Synthesis, Antibacterial, and Cytotoxic Evaluation of Certain 7-Substituted Norfloxacin Derivatives

Kuo-Chang Fang,[†] Yeh-Long Chen,[†] Jia-Yuh Sheu,[†] Tai-Chi Wang,[‡] and Cherng-Chyi Tzeng*[,†]

School of Chemistry, Kaohsiung Medical University, Kaohsiung City 807, Taiwan, Republic of China, and Department of Pharmacy, Tajen Institute of Technology, Pingtong 907, Taiwan, Republic of China

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We report herein the synthesis and biological evaluation of two series of 7-substituted norfloxacin derivatives. Most compounds tested in this study demonstrated better activity against methicillin-resistant *Staphylococcus aureus* than norfloxacin. Preliminary in vitro evaluation indicated that the 7-[4-(2-hydroxyiminoethyl)piperazin-1-yl] derivatives **3b**−**e** possess distinct cytotoxicity profiles as compared with their α-methylene-γ-butyrolactone counterparts, **4b**,**e**: i.e., excellent activities against the renal cancer subpanel. Among them, 1-ethyl-6-fluoro-7-{4-[2-(4-chlorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid **(3d)** demonstrated the most significant activities against renal cancer cell lines, with log GI_{50} values of −6.40 against CAK-1, −6.14 against RXF 393, and −7.54 against UO-31, compared with a mean log GI_{50} value of −5.03.

Introduction

Since the discovery of nalidixic acid by Lesher in 1962,[1] a number of analogues have been synthesized, and some of them, in particular the fluoroquinolone derivatives such as norfloxacin,[2] pefloxacin,[3] enoxacin,[4] ofloxacin,[5] and ciprofloxacin,[6] exhibited broader antibacterial spectrum and enhanced potencies. These agents were shown to be specific inhibitors of the bacterial DNA gyrase,[7−9] an enzyme which is responsible for negatively supercoiling covalently closed circular DNA, and also in catenation and decatenation reactions.[10]

The antibacterial activity of fluoroquinolones depends not only on the bicyclic heteroaromatic pharmacophore but also on the nature of the peripheral substituents and their spatial relationship. These substituents exert their influence on antibacterial activity by providing additional affinity for the bacterial enzymes, enhancing the cell penetration, or altering the pharmacokinetics.[11−13] The structure−activity relationships (SAR) of antibacterial quinolones have been the subject of extensive review. In general, the upper portion of the molecule which includes the C-3 carboxy and C-4 keto moieties is required for hydrogen-bonding interactions with DNA bases in the single-stranded regions of duplex DNA created by the action of the enzyme, and therefore it is essential. The lower portion of the molecule (i.e., N-1 and C-8) should be relatively small and lipophilic to enhance self-association.[11−13] Groups at C-5 and C-6 have also been optimized in which an amino and fluoro substituent, respectively, at C-5 and C-6 appear to be the best. The features for substitution at C-7 position were more difficult to formulate; therefore, the structural requirements were not precisely defined. Extensively investigated substituents are piperazin-1-yl and its 4-substituted derivatives.

Herein we report on the synthesis of certain norfloxacin derivatives with an additional functional moiety, such as a 4-hydroxyaminoalkyl on the C-7 piperazin-1-yl group, with the aim of providing extra hydrogen-bonding capacities with the target DNA gyrase and, therefore, to increase potency and broaden the antibacterial spectrum. A number of fluoroquinolones with an oxime or a substituted oxime attached to the pyrrolidine or piperazine ring at C-7 position were also synthesized and evaluated for antibacterial activities.[14−17] Indeed, although fluoroquinolones are generally classified as broad-spectrum antibacterial agents, their activities against the majority of methicillin-resistant staphylococci (especially *Staphylococcus aureus*) are limited. We have also prepared α-methylene-γ-butyrolactone-bearing analogues in hope that the α-methylene-γ-butyrolactone moiety will provide an extra covalent bonding capacity with DNA gyrase.[18−20] Due to structural and functional similarities between bacterial DNA gyrase and mammalian topoisomerase II, the cytotoxicities of these norfloxacin analogues were also evaluated.

Chemistry

Preparations of 6-fluoro-1,4-dihydro-7-[4-(2-hydroxyiminoethyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acids **3a**−**e** and their α-methylene-γ-butyrolactone analogues **4** are described in Scheme 1. Norfloxacin was treated with K_2CO_3 and bromoacetone to provide 6-fluoro-1,4-dihydro-4-oxo-7-[4-(2-oxopropyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**2a**)[21] which was then reacted with hydroxylamine to give exclusively (*E*)-6-fluoro-1,4-dihydro-7-[4-(2-hydroxyiminopropyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acid (**3a**). 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, **3b**−**e** were obtained by hydroxylamination of the respective **2b**−**e** which were synthesized via alkylation of norfloxacin with bromoacetophenone or its 4-substituted derivatives. However, compounds **3b**−**e** were found to be a mixture of *E*- and *Z*-isomers. The

* To whom correspondence should be addressed. Tel: (886) 7-3121101, ext 2198. Fax: (886) 7-3125339. E-mail: tzengch@cc.kmu.edu.tw.
[†] Kaohsiung Medical University.
[‡] Tajen Institute of Technology.

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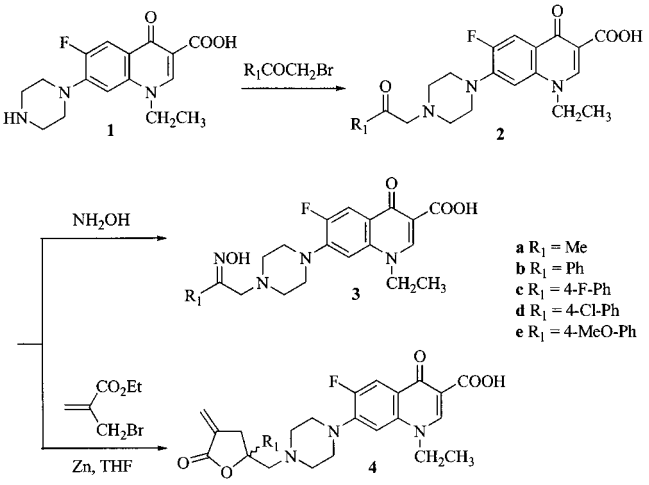
Table 1. In Vitro Antimicrobial Activity of Norfloxacin Derivatives

	MIC (μM)							
organism[a]	**3a**	**3b**	**3c**	**3d**	**3e**	**4b**	**2e**	Nf[b]
E. coli	0.51	0.86	6.38	6.16	1.62	98.9	0.83	0.63
M. ranae	32.0	55.3	>200	>200	104	>200	>200	9.80
P. aeruginosa	16.0	13.8	>200	>200	25.9	>200	13.4	1.22
K. pneumoniae	0.13	0.22	0.21	0.21	0.027	12.4	0.053	0.041
P. vulgaris	2.00	3.45	6.38	6.16	3.23	>200	0.83	0.16
S. aueus M-R	0.51	0.44	2.13	>200	0.41	1.54	6.70	4.89
S. pneumoniae E&A-R	30.0	>200	>200	>200	13.0	nd[c]	>200	19.6
E. faecalis V-R	30.0	>200	>200	>200	3.23	nd	>200	9.80

[a] Organisms selected are as follows: *Escherichia coli, Mycobacterium ranae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus* methicillin-resistant, *Staphylococcus pneumoniae* erythromycin- and ampicillin-resistant, *Enterococcus faecalis* vancomycin-resistant. [b] Nf, norfloxacin. [c] nd, not determined.

Scheme 1



carbon of 1′-CH_2 which is anti to the oxime moiety shifted downfield (δ 61.61 ppm for *E*-**3a** and 61.30 ppm for *E*-**3b**), while that of the syn isomer shifted upfield (δ 52.53 ppm for *Z*-**3b**).[22] The *Z*-form of **3b** was separated by silica gel column chromatography. Preliminary antibacterial assay indicated the same inhibitory activities between the mixture and the pure *Z*-isomer of **3b**, and separation of the respective **3c**−**e** was not attempted. Reformatsky-type condensation of **2b**[21] and **2e** respectively afforded 1-ethyl-6-fluoro-7-{4-[(2,3,4,5-tetrahydro-4-methylene-2-phenyl-5-oxofuran-2-yl)methyl]-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4b**) and its 4-methoxy derivative **4e**.

Results and Discussion

Two series of norfloxacin derivatives prepared for this study were tested in vitro against five susceptible strains and three resistant strains. The minimum inhibitory concentrations (MICs, μM) are presented in Table 1. The data for norfloxacin is included for comparison. The first information obtained in this study is that 6-fluoro-1,4-dihydro-7-[4-(2-hydroxyiminoethyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acids **3a**−**c** are less active than norfloxacin except for the inhibitory activity against methicillin-resistant *S. aureus*. A methyl substituent (**3a**, R_1 = Me) at the C-7 piperazinyl side chain is more favorable than a phenyl (**3b**, R_1 = Ph). Any substitution at the phenyl group of **3b** decreased the antibacterial activities with an exception of **3e** which exhibited the most significant activities against *K. pneumoniae*, methicillin-resistant *S. aureus*, erythromycin- and ampicillin-resistant *S. pneumoniae*, and vancomycin-resistant *E. faecalis*. However, its ketone precursor **2e** is relatively inactive against the growth of resistant strains. The α-methylene-γ-butyrolactone counterpart **4b** was inactive against most of the bacteria tested.

Table 2. Inhibitory Activity of Norfloxacin Derivatives in a Panel of Human Renal Cancer Cell Lines

	log GI_{50} (M)[a]			
compd	CAKI-1	RXF 393	UO-31	mean[c]
3a	>−4.0	>−4.0	−4.40	>−4.0
3b	−4.49	−4.44	−6.51	−4.53
3c	−5.72	−6.77	−6.88	−4.67
3d	−6.40	−6.14	−7.54	−5.03
3e	−5.16	nd[b]	−6.26	−4.50
4b	−5.73	nd	−5.80	−5.47
4e	−5.29	nd	−5.56	−5.19

[a] Data obtained from NCI's in vitro disease-oriented tumor cells screen.[23] [b] nd, not determined. [c] Mean values over all cell lines tested.

These norfloxacin derivatives **2**−**4** were also evaluated in the NCI human cancer cell line panel,[23] and the in vitro inhibitory activity for human renal cancer cell lines are presented in Table 2 as log GI_{50} values (the concentration of drug resulting in inhibition of cell growth to 50% of controls), together with the mean value (the average log GI_{50} value for the compound over all cell lines). Compound **3a** is inactive with the exception of its weak activity against renal UO-31. A more potent cytotoxicity was observed for its phenyl counterpart **3b** (log GI_{50} = −4.53), and the potency was further enhanced by the introduction of an electron-withdrawing substituent at the phenyl (**3c**, log GI_{50} = −4.67; **3d**, log GI_{50} = −5.03). Compounds **4b**,**e** which bear an alkylating α-methylene-γ-butyrolactone, are more cytotoxic than their respective hydroxyimino counterparts **3b**,**e**. However, it was especially surprising that the screening of **3b**−**e** revealed one of the most striking examples of subpanel specificity: excellent inhibitory activities against the renal cancer subpanel. Among them, **3d** demonstrated the most significant activities against renal cancer cell lines, with log GI_{50} values of −6.40 against CAK-1, −6.14 against RXF 393, and −7.54 against UO-31, compared with a mean log GI_{50} value of −5.03. Besides, the renal UO-31 cancer cell was found to be very sensitive to **3b**−**e** with log GI_{50} values of −6.51, −6.88, −7.54, and −6.26, respectively. However, these compounds are relatively inactive against leukemia cancer cells.

Conclusion

Two series of 7-substituted norfloxacin derivatives were synthesized and evaluated for antibacterial and

cytotoxic activities. Preliminary results indicated that most compounds tested in this study demonstrated better activity against methicillin-resistant *S. aureus* than norfloxacin. Among them, **3e** exhibited the most significant activities against *K. pneumoniae*, methicillin-resistant *S. aureus*, erythromycin- and ampicillin-resistant *S. pneumoniae*, and vancomycin-resistant *E. faecalis*. Although the alkylating α-methylene-γ-butyrolactone-bearing derivatives **4b**,**e** are more cytotoxic than their respective hydroxyimino counterparts **3b**,**e**, their cytotoxicity profiles are quite different. Compounds **3b**−**e** possess a distinct renal cancer subpanel specificity.

Experimental Section

Melting points were determined on a Fargo MP-ID melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H 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. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co. The elemental analyses were performed at the Instrument Center of National Science Council at National Cheng-Kung University and National Chung-Hsing University using a Heraeus CHN-O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

General Procedure for the Preparation of 1-Ethyl-6-fluoro-7-{4-[2-(4-substituted-phenyl)-2-oxoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic Acids 2c−**e**. A mixture of norfloxacin (0.5 g, 1.56 mmol), sodium bicarbonate (0.13 g, 1.56 mmol), potassium iodide (0.08 g, 0.5 mmol), and 2-bromo-4′-fluoroacetophenone (0.40 g, 1.86 mmol) in DMF (40 mL) was stirred at room temperature for 3 h, then poured into ice−water (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). The extract was washed with water, dried over Na_2SO_4, and evaporated. Recrystallization from EtOH−$CHCl_3$ (5:1) yielded 1-ethyl-6-fluoro-7-{4-[2-(4-fluorophenyl)-2-oxoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2c**) (61% yield): mp 208 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.41 (t, 3H, J = 7.0 Hz), 2.73 and 3.3 (two m, 8H), 3.94 (s, 2H), 4.58 (q, 2 H, J = 7.0 Hz), 7.18 (d, 1H, J = 7.0 Hz), 7.36 (m, 2H), 7.90 (d, 1H, J = 13.2 Hz), 8.11 (m, 2H), 8.94 (s, 1H), 15.30 (br s, 1H). Anal. ($C_{24}H_{23}F_2N_3O_4$) C, H, N.

1-Ethyl-6-fluoro-7-{4-[2-(4-chlorophenyl)-2-oxoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (2d). The general procedure using 2-bromo-4′-chloroacetophenone gave **2d** (67% yield): mp 207 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.41 (t, 3H, J = 7.2 Hz), 2.73 and 3.29 (two m, 8H), 3.95 (s, 2H), 4.58 (q, 2H, J = 7.2 Hz), 7.19 (d, 1H, J = 7.2 Hz), 7.59 and 8.02 (m, 4H), 7.92 (d, 1H, J = 13.2 Hz), 8.95 (s, 1H), 15.41 (br s, 1H). Anal. ($C_{24}H_{23}ClFN_3O_4\cdot 0.25H_2O$) C, H, N.

1-Ethyl-6-fluoro-7-{4-[2-(4-methoxyphenyl)-2-oxoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (2e). The general procedure using 2-bromo-4′-methoxyacetophenone gave **2e** (75% yield): mp 202 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.41 (t, 3H, J = 7.0 Mz), 2.73 and 3.3 (two m, 8H), 3.85 (s, 3H), 3.88 (s, 2H), 4.58 (q, 2H, J = 7.0 Mz), 7.02−7.2 (m, 3H), 7.87 (d, 1H, J = 13.8 Mz), 8.01 (m, 2H), 8.94 (s, 1H), 15.33 (br s, 1H). Anal. ($C_{25}H_{26}FN_3O_5$) C, H, N.

(*E*)-1-Ethyl-6-fluoro-7-[4-(2-hydroxyiminopropyl)-1-piperazinyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (3a). To a suspension of the 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(2-oxopropyl)-1-piperazinyl]quinoline-3-carboxylic acid[21] (**2a**; 0.38 g, 1 mmol) in absolute methanol (20 mL) was added a solution of hydroxylamine hydrochloride (0.14 g, 2 mmol) and sodium bicarbonate (0.17 g, 2 mmol) in water (2 mL). The mixture was stirred for 20 h at room temperature, then CH_2Cl_2 (50 mL) was added, and the layers were separated. The organic phase was washed successively with water and brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a solid which was purified by flash column chromatography (silica gel, with CH_2Cl_2−MeOH (10:1) as the eluent) and crystallization from CH_2Cl_2−MeOH (5:1) to give **3a** as an off-white amorphous solid (0.28 g, 72%): mp 218 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.42 (t, 3H, J = 7.2 Hz), 1.81 (s, 3H), 2.50 (m, 4H), 3.03 (s, 2H), 3.33 (m, 4H), 4.59 (q, 2H, J = 7.2 Hz), 7.18 (d, 1H, J = 7.3 Hz), 7.91 (d, 1H, J = 13.5 Hz), 8.95 (s, 1H), 10.58 (s, 1H), 15.32 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.19, 14.33, 49.04, 49.47, 49.51, 52.29, 61.61, 105.91, 107.06, 111.12 (J_{CF} = 22.8 Hz), 119.25 (J_{CF} = 7.5 Hz), 137.20, 145.50 (J_{CF} = 10.7 Hz), 148.48, 152.91 (J_{CF} = 247.3 Hz), 153.29, 166.11, 176.16 (J_{CF} = 3.0 Hz). Anal. ($C_{19}H_{23}FN_4O_4\cdot 0.5H_2O$) C, H, N.

General Procedure for the Preparation of 1-Ethyl-6-fluoro-4-oxo-7-{4-[2-hydroxyimino-2-(4-substituted-phenyl)ethyl]-1-piperazinyl}-1,4-dihydroquinoline-3-carboxylic Acids 3b−**e**. To a solution of 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-phenacyl-1-piperazinyl)quinoline-3-carboxylic acid[21] (**2b**; 0.44 g, 1 mmol) in absolute methanol (20 mL) was added a solution of hydroxylamine hydrochloride (0.14 g, 2 mmol) and sodium bicarbonate (0.17 g, 2 mmol) in water (2 mL). The mixture was heated at reflux for 36 h and allowed to cool to room temperature. CH_2Cl_2 (50 mL) was added, and the layers were separated. The organic phase was washed successively with water and brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a solid which was purified by flash column chromatography (silica gel, with CH_2Cl_2−MeOH (10:1) as the eluent) and crystallization from CH_2Cl_2−MeOH (5:1) to give 1-ethyl-6-fluoro-4-oxo-7-[4-(2-hydroxyimino-2-phenylethyl)-1-piperazinyl]-1,4-dihydroquinoline-3-carboxylic acid (**3b**; E/Z = 1:2.2) as an off-white amorphous solid (0.31 g, 68%): mp 203 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.39 (t, 3H, J = 7.2 Hz), 2.64 and 3.25 (two m, 8H), 3.43 and 3.74 (two s, 2 H), 4.56 (q, 2H, J = 7.2 Hz), 7.15 (d, 1H, J = 7.2 Hz), 7.36−7.81 (m, 5H), 7.89 (d, 1H, J = 13.2 Hz), 8.93 (s, 1H), 10.99 (s, *E*-form OH), 11.47 (s, *Z*-form OH), 15.07 (br s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.28, 48.98, 49.43, 49.51, 49.95, 52.16, 52.48, 61.30, 105.88, 105.94, 107.02,0.107.07, 110.76, 111.22, 119.13, 119.28, 126.23, 127.68, 127.95, 128.03, 128.27, 128.36, 138.45, 133.40, 136.03, 137.08, 145.35, 145.56, 148.36, 152.04, 152.93, 155.33, 166.04, 176.04, 176.09. Anal. ($C_{24}H_{25}FN_4O_4\cdot 1.5\ H_2O$) C, H, N.

The *Z*-form of **3b** was separated by silica gel column chromatography with CH_2Cl_2−MeOH (30:1) as the eluent: ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.39 (t, 3H, J = 7.0 Hz), 2.64 and 3.24 (two m, 8H), 3.74 (s, 2 H), 4.5 (q, 2H, J = 7.0 Hz), 7.15 (d, 1H, J = 7.0 Hz), 7.36 (m, 3H), 7.79 (m, 2H), 7.90 (d, 1H, J = 13.3 Hz), 8.93 (s, 1H), 11.47 (s, 1H), 15.28 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.31, 49.06, 49.50, 49.54, 49.98, 52.53, 106.08, 107.06, 111.09 (J_{CF} = 22.8 Hz), 119.30 (J_{CF} = 7.6 Hz), 126.28, 128.09, 128.52, 136.07, 137.18, 145.56 (J_{CF} = 10.6 Hz), 148.48, 152.95 (J_{CF} = 247.3 Hz), 153.02, 166.13, 176.19.

1-Ethyl-6-fluoro-7-{4-[2-(4-fluorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c, E/Z = 1:1.3): 66% yield (starting with 0.5 mmol of **2c**); mp 227 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.39 (t, 3H, J = 7.2 Hz), 2.62 and 3.25 (two m, 8H), 3.44 and 3.73 (two s, 2H), 4.57 (q, 2H, J = 7.2 Hz), 7.15 (d, 1H, J = 7.2 Hz), 7.42−7.83 (m, 4H),7.89 (d, 1H, J = 13.3 Hz), 8.93 (s, 1H), 11.19 (s, *E*-form OH), 11.62 (s, *Z*-form OH). Anal. ($C_{24}H_{24}F_2N_4O_4\cdot 0.5H_2O$) C, H, N.

1-Ethyl-6-fluoro-7-{4-[2-(4-chlorophenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3d, E/Z = 1:1.2): 73% yield (starting with 0.5 mmol of **2d**); mp 222 °C dec; ^{1}H NMR(200 MHz, DMSO-d_6) δ 1.39 (t, 3H, J = 7.0), 2.61 and 3.24 (two m, 8H), 3.44 and 3.73 (two s, 2H), 4.56 (q, 2H, J = 7.0 Hz), 7.24 and 7.78 (two m, 5H), 7.89 (d, 1H, J = 13.4 Hz), 8.93 (s, 1H), 11.10 (s, *E*-form OH), 11.49 (s, *Z*-form OH), 15.35 (br s, 1H). Anal. ($C_{24}H_{24}FClN_4O_4\cdot 0.25H_2O$) C, H, N.

1-Ethyl-6-fluoro-7-{4-[2-hydroxyimino-2-(4-methoxyphenyl)ethyl]-1-piperazinyl}-4-oxo-1,4-dihydroquinoline-

3-carboxylic acid (3e, *E*/*Z* = 1:1.3): 74% yield (starting with 0.5 mmol of **2e**); mp 221 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.39 (t, 3H, J = 7.2 Hz), 2.62 and 3.26 (two m, 8H), 3.42 and 3.71 (two s, 2H), 3.78 (s, 3H), 4.57 (q, 2H, J = 7.2 Hz), 6.92 (m, 2H),7.16 (d, 1H, J = 7.6 Hz),7.72 (m, 2H), 7.89 (d, 1H, J = 13.6 Hz), 8.93 (s, 1H), 10.97 (s, *E*-form OH), 11.28 (s, *Z*-form OH), 15.29 (br s, 1H). Anal. ($C_{25}H_{27}FN_4O_5 \cdot 0.25H_2O$) C, H, N.

1-Ethyl-6-fluoro-7-{4-[(2,3,4,5-tetrahydro-4-methylene-2-phenyl-5-oxofuran-2-yl)methyl]piperazinyl}-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (4b). To a suspension of **2b**[21] (0.44 g, 1 mmol) in dry THF (20 mL) were added activated Zn powder (0.13 g, 2 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl)acrylate (0.26 g, 1.3 mmol). The mixture was refluxed under nitrogen atmosphere for 15 h (TLC monitoring). After cooling, it was poured into an ice-cold 5% HCl solution (100 mL), neutralized with 1.0 N $NaHCO_3$, and extracted with CH_2Cl_2 (60 mL × 3). The CH_2Cl_2 extracts were combined and washed with H_2O, dried (Na_2SO_4), and then evaporated to give a residual solid which was purified by column chromatography on silica gel using CH_2Cl_2−MeOH (10:1). The proper fractions were combined and evaporated to furnish a residual solid which was crystallized from CH_2Cl_2−EtOH (5:1) to afford **4b** (0.42 g, 83% yield): mp 199−200 °C dec; ^{1}H NMR (200 MHz, CF_3COOD) δ 1.71 (t, 3H, J = 7.1 Hz), 3.28−3.75 (m, 7H), 3.98−4.42 (m, 5H), 4.82 (q, 2H, J = 7.1 Hz), 6.02 (br s, 1H,), 6.59 (br s, 1H), 7.39 (d, 1H, 6.6 Hz), 7.53 (m, 5H), 8.27 (d, 1H, J = 12.5 Hz), 9.28 (s, 1H, 2-H). Anal. ($C_{28}H_{28}FN_3O_5 \cdot 0.25H_2O$) C, H, N.

1-Ethyl-6-fluoro-7-{4-{[2,3,4,5-tetrahydro-2-(4-methoxyphenyl)-4-methylene-2-phenyl-5-oxofuran-2-yl]methyl}-piperazinyl}-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid (4e). From **2e** as described for **4b:** 88% yield; mp 191 °C; ^{1}H NMR (200 MHz, CF_3COOD) δ 1.76 (t, 3H, J = 7.0 Hz,), 3.28−3.79 (m, 7H), 4.02−4.42 (m, 8H), 4.84 (q, 2H, J = 7.0 Hz), 6.06 (br s, 1H), 6.62 (br s, 1H), 7.19−7.55 (m, 5H), 8.31 (d, 1H, J = 12.5 Hz), 9.32 (s, 1H) Anal. ($C_{29}H_{30}FN_3O_6$) C, H, N.

In Vitro Antibacterial Assay. **Determination of MIC:** 2 mg of each test compound was dissolved in an appropriate solvent (100% DMSO) and serially diluted with DMSO into the desired testing concentration ranges. Each series of testing solution (0.01 mL) was added into the 48-well plate with 0.99 mL of media broth containing 1−5 × 10^5 CFU/mL testing microorganism. Thus the final maximal concentration of DMSO was 1% and the initial concentration of testing solution was 300 μM. Media used were as follows: nutrient broth (NB, DIFCO) for *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*; Mueller−Hinton broth (DIFCO) for *Staphylococcus aureus*, methicillin-resistant (MRSA) and *Proteus vulgaris*; brain heart infusion broth (BHI, DIFCO) for *Mycobacterium ranae*; tryptic soy broth (DIFCO) containing of 7% calf serum for *Streptococcus pneumoniae* (EM & AM Research Clinical Isolates) and *Enterococcus faecalis* (VRE, Clinical Isolates). The plates were incubated for 20−72 h at 37 °C, then the MIC was determined by visual turbidity readout or by microscope observation of microorganism growth. Vehicle and reference agents were used in every test as the negative and positive controls, and the assays were performed in duplicate.

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Supporting Information Available: Table containing data for inhibition of cancer cell lines for **3a**−**e** and **4b,e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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