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Original article

Synthesis and cytotoxic evaluation of certain 4-anilino-2-phenylquinoline derivatives

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Abstract

The present report describes the synthesis and cell growth inhibition of certain 4-anilino-2-phenylquinoline derivatives. 4-(4-Acetylphenylamino)-6-methoxy-2-phenylquinoline (**11**), its oxime **15a**, and its methyloxime **15b**, exhibited significant cytotoxicity against all 60 cancer cells with mean GI_{50} values of 3.89, 3.02, and 3.89 μM, respectively, while 4-(4-acetylanilino)-6-methoxy-2-phenylquinoline-3-carboxylic acid (**9**) and its 3-carboxylic acid congeners **13a**, **13b**, **14a**, and **14b** were inactive, indicated free carboxylic acid at C(3) position is unfavorable. The steric hindrance exerted by the 3-carboxylate in **9**, **13**, and **14** may prevent the adjacent phenyl ring to lie coplanar with quinoline, which leads to the devoid of cytotoxicity. The comparable cytotoxicity of oxime **15a**, methyloxime **15b**, and the ketone precursor **11** implied a hydrogen-bonding accepting group at C(4) position of 4-anilino-moiety is crucial for cytotoxicity. Among these compounds, **11** is especially active against the growth of certain solid cancer cells such as NCI-H226 (non-small cell lung cancer), MDA-MB-231/ATCC (breast cancer), and SF-295 (CNS cancer) with GI_{50} values of 0.94, 0.04, and < 0.01 μM respectively.

Keywords: 4-Anilino-2-phenylquinoline derivatives; Cytotoxic activity; Anticancer agents

1. Introduction

Acridine derivatives, especially 9-anilinoacridines have been extensively studied as potential chemotherapeutic agents due to their capability of intercalating DNA leading to the inhibition of mammalian topoisomerase II [1–9]. Among them, 4′-(9-acridinylamino)methanesulfonyl-*m*-anisidine (amsacrine, *m*-AMSA), has been specifically relevant and has become a useful clinical drug for the treatment of leukemia and lymphoma [10–13]. 3-(9-Acridinylamino)-5-(hydroxymethyl)aniline (AHMA) was found to be superior to *m*-AMSA against the growth of certain solid tumors such as mammary adenocarcinoma, melanoma, and Lewis lung carcinoma in mice [7]. These studies, however, were focused only on the 9-anilinoacridine skeleton, with wide varieties of substituents on anilino and/or acridine chromophore. No attempt has been carried out concerning the replacement of acridine with its isosteric 2-phenylquinoline ring, which can be considered as an aza-analogue of antitumor 2-phenylnaphthalene skeleton, which consists of a large number of antitumor compounds [14–17]. A 2-phenyl derivative of quinoline-8-carboxamide had been shown to possess DNA-binding capability and a broad-spectrum activity in both leukemia and solid-tumor assays [15]. Certain 2-phenylquinoline and 2-phenylquinolone derivatives were also proved to possess potent antitumor activities [16,17]. Recently, we have synthesized certain bifunctional antitumor agents consisting of the alkylating α-methylene-γ-butyrolactone and the potential DNA-intercalating carrier such as flavone, xanthone, carbazole, and dibenzofuran [18]. These agents were evaluated for their cytotoxicities on the ground that through the intercalation, the α-methylene-γ-butyrolactone can specifically alkylate DNA molecule. The results were interesting because flavone derivatives were found to be more cytotoxic than their isomeric xanthone counterparts. Although the flavone skeleton is not a system with three fused aromatic rings required for a minimal DNA-intercalating ligand, its third phenyl ring

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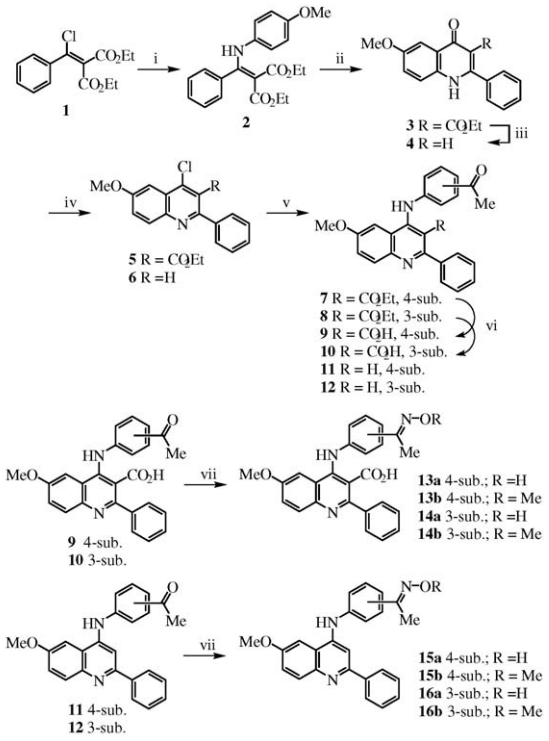

Scheme: (i)*p*-anisidine, K_2CO_3, DMF; (ii) Ph_2O, 230-250°C; (iii) 1N NaOH, 20% HCl; (iv) $POCl_3$ (v) 3- or 4-acetophenone, pyridine, EtOH; (vi) 1N NaOH, EtOH; (vii) NH_2OR HCl, K_2CO_3, EtOH.

Scheme 1. (i) *p*-anisidine, K_2CO_3, DMF; (ii) Ph_2O, 230–250 °C; (iii) 1 N NaOH, 20% HCl; (iv) $POCl_3$; (v) 3- or 4-acetophenone, pyridine, EtOH; (vi) 1 N NaOH, EtOH; (vii) NH_2OR HCl, K_2CO_3, EtOh.

is appended at C(2), which can accommodate itself in a virtually coplanar fashion to the chromophore [19].

Thus, we described herein the preparation and cell growth inhibition of certain 4-anilino-2-phenylquinoline derivatives whose structures belong to the common structural pattern of 2-phenylnaphthalene. Their oxime, methyloxime, and 3-carboxylate derivatives were also synthesized for evaluation. We expect oxime (H-bonding donor) and methyloxime (H-bonding acceptor) to form hydrogen bonding with DNA molecule during the intercalation process of 4-anilino-2-phenylquinoline while 3-carboxylate improves water solubility.

2. Chemistry

The preparation of 4-anilino-2-phenylquinoline and its 3-carboxylate derivatives is illustrated in the Scheme 1. Reaction of ethyl 3-chloro-2-(ethoxycarbonyl)-3-phenylpropenoate (**1**) [20] with *p*-anisidine gave diethyl 3-(4-methoxyanilino)-phenylidenemalonate (**2**) which was thermal cyclized to afford ethyl 6-methoxy-4-oxo-2-phenyl-1,4-dihydroquinoline-3-carboxylate (**3**). Chlorinated of **3** with $POCl_3$ gave ethyl 4-chloro-6-methoxy-2-phenylquinoline-3-carboxylate (**5**) which was treated with substituted anilines to give ethyl 4-(4-acetylanilino)-6-methoxy-2-phenylquinoline-3-carboxylate (**7**) and its 3-substituted isomer **8**. Hydrolysis of **7** and **8** with 1 N NaOH afforded their respective 3-caboxylic acid **9** and **10**. Treatment of **3** with 1 N NaOH followed by 3 N HCl provided 6-methoxy-2-phenyl-1*H*-quinolin-4-one (**4**), which was chlorinated to give 4-chloro-6-methoxy-2-phenylquinoline (**6**). Its reaction with substituted anilines afforded 4-(4-acetylanilino)-6-methoxy-2-phenylquinoline (**11**) and its 3-substituted isomer **12**. Reaction of **9** with $NH_2OH{\cdot}HCl$ gave exclusively (*E*)-oxime **13a** in 80% yield. 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, **15a** was obtained from **11** by the treatment with $NH_2OH{\cdot}HCl$. Reaction of **9** and **11** with $NH_2OMe{\cdot}HCl$ provided (*E*)-methyloxime **13b** and **15b** respectively. Compounds **14a** and **14b**, **16a** and **16b** were obtained, respectively, from **10** to **12** by the same reaction sequences.

3. Pharmacological results and discussion

All compounds were evaluated in vitro against a three-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) [21]. Results from Table 1 indicated all the 3-carboxylic acid derivatives **9**, **13a**, **13b**, **14a**, and **14b** are inactive. The steric hindrance exerted by the 3-carboxylate at C(3) of the quinoline moiety may prevent the adjacent phenyl ring to lie coplanar with bicyclic chromophore, which leads to devoid of cytotoxicity. Those active compounds were evaluated in 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 concentration, the concentration causing 50% cell growth inhibition (GI_{50}) compared with the control were calculated [22]. The 4-COMe group is more active than their respective 3-substituented couterparts (**11**, mean GI_{50} = 3.89 μM; **12**, 9.12 μM). The same cytotoxic SAR was observed for oxime (**15a**, 3.02 μM; **16a**, 8.51 μM) and methyloxime derivatives (**15b**, 3.89 μM; **16b**, 8.32 μM) in which 4-substituted anilino derivatives are preferred. Among these substituted moieties, the cytotoxicity of oxime (**15a**, 3.02 μM), methyloxime (**15b**, 3.89 μM), and their ketone precursor (**11**, 3.89 μM) are comparable indicated, a hydrogen-bonding accepting group at C(4) position of 4-anilino-moiety is crucial for cytotoxicity. The same cytotoxic SAR was observed for 3-substituted anilino derivatives in which the cytotoxicity of **16a**, (8.51 μM), **16b**, (8.32 μM), and **12**, (9.12 μM) are comparable.

Representative results of the individual cell cytotoxicity are summarized in Table 2. Compound **11** is especially active against the growth of certain solid cancer cells such as NCI-H226 (non-small cell lung cancer), MDA-MB-231/ATCC (breast cancer), and SF-295 (CNS cancer) with GI_{50} values of 0.94, 0.04, and < 0.01 μM respectively. Compounds **15a** and **15b** also showed selectivity against these three cancer

Table 1
Preliminary cytotoxic assay of 4-anilino-2-phenylquinoline derivatives

Compounds	Growth percentages [a]			Mean GI50 (μM) [b]
	NCI-H460 (Lung)	MCF7 (Breast)	SF-268 (CNS)	
9	111	84	133	nd [c]
11	2	7	27	3.89
12	2	1	1	9.12
13a	106	75	118	nd
13b	100	60	111	nd [b]
14a	97	84	94	nd
14b	85	73	72	nd
15a	3	12	32	3.02
15b	5	13	8	3.89
16a	1	15	10	8.51
16b	2	2	1	8.32

[a] 4-Anilino-2-phenylquinoline derivatives were evaluated in vitro against a three-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). In this protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single concentration (100 μM) and the culture incubated for 48 h. End-point determinations are made with alamar blue [21]. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduced the growth of any one of the cell lines to 32% or less are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range [22].

[b] Mean values over all 60 cell lines tested. Theses 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-H322 M, 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-3 M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, and UACC-257); ovarian cancer (IGROV1, 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).

[c] Not determined.

cells with GI_{50} values of less than 2.25 μM in each case. Among these cancer cells tested, MDA-MB-231/ATCC was found to be very sensitive to **11**, **15a**, and **15b** with GI_{50} values of 0.04, 0.73, and 1.23 μM respectively.

4. Conclusion

A number of 3-substituted-4-anilino- and 4-substituted-4-anilino-2-phenylquinoline derivatives were synthesized and evaluated for their cytotoxic activities. The preliminary cytotoxic assay indicated 4-substituted-4-anilino-derivatives are more active than their respective 3-substituted-4-anilino-counterparts, indicated position of substitution plays an important role in cytotoxicity. Substitution of the carboxylic acid at C(3) prevent the adjacent phenyl ring to lie coplanar with quinoline, which leads to devoid of cytotoxicity.

Table 2
In vitro cytotoxic assay (GI_{50}, μM) of selected 4-anilino-2-phenylquinoline derivatives

Cell line	Compounds					
	11	**12**	**15a**	**15b**	**16a**	**16b**
RPMI-8226	1.72	33.2	2.16	2.79	4.89	3.12
NCI-H226	0.94	16.1	2.05	2.25	3.18	15.7
COLO 205	16.4	6.58	4.91	7.02	21.4	33.1
SF-295	<0.01	12.7	1.53	2.02	2.05	3.82
SK-MEL-28	29.5	12.4	3.39	29.4	57.7	28.6
SK-OV-3	16.1	19.7	2.32	15.2	6.65	29.2
ACHN	2.54	5.78	14.0	3.27	7.30	14.1
DU-145	14.1	13.2	1.57	5.36	6.46	12.0
MDA-MB-231/ATCC	0.04	9.65	0.73	1.23	2.54	3.49

5. Experimental protocols

5.1. General

Melting points (m.p.) were determined on a Electrothermal IA9100 m.p. apparatus and are uncorrected. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. TLC was performed on silica gel 60 F-254 plates purchased from E. Merck and Co. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Chung-Hsing University using Heraeus CHN-O Rapid EA.

5.1.1. Diethyl 3-(4-methoxyanilino)phenylidenemalonate (*2*)

A mixture of ethyl 3-chloro-2-(ethoxycarbonyl)-3-phenylpropenoate (**1**) (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, CH_2Cl_2/MeOH 20:1). The mixture was evaporated under reduced pressure and then H_2O (100 ml) was added. This aqueous mixture was extracted with CH_2Cl_2 (100 ml × 3), dried ($MgSO_4$), and concentrated to yield a brown oil. The crude oil was purified by flash column chromatography (FC, silica gel; using CH_2Cl_2 as the eluent) to give **2** (2.88 g, 78%) as a yellow oil. ^1H NMR ($CDCl_3$) δ: 0.80 (*t*, 3H, *J* = 7.2 Hz, OCH_2CH_3), 1.32 (*t*, 3H, *J* = 7.2 Hz, OCH_2CH_3), 3.67 (*s*, 3H, OCH_3), 3.76 (*q*, 2H, *J* = 7.2 Hz, OCH_2CH_3), 4.26 (*q*, 2H, *J* = 7.2 Hz, OCH_2CH_3), 6.61 (*m*, 4H, Ar–H), 7.24 (*m*, 5H, Ar–H), 11.09 (*br s*, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 13.36, 14.25, 55.09, 59.94, 60.30, 96.34, 113.70 (2C), 125.47 (2C), 128.04 (2C), 128.56 (2C), 129.13, 131.70, 133.93, 156.55, 161.85, 167.49, 168.17. Anal. Calcd. for $C_{21}H_{23}NO_5$: C, 68.28; H, 6.28; N, 3.79. Found: C, 68.21; H, 6.29; N, 3.81.

5.1.2. Ethyl 6-methoxy-4-oxo-2-phenyl-1,4-dihydroquinoline-3-carboxylate (*3*)

A solution of **2** (3.70 g, 10 mmol) in Ph_2O (30 ml) was heated at 230–250 °C for 1 h (TLC monitoring, CH_2Cl_2/MeOH 5:1). The reaction mixture was cooled and then *n*-hexane (100 ml) was added. The resulting precipitate was collected and crystallized from MeOH to give **3** (2.65 g, 82%) as a white powder. M.p. 223–224 °C. ^1H NMR (TFA-*d*) δ: 0.80 (*t*, 3H, *J* = 7.2 Hz, OCH_2CH_3), 4.00 (*s*, 3H, OCH_3),

4.15 (*q*, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.40–7.92 (*m*, 8H, Ar–H). ^{13}C NMR (TFA-*d*) *δ*: 13.58, 57.60, 66.33, 104.89, 123.37, 129.36, 129.80 (2C), 130.92 (2C), 132.09, 132.36, 133.97, 134.78, 136.20, 159.04, 163.09, 171.19, 175.16. Anal. Calcd. for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.42; H, 5.34; N, 4.37.

*5.1.3. 6-Methoxy-2-phenyl-1H-quinolin-4-one (**4**)*

To a suspension of **3** (3.23 g, 10 mmol) in EtOH (20 ml) was added a solution of 1 N NaOH (40 ml) and the mixture heated at reflux for 6 h The mixture was evaporated under reduced pressure and then H_2O (200 ml) was added and neutralized with 20% HCl solution. The resulting precipitate was collected, refluxed with 20% HCl solution (200 ml) for 6 h (TLC monitoring, CH_2Cl_2/MeOH 5:1). The mixture was cooled to room temperature and the precipitate was collected, washed with H_2O, and then crystallized from MeOH to give **4** (2.13 g, 85%) as a white powder. M.p. 283–285 °C (lit. [11], 302–304 °C). ^{1}H NMR (TFA-*d*) *δ*: 4.31 (*s*, 3H, OCH_3), 7.78–8.11 (*m*, 8H, Ar–H), 8.34 (*d*, 1H, *J* = 8.0 Hz, H-8). ^{13}C NMR (TFA-*d*) *δ*: 57.56, 103.86, 106.15, 122.97, 123.20, 129.48 (2C), 130.22, 132.05 (2C), 132.89, 135.25, 137.40, 156.42, 161.75, 169.99.

*5.1.4. Ethyl 4-chloro-6-methoxy-2-phenylquinoline-3-carboxylate (**5**)*

A mixture of **3** (3.23 g, 10 mmol) and $POCl_3$ (30 ml) was refluxed for 12 h (TLC monitoring, CH_2Cl_2/MeOH 10:1). After cooling, the mixture was slowly poured in ice-water (150 ml), neutralized with NH_4OH. This aqueous mixture was extracted with CH_2Cl_2 (100 ml × 3), dried ($MgSO_4$), and concentrated to yield yellow solid. The crude product was purified by FC (using CH_2Cl_2 as the eluent) to give **5** (2.67 g, 78%) as a yellow needle crystals. M.p. 112–113 °C. ^{1}H NMR ($CDCl_3$) *δ*: 1.10 (*t*, 3H, *J* = 7.2 Hz, OCH_2CH_3), 3.90 (*s*, 3H, OCH_3), 4.25 (*q*, 2H, *J* = 7.2 Hz, OCH_2CH_3), 7.37–7.46 (*m*, 5H, Ar–H), 7.69 (*m*, 2H, Ar–H), 8.00 (*d*, 1H, *J* = 10.2 Hz, H-8). ^{13}C NMR ($CDCl_3$) *δ*: 13.54, 55.51, 61.89, 101.57, 124.08, 125.19, 126.90, 128.23 (4C), 128.77, 131.25, 138.34, 139.22, 143.93, 153.40, 158.92, 166.29. Anal. Calcd. for $C_{19}H_{16}ClNO_3$: C, 66.77; H, 4.72; N, 4.10. Found: C, 66.61; H, 4.77; N, 4.15.

*5.1.5. 4-Chloro-6-methoxy-2-phenylquinoline (**6**)*

Prepared from **4** by the same procedure as described for **5**. Compound **6** was obtained in a 92% yield. M.p. 103–104 °C. ^{1}H NMR (DMSO-d_6) *δ*: 3.93 (*s*, 3H, OCH_3), 7.38 (*d*, 1H, *J* = 2.8 Hz, H-5), 7.54 (*m*, 4H, Ar–H and H-7), 8.20 (*m*, 3H, Ar–H and H-8), 8.34 (*s*, 1H, H-3). ^{13}C NMR (DMSO-d_6) *δ*: 55.88, 101.67, 119.65, 124.28, 125.95, 127.59 (2C), 128.93 (2C), 129.78, 130.33, 135.97, 142.24, 142.42, 153.17, 158.74. Anal. Calcd. for $C_{16}H_{12}ClNO$: C, 71.25; H, 4.48; N, 5.19. Found: C, 71.47; H, 4.58; N, 5.30.

*5.1.6. Ethyl 4-(4-acetylanilino)-6-methoxy-2-phenylquinoline-3-carboxylate (**7**)*

A mixture of **5** (0.69 g, 2 mmol), 4-aminoacetophenone (0.27 g, 2 mmol), and pyridine (0.5 ml) in ethoxyethanol (20 ml) was refluxed for 3 h (TLC monitoring, CH_2Cl_2/MeOH 20:1). 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 purified by FC (using CH_2Cl_2 as the eluent) to give **7** (0.50 g, 58%) as a yellow powder. M.p. 156–157 °C. ^{1}H NMR ($CDCl_3$) *δ*: 0.76 (*t*, 3H, *J* = 7.2 Hz, OCH_2CH_3), 2.54 (*s*, 3H, C(=O)CH_3), 3.56 (*s*, 3H, OCH_3), 3.97 (*q*, 2H, *J* = 7.2 Hz, OCH_2CH_3), 6.84 (*m*, 2H, Ar–H), 6.92 (*d*, 1H, *J* = 2.4 Hz, H-5), 7.43 (*m*, 4H, Ar–H) 7.62 (*m*, 2H, Ar–H), 7.86 (*m*, 2H, Ar–H), 8.05 (*d*, 1H, *J* = 9.0 Hz, H-8), 8.37 (*br s*, 1H, NH). ^{13}C NMR ($CDCl_3$) *δ*: 13.09, 26.29, 55.33, 61.72, 103.42, 113.63, 117.43 (2C), 121.33, 124.05, 128.13 (2C), 128.29 (2C), 129.90 (2C), 130.64, 130.72, 131.65, 141.23, 144.73, 147.57, 155.95, 157.06, 157.32, 168.87, 196.50. Anal. Calcd. for $C_{27}H_{24}N_2O_4 \cdot 0.25H_2O$: C, 72.87; H, 5.88; N, 6.30. Found: C, 72.90; H, 5.62; N, 6.44.

*5.1.7. Ethyl 4-(3-acetylanilino)-6-methoxy-2-phenylquinoline-3-carboxylate (**8**)*

Prepared from **5** and 3-aminoacetophenone by the same procedure as described for **7**. Compound **8** in was obtained in a 52% yield. M.p. 128–129 °C. ^{1}H NMR ($CDCl_3$) *δ*: 0.74 (*t*, 3H, *J* = 7.2 Hz, OCH_2CH_3), 2.54 (*s*, 3H, C(=O)CH_3), 3.50 (*s*, 3H, OCH_3), 3.90 (*q*, 2H, *J* = 7.2 Hz, OCH_2CH_3), 6.96 (*d*, 1H, *J* = 2.2 Hz, H-5), 7.08 (*dd*, 1H, *J* = 9.2, 2.2 Hz, H-7), 7.31–7.47 (*m*, 5H, Ar–H), 7.61 (*m*, 4H, Ar–H), 8.12 (*d*, 1H, *J* = 9.2 Hz, H-8), 8.80 (*br s*, 1H, NH). ^{13}C NMR ($CDCl_3$) *δ*: 13.05, 26.73, 55.25, 61.58, 103.86, 115.02, 119.23, 120.42, 122.51, 123.93, 124.05, 128.16 (2C), 128.25 (2C), 128.34, 129.21, 131.16, 138.01, 141.14, 143.32, 144.13, 146.50, 156.13, 156.97, 168.98, 197.60. Anal. Calcd. for $C_{27}H_{24}N_2O_4 \cdot 0.25H_2O$: C, 72.87; H, 5.88; N, 6.30. Found: C, 72.84; H, 5.58; N, 6.20.

*5.1.8. 4-(4-Acetylanilino)-6-methoxy-2-phenylquinoline-3-carboxylic acid (**9**)*

To a suspension of **7** (0.88 g, 2 mmol) in EtOH (20 ml) was added 1 N NaOH solution (40 ml) and the mixture was heated at reflux for 2 h (TLC monitoring, CH_2Cl_2/MeOH 10:1). The mixture was cooled, evaporated under reduced pressure and then H_2O (200 ml) was added and neutralized with 20% HCl solution. The resulting precipitate was collected, washed with H_2O, and then crystallized from MeOH to give **9** (0.58 g, 71%). M.p. 198–199 °C. ^{1}H NMR (DMSO-d_6) *δ*: 2.57 (*s*, 3H, C(=O)CH_3), 4.00 (*s*, 3H, OCH_3), 7.44 (*m*, 2H, Ar–H), 7.63 (*m*, 5H, Ar–H), 7.77 (*dd*, 1H, *J* = 9.2, 2.6 Hz, H-7), 7.97 (*m*, 2H, Ar–H), 8.15 (*d*, 1H, *J* = 9.2 Hz, H-8), 8.22 (*d*, 1H, *J* = 2.6 Hz, H-5), 11.11 (*br s*, 1H, NH). ^{13}C NMR (DMSO-d_6) *δ*: 26.68, 56.64, 106.54, 112.27 (2C), 117.99 (2C), 122.95, 123.71, 128.41 (2C), 129.02 (2C), 129.36, 132.67, 132.79, 133.48, 133.97, 151.73, 157.21, 157.99, 158.75, 158.83, 165.25, 196.80. Anal. Calcd. for $C_{25}H_{20}N_2O_4 \cdot HCl \cdot 1.6H_2O$: C, 62.85; H, 4.86; N, 5.87. Found: C, 62.65; H, 5.20; N, 5.51.

*5.1.9. 4-(3-Acetylanilino)-6-methoxy-2-phenylquinoline-3-carboxylic acid (**10**)*

Prepared from **8** by the same procedure as described for **9**. Compound **10** was obtained in a 68% yield. M.p. 225–226 °C. ^{1}H NMR (DMSO-d_6) δ: 2.55 (*s*, 3H, C(=O)CH_3), 4.00 (*s*, 3H, OCH_3), 7.60 (*m*, 7H, Ar–H), 7.74 (*dd*, 1H, *J* = 9.2, 1.8 Hz, H-7), 7.89 (*m*, 2H, Ar–H), 8.13 (*d*, 1H, *J* = 9.2 Hz, H-8), 8.29 (*d*, 1H, *J* = 1.8 Hz, H-5), 11.03 (*br s*, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 26.86, 56.67, 103.58, 111.33, 118.12, 120.28, 122.40, 124.06, 126.20, 128.40 (2C), 128.93 (2C), 130.73, 132.58, 133.20, 137.25, 139.29, 150.80, 151.40, 158.06, 158.66, 158.81, 165.45, 197.41. Anal. Calcd. for $C_{25}H_{20}N_2O_4 \cdot H_2O$: C, 69.76; H, 5.15; N, 6.51. Found: C, 70.08; H, 5.13; N, 6.53.

*5.1.10. 4-(4-Acetylanilino)-6-methoxy-2-phenylquinoline (**11**)*

Prepared from **6** and 4-aminoacetophenone by the same procedure as described for **7**. Compound **11** was obtained in a 62% yield. M.p. 180–181 °C. ^{1}H NMR (DMSO-d_6) δ: 2.66 (*s*, 3H, C(=O)CH_3), 3.94 (*s*, 3H, OCH_3), 7.15 (*s*, 1H, 3-H), 7.25 (*dd*, 1H, *J* = 9.4, 2.0 Hz, H-7), 7.59 (*m*, 3H, Ar–H), 7.71 (*m*, 2H, Ar–H), 7.82 (*m*, 2H, Ar–H), 8.06 (*m*, 3H, Ar–H and H-5), 8.27 (*d*, 1H, *J* = 9.4 Hz, H-8), 10.82 (*br s*, 1H, NH), 13.79 (*br s*, 1H, HCl). ^{13}C NMR (DMSO-d_6) δ: 26.29, 56.55, 99.11, 102.26, 118.38, 121.78, 123.82 (2C), 125.65, 127.91 (2C), 129.32 (2C), 130.02 (2C), 131.37, 131.88, 134.80, 142.02, 150.91, 153.10, 153.25, 158.48, 197.42. Anal. Calcd. for $C_{24}H_{20}N_2O_2 \cdot HCl$: C, 71.19; H, 5.24; N, 6.92. Found: C, 71.10; H, 5.24; N, 6.92.

*5.1.11. 4-(3-Acetylanilino)-6-methoxy-2-phenylquinoline (**12**)*

Prepared from **6** and 3-aminoacetophenone by the same procedure as described for **7**. Compound **12** was obtained in a 58% yield. M.p. 129–130 °C. ^{1}H NMR ($CDCl_3$) δ: 2.54 (*s*, 3H, C(=O)CH_3), 3.91 (*s*, 3H, OCH_3), 6.85 (*s*, 1H, H-3), 7.35 (*m*, 5H, Ar–H), 7.71 (*m*, 4H, Ar–H), 8.20 (*m*, 2H, Ar–H), 8.59 (*d*, 1H, *J* = 9.2 Hz, H-8), 11.01 (*br s*, 1H, NH), 14.10 (*br s*, 1H, HCl). ^{13}C NMR ($CDCl_3$) δ: 26.67, 57.06, 98.21, 103.02, 118.42, 122.34, 124.28, 125.57, 126.45, 128.27 (2C), 129.06 (2C), 129.41, 129.99, 131.38, 131.53, 134.30, 138.27, 138.42, 150.64, 153.75, 158.45, 197.01. Anal. Calcd. for $C_{24}H_{20}N_2O_2 \cdot HCl \cdot 0.9H_2O$: C, 68.45; H, 5.50; N, 6.68. Found: C, 68.14; H, 5.58; N, 6.68.

*5.1.12. (E)-4-[4-(1-Hydroxyiminoethyl)anilino]-6-methoxy-2-phenylquinoline-3-carboxylic acid (**13a**)*

A mixture of **9** (206 mg, 0.50 mmol), $NH_2OH \cdot HCl$ (175 mg, 2.50 mmol), and K_2CO_3 (160 mg, 1.30 mmol) in EtOH (10 ml) was refluxed for 2 h (TLC monitoring, CH_2Cl_2/MeOH 10:1). The mixture was evaporated under reduced pressure and then H_2O (80 ml) was added. The resulting precipitate was collected by filtration, washed with H_2O, and then crystallized from MeOH to give **13a** (0.17 g, 80%) as a yellow powder. M.p. 240–241 °C. ^{1}H NMR (DMSO-d_6) δ: 2.08 (*s*, 3H, C(=N)CH_3), 3.61 (*s*, 3H, OCH_3), 6.80 (*m*, 2H, Ar–H), 7.19 (*d*, 1H, *J* = 2.2 Hz, H-5), 7.46 (*m*, 7H, Ar–H), 7.64 (*m*, 3H, Ar–H), 7.96 (*d*, 1H, *J* = 9.0 Hz, H-8), 8.79 (*br s*, 1H, NH), 10.97 (*br s*, 1H, NOH). ^{13}C NMR (DMSO-d_6) δ: 11.57, 55.48, 103.20, 117.16 (2C), 121.56, 122.84, 122.99, 126.38 (2C), 128.21 (2C), 128.49 (2C), 129.19, 129.78, 131.11, 140.41, 143.62, 143.75, 145.18, 152.80, 154.40, 157.22, 169.11. Anal. Calcd. for $C_{25}H_{21}N_3O_4 \cdot 0.9H_2O$: C, 68.09; H, 5.26; N, 9.53. Found: C, 68.39; H, 5.13; N, 9.16.

*5.1.13. (E)-6-Methoxy-4-[4-(1-methoxyiminoethyl)anilino]-2-phenylquinoline-3-carboxylic acid (**13b**)*

Prepared from **9** and $NH_2OCH_3 \cdot HCl$ by the same procedure as described for **13a**. Compound **13b** was obtained in a 84% yield. M.p. 225–226 °C. ^{1}H NMR (DMSO-d_6) δ: 2.11 (*s*, 3H, C(=N)CH_3), 3.68 (*s*, 3H, OCH_3), 3.87 (*s*, 3H, $NOCH_3$), 6.80 (*m*, 2H, Ar–H), 7.22 (*d*, 1H, *J* = 2.8 Hz, H-5), 7.50 (*m*, 6H, Ar–H), 7.69 (*m*, 2H, Ar–H), 8.00 (*d*, 1H, *J* = 9.2 Hz, H-8), 8.89 (*br s*, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 12.19, 55.45, 61.46, 103.11, 116.85 (2C), 121.97, 122.85, 123.17, 126.72 (2C), 127.78, 128.19 (2C), 128.49 (2C), 128.57, 131.16, 140.36, 143.48, 143.94, 145.84, 153.82, 154.33, 157.32, 169.02. Anal. Calcd. for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52. Found: C, 70.43; H, 5.45; N, 9.15.

*5.1.14. (E)-4-[3-(1-Hydroxyiminoethyl)anilino]-6-methoxy-2-phenylquinoline-3-carboxylic acid (**14a**)*

Prepared from **10** and $NH_2OH \cdot HCl$ by the same procedure as described for **13a**. Compound **14a** was obtained in a 75% yield. M.p. 229–230 °C. ^{1}H NMR (DMSO-d_6) δ: 2.04 (*s*, 3H, C(=N)CH_3), 3.53 (*s*, 3H, OCH_3), 6.77 (*m*, 1H, Ar–H), 6.94 (*d*, 1H, *J* = 2.8 Hz, H-5), 7.15 (*m*, 3H, Ar–H), 7.37 (*m*, 4H, Ar–H), 7.77 (*m*, 2H, Ar–H), 7.90 (*d*, 1H, *J* = 9.0 Hz, H-8), 8.95 (*br s*, 1H, NH), 11.10 (*br s*, 1H, NOH). ^{13}C NMR (DMSO-d_6) δ: 11.48, 54.99, 103.64, 114.78, 118.02, 118.28, 121.38, 122.00, 125.02, 127.60 (2C), 128.57 (2C), 128.70, 131.10, 137.41, 141.51, 142.57, 143.24, 144.62, 152.74, 154.61, 156.12, 170.64. Anal. Calcd. for $C_{25}H_{21}N_3O_4 \cdot HCl \cdot 0.5H_2O$: C, 63.48; H, 5.13; N, 8.88. Found: C, 63.49; H, 5.23; N, 8.50.

*5.1.15. (E)-6-Methoxy-4-[3-(1-methoxyiminoethyl)anilino]-2-phenylquinoline-3-carboxylic acid (**14b**)*

Prepared from **10** and $NH_2OCH_3 \cdot HCl$ by the same procedure as described for **13a**. Compound **14b** was obtained in a 72% yield. M.p. 193–194 °C. ^{1}H NMR (DMSO-d_6) δ: 2.07 (*s*, 3H, C(=N)CH_3), 3.83 and 3.86 (two *s*, each 3H, OCH_3 and $NOCH_3$), 7.24–7.64 (*m*, 10H, Ar–H), 7.89 (*m*, 2H, Ar–H), 7.95 (*d*, 1H, *J* = 9.2 Hz, H-8), 10.63 (*br s*, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 12.54, 56.50, 61.99 103.23, 110.94, 113.11, 118.89, 120.06, 122.33, 125.70, 126.63, 128.92 (2C), 129.69 (2C), 131.30, 132.59, 133.30, 137.02, 138.87, 151.71, 154.29, 158.23, 159.69, 160.42, 166.02. Anal. Calcd. for $C_{26}H_{23}N_3O_4 \cdot 0.25\ H_2O$: C, 70.01; H, 5.64; N, 9.42. Found: C, 70.06; H, 5.41; N, 9.31.

*5.1.16. (E)-4-[4-(1-Hydroxyiminoethyl)anilino]-6-methoxy-2-phenylquinoline (**15a**)*

Prepared from **11** and $NH_2OH{\cdot}HCl$ by the same procedure as described for **13a**. Compound **15a** was obtained in a 75% yield. M.p. 262–263 °C. ^{1}H NMR (DMSO-d_6) δ: 2.19 (*s*, 3H, C(=N)CH_3), 4.00 (*s*, 3H, OCH_3), 7.12 (*s*, 1H, H-3), 7.60 (m, 5H, Ar–H), 7.87 (m, 4H, Ar–H), 8.21 (m, 3H, Ar–H), 10.72 (br s, 1H, NH), 11.30 (br s, 1H, NOH). ^{13}C NMR (DMSO-d_6) δ: 11.45, 56.57, 98.98, 102.80, 118.38, 123.77, 124.25 (2C), 124.80, 126.88 (2C), 128.17 (2C), 129.17 (2C), 131.20, 133.40, 134.72, 135.97, 138.43, 151.34, 152.08, 152.34, 157.83. Anal. Calcd. for $C_{24}H_{21}N_3O_2{\cdot}HCl{\cdot}0.7H_2O$: C, 66.64; H, 5.59; N, 9.75. Found: C, 66.58; H, 5.51; N, 9.59.

*5.1.17. (E)-6-Methoxy-4-[4-(1-methoxyiminoethyl)anilino]-2-phenylquinoline (**15b**)*

Prepared from **11** and $NH_2OCH_3{\cdot}HCl$ by the same procedure as described for **13a**. Compound **15b** was obtained in a 76% yield. M.p. 108–109 °C. ^{1}H NMR (DMSO-d_6) δ: 2.21 (*s*, 3H, C(=N)CH_3), 3.93 and 3.95 (two *s*, each 3H, OCH_3 and $NOCH_3$), 7.45 (*m*, 6H, Ar–H), 7.59 (*s*, 1H, H-3), 7.75 (*m*, 3H, Ar–H), 7.93 (*d*, 1H, J = 9.2 Hz, H-8), 8.03 (*m*, 2H, Ar–H), 9.00 (*br s*, 1H, NH)·^{13}C NMR (DMSO-d_6) δ: 12.08, 55.75, 61.50, 100.15, 101.03, 120.01, 120.88 (2C), 121.65, 126.76 (2C), 127.05 (2C), 128.63 (2C), 128.80, 130.21, 131.10, 139.71, 142.18, 144.80, 146.84, 153.56, 154.26, 156.64. Anal. Calcd. for $C_{25}H_{23}N_3O_2{\cdot}H_2O$: C, 72.32; H, 6.07; N, 10.11. Found: C, 72.19; H, 6.04; N, 9.99.

*5.1.18. (E)-4-[3-(1-Hydroxyiminoethyl)anilino]-6-methoxy-2-phenylquinoline (**16a**)*

Prepared from **12** and $NH_2OH{\cdot}HCl$ by the same procedure as described for **13a**. Compound **16a** was obtained in a 77% yield. M.p. 250–251 °C. ^{1}H NMR (DMSO-d_6) δ: 2.19 (*s*, 3H, C(=N)CH_3), 3.95 (*s*, 3H, OCH_3), 7.46 (*m*, 8H, Ar–H), 7.75 (*m*, 2H, Ar–H), 7.90 (*d*, 1H, J = 9.0 Hz, H-8), 7.99 (*m*, 2H, Ar–H), 8.95 (*br s*, 1H, NH), 11.29 (*br s*, 1H, NOH). ^{13}C NMR (DMSO-d_6) δ: 11.67, 55.83, 99.24, 100.98, 119.05, 119.80, 120.94, 121.77, 122.23, 126.78, 128.78 (2C), 128.90 (2C), 129.63, 131.15, 138.32, 139.84, 141.06, 144.80, 147.58, 152.85, 154.35, 156.67. Anal. Calcd. for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 74.95; H, 5.62; N, 10.68.

*5.1.19. (E)-6-Methoxy-4-[3-(1-methoxyiminoethyl)anilino]-2-phenylquinoline (**16b**)*

Prepared from **12** and $NH_2OCH_3{\cdot}HCl$ by the same procedure as described for **13a**. Compound **16b** was obtained in a 78% yield. M.p. 115–116 °C. ^{1}H NMR (DMSO-d_6) δ: 2.24 (*s*, 3H, C(=N)CH_3), 3.95 and 4.03 (two *s*, each 3H, OCH_3 and $NOCH_3$), 7.03 (*s*, 1H, H-3), 7.65 (*m*, 7H, Ar–H), 7.88 (*m*, 3H, Ar–H), 8.27 (*b rs*, 1H, NH), 8.29 (d, 1H, J = 9.2 Hz, H-8), 10.99 (*br s*, 1H, HCl). ^{13}C NMR (DMSO-d_6) δ: 12.45, 56.66, 61.85, 98.84, 102.86, 118.09, 122.28, 122.61, 124.68, 125.53, 125.77, 128.40 (2C), 129.38 (2C), 130.22, 131.78, 132.37, 134.46, 137.67, 137.76, 150.97, 153.52, 153.67, 158.16. Anal. Calcd. for $C_{25}H_{23}N_3O_2{\cdot}HCl{\cdot}0.7H_2O$: C, 67.23; H, 5.64; N, 9.41. Found: C, 67.08; H, 5.72; N, 9.38.

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