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Synthesis and antiproliferative evaluation of certain 4-anilino-8-methoxy-2-phenylquinoline and 4-anilino-8-hydroxy-2-phenylquinoline derivatives

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Abstract—The present report describes the synthesis and antiproliferative evaluation of certain 4-anilino-8-methoxy-2-phenylquinoline and 4-anilino-8-hydroxy-2-phenylquinoline derivatives. The antiproliferative activity of 4′-COMe-substituted derivatives decreased in an order of 6-OMe (**1**, 3.89 μM) > 8-OMe (**8**, 10.47 μM) > 8-OH (**9**, 14.45 μM), indicating that the position of substitution at the quinoline ring is crucial. For 3′-COMe derivatives, the antiproliferative activity of 8-OH (**11**, 1.20 μM) is more potent than its 8-OMe counterpart (**10**, 8.91 μM), indicating that a H-bonding donating substituent is more favorable than that of a H-bonding accepting group. Comparison of 8-OH derivatives, the antiproliferative effect of COMe (**11**) is more potent than its oxime derivative (**15a**, 2.88 μM), which in turn is more potent than the methyloxime counterpart (**15b**, 5.50 μM). Compound **11** is especially active against the growth of certain solid cancer cells such as HCT-116 (colon cancer), MCF7, and MDA-MB-435 (breast cancer) with GI_{50} values of 0.07, <0.01, and <0.01 μM, respectively. Flow cytometric analyses revealed that growth inhibition by **11** and **15a** was due to accumulation in S-phase. This result is interesting because 2-phenylquinolone derivatives have been reported to be antimitotic agents which induced cell cycle arrest in G_2/M phase.

1. Introduction

Quinolin-4(1*H*)-one moiety is a characteristic component of a large number of antibacterial and/or anticancer agents.[1–5] The biological activity of these quinolone derivatives depends not only on the bicyclic heteroaromatic pharmacophore but also on the nature of the peripheral substituents and their spatial relationship. With a phenyl group appended on C-2 position of quinolin-4(1*H*)-one, a number of 2-phenylquinolone derivatives have been discovered to possess antimitotic activity.[6–8] Recently, we have synthesized certain AMSA analogs in which the tricyclic acridine was replaced with its isosteric furoquinoline for evaluation of their anticancer activity.[9–12] Further modification of AMSA has been explored by the replacement of acridine with its isomeric 2-phenylquinoline, an aza-analog of antitumor 2-phenylnaphthalene skeleton which consists of a large number of anticancer agents.[13–17] Among them, 4-(4-acetylanilino)-6-methoxy-2-phenylquinoline (**1**) and its oxime (**2a**) and methyloxime (**2b**) derivatives exhibited potent antiproliferative activity with a mean GI_{50} value of 3.89, 3.02, and 3.89 μM, respectively.[13] Structures of these antiproliferative agents can also be considered as the 4-anilino-substituted derivatives of antimitotic 2-phenylquinolin-4(1*H*)-ones. In continuation of our study to explore more potent anticancer drug candidates, we described herein the preparation and antiproliferative evaluation on 8-substituted isomers of **1**, **2a**, and **2b** (Chart 1). A number of carboxamide derivatives comprising polycyclic chromophores and a flexible cationic side chain are known to show antiproliferative effects[18–21] prompted us to prepare certain 4-(8-methoxy-2-phenylquinolin-4-ylamino)benzamides for evaluation. Selected compounds were further evaluated on their effect of cell cycle distribution.

2. Chemistry

The preparation of 4-anilino-2-phenylquinolines is illustrated in Schemes 1–3. Reaction of ethyl 3-chloro-2-

Keywords: 4-Anilino-2-phenylquinoline derivatives; Antiproliferative activity; Cytotoxicity; Anticancer agents.
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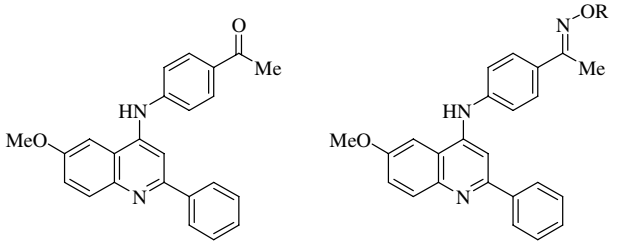
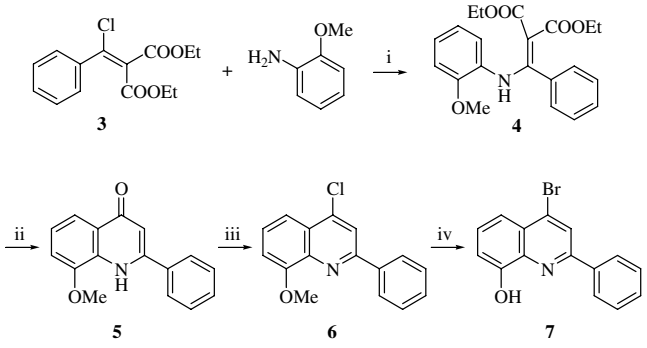


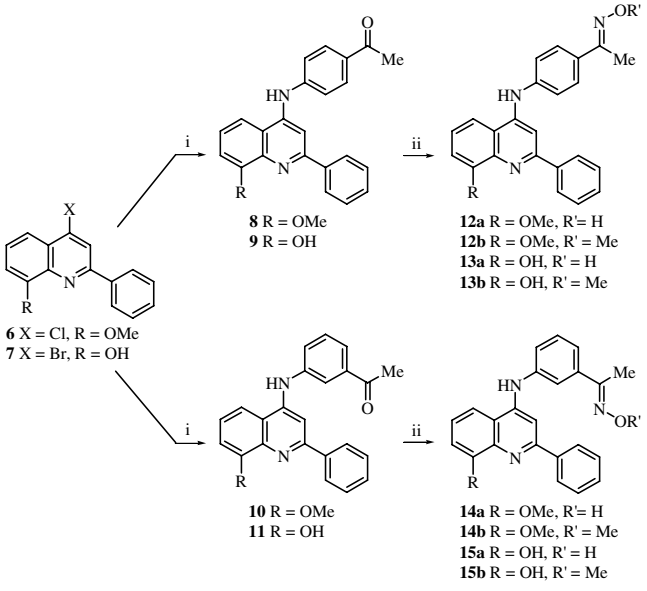
1 (Mean GI_{50} = 3.89 μM)

2a R = H (Mean GI_{50} = 3.02 μM)
2b R = Me (Mean GI_{50} = 3.89 μM)

Chart 1. Structures of 4-anilino-6-methoxy-2-phenylquinoline derivatives.

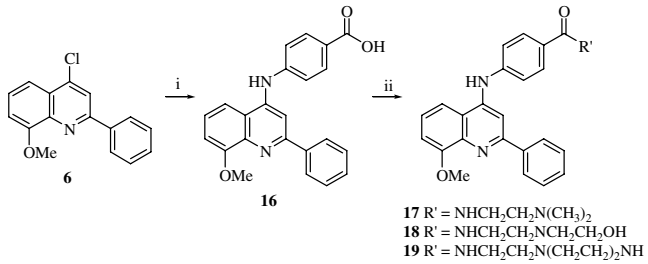


Scheme 1. Reagents and conditions: (i) K_2CO_3, DMF, 140 °C, 3 h (78%); (ii) a—Ph_2O, 260–280 °C, 0.5 h; b—4 N NaOH, reflux for 18 h; (c) 20% HCl, reflux for 5 h (83%); (iii) $POCl_3$, 80–90 °C, 1 h (95%); (iv) 48% HBr, reflux for 24 h (90%).



Scheme 2. Reagents and conditions: (i) 3- or 4-aminoacetophenone, pyridine, EtOH, reflux (49–86%); (ii) NH_2OR, K_2CO_3, EtOH, reflux for 0.5 h (70–94%).

(ethoxycarbonyl)-3-phenylpropenoate (**3**)[22] with o-anisidine gave 2-[(2-methoxyphenylamino)phenylmethylene]malonic acid diethyl ester (**4**), which was thermally cyclized to afford 8-methoxy-2-phenyl-1*H*-quinolin-4-one (**5**). Chlorination of **5** with $POCl_3$ gave 4-chloro-8-methoxy-2-phenylquinoline (**6**), which was reacted with 48% HBr to give 4-bromo-8-hydroxy-2-phenylquinoline (**7**) in a fairly good overall yield (Scheme 1). Treatment of **6** with 4-aminoacetophenone and 3-aminoacetophenone, respectively, afforded 4-(4-acetylanilino)-8-methoxy-2-phenylquinoline (**8**) and its 3-substituted isomer **10**, respectively, which were reacted with NH_2OH to give exclusively (*E*)-oximes **12a** and **14a**, respectively. The configuration of the oxime moiety was determined by through-space nuclear Overhauser effect spectroscopy (NOESY) which revealed coupling connectivity to CH_3 protons. Accordingly, (*E*)-oximes **13a** and **15a** were obtained from **9** and **11**, respectively, which in turn were prepared from **7** by the same reaction sequences. Reaction of **8** with NH_2OMe provided exclusively (*E*)-methyloxime **12b** in 72% yield. (*E*)-Methyloximes **13b**, **14b**, and **15b** were obtained, respectively, from **9**, **10**, and **11** by the same reaction sequences (Scheme 2). The configuration of the oxime and methyloxime moieties was further confirmed by the ^{13}C NMR spectra. The carbon of CH_3, which is *syn* to the OH moiety, shifted upfield (δ value of CH_3 is approximately 11.50 ppm for (*E*)-isomers), while that of the *anti*-isomer shifted downfield (δ value of CH_3 is approximately 18.75 ppm for (*Z*)-isomers)[23].



17 R' = $NHCH_2CH_2N(CH_3)_2$
18 R' = $NHCH_2CH_2NCH_2CH_2OH$
19 R' = $NHCH_2CH_2N(CH_2CH_2)_2NH$

Scheme 3. Reagents and conditions: (i) 4-aminobenzoic acid, pyridine, EtOH, reflux (49–52%); (ii) a—CDI, DMF, rt, 18 h; b—1°-amine, rt, 5 h (21–84%).

Reaction of **6** with 4-aminobenzoic acid afforded 4-(8-methoxy-2-phenylquinolin-4-ylamino)benzoic acid (**16**), which was converted to 4-(8-methoxy-2-phenylquinolin-4-ylamino)benzamides **17**–**19** in an overall yield of 21–84% (Scheme 3).

3. Pharmacological results and discussion

All compounds were evaluated in vitro against the full panel of 60 human tumor cell lines derived from nine cancer cell types (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer). For each compound, dose–response curves for each cell line were measured with five different drug concentrations, the concentration causing 50% cell growth inhibition (GI_{50}) compared with the control was calculated[24] and the results are summarized in Tables 1 and 2. The antiproliferative activity of 4′-COMe substituted derivatives decreasing in an order of 6-OMe (**1**, 3.89 μM) > 8-OMe (**8**, 10.47 μM) > 8-OH (**9**, 14.45 μM) indicating that the

Table 1. Antiproliferative assay of 4-substituted 4-anilino-2-phenylquinoline derivatives [GI_{50} (μM)[a,b]]

Cell lines	Compound								
	1	**2a**	**2b**	**8**	**9**	**12a**	**12b**	**13a**	**13b**
RPMI-8226	1.72	2.16	2.79	4.33	8.80	0.86	3.62	4.50	15.8
NCI-H226	0.94	2.05	2.25	5.88	28.2	14.4	3.59	14.7	22.4
HCT-116	1.54	1.52	1.76	1.23	5.79	1.79	1.54	13.2	22.2
SF-295	<0.01	1.53	2.02	22.9	37.2	2.89	1.97	5.24	12.7
SK-MEL-5	1.60	1.62	1.77	54.1	13.0	3.18	2.62	Nd[d]	24.0
SK-OV-3	16.1	2.32	15.2	38.3	57.1	14.9	20.3	27.4	18.8
UO-31	6.20	41.3	5.19	28.5	14.0	1.74	2.45	14.8	19.6
DU-145	14.1	1.57	5.36	>100	21.8	10.0	8.96	13.5	14.1
MCF7	2.47	1.47	5.01	54.1	Nd[d]	6.93	1.94	Nd[d]	Nd[d]
NCI/ADR-RES	2.78	2.25	2.61	2.69	5.53	5.79	3.43	8.55	14.5
MDA-MB-231/ATCC	0.04	0.73	1.23	2.52	24.0	12.4	2.87	2.46	15.0
MDA-MB-435	0.04	1.79	1.76	5.18	23.6	7.46	2.13	6.72	15.5
Mean[c]	3.89	3.02	3.89	10.47	14.45	6.02	4.26	10.71	16.59

[a] GI_{50}: drug molar concentration causing 50% cell growth inhibition.
[b] Data obtained from NCI's in vitro disease-oriented tumor cell screen.
[c] Mean values over all 60 cell lines tested. These cell lines are: leukemia (CCRF-CEM, HL-60 (TB), K-562, MOLT-4, PRMI-8226, and SR); non-small cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, and NCI-H522); colon cancer (COLC 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, and SW-620); CNS cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, and U251); melanoma (LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, and UACC-257); ovarian cancer (IGR0V1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3); renal cancer (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, and UO-31); prostate cancer (PC-3 and DU-145); and breast cancer (MCF7, MCF7/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N, and T-47D).
[d] Not determined.

Table 2. Antiproliferative assay of 3-substituted 4-anilino-2-phenylquinoline and 4-carboxamide derivatives

Cell lines	Compound									
	10	**11**	**14a**	**14b**	**15a**	**15b**	**16**	**17**	**18**	**19**
RPMI-8226	4.90	2.02	7.10	2.25	2.34	2.91	>100	8.11	24.5	18.0
NCI-H226	11.9	14.8	2.65	7.57	4.60	19.8	>100	19.3	58.1	>100
HCT-116	2.25	0.07	1.48	2.09	1.48	3.21	>100	3.88	34.6	30.9
SF-295	3.66	1.58	10.8	2.24	1.31	2.17	>100	22.9	40.9	33.1
SK-MEL-5	7.61	0.46	10.7	3.39	2.49	16.6	>100	17.3	38.6	31.0
SK-OV-3	32.4	1.13	14.5	>100	0.63	8.62	>100	29.4	86.7	>100
UO-31	4.95	1.36	3.92	6.23	8.83	4.58	>100	13.9	>100	39.4
DU-145	32.1	1.32	38.0	18.6	3.36	9.20	>100	16.4	58.7	71.0
MCF7	1.85	<0.01	0.38	>100	0.35	Nd	Nd	Nd	Nd	23.7
NCI/ADR-RES	7.01	0.10	4.62	3.43	0.98	2.02	>100	18.3	>100	>100
MDA-MB-231/ATCC	5.35	1.64	4.71	2.75	2.02	2.74	>100	4.15	25.5	19.9
MDA-MB-435	2.30	<0.01	3.19	2.07	0.25	1.97	>100	22.0	27.8	35.9
Mean	8.91	1.20	6.31	6.02	2.88	5.50	>100	14.45	28.84	41.69

position of substitution at quinoline ring is crucial. The same antiproliferative SAR was observed for oxime (**2a**, 3.02 μM > **12a**, 6.02 μM > **13a**, 10.71 μM) and methyloxime derivatives (**2b**, 3.89 μM > **12b**, 4.26 μM > **13b**, 16.59 μM) in which 6-OMe derivatives are preferred. For the substituent at C(4) position of anilino moiety, antiproliferative activity for oxime (**2a**, 3.02 μM), methyloxime (**2b**, 3.89 μM), and the ketone precursor (**1**, 3.89 μM) is comparably indicated, a H-bonding accepting group at C(4) position of 4-anilino-moiety is crucial. The same antiproliferative SAR was observed for 8-OH derivatives in which antiproliferative activity of **13a** (10.71 μM), **13b** (16.59 μM), and **9** (14.45 μM) is comparable (Table 1).

The antiproliferative activity of 3-substituted 4-anilino-2-phenylquinoline derivatives is summarized in Table 2. For 3′-COMe derivatives, the antiproliferative activity of 8-OH (**11**, 1.20 μM) is more potent than its 8-OMe counterpart (**10**, 8.91 μM) indicating that a H-bonding donating substituent is more favorable than that of a H-bonding accepting group. The same antiproliferative SAR was observed for oxime (**15a**, 2.88 μM > **14a**, 6.31 μM) and methyloxime derivatives (**15b**, 5.50 μM > **14b**, 6.02 μM). For 8-OH derivatives, the antiproliferative effect of 3′-COMe (**11**) is more potent than its oxime derivative (**15a**), which in turn is more potent than the methyloxime counterpart (**15b**). This antiproliferative SAR is not obvious for 8-OMe derivatives in which the cytotoxicity of oxime (**14a**), methyloxime (**14b**), and their ketone precursor (**10**) is comparable. Compound **11** is especially active against the growth of certain solid cancer cells such as HCT-116 (colon cancer), MCF7, and MDA-MB-435 (breast cancer) with GI_{50} values of 0.07, <0.01, and <0.01 μM, respectively. Among those cancer cells tested, breast cancer cells (MCF7, NCI/ADR-RES, MDA-MB-231/ATCC, and MDA-MB-435)

Table 3. Effects of selected 4-anilino-2-phenylquinolines on cell cycle progression

Compound	Sub-G_1 (%)	G_0/G_1 (%)	S (%)	G_2/M (%)
Control	4.57	29.6	52.0	18.4
11 (3 μg/mL)	6.5	26.7	67.9	5.4
11 (10 μg/mL)	11.2	22.3	70.1	7.7
15a (3 μg/mL)	6.0	28.9	65.4	4.8
15a (10 μg/mL)	8.6	25.6	69.6	4.9
16 (3 μg/mL)	3.2	29.7	53.5	16.9
16 (10 μg/mL)	4.1	30.0	48.7	21.3

were found to be the most sensitive to **11** and **15a** with GI_{50} values less than 2.02 μM in each case. 4-(8-Methoxy-2-phenylquinolin-4-ylamino)benzoic acid (**16**) was devoid of antiproliferative activity, while its carboxamide derivatives **17**–**19** were only weakly active.

For 8-OH derivatives, the 3′-COMe substituent is more active than their respective 4′-substituted couterparts (**11**, mean GI_{50} = 1.20 μM > **9**, 14.45 μM). The same antiproliferative SAR was observed for oxime (**15a**, 2.88 μM > **13a**, 10.71 μM) and methyloxime derivatives (**15b**, 5.50 μM > **13b**, 16.59 μM) in which 3′-substituted derivatives are preferred. This antiproliferative SAR is not obvious for 8-OMe derivatives in which 4′-substituted derivatives (**8**, 10.47 μM; **12a**, 6.02 μM; **12b**, 4.26 μM) and their respective 3′-substituted counterparts (**10**, 8.91 μM; **14a**, 6.31 μM; **14b**, 6.02 μM) are comparable.

Two of the most active compounds **11** and **15a** along with an inactive analog **16** were selected for evaluation of cell cycle progression (Table 3). Flow cytometric analyses indicated that **11** and **15a** induced cell cycle arrest in S phase. This result is interesting because 2-phenylquinolone derivatives have been reported to be antimitotic agents which induced cell cycle arrest in G_2/M phase. Thus, a substituent such as anilino group at C(4) position of 2-phenylquinoline altered the mode of pharmacological mechanism of antimitotic 2-phenylquinolone derivatives.

4. Conclusion

A number of 8-substituted 4-anilino-2-phenylquinoline derivatives were synthesized and evaluated for their antiproliferative activities. The results are: (1) for 4′-COMe-substituted derivatives, the antiproliferative activity decreased in an order of 6-OMe (**1**, 3.89 μM) > 8-OMe (**8**, 10.47 μM) > 8-OH (**9**, 14.45 μM); (2) for 3′-COMe derivatives, the antiproliferative activity of 8-OH (**11**, 1.20 μM) is more potent than its 8-OMe counterpart (10, 8.91 μM); (3) for 8-OH derivatives, the 3′-COMe derivative (**11**, 1.20 μM) is more active than its 4′-substituted isomer (**9**, 14.45 μM). Flow cytometric analyses indicated that **11** and **15a** induced cell cycle arrest in S-phase. This result is interesting because 2-phenylquinolone derivatives have been reported to be antimitotic agents which induced cell cycle arrest in G_2/M phase.

5. Experimental

5.1. General

TLC: precoated (0.2 mm) silica gel 60 F254 plates from EM Laboratories, Inc.; detection by UV light (254 nm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). Mp: Electrothermal IA9100 melting point apparatus; uncorrected. ^{1}H and ^{13}C NMR spectra: Varian-Unity-400 spectrometer at 400 and 100 MHz, chemical shifts δ in ppm with $SiMe_4$ as an internal standard (=0 ppm), coupling constants J in hertz. Elemental analyses were carried out on a Heraeus CHN-O-Rapid elemental analyzer, and results were within ±0.4% of calculated values.

5.1.1. 2-[(2-Methoxyphenylamino)phenylmethylene]malonic acid diethyl ester (4). A mixture of ethyl 3-chloro-2-(ethoxycarbonyl)-3-phenylpropenoate (**3**) (2.84 g, 10 mmol), *p*-anisidine (1.48 g, 12 mmol), K_2CO_3 (1.95 g, 14 mmol), and dry DMF (50 mL) was heated in 140 °C for 3 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (100 mL) was added. This aqueous mixture was extracted with CH_2Cl_2 (3× 100 mL), dried ($MgSO_4$), and concentrated. The crude oil thus obtained was purified by flash column chromatography (FC, silica gel; using CH_2Cl_2 as the eluent) to give **4** (2.88 g, 78%). ^{1}H NMR ($CDCl_3$) δ: 0.82 (t, 3H, J = 7.2 Hz, $OCH_2\mathit{CH_3}$), 1.33 (t, 3H, J = 7.2 Hz, $OCH_2\mathit{CH_3}$), 3.76 (s, 3H, OCH_3), 3.74 (q, 2H, J = 7.2 Hz, $O\mathit{CH_2}CH_3$), 4.26 (q, 2H, J = 7.2 Hz, $O\mathit{CH_2}CH_3$), 6.56–6.72 (m, 4H, Ar-H), 7.26 (m, 5H, Ar-H), 11.12 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 13.41, 14.36, 55.12, 60.08, 61.16, 97.05, 110.66, 115.41, 119.32, 121.09, 128.12 (2C), 128.68 (2C), 129.20, 131.82, 136.85, 148.16, 161.65, 167.27, 168.44. Anal. Calcd for $C_{21}H_{23}NO_5$: C, 68.27; H, 6.29; N, 3.79. Found: C, 68.19; H, 6.34; N, 3.68.

5.1.2. 8-Methoxy-2-phenyl-quinolin-4(1H)-one (5). A solution of **4** (3.68 g, 9.8 mmol) in Ph_2O (10 mL) was heated at 260–280 °C for 0.5 h (TLC monitoring). The reaction mixture was cooled and then *n*-hexane (150 mL) was added and stirred at room temperature for 6 h. The resulting precipitate was collected, dissolved in EtOH (20 mL), added NaOH (4 N, 40 mL), and the mixture was heated at reflux for 18 h. The solvent was evaporated under reduced pressure and then H_2O (100 mL) was added and neutralized with 20% HCl solution. The resulting precipitate was collected and refluxed with 20% HCl solution (200 mL) for 5 h (TLC monitoring). The mixture was cooled to room temperature and the precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **5** (2.04 g, 83%). Mp 170–171 °C. (lit.,[25] 168–170 °C). ^{1}H NMR ($CDCl_3$): 4.03 (3H, s, 8-OCH_3), 6.59 (1H, s, 3-H), 7.06 (1H, d, J = 7.6 Hz, 7-H), 7.27 (1H, m, Ar-H), 7.54 (3H, m, Ar-H), 7.69 (2H, m, Ar-H), 7.93 (1H, d, J = 8.0 Hz, 5-H), 8.84 (1H, br s, NH). ^{13}C NMR ($CDCl_3$): 56.07, 108.90, 110.56, 117.38, 123.18, 125.87, 126.44 (2C), 129.44 (2C), 130.65, 130.77, 134.55, 147.75, 148.40, 178.81. Anal. Calcd for $C_{16}H_{13}NO_2$: C, 76.47; H, 5.22; N, 5.58. Found: C, 76.39; H, 5.57; N, 5.31.

5.1.3. 4-Chloro-8-methoxy-2-phenylquinoline (6). A mixture of **5** (2.26 g, 9 mmol) and $POCl_3$ (27 mL) was heated at 80–90 °C for 1 h (TLC monitoring). After cooling, the mixture was slowly poured into ice water (150 mL) and neutralized with NH_4OH. The precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **6** (2.30 g, 95%). Mp 100–101 °C. (lit.,[26] 101–102 °C). ^1H NMR ($CDCl_3$): 4.10 (3H, s, 8-OCH_3), 7.12 (1H, d, J = 8.0 Hz, 7-H), 7.48–7.57 (4H, m, 6-H and Ar-H), 7.81 (1H, d, J = 8.4 Hz, 5-H), 8.01 (1H, s, 3-H), 8.15–8.17 (2H, m, Ar-H). ^{13}C NMR ($CDCl_3$): 56.32, 108.90, 115.64, 119.68, 126.41, 127.36, 127.58 (2C), 128.85 (2C), 129.62, 138.68, 140.97, 143.10, 155.69, 155.99. Anal. Calcd for $C_{16}H_{12}ClNO$: C, 71.24; H, 4.49; N, 5.19. Found: C, 71.11; H, 4.47; N, 5.20.

5.1.4. 4-Bromo-8-hydroxy-2-phenylquinoline (7). A mixture of **6** (0.26 g, 1 mmol) and 48% HBr (20 mL) was refluxed for 24 h (TLC monitoring). After cooling, the mixture was neutralized with saturated NaOH aqueous. The precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **7** (0.23 g, 90%). Mp 121–122 °C. ^1H NMR ($CDCl_3$): 7.23 (1H, dd, J = 7.6, 1.2 Hz, 7-H), 7.47–7.56 (4H, m, 6-H and Ar-H), 7.62 (1H, dd, J = 8.4, 1.2 Hz, 5-H), 8.09-8.12 (2H, m, Ar-H), 8.18 (1H, s, 3-H), 8.38 (1H, br s, 8-OH). ^{13}C NMR ($CDCl_3$): 111.12, 116.84, 123.48, 127.02, 127.36 (2C), 128.54, 128.96 (2C), 130.02, 134.97, 137.52, 138.39, 152.36, 154.72. Anal. Calcd for $C_{15}H_{10}BrNO$: C, 60.02; H, 3.36; N, 4.67. Found: C, 60.11; H, 3.40; N, 4.68.

5.1.5. 4-(4-Acetylanilino)-8-methoxy-2-phenylquinoline (8). A mixture of **6** (0.40 g, 1.5 mmol), 4-aminoacetophenone (0.41 g, 3 mmol), and pyridine (0.5 mL) in EtOH (20 mL) was added to a flask, which was placed in a sealed steel bomb. The bomb was heated at 200 °C for 18 h (TLC monitoring). The resulting mixture was evaporated under reduced pressure and then H_2O (100 mL) was added. The precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **8** (0.48 g, 86%). Mp 147–148 °C. ^1H NMR ($CDCl_3$): 2.60 (3H, s, $COCH_3$), 4.03 (3H, s, 8-OCH_3), 7.06 (1H, d, J = 7.2 Hz, 7-H), 7.40–7.51 (6H, m, Ar-H), 7.57 (1H, s, 3-H), 7.91 (1H, br s, NH), 7.95–8.01 (5H, m, Ar-H). ^{13}C NMR ($CDCl_3$): 26.39, 56.20, 103.08, 109.25, 113.38, 119.97 (2C), 120.51, 126.11, 127.40 (2C), 128.94 (2C), 129.70, 129.86, 130.31 (2C), 132.42, 139.29, 145.01, 147.52, 153.82, 155.34, 196.71. Anal. Calcd for $C_{24}H_{20}N_2O_2{\cdot}1.8H_2O$: C, 71.90; H, 5.94; N, 6.99. Found: C, 72.11; H, 5.54; N, 6.83.

5.1.6. 4-(4-Acetylanilino)-8-hydroxy-2-phenylquinoline (9). This compound was obtained from **7** and 4-aminoacetophenone as described for **8** and was crystallized from EtOH in 77% yield. Mp 175–176 °C. ^1H NMR ($CDCl_3$): 2.61 (3H, s, $COCH_3$), 6.91 (1H, br s, NH), 7.20 (1H, dd, J = 7.6, 2.0 Hz, 7-H), 7.35–7.52 (7H, m, Ar-H), 7.76 (1H, s, 3-H), 8.00–8.05 (4H, m, Ar-H), 10.36 (1H, br s, OH). ^{13}C NMR ($CDCl_3$): 26.43, 102.83, 109.92, 110.44, 119.31 (2C), 119.54, 126.69, 127.27 (2C), 128.81 (2C), 129.59, 130.53 (2C), 132.16, 139.05, 139.24, 145.05, 146.35, 152.99, 155.54, 196.67. Anal. Calcd for $C_{23}H_{18}N_2O_2{\cdot}0.1H_2O$: C, 77.54; H, 5.16; N, 7.86. Found: C, 77.27; H, 5.31; N, 7.89.

5.1.7. 4-(3-Acetylanilino)-8-methoxy-2-phenylquinoline (10). A mixture of **6** (0.54 g, 2 mmol), 3-aminoacetophenone (0.27 g, 2 mmol), and pyridine (0.5 mL) in EtOH (20 mL) was refluxed for 6 h (TLC monitoring). The mixture was then cooled and evaporated in vacuo to yield a yellow residue, treated with H_2O (50 mL), and the resulting precipitate was filtered and washed with H_2O. The crude product was crystallized from EtOH to give **10** (0.51 g, 70%). Mp 200–201 °C. ^1H NMR (DMSO-d_6): 2.62 (3H, s, $COCH_3$), 4.02 (3H, s, 8-OCH_3), 7.29 (1H, d, J = 7.6 Hz, 7-H), 7.45–7.65 (6H, m, Ar-H), 7.78 (2H, m, Ar-H), 7.96–8.03 (4H, m, Ar-H), 9.48 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 26.75, 56.08, 100.74, 108.98, 113.10, 119.73, 121.90, 124.28, 125.67, 126.89, 127.38 (2C), 128.76 (2C), 129.57, 129.84, 130.24, 138.42, 138.54, 140.46, 149.12, 154.21, 155.48, 197.71. Anal. Calcd for $C_{24}H_{20}N_2O_2{\cdot}H_2O$: C, 74.58; H, 5.75; N, 7.25. Found: C, 74.60; H, 5.58; N, 7.09.

5.1.8. 4-(3-Acetylanilino)-8-hydroxy-2-phenylquinoline (11). This compound was obtained from **7** and 3-aminoacetophenone as described for **10** and was crystallized from EtOH in 82% yield. Mp 201–202 °C. ^1H NMR (DMSO-d_6): 2.65 (3H, s, $COCH_3$), 6.95 (1H, s, 3-H), 7.45 (1H, d, J = 7.6 Hz, 7-H), 7.55–7.79 (7H, m, Ar-H), 7.89 (1H, m, Ar-H), 7.99 (1H, m, Ar-H), 8.10 (1H, m, Ar-H), 8.17 (1H, d, J = 8.4 Hz, 5-H) 10.80 (1H, br s, OH), 11.60 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 28.85, 99.82, 112.57, 116.25, 117.87, 124.50, 126.88, 127.60, 128.70 (2C), 128.82 (2C), 129.58, 130.36, 131.35, 132.68, 137.77, 138.28, 139.02, 148.37,152.97, 154.11, 197.30. Anal. Calcd for $C_{23}H_{18}N_2O_2{\cdot}0.1H_2O$: C, 77.54; H, 5.16; N, 7.86. Found: C, 77.20; H, 5.14; N, 7.79.

5.1.9. (*E*)-4-[4-(1-Hydroxyiminoethyl)anilino]-8-methoxy-2-phenylquinoline (12a). A mixture of **8** (0.52 g, 1.4 mmol), $NH_2OH{\cdot}HCl$ (0.49 g, 7.0 mmol), and K_2CO_3 (0.48 g, 3.5 mmol) in EtOH (10 mL) was refluxed for 0.5 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (80 mL) was added. The resulting precipitate was collected, washed with H_2O, and crystallized from EtOH to give **12a** (0.51 g, 94%). Mp 235–236 °C. ^1H NMR (DMSO-d_6): 2.19 (3H, s, (C=N)CH_3), 4.05 (3H, s, OCH_3), 7.26 (1H, s, 3-H), 7.43 (1H, d, J = 7.6, 7-H), 7.53–7.64 (6H, m, Ar-H), 7.78 (2H, m, Ar-H), 7.88 (2H, m, Ar-H), 8.21 (1H, d, J = 8.4 Hz, 5-H), 10.20 (1H, br s, NH), 11.25 (1H, s, NOH). ^{13}C NMR (DMSO-d_6): 11.30, 56.21, 100.18, 111.31, 114.06, 118.74, 123.09 (2C), 126.12, 126.71 (2C), 127.98 (2C), 128.62 (2C), 130.21, 133.71, 135.90, 138.59, 139.20, 147.68, 151.22, 152.24, 153.94. Anal. Calcd for $C_{24}H_{21}N_3O_2{\cdot}0.4H_2O$: C, 73.78, H, 5.64; N, 10.75. Found: C, 73.77; H, 5.63; N, 10.57.

The same procedure was used to convert each compound **9**, **10**, and **11** to **13a**, **14a**, and **15a**, respectively.

5.1.10. (*E*)-8-Hydroxy-4-[4-(1-hydroxyiminoethyl)anilino]-2-phenylquinoline (13a). Yield 75%. Mp 107–108 °C (EtOH). ^1H NMR (DMSO-d_6): 2.19 (3H, s, (C=N)CH_3), 7.12 (1H, d, J = 7.6, 7-H), 7.437–7.50 (6H, m, Ar-H), 7.58 (1H, s, 3-H), 7.75 (2H, m, Ar-H), 7.83 (1H, d, J = 8.4 Hz, 5-H), 8.22 (2H, m, Ar-H), 9.15 (1H, br s, NH), 10.62 (1H,

br s, OH), 11.14 (1H, s, NOH). ^{13}C NMR (DMSO-d_6): 11.38, 99.34, 111.13, 111.97, 119.48, 121.57 (2C), 125.47, 126.68 (2C), 127.26 (2C), 128.59 (2C), 129.26, 132.01, 138.52, 138.80, 141.04, 148.50, 152.43, 152.95, 154.30. Anal. Calcd for $C_{23}H_{19}N_3O_2 \cdot 1.7H_2O$: C, 69.04, H, 5.65; N, 10.50. Found: C, 69.01; H, 5.51; N, 10.35.

5.1.11. (*E*)-4-[3-(1-Hydroxyiminoethyl)anilino]-8-methoxy-2-phenylquinoline (14a). Purified by flash column chromatography (FC, silica gel; using MeOH/CH_2Cl_2 = 1/30 as the eluent) to give **14a** in 93% yield. Mp 201–202 °C. ^{1}H NMR (DMSO-d_6): 2.19 (3H, s, (C=N)CH_3), 4.00 (3H, s, OCH_3), 7.20 (1H, d, J = 7.6 Hz, 7-H), 7.41–7.56 (8H, m, Ar-H), 7.74 (1H, s, 3-H), 7.93 (1H, d, J = 8.0 Hz, Ar-H), 8.00–8.03 (2H, m, Ar-H), 9.02 (1H, br s, NH), 11.27 (1H, s, NOH). ^{13}C NMR (DMSO-d_6): 11.55, 55.80, 99.40, 109.11, 113.51, 119.03, 119.99, 120.86, 122.19, 124.84, 126.95 (2C), 128.64 (2C), 128.97, 129.48, 138.20, 139.82, 140.80, 140.87, 148.40, 152.67, 154.94, 155.59. Anal. Calcd for $C_{24}H_{21}N_3O_2 \cdot 0.2H_2O$: C, 74.46; H, 5.58; N, 10.86. Found: C, 74.42; H, 5.82; N, 10.49.

5.1.12. (*E*)-8-Hydroxy-4-[3-(1-hydroxyiminoethyl)anilino]-2-phenylquinoline (15a). Yield 75%. Mp 148–150 °C (EtOH). ^{1}H NMR (DMSO-d_6): 2.19 (3H, s, (C=N)CH_3), 6.90 (1H, d, J = 6.0 Hz, Ar-H), 7.46–7.81 (12H, m, Ar-H), 8.18 (1H, d, J = 8.0 Hz, 5-H), 10.91 (1H, br s, OH), 11.38 (1H, br s, NOH), 13.20 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 11.45, 99.22, 112.38, 114.35, 118.36, 123.03, 124.09, 126.60, 128.10 (2C), 128.64 (2C), 128.92, 129.70, 130.46, 138.42, 138.57, 139.37, 140.52, 140.69, 150.18, 152.32, 153.29. Anal. Calcd for $C_{23}H_{19}N_3O_2 \cdot 1.2H_2O$: C, 70.63, H, 5.53; N, 10.75. Found: C, 70.55; H, 5.36; N, 10.61.

5.1.13. (*E*)-8-Methoxy-4-[4-(1-methoxyiminoethyl)anilino]-2-phenylquinoline (12b). A mixture of **8** (0.31 g, 0.8 mmol), *O*-methylhydroxylamine·HCl (0.35 g, 4.2 mmol), and K_2CO_3 (0.29 g, 2.1 mmol) in EtOH (10 mL) was refluxed for 0.5 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (80 mL) was added. The resulting precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **12b** (0.23 g, 72%). Mp 181–182 °C. ^{1}H NMR (DMSO-d_6): 2.20 (3H, s, (C=N)CH_3), 3.92 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 7.21 (1H, d, J = 7.2 Hz, 7-H), 7.44–7.52 (6H, m, Ar-H), 7.62 (1H, s, 3-H), 7.73 (2H, m, Ar-H), 7.91 (1H, m, Ar-H), 8.05 (2H, m, Ar-H), 9.08 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 12.12, 55.82, 61.51, 100.39, 109.12, 113.57, 119.86, 120.83 (2C), 124.96, 127.03 (2C), 127.69 (2C), 128.63 (2C), 129.80, 130.23, 139.80, 140.92, 142.10, 147.71, 153.57, 155.02, 155.65. Anal. Calcd for $C_{25}H_{23}N_3O_2 \cdot 0.2H_2O$: C, 74.85; H, 5.89; N, 10.47. Found: C, 74.82; H, 6.07; N, 10.26.

The same procedure was used to convert each compounds **9**, **10**, and **11**, to **13b**, **14b**, and **15b**, respectively.

5.1.14. (*E*)-8-Hydroxy-4-[4-(1-methoxyiminoethyl)anilino]-2-phenylquinoline (13b). Yield 70%. Mp 154–155 °C (EtOH). ^{1}H NMR (DMSO-d_6): 2.21 (3H, s, (C=N)CH_3), 3.93 (3H, s, OCH_3), 7.21 (1H, d, J = 7.2 Hz, 7-H), 7.40 (1H, br s, NH), 7.45–7.54 (7H, m, Ar-H), 7.77 (2H, m, Ar-H), 7.92 (1H, d, J = 8.4 Hz, 5-H), 8.08 (2H, m, Ar-H), 9.85 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 12.13, 61.57, 99.78, 112.22 (2C), 112.88, 119.06, 122.43, 126.23, 127.13 (2C), 127.83 (2C), 128.66 (2C), 129.77, 129.99, 131.85, 140.53, 150.14, 151.47, 151.95, 153.47, 153.87. Anal. Calcd for $C_{24}H_{21}N_3O_2 \cdot 1.4H_2O$: C, 70.52; H, 5.88; N, 10.28. Found: C, 70.34; H, 5.62; N, 10.09.

5.1.15. (*E*)-8-Methoxy-4-[3-(1-methoxyiminoethyl)anilino]-2-phenylquinoline (14b). Yield 80%. Mp 163–164 °C (EtOH). ^{1}H NMR ($CDCl_3$): 2.24 (3H, s, (C=N)CH_3), 4.01 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 7.06 (1H, d, J = 7.6 Hz, 7-H), 7.34–7.44 (7H, m, Ar-H), 7.54 (2H, m, Ar-H), 7.67 (1H, m, Ar-H), 8.01–8.04 (2H, m, Ar-H), 9.19 (1H, br s, NH). ^{13}C NMR ($CDCl_3$): 12.65, 56.15, 62.05, 101.05, 108.29, 111.48, 119.71, 119.96, 122.02, 122.76, 125.33, 127.59 (2C), 128.56 (2C), 128.99, 129.71, 138.35, 139.24, 140.21, 140.34, 147.89, 154.10, 155.96, 156.76. Anal. Calcd for $C_{25}H_{23}N_3O_2 \cdot 0.2H_2O$: C, 74.85; H, 5.89; N, 10.48. Found: C, 74.90; H, 6.03; N, 10.15.

5.1.16. (*E*)-8-Hydroxy-4-[3-(1-methoxyiminoethyl)anilino]-2-phenylquinoline (15b). Yield 82%. Mp 107–108 °C (EtOH). ^{1}H NMR ($CDCl_3$): 2.20 (3H, s, (C=N)CH_3), 3.97 (3H, s, OCH_3), 7.17 (1H, s, 3-H), 7.21–7.49 (9H, m, Ar-H), 7.81 (2H, m, Ar-H), 8.08 (1H, d, J = 8.0 Hz, 5-H), 9.40 (1H, br s, NH), 10. 79 (1H, br s, OH). ^{13}C NMR ($CDCl_3$): 12.58, 62.03, 99.13, 112.84, 115.28, 118.03, 121.60, 123.89, 124.76, 126.87, 127.36 (2C), 129.29 (2C), 129.85, 131.08, 133.20, 134.75, 138.25, 138.52, 149.35, 152.62, 152.73, 153.82. Anal. Calcd for $C_{24}H_{21}N_3O_2 \cdot 1.7H_2O$: C, 70.21; H, 5.90; N, 10.24. Found: C, 69.95; H, 5.58; N, 0.03.

5.1.17. 4-(8-Methoxy-2-phenylquinolin-4-ylamino)benzoic acid (16). Obtained from **6** and 4-aminobenzoic acid as described for **9** and purified by FC (MeOH/CH_2Cl_2 = 1/10) to give **16** in 52% yield. Mp 236–237 °C. ^{1}H NMR (DMSO-d_6): 4.01 (3H, s, OCH_3), 7.23 (1H, d, J = 7.6 Hz, 7-H), 7.43–7.53 (6H, m, Ar-H), 7.79 (1H, s, 3-H), 7.89 (1H, d, J = 8.4 Hz, 5-H), 7.97 (2H, m, Ar-H), 8.10 (2H, m, Ar-H), 9.27 (1H, br s, NH) 12.60 (1H, br s, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): 55.86, 102.55, 109.21, 113.78, 118.92 (2C), 120.95, 124.03, 125.30, 127.15 (2C), 128.69 (2C), 129.14, 131.11 (2C), 139.62, 141.04, 146.00, 146.85, 155.01, 155.70, 167.18. Anal. Calcd for $C_{23}H_{18}N_2O_3 \cdot 0.6H_2O$: C, 72.46; H, 5.09; N, 7.35. Found: C, 72.33; H, 5.18; N, 7.28.

5.1.18. *N*-(2-Dimethylaminoethyl)-4-(8-methoxy-2-phenylquinolin-4-ylamino)benzamide (17). A mixture of **16** (0.37 g, 1.0 mmol) and CDI (0.97 g, 6.0 mmol) was stirred in dry DMF (10 mL) at room temperature for 2 h. To this mixture was added *N*,*N*-dimethylethylenediamine (0.61 g, 6.0 mmol), and the reaction mixture was stirred at room temperature for a further 24 h (TLC monitoring). The volatile components were then removed in vacuo, the residue was dissolved in CH_2Cl_2 (80 mL), washed with H_2O (2× 80 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to provide the crude product, which was purified by FC (MeOH/CH_2Cl_2 = 1/5) to give

17 (0.39 g, 84%). Mp 213–214 °C. ^{1}H NMR (DMSO-d_6): 2.20 (6H, s, $N(CH_3)_2$), 2.43 (2H, t, J = 6.8 Hz, CH_2N), 3.38 (2H, dd, J = 12.0, 6.8 Hz, NHCFb), 4.00 (3H, s, 8-OCH_3), 7.21 (1H, d, J = 8.0 Hz, 7-H), 7.42–7.53 (6H, m, Ar-H), 7.69 (1H, s, 3-H), 7.90 (3H, m, Ar-H), 8.07 (2H, m, Ar-H), 8.33 (1H, t, J = 5.6 Hz, CONH), 9.14 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 37.32, 45.24 (2C), 55.82, 58.28, 101.13, 109.14, 113.62, 119.78 (2C), 120.52, 125.08, 127.04 (2C), 128.39, 128.63 (2C), 128.68 (2C), 129.04, 139.71, 140.97, 144.01, 147.36, 154.98, 155.67, 165.64. Anal. Calcd for $C_{27}H_{28}N_4O_2 \cdot 0.2H_2O$: C, 73.00; H, 6.46; N, 12.62. Found: C, 73.27; H, 6.67; N, 12.36.

5.1.19. *N*-[2-(2-Hydroxyethylamino)ethyl]-4-(8-methoxy-2-phenylquinolin-4-ylamino)benzamide (18). Obtained from **16** and *N*-(2-hydroxyethyl)ethylenediamine as described for **17**. The crude product was purified by FC ($MeOH/CH_2Cl_2$ = 1/5) to give **18** in 51% yield. Mp 117–119 °C. ^{1}H NMR (DMSO-d_6): 2.62 (2H, t, J = 6.0 Hz, NCH_2CH_2OH), 2.71 (2H, t, J = 6.4 Hz, $CH_2NHCH_2CH_2OH$), 3.36 (2H, dd, J = 11.6, 6.0 Hz, $NHCH_2CH_2$), 3.47 (2H, t, J = 5.6 Hz, NCH_2CH_2OH), 4.00 (3H, s, 8-OCH_3), 4.53 (2H, br s, OH and NH), 7.23 (1H, d, J = 7.6 Hz, 7-H), 7.42–7.53 (6H, m, Ar-H), 7.69 (1H, s, 3-H), 7.91 (3H, m, Ar-H), 8.06 (2H, m, Ar-H), 8.38 (1H, t, J = 5.2 Hz, CONH), 9.15 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 40.76, 48.14, 50.96, 55.27, 59.91, 101.10, 109.15, 113.62, 119.77 (2C), 120.51, 125.05, 127.02 (2C), 128.51, 128.61 (2C), 128.70 (2C), 129.02, 139.70, 140.98, 143.98, 147.37, 154.97, 155.65, 165.82. Anal. Calc. for $C_{27}H_{28}N_4O_3 \cdot 1.3H_2O$: C, 67.55; H, 6.44; N, 11.67. Found: C, 67.31; H, 6.44; N, 11.56.

5.1.20. 4-(8-Methoxy-2-phenylquinolin-4-ylamino)-*N*-(2-piperazin-l-ylethyl)benzamide (19). Obtained from **16** and l-(2-aminoethyl)piperazine described for **17**. The crude product was purified by FC ($MeOH/CH_2Cl_2$ = 1/5) to give **19** in 52% yield. Mp 125–126 °C. ^{1}H NMR (DMSO-d_6): 2.36 (4H, m, piperazinyl-H), 2.44 (2H, t, J = 6.8 Hz, $NHCH_2C\underline{H_2}N$), 2.70 (4H, m, piperazinyl-H), 3.38 (2H, dd, J = 12.8, 6.4 Hz, $NHCH_2C\underline{H_2}N$), 4.00 (3H, s, 8-OCH_3), 4.09 (2H, br s, NH), 7.21 (1H, d, J = 8.0 Hz, 7-H), 7.42–7.52 (6H, m, Ar-H), 7.69 (1H, s, 3-H), 7.89 (3H, m, Ar-H), 8.07 (2H, m, Ar-H), 8.33 (1H, t, J = 5.6 Hz, CONH), 9.15 (1H, br s, NH). ^{13}C NMR (DMSO-d_6): 37.12 (2C), 46.03, 54.71, 56.26 (2C), 58.33, 101.58, 109.58, 114.07, 120.22 (2C), 120.97, 125.52, 127.48 (2C), 128.88, 129.07 (2C), 129.11 (2C), 129.49, 140.16, 141.41, 144.45, 147.80, 155.42, 156.11, 166.08. Anal. Calcd for $C_{29}H_{31}N_5O_2 \cdot 1.1H_2O$: C, 69.45; H, 6.69; N, 13.96. Found: C, 69.25; H, 6.77; N, 13.60.

5.2. Cell cycle analysis

Flow cytometry was used to measure cell cycle profile and apoptosis. For cell cycle analysis, K-562 cell treated with compounds (3 and 10 μM) for 24 h was harvested by centrifugation. After being washed with PBS, the cell was fixed with ice-cold 70% ethanol for 30 min, washed with PBS, and then treated with 1 mL of 1 mg/mL of RNase A solution at 37 °C for 30 min. Cells were harvested by centrifugation at 1000 rpm for 5 min and further stained with 250 μL DNA staining solution (10 mg propidium iodide [PI], 0.1 mg trisodium citrate, and 0.03 mL Triton X-100 dissolved in 100 mL H_2O) at room temperature for 30 min in the dark. After loading 500 μL PBS, the DNA contents of 10,000 events were measured by FACScan (Elite ESP, Beckman Coulter, Brea, CA) and the cell cycle profile was analyzed from the DNA content histograms by using WinCycle software. When cells were apoptotic, the containing DNA was digested by endonuclease and then the sub G_1 pick appeared. The percentage in sub G_1 was analyzed by gating on cell cycle dot blots using Windows Multiple Document Interface software (WinMDI).

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References and notes

1. Fang, K. C.; Chen, Y. L.; Sheu, J. Y.; Wang, T. C.; Tzeng, C. C. *J. Med. Chem.* **2000**, *43*, 3809.
2. Chen, Y. L.; Fang, K. C.; Sheu, J. Y.; Hsu, S. L.; Tzeng, C. C. *J. Med. Chem.* **2001**, *44*, 2374.
3. Tzeng, C. C.; Chen, Y. L. *Chin. Pharm. J.* **2002**, *54*, 229.
4. Sheu, J. Y.; Chen, Y. L.; Tzeng, C. C.; Hsu, S. L.; Fang, K. C.; Wang, T. C. *Helv. Chim. Acta* **2003**, *86*, 2481.
5. Zhao, Y. L.; Chen, Y. L.; Sheu, J. Y.; Chen, I. L.; Wang, T. C.; Tzeng, C. C. *Bioorg. Med. Chem.* **2005**, *13*, 3921.
6. Chen, K.; Kuo, S. C.; Hsieh, M. C.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **1997**, *40*, 3049.
7. Nakamura, S.; Kozuka, M.; Bastow, K. F.; Tokuda, H.; Nishino, H.; Suzuki, M.; Tatsuzaki, J.; Natschke, S. L. M.; Kuo, S. C.; Lee, K. H. *Bioorg. Med. Chem.* **2005**, *13*, 4396.
8. Lai, Y. Y.; Huang, L. J.; Lee, K. H.; Xiao, Z.; Bastow, K. F.; Yamori, T.; Kuo, S. C. *Bioorg. Med. Chem.* **2005**, *13*, 265.
9. Chen, I. L.; Chen, Y. L.; Tzeng, C. C.; Chen, I. S. *Helv. Chim. Acta* **2002**, *85*, 2214.
10. Chen, I. L.; Chen, Y. L.; Tzeng, C. C. *Chin. Pharm. J.* **2003**, *55*, 49.
11. Huang, Y. T.; Huang, D. M.; Guh, J. H.; Chen, I. L.; Tzeng, C. C.; Teng, C. M. *J. Biol. Chem.* **2005**, *280*, 2771.
12. Chen, Y. L.; Chen, I. L.; Wang, T. C.; Han, C. H.; Tzeng, C. C. *Eur. J. Med. Chem.* **2005**, *40*, 928.
13. Zhao, Y. L.; Chen, Y. L.; Chang, F. S.; Tzeng, C. C. *Eur. J. Med. Chem.* **2005**, *40*, 792.
14. Cheng, C. C. *Med. Hypothesis* **1986**, *20*, 157.
15. Atwell, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1989**, *32*, 396.
16. Kuo, S. C.; Lee, H. Z.; Juang, J. P.; Lin, Y. T.; Wu, T. S.; Chang, J. J.; Lednicer, D.; Paull, K. D.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **1993**, *36*, 1146.
17. Xia, Y.; Yang, Z. Y.; Xia, P.; Bastow, K. F.; Tachibana, Y.; Kuo, S. C.; Hamel, E.; Hackl, T.; Lee, K. H. *J. Med. Chem.* **1998**, *41*, 1155.

18. Deady, L. W.; Desneves, J.; Kaye, A. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *Bioorg. Med. Chem.* **2001**, *9*, 445.
19. Deady, L. W.; Rodemann, T.; Zhuang, L.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 1049.
20. Deady, L. W.; Roger, M. L.; Zhuang, L.; Baguley, B. C.; Denny, W. A. *Bioorg. Med. Chem.* **2005**, *13*, 1341.
21. Bu, X.; Chen, J.; Deady, L. W.; Smith, C. L.; Baguley, B. C.; Greenhalgh, D.; Yang, S.; Denny, W. A. *Bioorg. Med Chem.* **2005**, *13*, 3657.
22. Hormi, O. E. O.; Peltonen, C.; Heikkila, L. *J. Org. Chem.* **1990**, *55*, 2513.
23. Silverstein, R. M.; Webster, F. X. ^{13}C NMR Spectrometry. In *Spectrometric Identification of Organic Compounds*; Rose, N., Ed., 6th ed.; John Wiley and Sons: New York, 1998; pp 217–249.
24. Monks, A.; Scuderio, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langlay, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
25. Kuo, S. C.; Lee, H. Z.; Juang, J. P.; Lin, Y. T.; Wu, T. S.; Chang, J. J.; Lednicer, D.; Paull, K. D.; Lin, C. M.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **1993**, *63*, 1146.
26. Venturella, P.; Bellino, A. *J. Heterocycl. Chem.* **1975**, *12*, 669.