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Synthesis and biological relationships of 3′,6-substituted 2-phenyl-4-quinolone-3-carboxylic acid derivatives as antimitotic agents

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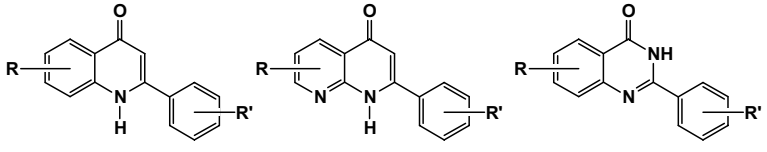
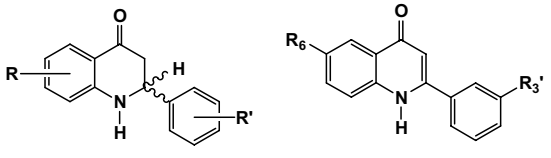
Abstract—As part of a continuing search for potential anticancer drug candidates in the 2-phenyl-4-quinolone series, 3′,6-substituted 2-phenyl-4-quinolone-3-carboxylic acid derivatives and their salts were synthesized and evaluated. Preliminary screening showed that carboxylic acid analogs containing a *m*-fluoro substituted 2-phenyl group displayed the highest in vitro anticancer activity. Activity decreased significantly if a chlorine or methoxy group replaced the fluorine atom. 3′-Fluoro-6-methoxy-2-phenyl-4-quinolone-3-carboxylic acid (**68**) had the highest in vitro cytotoxic activity among all tested carboxylic acid derivatives and their salts. The mechanism of action may be similar, but not identical, to that of tubulin binding drugs, such as navelbine and taxol. Compound **68** merits further investigation as a novel hydrophilic antimitotic agent.

1. Introduction

Quinolone derivatives were initially discovered as bacterial DNA gyrase inhibitors, and thus, developed as antibacterial agents.[1–5] Recently, DNA topoisomerase II has emerged as the pharmacological target for this class of quinolone compounds.[6–14] In our prior studies, substituted 2-phenyl-4-quinolones (**A**) were identified as novel antimitotic agents,[15,16] and structure–activity relationships (SAR) were established with many related



(A) (B) (C)



(D) (A-1)

R_6, R_3' = F, Cl, OCH_3

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synthetic analogs, including 2-phenylnaphthyridine-4-ones (**B**),[17] 2-phenyl-4-quinazolones (**C**),[18,19] and tetrahydro-2-phenyl-4-quinolones (**D**).[20] Among these analogs, many compounds, including 3′,6-disubstituted 2-phenyl-4-quinolones (**A-1**),[21] possessed potent cytotoxic activity against human tumor cell lines. However, most of these compounds were quite lipophilic, and therefore, not optimal for in vivo and clinical studies. Introducing a carboxylic acid group into the 2-phenyl-4-quinolone skeletone should increase polarity and might improve the pharmacokinetic properties of this cytotoxic compound class.

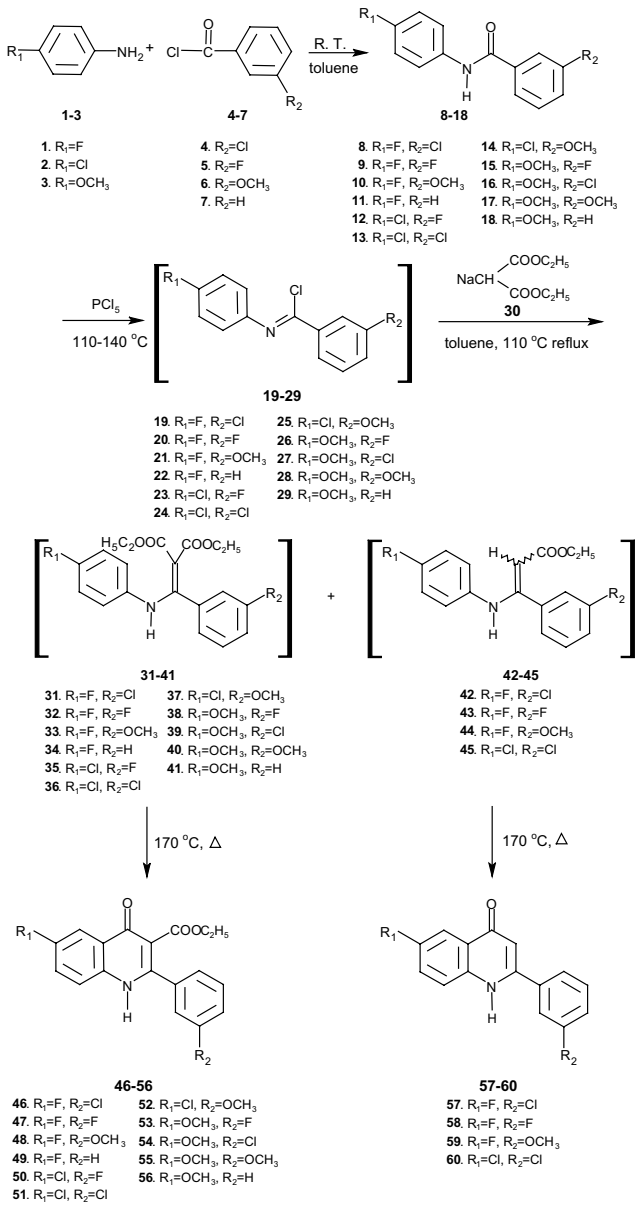
The design of target compounds was based on comparative Molecular Force Field (CoMFA) models derived from 104 known colchicine binding site antimitotic agents, including thiocolchicines, quinolones, and naphthyridinones,[22] and catalyst common-feature pharmacophore models, which were generated from 10 structurally diverse, colchicine binding site agents.[23] A closer examination of these pharmacophore models indicated that substitution at the 3-position did not interfere with any molecular area that is critical for antimitotic activity. Therefore, the antimitotic activities of 3-substituted and unsubstituted 2-phenyl-4-quinolones should be similar, and the effects of different phenyl substituents should also be comparable (3′,6-electron-rich substitution is favored in the parent series). Based on this hypothesis, 3′,6-substituted 2-phenyl-4-quinolone-3-carboxylic acids (**61**–**71**) and their salts (**72**–**82**) were designed as potential hydrophilic antimitotic agents. In this paper, the synthesis and in vitro cytotoxic activities of novel derivatives of 2-phenyl-4-quinolone with improved hydrophilicity are described.

2. Results and discussion

2.1. Chemistry

The synthesis of the key intermediates, ethyl 3′,6-disubstituted 2-phenyl-4-quinolone-3-carboxylates (**46**–**56**), is illustrated in Scheme 1.[24] Reaction of *p*-substituted anilines (**1**–**3**) with *m*-substituted benzoyl chlorides (**4**–**7**) yielded the corresponding *N*-(*p*-substituted phenyl)-3-substituted benzamides (**8**–**18**). Subsequent chlorination of compounds **8**–**18** with PCl_5 afforded carboximidoyl chlorides **19**–**29**, which, without further purification, were then treated with sodium diethylmalonate (**30**) to give the corresponding *N*-[1-(3-substituted phenyl)-2-diethoxycarbonylvinyl]-*N*-(4-substituted phenyl) amines (**31**–**41**).

These intermediates (**31**–**41**) were thermally cyclized to the corresponding ethyl 3′,6-disubstituted 2-phenyl-4-quinolone-3-carboxylates (**46**–**56**). During the purification of products **46**, **47**, **48**, and **51**, the 3′,6-disubstituted 2-phenyl-4-quinolones (**57**–**60**) were also obtained. Therefore, mono-ethoxycarbonyl vinyl derivatives (**42**–**45**) might have also been produced during the preparation of intermediates **31**, **32**, **33**, and **36**. Indeed, the mono-ethoxycarbonyl vinyl derivative (**42**) could be isolated and thermally cyclized to 3′-chloro-6-fluoro-2-phenyl-4-quinolone (**57**).



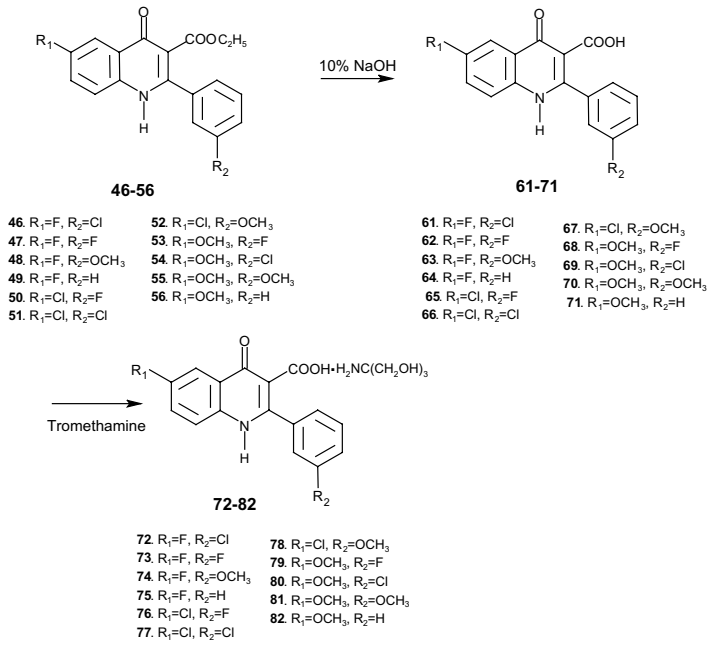
Scheme 1.

The synthetic route from key intermediates **46**–**56** to the target compounds **61**–**82** is illustrated in Scheme 2. As shown, compounds **46**–**56** were first hydrolyzed to the carboxylic acids (**61**–**71**), which were subsequently treated with tromethamine to afford the corresponding salts (**72**–**82**).

2.2. In vitro cytotoxic activity

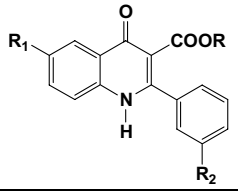
Initial screening was performed using the human lung cancer (A549) cell line. As shown in Table 1, the tested ethyl esters (**50**, **52**–**55**) were inactive. Among the tested 3-carboxylic acid derivatives (**61**–**71**), only compounds **62**, **65**, and **68**, which contain a fluorine atom at the *m*-position of the 2-phenyl group, showed significant activity.

3′-Fluoro-6-methoxy-2-phenyl-4-quinolone-3-carboxylic acid (**68**) was the most potent compound with an



Scheme 2.

Table 1. Cytotoxicity data for compounds **50**, **52**–**55**, **61**–**63**, and **65**–**71** against A549 lung cancer cells



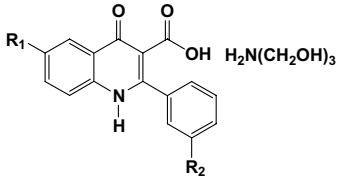
Compd	R	R_1	R_2	ED_{50} (μg/mL)[a]
50	CH_2CH_3	Cl	F	>20
52	CH_2CH_3	Cl	OCH_3	>20
53	CH_2CH_3	OCH_3	F	NA
54	CH_2CH_3	OCH_3	Cl	>20
55	CH_2CH_3	OCH_3	OCH_3	>20
61	H	F	Cl	20
62	H	F	F	0.5
63	H	F	OCH_3	>20
65	H	Cl	F	1.80
66	H	Cl	Cl	>20
67	H	Cl	OCH_3	>20
68	H	OCH_3	F	0.19
69	H	OCH_3	Cl	20
70	H	OCH_3	OCH_3	>20
71	H	OCH_3	H	>20

[a] Cytotoxicity expressed as ED_{50} against A549 (the concentration of compounds that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay).[22]

ED_{50} of 0.19 μg/mL. Activity decreased ($ED_{50} \geqslant 20$ μg/mL) when the *m*-position of the 2-phenyl group was substituted with OCH_3 (**63**, **67**, and **70**), Cl (**61**, **66**, and **69**), or H (**71**) rather than F.

The cytotoxic activity data for the tromethamine salts are presented in Table 2. The potencies followed the

Table 2. Cytotoxicity data for compounds **72**–**74** and **76**–**82** against A549 lung cancer cells



Compd	R_1	R_2	ED_{50} (μg/mL)[a]
72	F	Cl	17.6
73	F	F	4.3
74	F	OCH_3	NA
76	Cl	F	10.9
77	Cl	Cl	>20
78	Cl	OCH_3	>20
79	OCH_3	F	2.4
80	OCH_3	Cl	17.6
81	OCH_3	OCH_3	NA
82	OCH_3	H	>20

[a] Cytotoxicity expressed as ED_{50} against A549 (the concentration of compounds that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay).

general order: 3′-fluoro-derivatives (**73**, **76**, and **79**) ≫ 3′-chloro-derivatives (**72**, **77**, and **80**) > 3′-methoxy derivatives (**74**, **78**, and **81**). Similarly with the carboxylic acids, the salt (**79**) of compound **68** showed the best in vitro cytotoxic activity with an ED_{50} of 2.4 μg/mL.

The in vitro cytotoxic activity of compounds **68** and **79** was further tested in eight additional cancer cell lines (CAKI, HOS, KB, KB-VIN, SK-MEL-2, U87-MG, HCT-8, and 1A9). As shown in Table 3, these two compounds demonstrated marked inhibition against most of

Table 3. Activity of compounds **68** and **79** against HTCL replication

Compd	ED_{50} (μg/mL)[a]							
	CAKI[b]	HOS[b]	KB[b]	KB-VIN[b]	SK-MEL-2[b]	U87-MG[b]	HCT-8[b]	1A9[b]
68	10.0	0.14	0.13	0.13	0.16	1.50	0.14	0.03
79	NT	5.0	NT	NT	2.0	5.0	1.20	0.88

[a] Cell line/mean ED_{50} in microgram per milliliter (duplicates varied no more than 5%). Note: if inhibition is less than 50% at 10 μg/mL then inhibition values observed are shown in brackets. NT = not tested.

[b] Renal cancer (CAKI), bone (HOS), epidermoid carcinoma of the nasopharynx (KB), vincristine-resistant epidermoid carcinoma of the nasopharynx (KB-VIN), melanoma (SK-MEL-2), glioblastoma (C187-MG), ileocecal carcinoma (HCT-8), human ovarian cancer (1A9) cell lines.

the eight cancer cell lines, and most notably, were quite active against human ovarian cancer cells (1A9), which are known to have a high level of drug resistant P-glycoprotein. Compound **68** showed an ED_{50} of 0.03 μg/mL against 1A9 cells, and also was equally active against vincristine-sensitive and -resistant KB cells. Thus, this compound merits further anticancer development, particularly against drug resistant ovarian cancer and vincristine-resistant epidermal carcinoma of the nasopharynx.

Based on the excellent preliminary results, compound **68** was further evaluated against the JCI human cancer cell line panel,[25] which includes five breast cancer, six CNS, five colon, seven lung, one melanoma, five ovarian, two renal, six stomach, and two prostate cancer cell lines. Dose response curves at five different concentrations between 10^{-4} and 10^{-8} M, which were obtained from computer analysis. GI_{50} (50% growth inhibition), TGI (total growth inhibition), and LC_{50} (50% lethal concentration) were calculated and the corresponding mean graphs (fingerprints) obtained.

Compound **68** was active against most cancer cell lines, with a MG-MID (mean growth midpoint) for $\log GI_{50}$ of −6.22. The highest potency was found against HGC2998 cells ($\log GI_{50}$ −6.86), followed by OVCAR-4 ($\log GI_{50}$ −6.76) cells. When evaluated for TGI (Table 2B), compound **68** had a mean log value of −4.61, with marked inhibition against BSY-1 breast, SF-539 CNS, HGC2998 colon, NCI-H552 lung, OVCAR-4 and SK-OV-3 ovarian, RXF-631L renal, and DU-145 prostate cancer cell lines. The mean log value for the LC_{50} values was −4.05. Compound **68** showed impressive selective toxicity against the OVCAR-4 cell line with $\log LC_{50}$ value of −6.02, which is 100-fold different in comparison to the mean value. Therefore, compound **68** is an excellent lead compound and worthy of further development.

Most drugs with the same mechanism of action will show similar fingerprints against a cancer cell line database. Therefore, the fingerprint of compound **68** was submitted for data analysis by a computer pattern recognition (COMPARE) program, which has a database covering fingerprints of over 100 known anticancer agents with various action mechanisms. The three highest correlation coefficient (r) values that were statistically significant ($p < 0.05$) are presented in Table 4. Compound **68** was closest to navelbine ($r = 0.521$), followed by vindesine ($r = 0.461$), and then taxol ($r = 0.452$), which suggests that **68** shares a similar action mechanism with these drugs. A possible mechanism is tubulin binding and subsequent inhibition of microtubule organization. However, to prove this possibility, the tubulin binding activity of **68** must be examined. Compound **68** may have a different mode of action compared to the above tubulin binders, as the correlation coefficients were low. Further investigation is required to clarify the action mechanism of this novel compound.

Table 4. Results of COMPARE analysis of compound **68**

Rank	Compd	r[a]	Molecular targets/drug type
1	Navelbine	0.512	Tubulin
2	Vindesine	0.461	Tubulin
3	Taxol	0.452	Tubulin

[a] r: correlation coefficient.

3. Conclusions

In order to improve the pharmacokinetic properties of antimitotic 2-phenyl-4-quinolone derivatives, molecular modeling was used to design a series of 3-carboxylic acid analogs. Initial cytotoxicity screening in the A549 cancer cell line identified 3′-fluoro-6-methoxy-2-phenyl-4-quinolone-3-carboxylic acid (**68**) as the most active compound. In further in vitro cytotoxic evaluation, this compound showed impressive potency against OVCAR-4 cancer cells. Analysis by the COMPARE program suggested a similar, but not identical, mechanism of action to that of navelbine and taxol. Compound **68** is an attractive candidate for development as a novel anticancer agent.

4. Experimental

Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR-440 and Nicolet Impact 400 FT-IR spectrophotometers as KBr pellets. NMR spectra were obtained on a Bruker Advance DPX-200 FT-NMR spectrometer in DMSO-d_6 The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. MS spectra were measured with an HP 5995 GC–MS instrument. The UV spectra were recorded on a Shimadzu UV-160A UV–vis recording spectrophotometer as methanolic solutions. Elemental analyses (C, H, N) were performed

at China Medical University, Taiwan, and the results were within ±0.4% of the calculated values.

4.1. 3-Chlorophenyl-*N*-(4-fluorophenyl)benzamide (8)

p-Fluoroaniline (**1**) (11.11 g, 0.1 mol) in 100 mL of toluene at 20 ± 2 °C was added dropwise to *m*-chlorobenzoyl chloride (**4**) (8.81 g, 0.05 mol). After being stirred for 3 h, the resulting precipitate was collected and recrystallized from EtOH to give compound **9** as colorless needles (9.59 g, 77%); mp 134–136 °C; ^{1}H NMR (DMSO-d_6): δ 7.18 (2H, dd, J = 8.9 Hz, H-3′, H-5′), 7.36–7.46 (1H, m, H-4), 7.51–7.62 (1H, m, H-5), 7.74–7.84 (4H, m, H-2, H-6, H-2′, H-6′), 10.41 (1H, br, NH); IR (KBr) ν 3330 (NH), 1650 (C=O) cm^{-1}; MS (M^+) *m/z* 249.7; Anal. ($C_{13}H_9ClFNO$) C, H, N. Compounds **9**–**18** were prepared in an analogous manner.

4.2. 3-Fluorophenyl-*N*-(4-fluorophenyl)benzamide (9)

Obtained from compounds **1** and **5**; colorless needles (9.32 g, 80%); mp 147–148 °C; ^{1}H NMR (DMSO-d_6): δ 7.16 (2H, dd, J = 8.8 Hz, H-3′, H-5′), 7.34–7.43 (1H, m, H-4), 7.49–7.60 (1H, m, H-5), 7.75–7.86 (4H, m, H-2, H-6, H-2′, H-6′), 10.37 (1H, br, NH); IR (KBr) ν 3333 (NH), 1651 (C=O) cm^{-1}; MS (M^+) *m/z* 233.2; Anal. ($C_{13}H_9F_2NO$) C, H, N.

4.3. 3-Methoxyphenyl-*N*-(4-fluorophenyl)benzamide (10)

Obtained from compounds **1** and **6**; colorless needles (7.19 g, 75%); mp 104–105 °C; ^{1}H NMR (DMSO-d_6): δ 3.83 (3H, s, OCH_3), 7.11–7.23 (3H, m, H-4, H-3′, H-5′), 7.43 (1H, t, J = 7.7 Hz, H-5), 7.47–7.56 (2H, m, H-2, H-6), 7.79 (2H, dd, J = 9.1, 5.1 Hz, H-2′, H-6′), 10.27 (1H, br, NH); IR (KBr) ν 3305 (NH), 1650 (C=O) cm^{-1}; MS (M^+) *m/z* 245.2; Anal. ($C_{14}H_{12}FNO_2$) C, H, N.

4.4. *N*-(4-Fluorophenyl)benzamide (11)

Obtained from compounds **1** and **7**; colorless needles (8.28 g, 77%); mp 167–168 °C; ^{1}H NMR (DMSO-d_6): δ 7.18 (2H, dd, J = 9.0, 9.0 Hz, H-3′, H-5′), 7.47–7.58 (3H, m, H-4, H-2′, H-6′), 7.80 (2H, m, H-3, H-5), 7.95 (2H, m, H-2, H-6), 10.31(1H, br, NH); IR (KBr) ν 3344 (NH), 1655 (C=O) cm^{-1}; MS (M^+) *m/z* 215.2; Anal. ($C_{13}H_{10}FNO$) C, H, N.

4.5. 3-Fluorophenyl-*N*-(4-chlorophenyl)benzamide (12)

Obtained from compounds **2** and **5**; colorless needles (10.21 g, 82%); mp 156–158 °C; ^{1}H NMR (DMSO-d_6): δ 7.40 (2H, d, J = 8.9 Hz, H-3′, H5′), 7.43–7.48 (1H, m, H-5), 7.52–7.60 (1H, m, H-4), 7.71–7.82 (4H, m, H-2, H-6, H-2′, H-6′), 10.42 (1H, br, NH); IR (KBr) ν 3320 (NH), 1656 (C=O) cm^{-1}; MS (M^+) *m/z* 249.7; Anal. ($C_{13}H_9ClFNO$) C, H, N.

4.6. 3-Chloro-*N*-(4-chlorophenyl)benzamide (13)

Obtained from compounds **2** and **4**; colorless needles (10.64 g, 80%); mp 124–126 °C; ^{1}H NMR (DMSO-d_6): δ 7.38–7.43 (2H, d, J = 8.8 Hz, H-3′, H5′), 7.51–7.59 (1H, t, J = 7.6 Hz, H-5), 7.63–7.68 (1H, d, J = 8.3 Hz, H-4), 7.78–7.82 (2H, d, J = 8.8 Hz, H-2′, H-6′), 7.87–7.92 (1H, dt, J = 1.5, 7.6 Hz, H-6), 7.98-8.00 (1H, t, J = 1.6 Hz, H-2), 10.46 (1H, br, NH); IR (KBr) ν 3353 (NH), 1652 (C=O) cm^{-1}; MS (M^+) *m/z* 266.1; Anal. ($C_{13}H_9Cl_2NO$) C, H, N.

4.7. 3-Methoxyphenyl-*N*-(4-chlorophenyl)benzamide (14)

Obtained from compounds **2** and **6**; colorless needles (9.92 g, 76%); mp 123–124 °C; ^{1}H NMR (DMSO-d_6): δ 3.99 (3H, s, OCH_3), 7.13–7.16 (1H, dd, J = 2.4, 8.3 Hz, H-4), 7.38–7.41 (2H, d, J = 8.8 Hz, H-2′, H-6′), 7.43–7.44 (1H, t, H-5), 7.44–7.45 (1H, s, H-2), 7.50–7.52 (1H, d, J = 7.8 Hz, H-6), 7.74–7.77 (2H, d, J = 11.7 Hz, H-6); IR (KBr) ν 3300 (NH), 1649 (C=O) cm^{-1}; MS (M^+) *m/z* 261.7; Anal. ($C_{14}H_{12}ClNO_2$) C, H, N.

4.8. 3-Fluorophenyl-*N*-(4-methoxyphenyl)benzamide (15)

Obtained from compounds **3** and **5**; colorless needles (10.78 g, 88%); mp 161–163 °C; ^{1}H NMR (DMSO-d_6): δ 3.64 (3H, s, OCH_3), 6.83 (2H, d, J = 9.1 Hz, H-3′, H-5′), 7.26–7.36 (1H, m, H-4), 7.48 (1H, m, H-5), 7.56 (2H, d, J = 9.1 Hz, H-2′, H-6′), 7.62–7.72 (2H, m, H-2, H-6), 10.10 (1H, br, NH); IR (KBr) ν 3320 (NH), 1650 (C=O) cm^{-1}; MS (M^+) *m/z* 245.2; Anal. ($C_{14}H_{12}FNO_2$) C, H, N.

4.9. 3-Chlorophenyl-*N*-(4-methoxyphenyl)benzamide (16)

Obtained from compounds **3** and **4**; colorless needles (10.57 g, 81%); mp 146–148 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (3H, s, OCH_3), 6.93 (2H, d, J = 9.0 Hz, H-3′, H-5′), 7.47–7.51 (1H, m, H-5), 7.58–7.62 (1H, m, H-4), 7.71 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.93 (1H, d, J = 7.4 Hz, H-6), 8.02 (1H, m, H-2), 10.25 (1H, br, NH); IR (KBr) ν 3345 (NH), 1650 (C=O) cm^{-1}; MS (M^+) *m/z* 261.7; Anal. ($C_{14}H_{12}ClNO_2$) C, H, N.

4.10. 3-Methoxyphenyl-*N*-(4-methoxyphenyl)benzamide (17)

Obtained from compounds **3** and **6**; colorless needles (10.02 g, 78%); mp 107–109 °C; ^{1}H NMR (DMSO-d_6): δ 3.74 (3H, s, 4′-OCH_3), 3.83 (3H, s, 3-OCH_3), 6.93 (2H, d, J = 9.1 Hz, H-3′, H-5′f), 7.50 (1H, m, H-2), 7.13 (1H, d, J = 9.1 Hz, H-4), 7.42 (1H, d, J = 7.7 Hz, H-5), 7.55 (1H, d, J = 7.7 Hz, H-6), 7.69 (2H, d, J = 9.0 Hz, H-2′, H-6′), 10.11 (1H, br, NH); IR (KBr) ν 3304 (NH), 1644 (C=O) cm^{-1}; MS (M^+) *m/z* 257.3; Anal. ($C_{15}H_{15}NO_3$) C, H, N.

4.11. *N*-(4-Methoxyphenyl)benzamide (18)

Obtained from compounds **3** and **7**; colorless needles (9.65 g, 85%); mp 153–155 °C; ^{1}H NMR (DMSO-d_6): δ 3.74 (3H, s, OCH_3), 6.93 (2H, d, J = 9.0 Hz, H-3′, H-5′), 7.50–7.57 (3H, m, H-3, H-4, H-5), 7.71 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.97 (2H, dd, J = 7.4, 1.6 Hz, H-2, H-6), 10.16 (1H, br, NH); IR (KBr) ν 3332 (NH),

1649 (C=O) cm^{-1}; MS (M^+) *m/z* 227.3; Anal. ($C_{14}H_{13}NO_2$) C, H, N.

4.12. *N*-[1-(3-Chlorophenyl)-2,2-diethoxycarbonylvinyl]-*N*-(4-fluorophenyl)amine (31), *N*-[1-(3-chlorophenyl)-2-ethoxy-carbonylvinyl]-*N*-(4-fluorophenyl)amine (42), ethyl 3′-chloro-6-fluoro-phenyl-4-quinolone-3-carboxylate (46) and 3′-chloro-6-fluoro-2- phenyl-4-quinolone (57)

PCl_5 (2.24 g, 0.01 mol) was added to compound **8** (2.50 g, 0.01 mol) and the mixture was heated to 110 °C, stirred for 1 h, and then evaporated under vacuum to yield carboximidoyl chloride (**19**) as viscous liquid. Meanwhile, diethyl malonate (4.8 g, 0.03 mol) was introduced dropwise into the solution of sodium (0.72 g, 0.03 mol) in absolute ethanol (50 mL), at 20 ± 2 °C. The mixture was stirred for 1 h at 50 ± 2 °C. Subsequent removal of ethanol under vacuum afforded sodium diethyl malonate (**30**) as a gel. Then compound **19** was dissolved in toluene (20 mL) and added into a suspension of **30** in toluene (20 mL). The resulting mixture was allowed to react 4 h at 110 °C. The reaction mixture was filtered and the filtrate was concentrated and extracted with diethyl ether, washed with water, dried over $MgSO_4$, and concentrated under vacuum to yield **31** and **42** as yellowish, viscous liquid. Without further purification, the viscous liquid was heated for 4 h at 170 °C to give a yellowish solid that was purified further by column chromatography (silica gel, 100:1 $CHCl_3$–EtOH) to produce compounds **46** (1.21 g, 35%) and **57** (0.82 g, 30%).

After the initial reaction, compounds **31** and **42** could also be separated by chromatography on silica gel using $CHCl_3$ as eluant to give pure.

N-[1-(3-Chlorophenyl)-2,2-diethoxycarbonylvinyl]-*N*-(4-fluorophenyl)amine (**31**) (1.84 g, 47%) and *N*-[1-(3-chlorophenyl)-2-ethoxycarbonylvinyl]-*N*-(4-fluorophenyl)-amine (**42**) (1.09 g, 34%). Compounds **31** (1.17 g, 3 mmol) and **42** (0.96 g, 3 mmol) were then heated separately for 4 h at 170 °C to give yellowish solids, which were purified by individual column chromatography (silica gel, $CHCl_3$–EtOH) to produce compounds **46** (0.84 g, 81%) and **57** (0.60 g, 73%).

4.13. Compound 31

Mp 70–72 °C; ^{1}H NMR (DMSO-d_6): δ 0.92 (3H, t, J = 7.2 Hz, CH_3), 1.30 (3H, t, J = 7.2 Hz, CH_3), 3.90 (2H, q, J = 7.2 Hz, CH_2), 4.28 (2H, q, J = 7.2 Hz, CH_2), 6.67–6.78 (2H, m, H-3, H-5), 6.81–6.83 (2H, m, H-2, H-6), 7.20–7.28 (2H, m, H-4′, H-5′), 7.77–7.90 (2H, m, H-2′, H-6′), 11.30 (1H, br, NH); IR (KBr) v 1720, 1725 (C=O) cm^{-1}; MS (M^+) *m/z* 391.8; Anal. ($C_{20}H_{19}ClFNO_4$) C, H, N.

4.14. Compound 42

Mp 52–54 °C; ^{1}H NMR (DMSO-d_6): δ 1.31 (3H, t, J = 7.2 Hz, CH_3), 4.20 (2H, q, J = 7.2 Hz, CH_2), 4.99 (1H, s, CH_2), 6.64–6.67 (2H, m, H-3, H-5), 6.78–6.83 (2H, m, H-2, H-6), 7.16–7.18 (2H, m, H-4′, H-5′), 7.27–7.36 (1H, m, H-6′), 7.79–7.80 (1H, m, H-2′), 12.56 (1H, br, NH); IR (KBr) v 1720 (C=O) cm^{-1}; MS (M^+) *m/z* 319.8; Anal. ($C_{17}H_{15}ClFNO_2$) C, H, N.

4.15. Compound 46

Colorless needles; mp 215–217 °C; ^{1}H NMR (DMSO-d_6): δ 0.92 (3H, t, J = 7.2 Hz, CH_3), 3.96 (2H, q, J = 7.2 Hz, CH_2), 7.44–7.61 (3H, m, aromatic), 7.71–7.92 (4H, m, aromatic), 12.30 (1H, br, NH); IR (KBr) v 1723, 1615 (C=O) cm^{-1}; MS (M^+) *m/z* 345.8; Anal. ($C_{18}H_{13}ClFNO_3$) C, H, N.

4.16. Compound 57

Amorphous colorless; mp 271–289 °C (dec); ^{1}H NMR (DMSO-d_6): δ 6.38 (1H, s, H-3), 7.57–7.81 (6H, m, H-5, H-7, H-8, H-4′, H-5′, H-6′), 7.92 (1H, s, H-2′), 11.90 (1H, br, NH); IR (KBr) v 1630 (C=O) cm^{-1}; MS (M^+) *m/z* 273.7; Anal. ($C_{15}H_9ClFNO$) C, H, N.

Compounds **47**–**60** were prepared in an analogous manner without the intermediate purification step.

4.17. Ethyl 3′,6-difluoro-2-phenyl-4-quinolone-3-carboxylate (47) and 3′,6-difluoro-2-phenyl-4-quinolone (58)

Obtained from compound **9** (2.33 g, 0.01 mol).

4.18. Compound 47

Colorless needles (1.21 g, 38%); mp 209–220 °C (dec); ^{1}H NMR (DMSO-d_6): δ 0.93 (3H, t, J = 7.2 Hz, CH_3), 3.98 (2H, q, J = 7.2 Hz, CH_2), 7.38–7.49 (3H, m, aromatic), 7.59–7.78 (4H, m, aromatic), 12.28 (1H, br, NH); IR (KBr) v 3228 (NH), 1722, 1612 (C=O) cm^{-1}; MS (M^+) *m/z* 329.3; Anal. ($C_{18}H_{13}F_2NO_3$) C, H, N.

4.19. Compound 58

Amorphous colorless (0.54 g, 21%); mp 252–278 °C (dec); ^{1}H NMR (DMSO-d_6): δ 6.43 (1H, s, H-3), 7.40 (1H, dd, J = 8.2, 7.4 Hz, H-7), 7.57–7.85 (6H, m, H-5, H-8, H-2′, H-4′, H-5′, H-6′), 11.87 (1H, br, NH); IR (KBr) v 1635 (C=O) cm^{-1}; MS (M^+) *m/z* 257.2; Anal. ($C_{15}H_9F_2NO$) C, H, N.

4.20. Ethyl 3′-methoxy-6-fluoro-2-phenyl-4-quinolone-3-carboxylate (48) and 3′-methoxy-6-fluoro-2-phenyl-4-quinolone (59)

Obtained from compound **10**.

4.21. Compound 48

Colorless needles (1.09 g, 32%); mp 214–216 °C; ^{1}H NMR (DMSO-d_6): δ 0.95 (3H, t, J = 7.2 Hz, CH_3), 3.86 (3H, s, OCH_3), 4.00 (2H, q, J = 7.2 Hz, CH_2), 7.12–7.15 (3H, m, H-2′, H-4′, H-6′), 7.47 (1H, t, J = 4.1 Hz, H-5′), 7.63 (1H, ddd, J = 8.4, 2.9 Hz, H-7), 7.75–7.79 (2H, m, H-5, H-8), 12.19 (1H, br, NH); IR (KBr) v 1711 (C=O) cm^{-1}; MS (M^+) *m/z* 341.3; Anal. ($C_{19}H_{16}FNO_4$) C, H, N.

4.22. Compound 59

Amorphous colorless (0.67 g, 25%); mp 236 °C (dec); ^{1}H NMR (DMSO-d_6): δ 3.86 (3H, s, OCH_3), 6.43 (1H, s, H-3), 7.53–7.63 (3H, m, H-7, H-4′, H-5′), 7.68–7.91 (4H, m, H-5, H-8, H-2′, H-6′), 11.91 (1H, br, NH); IR (KBr) ν 1631 (C=O) cm^{-1}; MS (M^+) m/z 269.3; Anal. ($C_{16}H_{12}FNO_2$) C, H, N.

4.23. Ethyl 3′,6-dichloro-phenyl-4-quinolone-3-carboxylate (51) and 3′,6-dichloro-2-phenyl-4-quinolone (60)

Obtained from compound **13**.

4.24. Compound 51

Colorless needles (4.89 g, 45%); mp 210–228 °C (dec); ^{1}H NMR (DMSO-d_6): δ 0.93 (3H, t, J = 7.1 Hz, CH_3), 3.99 (2H, q, J = 7.1 Hz, CH_2), 7.50–7.77 (6H, m, H-7, H-8, H-2′, H-4′, H5′, H-6′), 8.04 (1H, s, H-5), 12.31 (1H, br, NH); IR (KBr) ν 1720, 1631 (C=O) cm^{-1}; MS (M^+) m/z 362.2; Anal. ($C_{18}H_{13}Cl_2NO_3$) C, H, N.

4.25. Compound 60

Amorphous colorless (0.87 g, 30%); mp 275–286 °C (dec); ^{1}H NMR (DMSO-d_6): δ 6.47 (1H, s, H-3), 7.59–7.80 (5H, m, H-2′, H-4′, H-5′, H-6′, H-8), 7.93 (1H, m, H-7), 7.80 (1H, m, H-5); IR (KBr) ν 1636 (C=O) cm^{-1}; MS (M^+) m/z 290.1; Anal. ($C_{15}H_9Cl_2NO$) C, H, N.

4.26. Ethyl 6-fluoro-2-phenyl-4-quinolone-3-carboxylate (49)[26]

Obtained from compound **11**; colorless needles (4.29 g, 46%); mp 277–279 °C; ^{1}H NMR (DMSO-d_6): δ 0.89 (3H, t, J = 7.0 Hz, CH_3), 3.96 (2H, q, J = 7.0 Hz, CH_2), 7.56–7.67 (5H, m, H-7, H-8, H-3′, H-4′, H-5′), 7.73–7.79 (3H, m, H-5, H-2′, H-6′), 12.25 (1H, br, NH); IR (KBr) ν 3232 (NH), 1720 (C=O) cm^{-1}; MS (M^+) m/z 311.3; Anal. ($C_{18}H_{14}FNO_3$) C, H, N.

4.27. Ethyl 3′-fluoro-6-chloro-2-phenyl-4-quinolone-3-carboxylate (50)

Obtained from compound **12**; colorless needles (5.91 g, 57%); mp 237–238 °C; ^{1}H NMR (DMSO-d_6): δ 0.92 (3H, t, J = 7.1 Hz, CH_3), 3.98 (2H, q, J = 7.1 Hz, CH_2), 7.37–7.67 (4H, m, H-2′, H-4′, H-5′, H-6′), 7.72–7.76 (2H, m, H-7, H-8), 8.05 (1H, d, J = 2.2 Hz, H-5); IR (KBr) ν 1715, 1630 (C=O) cm^{-1}; MS (M^+) m/z 345.8; Anal. ($C_{18}H_{13}ClFNO_3$) C, H, N.

4.28. Ethyl 3′-methoxy-6-chloro-2-phenyl-4-quinolone-3-carboxylate (52)

Obtained from compound **14**; colorless needles (5.80 g, 54%); mp 211–236 °C (dec); ^{1}H NMR (DMSO-d_6): δ 0.80 (3H, t, J = 7.2 Hz, CH_3), 3.60 (3H, s, OCH_3), 3.86 (2H, q, J = 7.2 Hz, CH_2), 6.66 (1H, m, H-6′), 6.95–7.10 (3H, m, H-2′, H-4′, H-5′), 7.49 (1H, dd, J = 8.9, 2.3 Hz, H-7), 7.78 (1H, d, J = 8.9 Hz, H-8), 7.87 (1H, d, J = 2.3 Hz, H-5); IR (KBr) ν 1712 (–C=O) cm^{-1}; MS (M^+) m/z 357.8; Anal. ($C_{19}H_{16}ClNO_4$) C, H, N.

4.29. Ethyl 3′-fluoro-6-methoxy-2-phenyl-4-quinolone-3-carboxylate (53)

Obtained from compound **15**; colorless needles (6.44 g, 63%); mp 211–214 °C; ^{1}H NMR (DMSO-d_6): δ 0.93 (3H, t, J = 7.2 Hz, CH_3), 3.85 (3H, s, OCH_3), 3.98 (2H, q, J = 7.1 Hz, CH_2), 7.34–7.48 (4H, m, H-2′, H-4′, H-5′, H-6′), 7.51 (1H, d, J = 2.9 Hz, H-5), 7.54–7.57 (1H, m, H-7), 7.64 (1H, d, J = 9.1 Hz, H-8), 12.13 (1H, br s, NH); IR (KBr) ν 1709, 1632 (C=O) cm^{-1}; MS (M^+) m/z 341.3; Anal. ($C_{19}H_{16}FNO_4$) C, H, N.

4.30. Ethyl 3′-chloro-6-methoxy-2-phenyl-4-quinolone-3-carboxylate (54)

Obtained from compound **16**; colorless needles (6.65 g, 62%); mp 193–210 °C (dec); ^{1}H NMR (DMSO-d_6): δ 0.90 (3H, t, J = 7.1 Hz, CH_3), 3.82 (3H, s, OCH_3), 3.96 (2H, q, J = 7.1 Hz, CH_2), 7.37–7.52 (4H, m, H-2′, H-4′, H-5′, H-6′), 7.48 (1H, d, J = 2.9 Hz, H-5), 7.36 (1H, dd, J = 9.0, 2.9 Hz, H-7), 7.57 (1H, d, J = 9.1 Hz, H-8), 12.12 (1H, br s, NH); IR (KBr) ν 1712 (C=O) cm^{-1}; MS (M^+) m/z 357.8; Anal. ($C_{19}H_{16}ClNO_4$) C, H, N.

4.31. Ethyl 3′,6-dimethoxy-2-phenyl-4-quinolone-3-carboxylate (55)

Obtained from compound **17**; colorless needles (6.25 g, 59%); mp 242–243 °C (dec); ^{1}H NMR (DMSO-d_6): δ 0.93 (3H, t, J = 7.2 Hz, CH_3), 3.79 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.97 (2H, q, J = 7.1 Hz, CH_2), 7.08–7.13 (3H, m, H-2′, H-4′, H-6′), 7.35 (1H, dd, J = 9.1, 2.9 Hz, H-7), 7.44 (1H, t, J = 7.9 Hz, H-5′), 7.51 (1H, d, J = 2.8 Hz, H-5), 7.65 (1H, d, J = 9.2 Hz, H-8), 12.10 (1H, s, NH); IR (KBr) ν 1731 (C=O) cm^{-1}; MS (M^+) m/z 353.4; Anal. ($C_{20}H_{19}NO_5$) C, H, N.

4.32. Ethyl 6-methoxy-2-phenyl-4-quinolone-3-carboxylate (56)[24]

Obtained from compound **18**; colorless needles (5.72 g, 59%); mp 225–227 °C; ^{1}H NMR (DMSO-d_6): δ 0.89 (3H, t, J = 7.1 Hz, CH_3), 3.84 (3H, s, OCH_3), 3.94 (2H, q, J = 7.1 Hz, CH_2), 7.36 (1H, dd, J = 9.0, 2.9 Hz, H-7), 7.51 (1H, d, J = 2.9 Hz, H-5), 7.54 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.65 (1H, d, J = 9.0 Hz, H-8), 12.08 (1H, br, NH); IR (KBr) ν 1716 (C=O) cm^{-1}; MS (M^+) m/z 323.3; Anal. ($C_{19}H_{17}NO_4$) C, H, N.

4.33. 3′-Chloro-6-fluoro-2-phenyl-4-quinolone-3-carboxylic acid (61)

Compound **46** (1.04 g, 3 mmol) was stirred in 10% NaOH (100 mL) until completely dissolved (ca. 1 h). The solution was cooled to 5 ± 2 °C and acidified with dil HCl. The resulting precipitate was collected by filtration, washed with water, and recrystallized from EtOH to afford the desired carboxylic acid; amorphous colorless (0.86 g, 90%); mp 263 °C; ^{1}H NMR (DMSO-d_6): δ 7.46 (1H, d, J = 7.4 Hz, H-6′), 7.52 (1H, d, J = 7.5 Hz,

H-5′), 7.58–7.61 (2H, m, H-2′, H-4′), 7.76 (1H, ddd, $J = 2.8$, 8.8 Hz, H-7), 7.85 (1H, dd, $J = 9.0$, 4.6 Hz, H-8), 7.90 (1H, dd, $J = 9.3$, 2.8 Hz, H-5); IR (KBr) ν 3454 (NH), 1631 (C=O) cm^{-1}; MS (M^+) m/z 317.7; Anal. ($C_{16}H_9ClFNO_3$) C, H, N.

Compounds **62**–**71** were prepared in an analogous manner.

4.34. 3′,6-Difluoro-2-phenyl-4-quinolone-3-carboxylic acid (62)

Obtained from compound **47**; amorphous colorless (0.78 g, 86%); mp 272 °C (dec); ^1H NMR (DMSO-d_6): δ 7.27–7.33 (3H, m, H-2′, H-4′, H-6′), 7.46–7.51 (1H, m, H-5′), 7.70 (1H, ddd, $J = 3.0$, 8.8, 8.7 Hz, H-7), 7.84 (1H, dd, $J = 9.0$, 4.7 Hz, H-8), 7.91 (1H, dd, $J = 9.3$, 3.0 Hz, H-5); IR (KBr) ν 1677, 1620 (C=O) cm^{-1}; MS (M^+) m/z 301.2; Anal. ($C_{16}H_9F_2NO_3$) C, H, N.

4.35. 6-Fluoro-3′-methoxy-2-phenyl-4-quinolone-3-carboxylic acid (63)

Obtained from compound **48**; amorphous colorless (0.84 g, 89%); mp 231 °C; ^1H NMR (DMSO-d_6): δ 3.97 (3H, s, OCH_3), 7.06–7.11 (3H, m, H-2′, H-4′, H-6′), 7.42 (1H, m, H-5′), 7.77 (1H, dd, $J = 2.9$, 8.6 Hz, H-7), 7.86–7.93 (2H, m, H-5, H-8); IR (KBr) ν 3450 (NH), 1679, 1617 (C=O) cm^{-1}; MS (M^+) m/z 313.3; Anal. ($C_{17}H_{12}FNO_4$) C, H, N.

4.36. 6-Fluoro-2-phenyl-4-quinolone-3-carboxylic acid (64)

Obtained from compound **49**; amorphous colorless (0.76 g, 89%); mp 297 °C; ^1H NMR (DMSO-d_6): δ 7.52 (5H, m, H-7, H-8, H-3′, H-4′, H-5′), 7.74–7.95 (3H, m, H-5, H-2′, H-6′), 13.08 (1H, br, NH), 15.50 (1H, br, OH); IR (KBr) ν 1674 (C=O) cm^{-1}; MS (M^+) m/z 283.3; Anal. ($C_{16}H_{10}FNO_3$) C, H, N.

4.37. 3′-Fluoro-6-chloro-2-phenyl-4-quinolone-3-carboxylic acid (65)

Obtained from compound **50**; amorphous colorless (1.40 g, 88%); mp > 300 °C; ^1H NMR (DMSO-d_6): δ 7.37–7.58 (4H, m, H-2′, H-4′, H-5′, H-6′), 7.72–7.76 (2H, m, H-7, H-8), 8.05 (1H, d, $J = 2.2$ Hz, H-5), 12.38 (1H, br, NH); IR (KBr) ν 3430 (NH), 1687, 1635 (C=O) cm^{-1}; MS (M^+) m/z 317.7; Anal. ($C_{16}H_9ClFNO_3$) C, H, N.

4.38. 3′,6-Dichloro-2-phenyl-4-quinolone-3-carboxylic acid (66)

Obtained from compound **51**; amorphous colorless (1.52 g, 91%); mp 260 °C (dec); ^1H NMR (DMSO-d_6): δ 7.47 (1H, d, $J = 7.58$ Hz, H-4′), 7.53 (1H, dd, $J = 7.9$, 7.9 Hz, H-5′), 7.59–7.63 (2H, m, H-2′, H-6′), 7.80 (1H, d, $J = 8.9$ Hz, H-8), 7.89 (1H, dd, $J = 8.9$, 2.6 Hz, H-7), 8.20 (1H, d, $J = 2.4$ Hz, H-5); IR (KBr) ν 3452 (NH), 1680, 1635 (C=O) cm^{-1}; MS (M^+) m/z 334.2; Anal. ($C_{16}H_9Cl_2NO_3$) C, H, N.

4.39. 3′-Methoxy-6-chloro-2-phenyl-4-quinolone-3-carboxylic acid (67)

Obtained from compound **52**; amorphous colorless (1.46 g, 89%); mp 243 °C (dec); ^1H NMR (DMSO-d_6): δ 7.05 (3H, m, H-2′, H-4′, H-6′), 7.40 (1H, dd, $J = 8.3$ Hz, H-5′), 7.80 (1H, d, $J = 8.9$ Hz, H-8), 7.91 (1H, dd, $J = 8.8$, 2.4 Hz, H-7), 8.19 (1H, d, $J = 2.4$ Hz, H-5), 13.01 (1H, br s, NH), 15.37 (1H, br s, OH); IR (KBr) ν 3450 (NH), 1675 (C=O) cm^{-1}; MS (M^+) m/z 329.7; Anal. ($C_{17}H_{12}ClNO_4$) C, H, N.

4.40. 3′-Fluoro-6-methoxy-2-phenyl-4-quinolone-3-carboxylic acid (68)

Obtained from compound **53**; amorphous colorless (1.46 g, 93%); mp 268 °C (dec); ^1H NMR (DMSO-d_6): δ 3.76 (3H, s, OCH_3), 7.07–7.12 (2H, m, H-2′, H-4′), 7.17 (1H, d, $J = 7.9$ Hz, H-6′), 7.28 (1H, dd, $J = 2.9$, 9.2 Hz, H-7), 7.31–7.42 (1H, m, H-5′), 7.56 (1H, d, $J = 2.9$ Hz, H-5), 7.65 (1H, d, $J = 9.0$ Hz, H-8); IR (KBr) ν 1680, 1625 (C=O) cm^{-1}; MS (M^+) m/z 313.3; Anal. ($C_{17}H_{12}FNO_4$) C, H, N.

4.41. 3′-Chloro-6-methoxy-2-phenyl-4-quinolone-3-carboxylic acid (69)

Obtained from compound **54**; amorphous colorless (1.48 g, 90%); mp 223 °C (dec); ^1H NMR (DMSO-d_6): δ 3.91 (3H, s, OCH_3), 7.43–7.66 (6H, m, H-5, H-7, H-2′, H-4′, H-5′, H-6′), 7.77 (1H, d, $J = 9.1$ Hz, H-8), 13.15 (1H, br s, NH); IR (KBr) ν 3444 (NH), 1679, 1624 (C=O) cm^{-1}; MS (M^+) m/z 329.7; Anal. ($C_{17}H_{12}ClNO_4$) C, H, N.

4.42. 3′,6-Dimethoxy-2-phenyl-4-quinolone-3-carboxylic acid (70)

Obtained from compound **55**; amorphous colorless; mp > 300 °C; ^1H NMR (DMSO-d_6): δ 3.90 (3H, s, OCH_3), 7.03–7.08 (3H, m, H-4′, H-5′, H-6′), 7.37–7.49 (2H, m, H-7, H-2′), 7.59 (1H, m, H-5), 7.75 (1H, d, $J = 9.0$ Hz, H-8), 13.03 (1H, br, NH), 16.02 (1H, br, OH); IR (KBr) ν 3455 (NH), 1677, 1624 (C=O) cm^{-1}; MS (M^+) m/z 325.3; Anal. ($C_{18}H_{15}NO_5$) C, H, N.

4.43. 6-Methoxy-2-phenyl-4-quinolone-3-carboxylic acid (71)

Obtained from compound **56**; amorphous colorless; mp > 300 °C; ^1H NMR (DMSO-d_6): δ 3.91 (3H, s, OCH_3), 7.49–7.50 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.54 (1H, d, $J = 2.9$ Hz, H-7), 7.65 (1H, d, $J = 2.8$ Hz, H-5), 7.79 (1H, d, $J = 9.1$ Hz, H-8); IR (KBr) ν 3450 (NH), 1672, 1619 (C=O) cm^{-1}; MS (M^+) m/z 295.3; Anal. $C_{17}H_{13}NO_4$) C, H, N.

4.44. 3′-Chloro-6-fluoro-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (72)

A solution of compound **61** (0.32 g, 1 mmol) in butyl chloride (20 mL) was treated with a methanolic solution (10 mL) of tromethamine (0.12 g, 1 mmol) and an addi-

tional 20 mL of butyl chloride to yield 0.41 g (93%) of a white solid precipitate, which was dried overnight at 50 °C under reduced pressure; mp 241 °C (dec); ^{1}H NMR (DMSO-d_6): δ 3.46 (CH_2OH), 5.16 (3H, br, OH), 7.26–7.33 (4H, m, H-2′, H-4′, H-5′, H-6′), 7.49 (1H, ddd, J = 8.7, 3.0 Hz, H-7), 7.74 (1H, dd, J = 9.2, 5.2 Hz, H-8), 7.79 (1H, dd, J = 8.8, 3.1 Hz, H-5); IR (KBr) ν 3432, 3228 cm^{-1} (NH); MS (M^+) m/z 438.8; Anal. ($C_{20}H_{20}ClFN_2O_6$) C, H, N.

Salts **73**–**82** were obtained in a similar manner.

4.45. 3′,6-Difluoro-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (73)

Obtained from compound **62**; amorphous colorless (0.40 g, 94%); mp > 300 °C; ^{1}H NMR (DMSO-d_6): δ 3.49 (CH_2OH), 5.08 (br, OH), 7.04–7.15 (3H, m, H-2′, H-4′, H-6′), 7.31 (1H, m, H-5′), 7.48 (1H, ddd, J = 9.0, 3.0 Hz, H-7), 7.69–7.82 (2H, m, H-5, H-8); IR (KBr) ν 3391 cm^{-1} (NH); MS (M^+) m/z 422.4; Anal. ($C_{20}H_{20}F_2N_2O_6$) C, H, N.

4.46. 3′-Methoxy-6-fluoro-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (74)

Obtained from compound **63**; amorphous colorless (0.40 g, 93%); mp 222 °C; ^{1}H NMR (DMSO-d_6): δ 3.50 (CH_2OH), 3.75 (3H, s, OCH_3), 5.11 (3H, br, OH), 6.84–6.89 (3H, m, H-2′, H-4′, H-6′), 7.20 (1H, m, H-5′), 7.48 (1H, ddd, J = 9.2, 2.9 Hz, H-7), 7.67–7.80 (2H, m, H-5, H-8); IR (KBr) ν 3384 cm^{-1} (NH); MS (M^+) m/z 434.4; Anal. ($C_{21}H_{23}FN_2O_7$) C, H, N.

4.47. 6-Fluoro-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (75)

Obtained from compound **64**; amorphous colorless (0.36 g, 89%); mp > 300 °C; ^{1}H NMR (DMSO-d_6): δ 3.71 (3 × CH_2OH), 5.12 (3H, br, OH), 7.26–7.36 (5H, m, H-7, H-8, H-3′, H-4′, H-5′), 7.58–7.91 (3H, m, H-5, H-2′, H-6′); IR (KBr) ν 3378 cm^{-1} (NH); MS (M^+) m/z 404.4; Anal. ($C_{20}H_{21}FN_2O_6$) C, H, N.

4.48. 3′-Fluoro-6-chloro-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (76)

Obtained from compound **65**; amorphous colorless (0.41 g, 93%); mp 262 °C (dec); ^{1}H NMR (DMSO-d_6): δ 3.60 (3 × CH_2OH), 5.15 (3H, br, OH), 7.06–7.15 (3H, m, H-4′, H-5′, H-6′), 7.31 (1H, dd, J = 7.3 Hz, H-2′), 7.59 (1H, dd, J = 9.0, 2.4 Hz, H-7), 7.68 (1H, d, J = 8.9 Hz, H-8), 8.11 (1H, d, J = 2.3 Hz, H-5); IR (KBr) ν 3375 cm^{-1} (NH); MS (M^+) m/z 438.8; Anal. ($C_{20}H_{20}ClFN_2O_6$) C, H, N.

4.49. 3′,6-Dichloro-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (77)

Obtained from compound **66**; amorphous colorless (0.43 g, 94%); mp 272 °C (dec); ^{1}H NMR (DMSO-d_6): δ 3.60 (3 × CH_2OH), 5.15 (3H, br, OH), 7.25–7.33 (4H, m, H-2′, H-4′, H-5′, H-6′), 7.58 (1H, dd, J = 8.8, 2.4 Hz, H-7), 7.69 (1H, d, J = 8.8 Hz, H-8), 8.12 (1H, d, J = 2.4 Hz, H-5); IR (KBr) ν 3228 cm^{-1} (NH); MS (M^+) m/z 455.3; Anal. ($C_{20}H_{20}Cl_2N_2O_6$) C, H, N.

4.50. 3′-Methoxy-6-chloro-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (78)

Obtained from compound **67**; amorphous colorless (4.1 g, 92%); mp 261 °C (dec); ^{1}H NMR (DMSO-d_6): δ 3.75 (3H, s, OCH_3), 5.10 (3H, br, OH), 6.82–6.90 (3H, m, H-2′, H-4′, H-6′), 7.16–7.20 (1H, m, H-5′), 7.57 (1H, dd, J = 8.8, 2.4 Hz, H-7), 7.67 (1H, d, J = 8.8 Hz, H-8), 8.11 (1H, d, J = 2.3 Hz, H-5); IR (KBr) ν 3379 (NH) cm^{-1}; MS (M^+) m/z 450.9; Anal. ($C_{21}H_{23}ClN_2O_7$) C, H, N.

4.51. 3′-Fluoro-6-methoxy-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (79)

Obtained from compound **68**; amorphous colorless (0.41 g, 95%); mp 268-269 °C; ^{1}H NMR (DMSO-d_6): δ 3.81 (3 × CH_2OH), 3.86 (3H, s, OCH_3), 4.78 (3H, br, OH), 7.08–7.34 (5H, m, H-2′, H-3′, H-4′, H-5′), 7.54 (1H, d, J = 2.8 Hz, H-5), 7.63 (1H, d, J = 9.8 Hz, H-8); IR (KBr) ν 3256 (br, NH) cm^{-1}; MS (M^+) m/z 434.4; Anal. ($C_{21}H_{23}FN_2O_7$) C, H, N.

4.52. 3′-Chloro-6-methoxy-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (80)

Obtained from compound **69**; amorphous colorless (0.42 g, 94%); mp 144 °C; ^{1}H NMR (DMSO-d_6): δ 3.85 (3 × CH_2OH), 3.87 (3H, s, OCH_3), 4.81 (3H, br, OH), 7.27–7.37 (5H, m, H-2′, H-4′, H-5′, H-6′, H-7), 7.56 (1H, d, J = 2.6 Hz, H-5), 7.66 (1H, d, J = 9.1 Hz, H-8); IR (KBr) ν 3341 cm^{-1} (NH), 1621 cm^{-1} (–C=O); MS (M^+) m/z 450.9; Anal. ($C_{21}H_{23}ClN_2O_7$) C, H, N.

4.53. 3′,6-Dimethoxy-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (81)

Obtained from compound **70**; amorphous colorless (0.42 g, 93%); mp 225 °C; ^{1}H NMR (DMSO-d_6): δ 3.74 (3 × CH_2OH), 4.85 (3H, br, OH), 6.83–6.91 (3H, m, H-4′, H-5′, H-6′), 7.17–7.29 (2H, m, H-7, H-2′), 7.52 (1H, m, H-5), 7.63 (1H, d, J = 9.0 Hz, H-8); IR (KBr) ν 3252 cm^{-1} (NH); MS (M^+) m/z 446.5; Anal. ($C_{22}H_{26}N_2O_8$) C, H, N.

4.54. 6-Methoxy-2-phenyl-4-quinolone-3-carboxylic acid tromethamine salt (82)

Obtained from compound **71**; amorphous colorless (0.39 g, 93%); mp > 300 °C; ^{1}H NMR (DMSO-d_6): δ 3.37 (3 × CH_2OH), 3.85 (3H, s, OCH_3), 4.88 (3H, br, OH), 7.22–7.33 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′, H-7), 7.52 (1H, d, J = 2.9 Hz, H-5), 7.61 (1H, d, J = 9.0 Hz, H-8); IR (KBr) ν 3382 cm^{-1} (NH); MS (M^+) m/z 416.4; Anal. ($C_{21}H_{24}N_2O_7$) C, H, N.

5. Preliminary cytotoxicity assay

Compounds were assayed for in vitro cytotoxicity in a panel of human tumor cell lines at the School of

Pharmacy, University of North Carolina at Chapel Hill, according to procedures described previously.[19,25,27,28] The cell lines included human ovarian cancer (1A9), renal cancer (CAKI), ileocecal carcinoma (HCT-8), lung carcinoma (A549), glioblastoma (U-87-MG), bone (HOS), epidermoid carcinoma of the nasopharynx (KB), P-gp-expressing epidermoid carcinoma of the nasopharynx (KB-VIN), and melanoma (SKMEL-2) cell line. The cytotoxic effects of each compound were obtained as EC_{50} values, which represent the drug concentrations required to cause 50% inhibition.

6. Evaluation against human cancer cell line panel

6.1. Cell lines

Compound **68** was evaluated against the human cancer cell line panel at JCI, according to procedures described previously.[25] Human breast cancer MDA-MB-231 was purchased from American type culture collection (Rockville, MD) and the following human cancer cell lines[29] were generously distributed by the National Cancer Institute (Frederick, MD): lung cancer, NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, and DMS114; colon cancer, HGC-2998, KM-12, HT-29, HCT-15, and HCT-116; ovarian cancer, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SKOV-3; breast cancer, MCF-7; renal cancer, RXF-631L, and ACHN; melanoma, LOX-IMVI; brain tumor, U251, SF-295, SF-539, SF-268, SNB-75, and SNB-78, and prostate cancer, DU-145 and PC-3. Human stomach cancer, MKN-1, MKN-7, MKN-28, MKN-45, MKN-74, and St-4, and human breast cancer BSY-1, HBC-4, and HBC-5 were described elsewhere.[30,31] The cells were cultured in RPM1 1640 supplemented with 5% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 mg/mL) at 37 °C in humidified air containing 5% CO_2.

6.2. Human cancer cell line panel and the database

To evaluate drugs for the cell growth inhibition profile, a human cell line panel was combined with a database. The system as a whole was developed according to the method of the National Cancer Institute,[27,32] with modification. The cell line panel consisted of 38 human cancer cell lines, described above. With this system, the antiproliferative effect of more than 200 standard compounds, including various anticancer drugs, was examined and a new database established, as described below.

6.3. Measurements of cell growth inhibition and data analysis

The details of measuring cell growth inhibition are described elsewhere.[28,33] Briefly, the cells were plated at proper density in 96-well plates in RPMI 1640 with 5% fetal bovine serum and allowed to attach overnight. The cells were exposed to drugs for 48 h, then the cell growth was determined according to the sulforhodamine B assay, described by Skehan et al.[34] Data calculations were made according to the method described previously.[28] Absorbance for the control well (C) and the tests well (T) were measured at 525 nm. Moreover, at time 0 (addition of drugs), absorbance for the test well (T_0) was also measured. Using these measurements, cell growth inhibition (percentage of growth) by each concentration of drug was calculated as: % growth $= 100 \times [(T - T_0)/(C - T_0)]$, when $T > T_0$ and 50% growth inhibition parameter (GI_{50}) was determined. The GI_{50} was calculated as $100 \times [(T - T_0)/(C - T_0)] = 50$. The mean graph, which shows the differential growth inhibition of the drug in the cell line panel, was drawn based on a calculation using a set of GI_{50}.[27,32] To analyze the correlation between the mean graphs of drug A and drug B, the COMPARE computer algorithm was developed according to the method described by Paull et al.[32] Peason correlation coefficients were calculated using the following formula: $\gamma = (\sum(X_i - X_m)(Y_i - Y_m))/(\sum(X_i - X_m)^2 \sum(Y_i - Y_m)^2)^{1/2}$, where X_i and Y_i are log-GI_{50} of drug A and drug B, respectively, against each cell line and X_m and Y_m are the mean values of X_i and Y_i, respectively.

Computer processing of the GI_{50}s produced the mean graph. The $\log GI_{50}$ for each cell line is indicated. One scale represents one logarithm difference. Other calculated values are MG-MID, the mean of $\log GI_{50}$ values for 39 cell lines; Delta, the logarithm of difference between the MG-MID and the $\log GI_{50}$ of the most sensitive cell line; and range, the logarithm of difference between the $\log GI_{50}$ of the most resistant cell line and the $\log GI_{50}$ of the most sensitive one.

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