mmol) was added, the heating was continued for 30 min, the mixture was allowed to cool to room temperature, and methyl iodide (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 and washed with 100 mL water, and the product was dried and crystallized from dioxane in 80% yield, m.p. 327–330°C; IR, cm^{-1}: 3403 (br, NH), 3036 (CH aryl), 2925 (CH alkyl), 1687 (CO), 1652 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.65–1.68 (t, 2H, CH_2), 2.30–2.36 (t, 2H, CH_2), 2.73–2.77 (m, 2H, CH_2), 2.84 (s, 3H, SCH_3), 7.18–7.23 (d, 2H, Ar-H), 7.31–7.35 (d, 2H, Ar-H), 7.43–7.48 (d, 2H, Ar-H), 7.56–7.63 (d, 2H, Ar-H), 8.34 (s, 1H, CH), 9.20 (br, NH, D_2O exchangeable); MS: [M^+], m/z 480, (100%), [M^+-SCH_3], m/z 433 (44%); $C_{25}H_{19}Cl_2N_3OS$ (480.4); Requires (Found): C, 62.50 (62.47); H, 3.98 (3.94); N, 8.75 (8.79).

2-Arylamino-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,8,9-tetra-hydropyrimido[4,5-*b*]quinoline (6a–c or 7a,b): General Procedure

Into a warm solution of **5** (4.79 g, 10 mmol) in methanol (100 mL) was added the freshly distilled amine (10 mmol). The reaction mixture was stirred under reflux for 5 h, then allowed to cool to 0°C for 12 h. The solid obtained was filtered, washed with water (100 mL), dried, and recrystallized from appropriate solvent to produce (**6a–c**) and (**7a**,**b**).

5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2-phenylamino-6,7,8,9-tetra-hydropyrimido[4,5-b]quinolin-4-one (6a)

It was obtained from aniline (0.93 g, 10 mmol) as yellow crystals, crystallized from dioxane; in 76% yield, m. p. 215–217°C; IR, cm^{-1}: 3400 (brs NH), 3030 (CH aryl), 2915 (CH alkyl), 1683 (C=O), 1600 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.63–1.67 (t, 2H, CH_2) 2.28–2.35 (t, 2H, CH_2), 2.70–2.74 (m, 2H, CH_2), 6.97–7.13 (m, 3H, Ar-H), 7.19–7.25 (d, 2H, Ar-H), 7.37–7.43 (m, 2H, Ar-H), 7.47–7.53 (dd, 4H, Ar-H), 7.60–7.64 (d, 2H, Ar-H), 8.30 (s, 1H, CH), 9.20 (br, NH, D_2O exchangeable), 10.30 (br, NH, D_2O exchangeable); MS: [M^+], m/z 525, (73%), [M^++1], m/z 526 (29%); $C_{30}H_{22}Cl_2N_4O$ (525.4); Requires (Found): C, 68.57 (68.49); H, 4.22 (4.19); N, 10.66 (10.57).

2-(4-Chlorophenylamino)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,7,-8,9-tetrahydropyrimido[4,5-b]quinolin-4-one (6b)

It was obtained from 4-chloro-aniline (1.27 g, 10 mmol) as yellow crystals, crystallized from ethanol; in 73% yield, m.p. 221–223°C; IR, cm^{-1}: 3395 (brs, NH), 3039 (CH aryl), 2924 (CH alkyl), 1685 (C=O), 1605 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.65–1.68 (t, 2H, CH_2) 2.25–

2.34 (t, 2H, CH_2), 2.66–2.72 (m, 2H, CH_2), 7.00–7.18 (dd, 4H, Ar-H), 7.21–7.26 (d, 2H, Ar-H), 7.33–7.40 (2d, 4H, Ar-H), 7.63–7.69 (d, 2H, Ar-H), 8.33 (s, 1H, CH), 9.50 (br, NH, D_2O exchangeable), 10.00 (br, NH, D_2O exchangeable); MS: [M^+], m/z 559, (100%), [M^++1], m/z 560 (27%); $C_{30}H_{21}Cl_3N_4O$ (559.9); Requires (Found): C, 64.35 (64.32); H, 3.78 (3.74); N, 10.01 (9.97).

5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2-(4-methoxyphenylamino)-6,7,8,9-tetrahydropyrimido[4,5-b] quinolin-4-one (6c)

The compound was obtained from 4-anisidine (1.23 g, 10 mmol) as pale yellow crystals, crystallized from ethanol; in 77% yield, m.p. 217–219°C; IR, cm^{-1}: 3420 (brs, NH), 3045 (CH aryl), 2928 (CH alkyl), 1683 (C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.63–1.68 (t, 2H, CH_2) 2.28–2.33 (t, 2H, CH_2), 2.68–2.73 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3), 6.96–7.14 (dd, 4H, Ar-H), 7.20–7.26 (d, 2H, Ar-H), 7.30–7.34 (d, 2H, Ar-H), 7.43–7.48 (d, 2H, Ar-H), 7.62–7.66 (d, 2H, Ar-H), 8.43 (s, 1H, CH), 9.60 (br, NH, D_2O exchangeable), 10.30 (br, NH, D_2O exchangeable); MS: [M^+], m/z 555, (100%); $C_{31}H_{24}Cl_2N_4O_2$ (555.4); Requires (Found): C, 67.03 (67.00); H, 4.35 (4.37); N, 10.08 (10.11).

5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2-piperazino-6,7,8,9-tetra-hydropyrimido[4,5-b]quinolin-4-one (7a)

The compound was obtained from piperazine (0.86 g, 10 mmol) as pale yellow crystals, crystallized from ethanol in 70% yield, m.p. 201–203°C; IR, cm^{-1}: 3380 (br, NH), 3053 (CH aryl), 2930 (CH alkyl), 1684 (C=O), 1590 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.65–1.69 (t, 2H, CH_2) 2.24–2.32 (t, 2H, CH_2), 2.67–2.73 (m, 2H, CH_2), 3.26–3.31 (t, 2H, CH_2), 3.38–3.41 (m, 4H, CH_2), 3.46–3.50 (t, 2H, CH_2), 6.98–7.16 (d, 2H, Ar-H), 7.28–7.32 (d, 2H, Ar-H), 7.36–7.40 (d, 2H, Ar-H), 7.51–7.55 (d, 2H, Ar-H), 8.35 (s, 1H, CH), 9.60 (br, NH, D_2O exchangeable), 10.50 (br, NH, D_2O exchangeable); MS: [M^+], m/z 518, (100%), [M^++1], m/z 519 (26%); $C_{28}H_{25}Cl_2N_5O$ (518.4); Requires (Found): C, 64.86 (64.79); H, 4.86 (4.84); N, 13.51 (13.48).

5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2-morpholino-6,7,8,9-tetra-hydropyrimido[4,5-b]quinolin-4-one (7b)

The compound was obtained from morpholine (0.87 g, 10 mmol) as yellow crystals, crystallized from ethanol; in 62% yield, m.p. 187–190°C; IR, cm^{-1}: 3415 (brs, NH), 3036 (CH aryl), 2929 (CH alkyl), 1688 (C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.68–1.75 (t, 2H, CH_2) 2.26–

2.32 (t, 2H, CH_2), 2.67–2.73 (m, 2H, CH_2), 3.27–3.32 (t, 2H, CH_2), 3.40–3.42 (t, 2H, CH_2), 3.84–3.88 (m, 4H, $2CH_2$), 6.96–7.12 (d, 2H, Ar-H), 7.24–7.29 (d, 2H, Ar-H), 7.38–7.44 (d, 2H, Ar-H), 7.56–7.61 (d, 2H, Ar-H), 8.36 (s, 1H, CH), 9.60 (br, NH, D_2O exchangeable); MS: [M^+], m/z 519, (68%), [M^++1], m/z 520 (30%); $C_{28}H_{24}Cl_2N_4O_2$(519.4); Requires (Found): C, 64.74 (64.69); H, 4.66 (4.64); N, 10.78 (10.88).

2-(*o*-Carboxyphenylamino)-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-6,-7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-4-one (8)

The compound was obtained from anthranilic acid (1.37 g, 10 mmol) as yellow powder, crystallized from ethanol; in 75% yield, m.p. 243–245°C; IR, cm^{-1}; 3520 (br, OH), 3390 (br, NH), 3027 (CH aryl), 2919 (CH alkyl), 1718 (C=O), 1685 (C=O), 1600 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm); 1.66–1.71 (t, 2H, CH_2) 2.23–2.31 (t, 2H, CH_2), 2.69–2.76 (m, 2H, CH_2), 6.97–7.14 (dd, 4H, Ar-H), 7.20–7.25 (d, 2H, Ar-H), 7.36–7.40 (m, 2H, Ar-H), 7.47–7.53 (d, 1H, Ar-H), 7.55–7.61 (d, 2H, Ar-H), 7.66–7.74 (d, 1H, Ar-H), 8.30 (s, 1H, CH), 9.20, 10.30 (2br, 2NH, D_2O exchangeable), 12.50 (brs, OH, D_2O exchangeable); MS: [M^+], m/z 569, (100%); $C_{31}H_{22}Cl_2N_4O_3$ (569.4); Requires (Found): C, 65.38 (65.29); H, 3.89 (3.92); N, 9.84 (9.87).

12-(4-Chlorophenyl)-8-(4-chlorophenylmethylene)-8,9,10,11-terahydroquino[2′,-3′:4,5]pyrimido[2,1-*b*]quinazoline-13,14-dione (9)

A solution of **9** (2.84, 5 mmol) in glacial acetic acid (40 mL) and a 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) and neutralized with ammonia solution. The solid precipitate was filtered off, washed with water, dried, and crystallized from DMF as yellow crystals; in 76% yield, m.p. 229–231°C; IR, cm^{-1}: 3028 (CH aryl), 2927 (CH alkyl), 1705 (C=O), 1692 (C=O), 1620 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.67–1.73 (t, 2H, CH_2), 2.26–2.34 (t, 2H, CH_2), 2.69–2.76 (m, 2H, CH_2), 6.99–7.17 (dd, 4H, Ar-H), 7.21–7.25 (d, 2H, Ar-H), 7.33–7.40 (m, 2H, Ar-H), 7.50–7.54 (d, 1H, Ar-H), 7.60–7.65 (d, 2H, Ar-H), 7.72–7.78 (d, 1H, Ar-H), 8.43 (s, 1H, CH), 10.25 (br, NH, D_2O exchangeable); MS: [M^+], m/z 551, (100%); $C_{31}H_{20}Cl_2N_4O_2$ (551.4); Requires (Found): C, 67.52 (67.49); H, 3.65 (3.59); N, 10.16 (10.12).

4-Chloro-5-(4-chlorophenyl)-9-(4-chlorophenylmethylene)-2-methylthio-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinoline (10)

A solution of **5** (4.79 g, 10 mmol) in dry dioxane (30 mL) was treated with 10 mL of phosphorus oxychloride, and the mixture was stirred under reflux for 3 h. The reaction mixture was allowed to cool to room temperature, and poured into cold water (100 mL), whereby a solid was separated, filtered off, and crystallized from ethanol (yellow powder), in 80% yield, m.p. 360–362°C; IR, cm^{-1}; 3049 (CH aryl), 2919 (CH alkyl), 1600 (C=N), 1160 (C-Cl); ^{1}H-NMR ($CDCl_3$, δ, ppm); 1.65–1.69 (t, 2H, CH_2) 2.31–2.39 (t, 2H, CH_2), 2.65–2.72 (m, 2H, CH_2), 2.84 (s, 3H, SCH_3), 6.91–7.11 (d, 2H, Ar-H), 7.15–7.26 (d, 2H, Ar-H), 7.34–7.40 (d, 2H, Ar-H), 7.42–7.53 (d, 2H, Ar-H), 8.25 (s, 1H, CH); MS: [M^+], m/z 498, (78%), [M^++1], m/z 499 (29%); $C_{25}H_{18}Cl_3N_3S$(498.8); Requires (Found): C, 60.19 (60.17); H, 3.63 (3.65); N, 8.42 (8.43).

5-(4-Chlorophenyl)-9-(4-chlorophenylmethylene)-2,4-dihydrazino-6,7,8,9-tetra-hydropyrimido[4,5-*b*]quinoline (11)

A mixture of **6** (4.98 g, 10 mmol) and hydrazine hydrate (99–100%, 20 mL) was stirred under reflux in dioxane (50 mL) and ethanol (20 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 pale yellow powder; in 90% yield, m.p. 298–300°C; IR, cm^{-1}: 3438–3350 (brs, NH), 3035 (CH aryl), 2923 (CH alkyl), 1625 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm); 1.67–1.75 (t, 2H, 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), 7.05–7.09 (d, 2H, Ar-H), 7.14–7.18 (d, 2H, Ar-H), 7.27–7.34 (d, 2H, Ar-H), 7.40–7.45 (d, 2H, Ar-H), 8.32 (s, 1H, CH), 8.54–8.80 (br, NH_2, D_2O exchangeable), 10.70, 11.60 (two, brs, 2NH, D_2O exchangeable); $C_{24}H_{21}Cl_2N_7$ (478.4); Requires (Found): C, 60.25(60.23); H, 4.43 (4.45); N, 20.50 (20.52).

6-(4-Chlorophenylmethylene)-10-(4-chlorophenyl)-6,7,8,9-tetrahydro-*s*-triazolo-[3′,4′:6,1]-*s*-triazolo[3″,4″,:2,3]pyrimido[4,5-*b*]quinoline (12a)

A mixture of compound **11** (2.39 g, 5 mmol) and formic acid (30 mL) was heated under reflux for 18 h. The reaction mixture was allowed to cool to room temperature and poured into water (100 mL). The solid formed was collected by filtration, washed with ethanol (20 mL), dried, and crystallized from ethanol; in 69 % yield, m.p. 289–291°C; IR, cm^{-1}: 3025 (CH aryl), 2943 (CH alkyl), 1605 (C=N), 1560 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.69–1.73 (m, 2H, CH_2), 2.36–2.40 (t, 2H, CH_2),

2.77–2.83 (t, 2H, CH_2), 7.14–7.18 (d, 2H, Ar-H), 7.40–7.47 (dd, 4H, Ar-H), 7.52–7.56 (d, 2H, Ar-H), 8.15 (s, 1H, CH), 8.25 (s, 1H, triazole), and 8.67 (s, 1H, triazole). ^{13}C-NMR (DMSO-d_6) ppm: δ 22.83, 26.36, 27.09 (3C, 3CH_2), 108.6, 128.1, 128.2, 128.4, 129.3, 130.9, 131.3, 131.5, 132.4, 132.6, 134.3, 135.8, 136.4, 143.1, 144.5, 152.6, 154.6, 155.7, 158.7 (23 sp^2 carbon atoms); $C_{26}H_{17}Cl_2N_7$ (498.4); Requires (Found): C, 62.65 (62.57); H, 3.44 (3.41); N, 14.23 (14.19).

6-(4-Chlorophenylmethylene)-10-(4-chlorophenyl)-6,7,8,9-tetrahydro-3,14-dimethyl-*s*-triazolo[3′,4′:6,1]-*s*-triazolo [3″,4″,:2,3]pyrimido[4,5-*b*]quinoline (12b)

A mixture of **11** (2.39 g, 5 mmol) and glacial acetic acid (80 mL) was stirred under reflux for 20 h (TLC control). The reaction mixture was allowed to cool to room temperature and poured into water (100 mL). The solid formed was collected by filtration, washed with ethanol (20 mL), dried, and crystallized from DMF; in 72 % yield, m.p. 256–258°C; IR,cm^{-1}: 3038 (CH aryl), 2972 (CH alkyl), 1698 (CO), 1636 (C=N), 1565 (C=C). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.62–1.66 (m, 2H, CH_2), 2.27–2.32 (t, 2H, CH_2), 2.41 (s, 3H, CH_3), 2.44 (s, 3H CH_3), 2.76-2.79 (t, 2H, CH_2), 7.09–7.14 (d, 2H, Ar-H), 7.40–7.45 (d, 2H, Ar-H), 7.48–7.52 (dd, 4H, Ar-H), 8.12 (s, 1H, CH); $C_{28}H_{21}Cl_2N_7$ (526.4); Requires (Found): C, 63.88 (63.86); H, 4.02 (3.99); N, 18.63 (18.66).

3-Amino-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-5-oxo-7,8,9,10-tetra-hydrothiazolo[3′,2′:1,2]pyrimido[4,5-*b*] quinoline-2-carbonitrile (13)

A mixture of compound **4** (4.65 g, 10 mmol), bromomalononitrile (1.45 g, 10 m mol) and anhydrous potassium carbonate (2.76 g, 20 mmol) was stirred under reflux in 30 mL ethanol for 12 h (TLC control). The reaction mixture was allowed to cool to room temperature and then poured into cold water (100 mL). The precipitate was filtered off, dried, and crystallized from benzene as brown crystals, crystallized from ethanol; in 68% yield, m.p. 262–264°C; IR, cm^{-1}: 3410 (brs, NH), 3053 (CH aryl), 2915 (CH, alkyl), 2215 (CN), 1686 (CO), 1610 (C=N), 1520 (C=C); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.63, 1.74 (m, 2H, CH_2), 2.25–2,34 (m, 2H, CH_2), 2.72–2.78 (m, 2H, CH_2), 7.10–7.19 (dd, 4H, Ar-H), 7.28–7.33 (d, 2H, Ar-H), 7.35–7.43 (d, 2H, Ar-H), 8.19 (s, 1H, CH), 8.90 (br, NH_2, D_2O exchangeable); ^{13}C-NMR; 24.30, 26.97, 28.06 (3C, 3CH_2), 68.50 (1C, CN), 108.5, 125.1, 127.8, 128.1, 128.7, 128.8, 129.3, 129.5, 130.8,

131.0, 132.6, 135.3, 135.6, 135.9, 141.6, 147.9, 152.8, 158.7 (22C, sp^2 carbon atoms) and 163.88 (CO); MS: [M^+], m/z 530, (100%), [M^{+2}-H_2N-C=C-CN], m/z 464 (17%); $C_{27}H_{17}Cl_2N_5OS$ (530.5); Requires (Found): C, 61.14 (61.18); H, 3,23 (3.19); N, 13.20 (13.16).

8-(4-Chlorophenylmethylene)-12-(4-chlorophenyl)-8,9,10,11-tetrahydropyrim-ido[4″,5″,:4′,5′]thiazolo[3′,2′:1,2]pyrimido [4,5-*b*]quinoline-4,13-dione (14a)

A mixture of compound **13** (2.65 g, 5 mmol), formic acid (10 mL), and a catalytic amount of concentrated hydrochloric acid was heated under reflux for 16 h. The reaction mixture was allowed to cool to room temperature, and poured into cold water (100 mL). The formed solid was collected by filtration, washed by ethanol (20 mL), dried, and crystallized from DMF (yellow) in 79% yield, m.p 233–235°C; IR, cm^{-1}: 3380 (brs, NH), 3064 (CH aryl), 2940 (CH alkyl), 1690, 1679 (2CO), 1615 (C=N), 1525 (C=C); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.65–1.73 (m, 2H, CH_2), 2.28–2.36 (m, 2H, CH_2), 2.82–2.87 (m, 2H, CH_2), 7.15–7.22 (d, 2H, Ar-H), 7.24–7.29 (dd, 4H, Ar-H), 7.51–7.55 (d, 2H, Ar-H), 8.22 (s, 1H, CH), 8.88 (s, 1H, CH pyrimidine) and 11.20 (brs, NH, D_2O exchangeable); $C_{28}H_{17}Cl_2N_5O_2S$ (558.4); Requires (Found): C, 60.23 (60.26); H, 3.06 (2.99); N, 12.54 (12.49).

8-(4-Chlorophenylmethylene)-12-(4-chlorophenyl)-2-methyl-8,9,10,11-tetra-hydropyrimido[4″,5″,:4′,5′]thiazolo[3′,2′:1,2] pyrimido[4,5-*b*]quinoline-4,13-dione (14b)

A mixture of compound **13** (5.30 g, 0.01 mol) and acetic acid (80 mL) was heated under reflux for 20 h. The reaction mixture was allowed to cool to room temperature and poured into cold water (100 mL). The formed solid was collected by filtration, washed with ethanol (20 mL), dried, and crystallized from ethanol as yellow powder; in 77% yield, m.p. 223–225°C; IR, cm^{-1}: 3400 (brs, NH), 3054 (CH aryl), 2932 (CH alkyl), 1689, 1676 (2CO), 1625 (C=N), 1510 (C=C); ^{1}H-NMR (DMSO-d_6, δ, ppm): δ 1.65–1.76 (m, 2H, CH_2), 2.25–2.28 (t, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.75–2.79 (t, 2H, CH_2), 7.10-7.14 (d, 2H, Ar-H), 7.29-7.34 (dd, 4H, Ar-H), 7.42–7.44 (d, 2H, Ar-H), 8.02 (s, 1H, CH), 10.00 (brs, NH, D_2O exchangeable); $C_{29}H_{19}Cl_2N_5O_2S$ (572.5); Requires (Found): C, 60.85 (60.78); H, 3.34 (3.29); N, 12.34 (12.29).

4-Amino-8-(4-chlorophenylmethylene)-12-(4-chlorophenyl)-8,9,10,11-tetrahydro-pyrimido[4″,5″,:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolin-13-one (15)

Method A

A mixture of compound **13** (5.30 g, 0.01 mol), formamide (10 mL), and formic acid (2 mL) was stirred under reflux in DMF (50 mL) for 6 h. The reaction mixture was allowed to cool to room temperature, poured into water (100 mL), and neutralized with ammonia solution. The precipitate was collected by filtration, washed with water and ethanol, dried, and crystallized from dioxane in 64% yield, m.p. 295°C (dec.); IR, cm^{-1}: 3390 (brs, NH), 3073 (CH aryl), 2934 (CH alkyl), 1682 (CO), 1630 (C=N), 1525 (C=C); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.65–1.69 (t, 2H, CH_2), 2.28–2.33 (m, 2H, CH_2), 2.76–2.81 (t, 2H, CH_2), 7.11–7.16 (d, 2H, Ar-H), 7.28–7.34 (d, 2H, Ar-H), 7.41–7.44 (d, 2H, Ar-H), 7.50–7.54 (d, 2H, Ar-H), 8.05 (s, 1H, CH), 8.25 (s, 1H, CH pyrimidine), and 8.90 (br, NH_2, D_2O exchangeable); $C_{28}H_{18}Cl_2N_6OS$ (557.4); Requires (Found): C, 60.32 (60.29); H, 3.25 (3.22); N, 15.08 (15.11).

Method B

To a warmed ethanolic sodium ethoxide solution (prepared by dissolving (0.23 g, 10 mmol), sodium metal in 50 mL absolute ethanol) compound **21** (2.78 g, 5 mmol) was added. The mixture was stirred under reflux for 5 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL), and neutralized with acetic acid. The precipitate was filtered off, dried, and crystallized from dioxane in 63% yield, with identical data.

4-Amino-8-(4-chlorophenylmethylene)-12-(4-chlorophenyl)-8,9,10,11-tetrahydro-pyrimido[4″,5″,:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-*b*]quinolin-2-(oxo/or thioxo)-13-ones (16a,b): General Procedure

A mixture of compound **13** (5.30 g, 10 mmol) and urea or thiourea (0.01 mol) was heated at 180°C in a test tube on a sand-bath for 4 h. The mixture was allowed to cool to room temperature; the product was solidified by cooling and adding methanol (50 mL). The precipitate was collected by filtration and crystallized from the proper solvent to produce (**16a**,**b**).

4-Amino-8-(4-chlorophenylmethylene)-12-(4-chlorophenyl)-8,9,10,11-tetrahydro-pyrimido[4″,5′,:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-2,13-dione (16a)

The compound was obtained from urea (0.61 g, 0.01 mol) as a dark brown substance and crystallized from ethanol, in 82% yield, m.p. 216–218°C; IR, cm^{-1}: 3400 (brs, NH), 3079 (CH aryl), 2938 (CH alkyl), 1694, 1686 (2CO), 1654 (C=N), 1530 (C=C); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.64–1.69 (m, 2H, CH_2), 2.29–2.32 (t, 2H, CH_2), 2.76–2.78 (t, 2H, CH_2), 7.06–7.12 (d, 2H, Ar-H), 7.39–7.41 (d, 2H, Ar-H), 7.43–7.47 (dd, 4H, Ar-H), 8.02 (s, 1H, CH), 8.75 (brs, NH_2, D_2O exchangeable), 9.50 (brs, NH, D_2O exchangeable); $C_{28}H_{18}Cl_2N_6O_2S$ (573.4); Requires (Found): C, 58.64 (58.65); H, 3.16 (3.12); N, 14.66 (14.62).

4-Amino-8-(4-chlorophenylmethylene)-12-(chlorophenyl)-8,9,10,11-tetrahydro-pyrimido[4″,5′,:4′,5′]thiazolo[3′,2′:1,2]pyrimido[4,5-b]quinolin-2-thioxo-13-one (16b)

The compound was obtained from thiourea (0.77 g, 0.01 mol) as brown crystals and crystallized from ethanol; in 80% yield, m.p. 240–242°C (dec.); IR, cm^{-1}: 3397 (brs, NH), 3084 (CH aryl), 2929 (CH alkyl), 1683 (CO), 1658 (C=N), 1526 (C=C); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.65–1.69 (m, 2H, CH_2), 2.31–2.36 (t, 2H, CH_2), 2.69–2.74 (t, 2H, CH_2), 7.09–7.18 (dd, 4H, Ar-H), 7.21–7.29 (d, 2H, Ar-H), 7.35–7.39 (d, 2H, Ar-H), 8.16 (s, 1H, CH), 8.82 (br, NH_2) and 11.50 (br, NH). $C_{28}H_{18}Cl_2N_6OS_2$ (589.5); Requires (Found): C, 57.04 (57.03); H, 3.08 (2.99); N, 14.26 (14.19).

8-(4-Chlorophenylmethylene)-12-(4-chlorophenyl)-4-imino-8,9,10,11-tetrahydro-quino[2″,3″,:4′,5′]pyrimido[2′,1′:2,3]thiazolo[4,5-*d*][1,3]thiazine-13-one (17)

A mixture of compound **13** (2.65 g,5 m mol) and carbon disulfide (excess 10 mL) was heated under reflux on a water-bath (80°C) in 40 mL pyridine for 10 h (TLC control). The reaction mixture was allowed to cool to 0°C for 12 h, the precipitate was filtered off, washed with ethanol (40 mL), dried, and crystallized from ethanol; in 64% yield, m.p. 272–275°C (dec.); IR,cm^{-1}: 3420 (brs, NH), 3085 (CH aryl), 2932 (CH alkyl), 1678 (CO), 1625 (C=N), 1520 (C=C); ^{1}H-NMR (DMSO-d_6) ppm: δ 1.63–1.74 (m, 2H, CH_2), 2.33–2.38 (t, 2H, CH_2), 2.71–2.81 (t, 2H, CH_2), 7.05–7.11 (d, 2H, Ar-H), 7.19–7.22 (d, 2H, Ar-H), 7.36–7.41 (d, 2H, Ar-H), 7.46–7.51 (d, 2H, Ar-H), 8.12 (s, 1H, CH), and 9.75, 10.50 (2brs, 2NH, D_2O exchangeable); ^{13}C-NMR; 22.23, 26.65, 27.33 (3C, 3CH_2), 108.6, 125.2, 127.6, 128.4, 128.6, 128.9, 129.4, 129.8, 130.6, 131.3, 132.7,

135.6, 135.8, 135.5, 141.4, 147.6, 152.8, 154.3, 155.3 (23C, sp^2 carbon atoms) and 163.5 (CO), 176.8 (C=S); $C_{28}H_{17}Cl_2N_5OS_3$ (606.5); Requires (Found): C, 55.44 (55.39); H, 2.82 (2.84); N, 11.55 (11.49).

3-Ethoxymethyleneamino-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-5-oxo-7,8,9,10-tetrahydrothiazolo [3′,2′:1,2]pyrimido[4,5-*b*]quinoline-2-carbonitrile (18)

A mixture of compound **13** (2.65 g, 5 mmol) and triethyl orthoformate (2.96 g, 20 mmol) was stirred under reflux in acetic anhydride (30 mL) for 6 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL), and neutralized with ammonia solution. The precipitate was collected by filtration, washed with water, dried and crystallized from ethanol in 77% yield, m.p. 191–193°C; IR, cm^{-1}: 3066 (CH aryl), 2922 (CH alkyl), 2215 (CN), 1690 (CO), 1620 (C=N), 1510 (C=C). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.24–1.28 (t, 3H, CH_3), 1.65–1.71 (t, 2H CH_2), 2.26–2.32 (t, 2H, CH_2) 2.70–2.76 (t, 2H, CH_2), 4.39–4.45 (q, 2H, CH_2), 7.14–7.18 (d, 2H, Ar-H), 7.28–7.33 (d, 2H, Ar-H), 7.41–7.45 (d, 2H, Ar-H), 7.50–7.56 (d, 2H, Ar-H), 8.20 (s, 1H, CH), 8.50 (s, 1H, CH); $C_{30}H_{21}Cl_2N_5O_2S$ (586.5); Requires (Found): C, 61.43 (61.39); H, 3.61 (3.58); N, 11.94 (11.78).

6-(4-Chlorophenyl)-10-(4-chlorophenylmethylene)-3-hydrazinomethyleneamino-5-oxo-7,8,9,10-tetrahydrothiazolo [3′,2′:1,2]pyrimido[4,5-*b*]quinoline-2-carbonitrile (19)

A mixture of compound **18** (2.93 g, 5 mmol) and hydrazine hydrate (99–100%) (20 mL) was stirred under reflux in a mixture of dioxane/ethanol (80 mL/20 mL) for 12 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL), and neutralized by acetic acid. The precipitate was collected by filtration, washed with water, dried, and crystallized from ethanol; in 69% yield, m.p. 299–301°C; IR, cm^{-1}: 3420 (brs, NH), 3054 (CH aryl), 2926 (CH alkyl), 2216 (CN), 1683 (CO), 1630 (C=N), 1505 (C=C).^{1}H-NMR (DMSO-d_6, δ, ppm): 1.68–1.76 (t, 2H, CH_2), 2.27–2.37 (m, 2H, CH_2), 2.69–2.76 (t, 2H, CH_2), 7.18–7.21 (d, 2H, Ar-H), 7.31–7.35 (dd, 4H, Ar-H), 7.40–7.56 (d, 2H, Ar-H), 8.06 (s, 1H, CH), 8.45 (s, 1H, CH), 8.80 (br, NH_2, D_2O exchangeable) and 10.00 (br, NH, D_2O exchangeable); $C_{28}H_{19}Cl_2N_7OS$ (572.4); Requires (Found): C, 58.74 (58.75); H, 3.34 (3.32); N, 17.13 (17.11).

3-Amino-8-(4-chlorophenylmethylene)-12-(4-chlorophenyl)-4-imino-8,9,10,11-tetrahydropyrimido[4″,5″,:4′,5′]thiazolo [3′,2′:1,2]pyrimido[4,5-*b*]quinolin-13-one (20)

Compound **19** (2.86 g, 5 mmol) was added to a warmed ethanolic sodium ethoxide solution (prepared by dissolving (0.23 g, 10 mmol) sodium metal in 50 mL absolute ethanol). The mixture was stirred under reflux for 8 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL), and neutralized with acetic acid. The precipitate was filtered off, dried, and crystallized from benzene in 59 % yield, m.p. 267–270°C; IR, cm^{-1}: 3400 (brs, NH), 3091 (CH, aryl), 2937 (CH, alkyl), 1692 (CO), 1635 (C=N), 1546 (C=C); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.69–1.75 (t, 2H, CH_2), 2.28–2.33 (t, 2H, CH_2), 2.68–2.76 (t, 2H, CH_2), 7.10–7.15 (d, 2H, Ar-H), 7.23–7.28 (d, 2H, Ar-H), 7.40–7.43 (d, 2H, Ar-H), 7.44–7.46 (d, 2H, Ar-H), 8.28 (s, 1H, CH), 8.49 (s, 1H, CH pyrimidine), 8.85 (br, 2H, NH_2), and 11.30 (br, NH); $C_{28}H_{19}Cl_2N_7OS$ (572.4); Requires (Found): C, 58.74 (58.69); H, 3.34 (3.31); N, 17.13 (17.09).

3-Aminomethyleneamino-6-(4-chlorophenyl)-10-(4-chlorophenylmethylene)-5-oxo-7,8,9,10-tetrahydrothiazolo [3′,2′:1,2]pyrimido[4,5-*b*]quinoline-2-carbonitrile (21)

A mixture of compound **18** (2.93 g, 5 mmol) and ammonia solution (40–50%, 10 mL) was stirred under reflux in 50 mL ethanol for 12 h. The reaction mixture was allowed to cool to room temperature, poured into cold water (100 mL), and neutralized with acetic acid. The precipitate was filtered off, washed with water, dried, and crystallized from DMF in 55 % yield, m.p. 287–290°C (dec.); IR, cm^{-1}: 3395 (br, NH_2), 3061 (CH aryl), 2929 (CH alkyl), 2217 (CN), 1690 (CO), 1620 (C=N), 1530 (C=C).^{1}H-NMR (DMSO-d_6, δ, ppm): 1.65–1.71 (t, 2H, CH_2), 2.26–2.31 (t, 2H, CH_2), 2.71–2.78 (t, 2H, CH_2), 6.97–7.00 (d, 2H, Ar-H), 7.11–7.16 (d, 2H, Ar-H), 7.23–7.31 (d, 2H, Ar-H), 7.40–7.44 (d, 2H, Ar-H), 8.08 (s, 1H, CH), 8.54 (s, 1H, CH), and 9.05 (br, NH_2, D_2O exchangeable); $C_{28}H_{18}Cl_2N_6OS$ (557.4); Requires (Found): C, 60.32 (60.29); H, 3.25 (3.21); N, 15.08 (15.11).

Pharmacological Screening

Animals

Male Sprague-Dawley rats (150–200 g) were used in the study of antioxidant activity, but adult females were used in anti-inflammatory activity. Both sex of Swiss mice weighing 25–30 g were used in analgesic

activity, taking into account international principles and local regulations concerning the care and use of laboratory animals.[16] The animals had free access to standard commercial diet and water *ad libitum*, and were kept in rooms maintained at 22 ± 1°C with a 12 h light/dark cycle.

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), 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was purchased from Wak. All other chemicals were of the highest quality available.

Antioxidant Sreening

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 2.500 rpm for 10 min to obtain a constantly packed cell preparation. Erythrocyte hemolysis was mediated by peroxyl radicals in this assay system.[17] 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 8 volumes of PBS, and centrifuged at 2,500 rpm for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with 8 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 the equation (1-A/B) × 100%. The data were expressed as mean standard deviation. L-Ascorbic acid was used as a positive control.

Antioxidant Activity Screening Assay ABTS Method

For each of the investigated compounds, 2 mL of ABTS solution (60 μM) were 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) were 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[18]:

$$\text{The \% inhibition} = \frac{A_{control} - A_{test}}{A_{Control}} \times 100$$

Ascorbic acid (Vitamin C) was used as standard antioxidant (positive control). A blank sample was run without ABTS and using MeOH/Phosphate buffer (1:1) instead of the sample. A negative control sample was run with MeOH/phosphate buffer (1:1) instead of the tested compound.

Bleomycin-Dependent DNA Damage

The assay was done according to Aeschlach et al.[19] 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 (0.1M). The color was developed by adding 0.5 mL thiobarbituric acid (TBA)(1%, w/v) and 0.5 mL HCI (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.

Anti-Inflammatory Activity (Carrageenan Induced Rat Hind Paw Edema Model)

The method adopted resembles essentially that described by Winter et al.[20] (distilled water was selected as vehicle to suspend the standard drugs and the test compounds). The albino rats weighing between 150–180 g were starved for 18 h prior to the experiment. The animals were weighed, marked for identification, and divided into 14 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 first group was kept as control and was given the respective volume of the solvent (0.5 mL distilled water). Groups 2–13 were orally administered aqueous suspension of the synthesized compounds in doses 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.[21] 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 carrageenin injection (mL), and E_c, E_t are the edema rat of control group and treated group, respectively.

Analgesic Activity Using Hot-Plate Test

The experiment was carried out as described by Turner[22] using a hot-plate apparatus, maintained at 53 ± 0.5°C. The mice were divided into 14 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). The first group was kept as normal control. The aqueous suspension of the synthesized compounds was orally administered to the mice of the groups (2–13) at doses of 20 mg/kg. The mice of the last group were orally treated with diclofenac sodium in a dose of 20 mg/kg. The reaction time was again measured at 15 min and repeated at 30, 60, and 90 min after treatment. To avoid tissue damage to the mice paws, cut-off time for the response to the thermal stimulus was set at 60 seconds. The reaction time was calculated for each synthesized compounds and drug-treated group.

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.[23] The mice were divided into 14 groups (6 in each group), starved for 16 h, and pretreated as follows: The first group, which served as control positive, orally received distilled water in appropriate volumes. The last group orally received diclofenac sodium in a dose of 20 mg/kg. The remaining groups received the aqueous suspension of synthesized compounds orally at doses (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 writhes was recorded, and the percentage protection was calculated using the

following ratio (%) protection = (control mean – treated mean/control mean) × 100.

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