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Original article

Synthesis and *in vitro* antibacterial activity of a series of novel gatifloxacin derivatives

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ABSTRACT

A series of novel gatifloxacin (GTFX) derivatives were designed, synthesized and characterized by ^{1}H NMR, ^{13}C NMR, MS and HRMS. These derivatives were evaluated for *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains. Our results reveal that most of the target compounds show good potency in inhibiting the growth of *Staphylococcus aureus* including MRSA and *Staphylococcus epidermidis* including MRSE. Compounds **8**, **14** and **20** have useful activity against all of the tested Gram-positive and Gram-negative strains (MICs: 0.06–4 μg/mL). In particular, **20** possessing a broad antimicrobial spectrum (MICs: 0.06–1 μg/mL) was found to be 2–32-folds more potent than the reference drug levofloxacin and parent GTFX against *Pseudomonas aeruginosa*.

1. Introduction

Since the discovery of norfloxacin by Koga et al. in the early 1980s [1], fluoroquinolones have become an important class of antibiotics for the treatment of various bacterial infections in both community and hospital settings. These antibiotics exert their antimicrobial effect by inhibition of two type II bacterial topoisomerase enzymes, DNA gyrase and topoisomerase IV, which are essential cellular enzymes that catalyze the double strand breakage of DNA to allow strand passage and thereby control the topology and conformation of DNA [2–4].

Structure–activity relationship (SAR) studies of fluoroquinolone antibacterial agents showed that the basic group at the C-7 position is the most adaptable site for chemical change and an area that greatly influences their potency, spectrum and safety [5,6]. Piperazinyl and pyrrolidinyl type side chains have been proven to be the optimal substituents. For example, many of the early fluoroquinolones (ciprofloxacin and ofloxacin), have a piperazinyl group at the C-7 position, and introduction of the noted pyrrolidine derivatives to the fluoroquinolones (trovafloxacin and moxifloxacin/MXFX) resulted in a dramatic improvement of Gram-positive activity.

Considering that the activity of most fluoroquinolones currently on the market or under development against clinically important Gram-positive pathogens, including Staphylococci, Streptococci, and Enterococci, is relatively moderate, which has not only limited their use in infections caused by these strains, but is also believed to be one of the reasons for the rapidly developing quinolone resistance. Thus, recent efforts have been directed toward the synthesis of new quinolones that can provide improved Gram-positive antibacterial activity, while retaining the good Gram-negative activity of early fluoroquinolones [7,8].

Recently, oxime-functionalized pyrrolidine derivatives as novel C-7 substituents have attracted great attention and led to the discovery of some new fluoroquinolones, such as gemifloxacin, zabofloxacin (DW224a) and DW286. All of the three compounds show excellent (especially Gram-positive) antibacterial activity and pharmacokinetic profiles [9–11].

It was reported that linking a hydroxyl group to the nitrogen atom of the C-7 side chain of norfloxacin or ciprofloxacin causes increased *in vitro* and *in vivo* antibacterial activity [12,13]. Moreover, we had previously synthesized a series of MXFX derivatives and found the compound with an 3-oxobutyl group on the nitrogen atom of the C-7 side chain of MXFX, has better *in vitro* and *in vivo* antibacterial activity than the corresponding hydroxyl analog [14].

These research results intensified our interest, it was decided to make structural modifications on gatifloxacin (GTFX, Scheme 1), by introduction of diversified substituents to the nitrogen atom of the piperazine ring. These substituents include hydroxyl or methylene-/ethylene-linked cyano, carbalkoxy, acyl, oxime, alkyloxime and hydrazone. In order to clarify rationale of the design idea, we also initially conducted molecular docking to predict the binding

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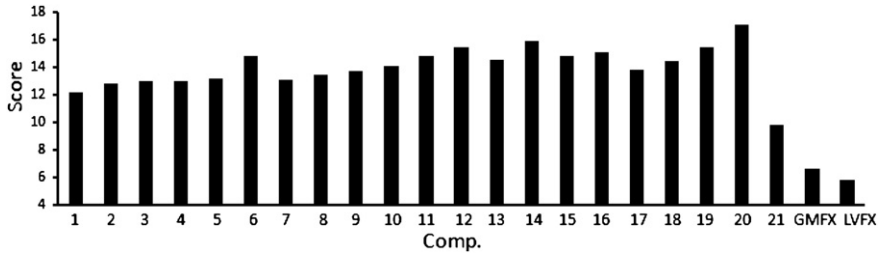


Fig. 1. Surflex-Dock scores for the novel quinolones **1**–**21**.

affinity of the target quinolone–enzyme–DNA complexes [15]. The scores (Fig. 1) reflecting the fitting quality of ligand–receptor complexes show that all of the designed derivatives (**1**–**21**) are much more potent than LVFX and GTFX against *Streptococcus pneumoniae*. We report herein the synthesis of a series of novel GTFX derivatives and their antibacterial activity. A SAR study is also explored to facilitate the further development of the fluoroquinolones.

2. Results and discussion

2.1. Chemistry

Synthetic pathways to the novel GTFX derivatives **1**–**21** are depicted in Scheme 1. Nucleophilic substitution or addition of GTFX with requisite chlorinated or propenyl reactants in the presence of triethylamine gave the target compounds **1**–**6** (53–82%), and the ketones **7**, **8** were prepared as the same procedure. Condensation of **7**, **8** with substituted amines formed Schiff's bases **9**–**20** (52–91%) in refluxing methanol, and as far as the amine hydrochlorides were concerned, $NaHCO_3$ was additionally acquired for neutralization. Introduction of a hydroxyl group to the C-7 side chain of GTFX by oxidation rearrangement of the ketone **8** in H_2O_2 (30%)–NaOH (2 N) system yields **21** (63%). Table 1 shows structures, purities, melting points and the toxicity (CC_{50}) of the GTFX derivatives.

Since the imino moiety can exist in the E or Z configuration, it was necessary to determine the geometries of the Schiff's bases **9**–**20**. However, it was a pity that we were not successful in preparing X-ray quality single crystals.

2.2. Pharmacology

The GTFX derivatives **1**–**21** were evaluated for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques [16]. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth. MICs of the synthesized compounds along with the standard drugs levofloxacin (LVFX) and GTFX for comparison are reported in Table 2.

Generally, all of the target compounds **1**–**21** have potent antibacterial activity against the tested strains. Compouds **8**, **10**, **12**, **14**, **20** and **21** show good potency in inhibiting the growth of *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA) and *Staphylococcus epidermidis* including methicillin-resistant *S. epidermidis* (MRSE) (MICs: 0.06–0.5 μg/mL). Among them, compounds **8**, **14** and **20** possess also useful activity against the other Gram-positive strains including *S. pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium* and all of the tested Gram-negative strains including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (MICs: 0.5–4 μg/mL).

The most active compound **20** has a broad antimicrobial spectrum against all of the tested strains (MICs: 0.06–1 μg/mL) which is generally better than the reference drug LVFX and parent GTFX (MICs: 0.06–16 μg/mL). In particular, **20** (MICs: 0.25–0.5 μg/mL) is

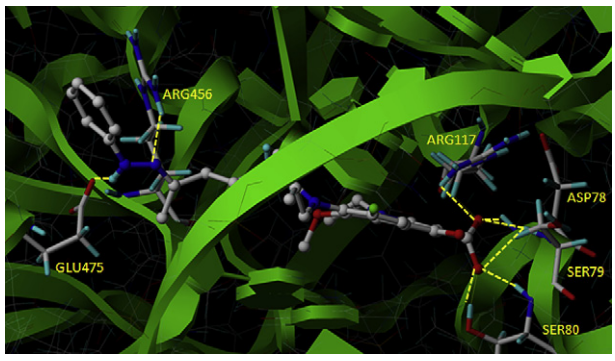
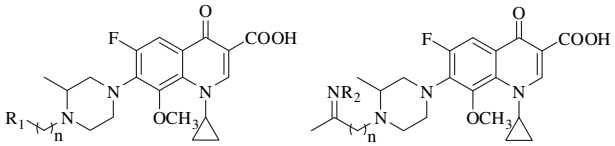


Fig. 2. Predicted three-dimensional conformations of compound **20** docked in 3FOF.

Table 1
Structures, melting points, purities and cytotoxicity of GTFX derivatives **1**–**21**.



Compd.	R_1	R_2	n	m.p. (°C)	Purities (%)	CC_{50}[a] (μg/mL)
1	CN	–	1	181–183	99.41	353.55
2	CN	–	2	180–182	99.45	500
3	$COOCH_3$	–	1	195–197	98.11	500
4	$COOCH_3$	–	2	152–153	94.08	>1000
5	$COOC_2H_5$	–	1	184–186	99.37	1000
6	$COOC_2H_5$	–	2	141–142	99.45	NT[b]
7	$COCH_3$	–	1	146–148	97.33	500
8	$COCH_3$	–	2	134–136	96.63	9.84
9	–	OCH_3	1	151–153	96.54	49.61
10	–	OC_2H_5	1	144–146	99.96	31.25
11	–	$NHCONH_2$	1	216–218	93.31	500
12	–	$NHCOCH_3$	1	205–207	95.85	707.11
13	–	$NHCOC_5H_4N$	1	178–180	95.54	250
14	–	NHC_6H_5	1	161–163	96.56	11.05
15	–	OH	1	211–213	97.56	176.78
16	–	OH	2	198–200	95.67	44.19
17	–	OCH_3	2	180–182	96.95	157.49
18	–	OC_2H_5	2	177–179	97.82	88.39
19	–	$NHCONH_2$	2	181–183	93.56	31.25
20	–	NHC_6H_5	2	176–178	96.54	62.5
21	OH	–	0	227–229	97.12	19.69

[a] CC_{50}: The 50% cellular cytotoxic concentration.
[b] Not tested.

Table 2
In vitro antibacterial activity of GTFX derivatives **1**–**21** against selected strains.

Strains	MIC(μg/mL)																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	LVFX	GTFX
S.a.	1	2	8	8	4	128	0.25	0.06	0.5	0.125	4	0.25	2	0.125	0.25	2	0.5	64	0.5	0.06	0.125	0.25	0.125
MRSA	1	4	128	4	>128	>128	16	0.5	>128	0.25	2	0.25	32	0.125	>128	32	>128	16	2	0.25	0.25	0.06	0.125
MSSA	1	4	32	4	4	>128	1	0.25	0.25	0.25	>128	0.25	2	0.125	0.25	32	2	128	1	0.125	0.25	0.25	0.06
MRSE	0.25	2	32	>128	4	>128	2	0.25	0.5	0.25	2	0.25	2	0.25	0.25	0.25	2	0.5	1	0.06	0.25	0.5	0.25
MSSE−1	0.25	0.25	32	>128	4	>128	0.25	0.25	0.5	0.25	2	0.25	0.25	0.25	0.25	0.25	0.5	0.5	0.25	0.06	0.25	0.06	0.25
MSSE−2	0.25	2	32	>128	4	>128	0.25	0.25	4	0.25	1	0.25	0.25	0.25	0.25	0.25	1	0.5	0.25	0.25	0.25	2	8
*S.p.*1	8	32	>128	>128	>128	>128	32	4	64	>128	64	8	32	0.25	128	32	>128	128	8	0.5	16	16	4
*S.p.*2	1	4	>128	>128	4	>128	2	0.25	2	2	2	0.25	4	0.25	0.25	4	>128	>128	2	0.25	2	4	0.25
*E.fm.*1	8	32	>128	>128	>128	>128	32	4	>128	>128	>128	16	64	4	>128	64	>128	>128	8	0.5	16	4	2
*E.fm.*2	8	32	>128	>128	>128	>128	32	4	>128	>128	>128	16	64	4	>128	64	>128	>128	8	0.5	16	8	4
*E.fs.*1	8	64	>128	>128	>128	>128	32	4	>128	>128	>128	16	128	4	>128	>128	>128	>128	8	0.5	16	8	4
*E.fs.*2	8	32	>128	>128	>128	>128	128	4	>128	>128	>128	16	128	4	>128	>128	>128	>128	8	1	16	8	2
*E.co.*1	4	16	128	>128	128	128	8	0.5	64	128	>128	8	16	0.5	128	16	128	>128	2	0.25	4	2	2
*E.co.*2	8	32	>128	>128	>128	>128	128	4	>128	>128	>128	16	128	4	>128	64	>128	>128	8	1	16	8	4
*E.co.*3	8	64	>128	>128	>128	>128	32	0.5	>128	>128	>128	16	64	0.25	>128	64	>128	>128	4	1	16	4	4
*K.p.*1	2	32	128	>128	>128	>128	16	0.5	>128	>128	>128	0.25	16	0.25	>128	64	>128	128	1	0.5	2	16	0.125
*K.p.*2	2	32	>128	>128	>128	>128	2	0.5	>128	>128	>128	4	32	0.125	>128	8	>128	>128	2	1	16	8	2
*P.a.*1	2	8	128	>128	128	128	8	1	32	>128	>128	4	4	2	32	8	128	>128	4	0.5	4	4	1
*P.a.*2	0.25	4	128	>128	>128	>128	2	0.25	64	>128	64	0.25	4	0.25	32	4	128	128	0.25	0.5	2	16	8
*P.a.*3	32	128	128	>128	>128	>128	128	4	>128	>128	>128	64	128	4	>128	>128	>128	>128	16	0.5	32	8	4
*P.a.*4	2	16	>128	>128	>128	>128	16	0.5	>128	>128	>128	2	32	0.25	>128	8	>128	>128	2	0.25	4	1	1

LVFX: Levofloxacin; GTFX: Gatifloxacin.
S.a., *Staphylococcus aureus* ATCC25923; MRSA, Methicillin-resistant *Staphylococcus aureus* 08-1; MSSA, Methicillin-sensitive *Staphylococcus aureus* 08-1; MRSE, Methicillin-resistant *Staphylococcus epidermidis* 09-4; MSSE-1, Methicillin-sensitive *Staphylococcus epidermidis* 09-3; MSSE-2, Methicillin-sensitive *Staphylococcus epidermidis* 09-6; *S.p.*1, *Streptococcus pneumonia* 08-2; *S.p.*2, *Streptococcus pneumonia* 08-4; *E.fm.*1, *Enterococcus faecium* 08-2; *E.fm.*2, *Enterococcus faecium* 08-7; *E.fs.*1, *Enterococcus faecalis* 08-10; *E.fs.*2, *Enterococcus faecalis* 08-12; *E.co.*1, *Escherichia coli* ATCC25922; *E.co.*2, *Escherichia coli* 08-21; *E.co.*3, *Escherichia coli* 08-22; *K.p.*1, *Klebsiella pneumoniae* 09-22; *K.p.*2, *Klebsiella pneumoniae* 09-23; *P.a.*1, *Pseudomonas aeruginosa* ATCC27853; *P.a.*2, *Pseudomonas aeruginosa* 09-32; *P.a.*3, *Pseudomonas aeruginosa* 09-33; *P.a.*4, *Pseudomonas aeruginosa* 09-34.

2–32-folds more potent than the two reference drugs (MICs: 1–16 μg/mL) against *P. aeruginosa*.

Some compounds were further examined for cytotoxicity (CC_{50}: The 50% cellular cytotoxic concentration) in a mammalian Vero cell line. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan product and the results are reported in Table 1. The tested compounds showed CC_{50} values ranging from 9.84 to >1000 μg/mL.

3. Conclusion and discussion

In summary, a series of novel GTFX derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains. Compounds **8**, **14** and **20** have good activity against all of the tested Gram-positive and Gram-negative strains (MICs: 0.06–4 μg/mL). It is worth noting that the most active compound **20** (MICs: 0.25–0.5 μg/mL) is 2–32-folds more potent than the two reference drugs against *P. aeruginosa*.

Overall the tested activity of the GTFX derivatives (except for **14** and **20**) against *S. pneumoniae* is not in agreement with what we predicted based only on inhibition of topoisomerase IV. It suggests that both of the topoisomerase IV and DNA gyrase in Gram-positive bacteria could be the targets of the fluoroquinolones.

Antibacterial activity of the GTFX derivatives is dependant mainly on the substituents on the nitrogen atom of the C-7 side chain. The relative contribution of the substituents to antibacterial activity is as follows: 1) phenylhydrazone > ketone > semicarbazone > oxime > methyloxime > ethyloxime, as for the 3-oxo/imino butyl groups ($n = 2$); 2) phenylhydrazone > acetylhydrazone > ketone ≈ ethyloxime > oxime > isonicotinoylhydrazone ≈ methyloxime > semicarbazone, as for 2-oxo/imino propyl groups ($n = 1$). In addition, antibacterial activity of the remaining groups is in the order: hydroxy > cyanomethyl > cyanoethyl > (ethoxyl carbonyl)methyl > (methoxylcarbonyl)methyl ≈ (2-methoxylcarbonyl)>ethyl > (2-ethoxylcarbonyl)ethyl.

4. Experimental protocols

4.1. Chemistry

Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury-400 or a Varian NMR-600 spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane as an internal standard. Fast Atom Bombardment (FAB) mass spectra and high-resolution mass spectra (HRMS) were obtained on a MICROMASS AutoSpec Ultima-TOF mass spectrometer. Chemical purities were determined by HPLC using Agilent Technologies 1200 Series HPLC system consisting of a G1311A quaternary pump, G1329A auto sampler, G1316A oven, and G1315D diode array detector. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F_{254}).

4.2. Synthesis

4.2.1. General procedure for the preparation of compounds ***1***–***8***

A mixture of 1-cyclopropyl-6-fluoro-8-methoxyl-7-(3-methyl-1-piperidin-1-yl)-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid (gatifloxacin, 10.0 mmol), chlorinated or propenyl reactants (20.0 mmol) and triethylamine (20.0 mmol) in anhydrous ethanol (25 mL) was stirred for 5–8 h at 50 °C under an atmosphere of nitrogen. The precipitate obtained was filtered and recrystallized from methanol to give the title compounds **1**–**8** as off-white solids.

*4.2.1.1. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(cyanomethyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **1***. 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.99–1.03 (2H, m, cyclopropyl CH_2), 1.19–1.25 (5H, m, CH_3, cyclopropyl CH_2), 2.88–2.98 (3H, m), 3.16–3.22 (1H, m), 3.44–3.61 (4H, m), 3.82 (3H, s, OCH_3), 3.97–4.05 (2H, m), 7.89 (1H, d, $J = 12.0$ Hz, C_5-H), 8.82 (1H, s, C_2-H), 14.68 (1H, s, COOH). ^{13}C NMR (600 MHz, DMSO-d_6) δ ppm: 8.90, 8.95, 15.95, 40.75, 41.79, 50.08, 52.20, 54.24, 56.54, 62.82, 106.48 (d, $J = 23$ Hz), 106.52, 115.28, 120.98 (d, $J = 9$ Hz), 134.06, 138.67 (d, $J = 12$ Hz), 145.86, 150.51, 154.61 (d, $J = 247$ Hz), 165.58, 176.21.FAB-MS: *m/z* 415 $(M + H)^+$. HRMS-FAB: *m/z* Calcd. for $C_{21}H_{24}FN_4O_4$ $(M + H)^+$: 415.1782; Found 415.1795.

*4.2.1.2. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(2-cyanoethyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **2***. 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.10–1.22 (7H, m, 2 × cyclopropyl CH_2, CH_3), 2.55–3.45 (11H, m), 3.76 (3H, s, OCH_3), 3.99–4.04 (1H, m), 7.84 (1H, d, $J = 12.0$ Hz, C_5-H), 8.80 (1H, s, C_2-H), 14.75 (1H, s, COOH). FAB-MS: *m/z* 429 $(M + H)^+$. HRMS-FAB: *m/z* Calcd. for $C_{22}H_{26}FN_4O_4$ $(M + H)^+$: 429.1938; Found 429.1924.

*4.2.1.3. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(2-methoxy-2-oxoethyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **3***. 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.99–1.22 (7H, m, 2 × cyclopropyl CH_2, CH_3), 2.96–3.12 (4H, m), 3.41–3.53 (5H, m), 3.75, 3.77 (6H, 2s, 2 × OCH_3), 4.00–4.03 (1H, m), 7.86 (1H, d, $J = 12.0$ Hz, C_5-H), 8.80 (1H, s, C_2-H), 14.76 (1H, s, COOH). ^{13}C NMR (600 MHz, DMSO-d_6) δ ppm: 8.88, 8.94, 15.87, 40.77, 50.55, 51.07, 51.84, 53.83, 54.14, 57.14, 62.87, 106.47 (d, $J = 9$ Hz), 106.50, 120.74 (d, $J = 9$ Hz), 134.12, 138.99 (d, $J = 12$ Hz), 145.75, 150.47, 155.45 (d, $J = 248$ Hz), 165.62, 170.92, 176.27. FAB-MS: *m/z* 448 $(M + H)^+$. HRMS-FAB: *m/z* Calcd. for $C_{22}H_{27}FN_3O_6$ $(M + H)^+$: 448.1884; Found 448.1854.

*4.2.1.4. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(3-methoxy-3-oxopropyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **4***. 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.96–1.03 (2H, m, cyclopropyl CH_2), 1.12–1.28 (5H, m, CH_3, cyclopropyl CH_2), 2.51–2.55 (3H, m), 2.64–2.79 (2H, m), 2.90–3.18 (3H, m), 3.37–3.42 (3H, m), 3.70, 3.74 (6H, 2s, 2 × OCH_3), 4.00–4.03 (1H, m), 7.84 (1H, d, $J = 12.0$ Hz, C_5-H), 8.80 (1H, s, C_2-H), 14.78 (1H, ;s, COOH). ^{13}C NMR (600 MHz, DMSO-d_6) δ ppm: 8.87, 8.97, 15.37, 30.80, 40.75, 48.40, 50.53, 50.70, 51.24, 54.36, 56.99, 62.88, 106.39, 106.47 (d, $J = 24$ Hz), 106.48, 120.66 (d, $J = 9$ Hz), 134.07, 139.00 (d, $J = 12$ Hz), 145.68, 150.40, 155.32 (d, $J = 249$ Hz), 165.57, 172.55, 176.22. FAB-MS: *m/z* 462 $(M + H)^+$. HRMS-FAB: *m/z* Calcd. for $C_{23}H_{29}FN_3O_6$ $(M + H)^+$: 462.2040; Found 462.2047.

*4.2.1.5. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(2-ethoxy-2-oxoethyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **5***. 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.99–1.24 (7H, m, 2 × cyclopropyl CH_2, CH_3), 1.30 (3H, t, $J = 7.0$ Hz, $OCH_2\underline{CH_3}$), 2.98–3.13 (4H, m), 3.40–3.50 (5H, m), 3.77 (3H, s, OCH_3), 4.01–4.03 (1H, m), 4.20 (2H, q, $J = 7.0$ Hz, $O\underline{CH_2}CH_3$), 7.84 (1H, d, $J = 12.0$ Hz, C_5-H), 8.79 (1H, s, C_2-H), 14.76 (1H, s, COOH). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 9.41, 9.51, 14.24, 16.17, 40.50, 50.82, 52.56, 54.61, 54.86, 57.40, 60.49, 62.53, 107.61, 108.02 (d, $J = 24$ *Hz*), 121.57 (d, $J = 9$ *Hz*), 133.89, 139.31 (d, $J = 11$ *Hz*), 145.28, 149.76, 155.98 (d, $J = 252$ *Hz*), 166.68, 170.55, 176.92. FAB-MS: *m/z* 462 $(M + H)^+$. HRMS-FAB: *m/z* Calcd. for $C_{23}H_{29}FN_3O_6$ $(M + H)^+$: 462.2040; Found 462.2032.

*4.2.1.6. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(3-ethoxy-3-oxopropyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **6***. 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.96–1.21

(7H, m, 2 × cyclopropyl CH_2, CH_3), 1.28 (3H, t, J = 7.0 Hz, $OCH_2\underline{CH_3}$), 2.51–2.52 (3H, m), 2.65–2.77 (2H, m), 2.92–3.15 (3H, m), 3.37–3.42 (3H, m), 3.74 (3H, s, OCH_3), 4.00–4.03 (1H, m), 4.16 (2H, q, J = 7.0 Hz, $O\underline{CH_2}CH_3$), 7.84 (1H, d, J = 12.0 Hz, C_5-H), 8.79 (1H, s, C_2-H), 14.78 (1H, s, COOH). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 9.44, 9.59, 14.23, 15.82, 31.38, 40.48, 48.88, 50.81, 51.42, 55.11, 57.31, 60.56, 62.68, 107.80, 108.13 (d, J = 23 Hz), 121.70, 133.95, 139.25, 145.21, 149.84, 155.95 (d, J = 250 Hz), 166.72, 172.56, 177.00. FAB-MS: m/z 476 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{24}H_{31}FN_3O_6$ $(M + H)^+$: 476.2197; Found 476.2181.

4.2.1.7. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(2-oxopropyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***7***. 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.97–1.00 (2H, m, cyclopropyl CH_2), 1.10 (3H, d, J = 6.4 Hz, CCH3), 1.18–1.24 (2H, m, cyclopropyl CH_2), 2.20 (3H, s, $COCH_3$), 2.67–2.90 (3H, m), 3.11–3.19 (2H, m), 3.40–3.59 (4H, m), 3.77 (3H, s, OCH_3), 4.00–4.04 (1H, m), 7.86 (1H, d, J = 12.0 Hz, C_5-H), 8.80 (1H, s, C_2-H), 14.77 (1H, s, COOH). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 9.43, 9.56, 16.11, 27.80, 40.50, 50.72, 52.97, 55.66, 57.29, 62.62, 63.90, 107.73, 108.13 (d, J = 23.5 *Hz*), 121.75, 133.92, 139.22, 145.30, 149.82, 155.98 (d, J = 252 *Hz*), 166.70, 176.99. FAB-MS: m/z 432 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{22}H_{27}FN_3O_5$ $(M + H)^+$: 432.1935; Found 432.1905.

4.2.1.8. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(3-oxobutyl)-3-methylpiperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***8***. 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.96–1.24 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 2.20 (3H, s, $COCH_3$), 2.48–2.68 (5H, m), 2.90–3.16 (3H, m), 3.37–3.42 (3H, m), 3.74 (3H, s, OCH_3), 4.00–4.03 (1H, m), 7.83 (1H, d, J = 12.0 Hz, C_5-H), 8.78 (1H, s, C_2-H), 14.77 (1H, br, COOH). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 9.42, 9.58, 15.73, 30.30, 40.36, 40.48, 47.96, 50.78, 51.44, 55.46, 57.32, 62.66, 107.72, 108.10 (d, J = 23 Hz), 121.66, 133.94, 139.40, 145.23, 149.82, 155.96 (d, J = 249 Hz), 166.70, 176.97, 207.78. FAB-MS: m/z 446 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{23}H_{29}FN_3O_5$ $(M + H)^+$: 446.2091; Found 446.2089.

4.2.2. General procedure for the preparation of compounds ***9–20***

To a stirring solution of **7**, **8** (6.50 mmol) dissolved in methanol (25 mL) was added dropwise a solution of substituted amine hydrochlorides (10.0 mmol) and sodium bicarbonate (10.0 mmol) in water (15 mL) at room temperature. The reaction mixture was heated to refluxing and stirred for 2–3 h and concentrated under reduced pressure. The residue was purified via silica gel column chromatography (chloroform/methanol, 10:1, v/v) to give the title compounds **9**–**11** and **15**–**19** as off-white solids.

A refluxing mixture of **7**, **8** (2.32 mmol), substituted amines (3.48 mmol) in methanol (6.50 mL) was stirred for 4–6 h and concentrated under reduced pressure. The residue was purified via silica gel column chromatography (chloroform/methanol, 10:1, v/v) to give the title compounds **12**–**14** and **20** as off-white solids.

4.2.2.1. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(methoxyimino) propyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***9***. 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$+D_2O) δ_H 0.97–1.26 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 1.92 (3H, s, CH_3), 2.39–2.43 (1H, m), 2.64–2.85 (3H, m), 3.08–3.13 (1H, m), 3.36–3.50 (4H, m), 3.76, 3.87 (6H, 2s, 2 × OCH_3), 3.99–4.03 (1H, m), 7.87 (1H, d, J = 12.0 Hz, C_5-H), 8.81 (1H, s, C_2-H), 14.79 (1H, s, COOH). FAB-MS: m/z 461 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{23}H_{30}FN_4O_5$ $(M + H)^+$: 461.2200; Found 461.2234.

4.2.2.2. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(ethoxyimino) propyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***10***. 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.96–1.23 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 1.26 (3H, t, J = 7.0 Hz, $OCH_2\underline{CH_3}$), 1.92 (3H, s, CH_3), 2.39–2.44 (1H, m), 2.63–2.66 (1H, m), 2.83–2.86 (2H, m), 3.08–3.13 (1H, m), 3.37–3.50 (4H, m), 3.76 (3H, s, OCH_3), 4.00–4.03 (1H, m), 4.10 (2H, q, J = 7.0 Hz, $O\underline{CH_2}CH_3$), 7.85 (1H, d, J = 12.0 Hz, C_5-H), 8.80 (1H, s, C_2-H), 14.79 (1H, s, COOH). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 9.41, 9.54, 13.13, 14.63, 15.91, 40.50, 50.93 51.39, 55.91, 57.46, 57.70, 62.43, 68.98, 107.63, 108.08 (d, J = 23 Hz), 121.57, 133.89, 139.50, 145.25, 149.77, 155.48, 156.03 (d, J = 250 Hz), 166.70, 176.93. FAB-MS: m/z 475 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{24}H_{32}FN_4O_5$ $(M + H)^+$: 475.2357; Found 475.2362.

4.2.2.3. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(carbamoylhydrazono)propyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***11***. 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.97–1.26 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 1.91 (3H, s, CH_3), 2.39–2.45 (1H, m), 2.64–2.89 (3H, m), 3.08–3.14 (1H, m), 3.38–3.57 (4H, m), 3.76 (3H, s, OCH_3), 4.00–4.03 (1H, m), 7.51 (1H, s, NH), 7.87 (1H, d, J = 12.0 Hz, C_5-H), 8.81 (1H, s, C_2-H), 14.76 (1H, s, COOH). ^{13}C NMR (600 MHz, DMSO-d_6) δ ppm: 8.86, 8.95, 14.18, 15.33, 40.75, 50.54, 51.14, 55.37, 57.00, 60.35, 62.88, 106.50, 106.62, 120.68 (d, J = 9 Hz), 134.12, 139.03 (d, J = 12 Hz), 145.72, 147.37, 150.47, 155.47 (d, J = 247 Hz), 157.13, 165.63, 176.27. FAB-MS: m/z 489 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{23}H_{30}FN_6O_5$ $(M + H)^+$: 489.2262; Found 489.2250.

4.2.2.4. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(acetylhydrazono) propyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***12***. 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.97–1.24 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 1.91 (3H, s, CH_3), 2.28 (3H, s, $COCH_3$), 2.45–2.49 (1H, m), 2.69–2.96 (3H, m), 3.08–3.13 (1H, m), 3.35–3.57 (4H, m), 3.77 (3H, s, OCH_3), 4.02–4.03 (1H, m), 7.87 (1H, d, J = 12.0 Hz, C_5-H), 8.28 (1H, s, NH), 8.81 (1H, s, C_2-H), 14.77 (1H, s, COOH). FAB-MS: m/z 488 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{24}H_{31}FN_5O_5$ $(M + H)^+$: 488.2309; Found 488.2315.

4.2.2.5. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(iso-nicotinoylhydrazono)propyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***13***. 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.97–1.30 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 2.18 (3H, s, CH_3), 2.50–2.66 (1H, m), 2.95–3.28 (4H, m), 3.38–3.51 (3H, m), 3.63 (3H, s, OCH_3), 3.75–3.81 (1H, m), 3.95–3.98 (1H, m), 7.89 (1H, d, J = 11.6 Hz, C_5-H), 7.77–7.78 (2H, m, pyridine-2H), 8.79–8.81 (2H, m, pyridine-2H), 8.82 (1H, s, C_2-H), 13.42 (1H, s, NH), 14.59 (1H, s, COOH). FAB-MS: m/z 551 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{28}H_{32}FN_6O_5$ $(M + H)^+$: 551.2418; Found 551.2418.

4.2.2.6. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(phenylhydrazono) propyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***14***. 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.95–1.27 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 1.97 (3H, s, CH_3), 2.44–3.18 (5H, m), 3.42–3.64 (4H, m), 3.78 (3H, s, OCH_3), 3.99–4.04 (1H, m), 6.84–6.87 (1H, m, Ph-1H), 7.01–7.23 (2H, m, Ph-2H), 7.25–7.26 (2H, m, Ph-2H), 7.89 (1H, d, J = 12.0 Hz, C_5-H), 8.81 (1H, s, C_2-H), 14.79 (1H, s, COOH). FAB-MS: m/z 522 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{28}H_{33}FN_5O_4$ $(M + H)^+$: 522.2517; Found 522.2509.

4.2.2.7. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(hydroxyimino) propyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***15***. 91% yield. 1H NMR (400 MHz, C_5D_5N) δ_H 0.97–1.03 (4H, m, 2 × cyclopropyl CH_2), 1.16 (3H, d, J = 6.0 Hz, CCH_3), 2.26 (3H, s, CH_3), 2.52–2.66 (1H, m), 2.75–2.77 (1H, m), 3.00–3.25 (3H, m), 3.37–3.49 (4H, m), 3.73 (3H, s, OCH_3), 3.97–3.98 (1H, m), 8.06 (1H, d, J = 12.0 Hz, C_5-H), 8.88 (1H, s, C_2-H), 12.89 (1H, br, COOH). ^{13}C NMR (600 MHz, C_5D_5N) δ ppm: 9.50, 9.60, 12.92, 15.64, 19.23, 41.00, 50.89, 51.77, 56.59, 57.31, 58.25, 62.83, 107.93,

108.14, 122.08, 134.40, 139.60, 146.05, 150.23, 154.07, 156.79 (d, $J = 247$ Hz), 166.70, 177.42. FAB-MS: m/z 447 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{22}H_{28}FN_4O_5$ $(M + H)^+$: 447.2044; Found 447.2027.

4.2.2.8. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[3-(hydroxyimino) butyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***16***. 70% yield. ^{1}H NMR (400 MHz, C_5D_5N) δ_H 0.96–1.14 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 2.13 (3H, s, CH_3), 2.49–2.65 (5H, m), 2.94–3.12 (3H, m), 3.12–3.40 (3H, m), 3.71 (3H, s, OCH_3), 3.96–3.99 (1H, m), 8.06 (1H, d, $J = 12.0$ Hz, C_5-H), 8.88 (1H, s, C_2-H), 12.47 (1H, s, NOH), 15.47 (1H, s, COOH). FAB-MS: m/z 461 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{23}H_{30}FN_4O_5$ $(M + H)^+$: 461.2200; Found 461.2210.

4.2.2.9. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[3-(methoxyimino) butyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***17***. 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.01–1.64 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 1.90 (3H, s, CH_3), 2.73–3.01 (2H, m), 3.09–3.62 (7H, m), 3.83, 3.86 (6H, 2s, 2 × OCH_3), 4.00–4.12 (2H, m), 4.26–4.49 (1H, m), 7.85 (1H, d, $J = 12.0$ Hz, C_5-H), 8.82 (1H, s, C_2-H), 14.53 (1H, s, COOH). ^{13}C NMR (600 MHz, $CDCl_3$) δ ppm: 9.57, 9.67, 14.48, 15.16, 29.48, 40.37, 47.20, 49.30, 52.11, 54.56, 59.92, 61.59, 64.06, 108.25, 108.16, 122.84, 133.99, 137.45, 145.68, 150.17, 150.23, 153.50 (d, $J = 250$ Hz), 166.32, 176.86. FAB-MS: m/z 475 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{24}H_{32}FN_4O_5$ $(M + H)^+$: 475.2357; Found 475.2367.

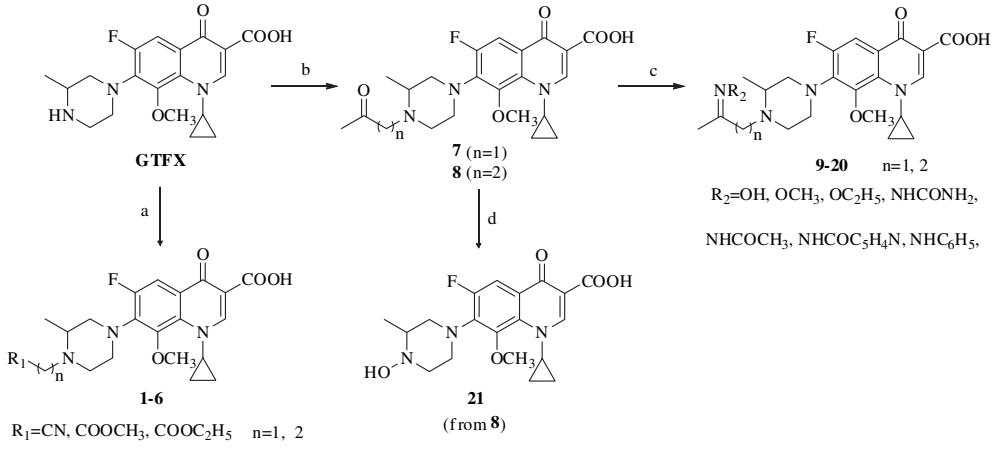
4.2.2.10. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[3-(ethoxyimino) butyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***18***. 67% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.00–1.64 (7H, m, 2 × cyclopropyl CH_2, $OCH_2\underline{CH_3}$), 1.62 (2H, d, $J = 6.0$ Hz, CCH_3), 1.91 (3H, s, CH_3), 2.73–2.99 ($\overline{2H}$, m), 3.14–3.80 (7H, m), 3.79 (3H, s, OCH_3), 4.01–4.05 (2H, m), 4.05–4.11 (2H, m, $J = 7.0$ Hz, $O\underline{CH_2}CH_3$), 4.32–4.44 (1H, m), 7.76 (1H, d, $J = 12.0$ Hz, C_5-H), 8.81 ($\overline{1H}$, s, C_2-H), 12.93 (1H, s, COOH). ^{13}C NMR (600 MHz, $CDCl_3$) δ ppm: 9.58, 9.69, 14.47, 14.59, 15.33, 29.53, 40.38, 47.22, 49.31, 51.50, 53.68, 59.83, 64.09, 69.30, 108.13, 108.18 (d, $J = 24$ Hz), 122.81, 134.06, 137.46, 145.65, 150.17, 152.03, 156.83 (d, $J = 249$ Hz), 166.32, 176.85. FAB-MS: m/z 489 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{25}H_{34}FN_4O_5$ $(M + H)^+$: 489.2513; Found 489.2524.

4.2.2.11. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[3-(carbamoylhydrazono)butyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***19***. 68% yield. ^{1}H NMR (400 MHz, C_5D_5N) δ_H 0.96–1.25 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 2.03 (3H, s, CH_3), 2.81–3.42 (8H, m), 3.58–3.76 (3H, m), 3.83 (3H, s, OCH_3), 3.95–3.98 (1H, m), 8.09 (1H, d, $J = 12.0$ Hz, C_5-H), 8.89 (1H, s, C_2-H). FAB-MS: m/z 503 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{24}H_{32}FN_6O_5$ $(M + H)^+$: 503.2418; Found 503.2425.

4.2.2.12. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[3-(phenylhydrazono)butyl]-3-methylpiperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***20***. 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.96–1.36 (7H, m, 2 × cyclopropyl CH_2, CCH_3), 1.94 (3H, s, CH_3), 2.67–3.51 (11H, m), 3.76 (3H, s, OCH_3), 3.99–4.03 (1H, m), 6.82–6.86 (1H, m, Ph-1H), 6.99–7.04 (2H, m, Ph-2H), 7.22–7.26 (2H, m, Ph-2H), 7.86 (1H, d, $J = 12.0$ Hz, C_5-H), 8.80 (1H, s, C_2-H), 14.73 (1H, s, COOH). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 9.44, 9.59, 14.89, 15.81, 34.81, 40.49, 50.38, 50.40, 51.46, 55.06, 57.24, 62.72, 107.77, 108.12 (d, $J = 23$ Hz), 112.88, 119.75, 121.63, 122.53, 129.17, 133.96, 139.28, 145.20, 145.68, 149.83, 155.93 (d, $J = 250$ Hz), 166.73, 176.99. FAB-MS: m/z 536 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{29}H_{35}FN_5O_4$ $(M + H)^+$: 536.2673; Found 536.2651.

4.2.3. 1-Cyclopropyl-6-fluoro-8-methoxy-7-(4-hydroxy-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***21***

A suspension of **8** (1.0 g, 2.24 mmol) in water (10.0 mL) was adjusted pH 9–10 with 2 N sodium hydroxide solution, and then added 30% hydrogen peroxide (3.0 mL). The reaction mixture was stirred for 12 h at room temperature and filtered. The resulting precipitate was recrystallized from methanol to give the title compound **21** as a white solid. 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.98–1.03 (2H, m, cyclopropyl CH_2), 1.22–1.34 (5H, m, cyclopropyl CH_2, CCH_3), 2.90–3.19 (3H, m), 3.39–3.56 (4H, m), 3.79 (3H, s, OCH_3), 4.00–4.04 (1H, m), 7.89 (1H, d, $J = 12.4$ Hz, C_5-H), 8.82 (1H, s, C_2-H), 14.70 (1H, s, COOH). ^{13}C NMR (600 MHz, DMSO-d_6) δ ppm: 8.89, 8.93, 16.58, 40.78, 49.87, 55.90, 58.28, 61.55, 62.67, 106.50, 106.66, 120.86, 134.06, 138.50,145.75, 150.51, 155.41 (d, $J = 249$ Hz), 165.62, 176.30. FAB-MS: m/z 392 $(M + H)^+$. HRMS-FAB: m/z Calcd. for $C_{19}H_{23}FN_3O_5$ $(M + H)^+$: 392.1622; Found 392.1625.



Scheme 1. Synthesis of GTFX derivatives **1**–**21**.

4.3. Molecular modeling

The target quinolone–enzyme–DNA complexes were evaluated by employing SYBYL X1.2 [16] software on a windows workstation. The crystal structure data (3FOF) [4] was obtained from the protein data bank. The surflex-dock module of the SYBYL software suite was then implemented and the protomol was generated using a ligand-based approach. The ligand was extracted from DNA-bound crystal structure. For the purposes of these experiments, "proto_thresh" was set to 0.5 and "proto_bloat" was left at the default (0). Two factors "Multistart 5" and "Random 5" were selected to help optimize ligand targeted docking. All other parameters were left at the default values [17]. Surflex-dock returns an affinity score reported as $-\log(K_d)$ that takes into account hydrophobic, polar complementary, entropic, and solvation terms and a "crash" score that represents "inappropriate" penetration of a potential ligand into a binding site [16]. The predicted three-dimensional conformation of compound **20** is shown in Fig. 2.

4.4. MIC determination

All compounds were screened for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains, by means of standard two-folds serial dilution method using agar media [18]. Minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give complete inhibition of bacterial growth after incubation at 35 °C for 18–24 h.

4.5. Cytotoxicity

Some compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line [19]. The Vero cells were maintained in culture medium (Minimum Essential Medium with Earle's salt, supplemented with 10% fetal bovine serum) at 37 °C under 5% CO_2. Cells were seeded in 96-well plate at the plating density of 1×10^4 cells per well and allowed to recover for 24 h. Culture medium was replaced by assay medium containing the compound to be tested or drug-free. After 72 h of exposure, cells were harvested and cell viability was assessed by MTT assay. The CC_{50} values were calculated by Bliss analyses.

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References

[1] H. Koga, A. Itoh, S. Murayama, S. Suzue, T. Irikura, J. Med. Chem. 23 (1980) 1358–1363.
[2] S. Emami, A. Shafiee, A. Foroumadi, Mini-Rev. Med. Chem. 6 (2006) 375–386.
[3] J.M. Berger, S.J. Gamblin, S.C. Harrison, J.C. Wang, Nature 379 (1996) 225–232.
[4] I. Laponogov, M.K. Sohi, D.A. Veselkov, X.S. Pan, R. Sawhney, A.W. Thompson, K.E. McAuley, L.M. Fisher, M.R. Sanderson, Nat. Struct. Mol. Biol. 16 (2009) 667–669.
[5] A. Bryskier, J.F. Chantot, Drugs 49 (Suppl. 2) (1995) 16–28.
[6] Y. Chai, M.L. Liu, B. Wang, X.F. You, L.S. Feng, Y.B. Zhang, J. Cao, H.Y. Guo, Bioorg. Med. Chem. Lett. 20 (2010) 5195–5198.
[7] C.Y. Hong, Y.K. Kim, Y.H. Lee, J.H. Kwak, Bioorg. Med. Chem. Lett. 8 (1998) 221–226.
[8] V. Cecchetti, A. Fravolini, M.C. Lorenzini, O. Tabarrini, P. Terni, T. Xin, J. Med. Chem. 39 (1996) 436–445.
[9] C.Y. Hong, Y.K. Kim, J.H. Chang, S.H. Kim, H. Choi, D.H. Nam, Y.Z. Kim, J.H. Kwak, J. Med. Chem. 40 (1997) 3584–3593.
[10] A.R. Kwon, Y.H. Min, J.M. Ryu, D.R. Choi, M.J. Shim, E.C. Choi, J. Antimicrob. Chemother. 58 (2006) 684–688.
[11] D.R. Choi, J.H. Shin, J. Yang, S.Y. Yoon, Y.H. Jung, Bioorg. Med. Chem. Lett. 14 (2004) 1273–1277.
[12] T. Uno, T. Okuno, M. Taguchi, K. Iuchi, Y. Kawahata, M. Sotomura, G. Tsukamoto, J. Heterocyclic. Chem. 26 (1989) 393–396.
[13] T. Uno, H. Kondo, Y. Inoue, Y. Kawahata, M. Sotomura, K. Iuchi, G. Tsukamoto, J. Med. Chem. 33 (1990) 2929–2932.
[14] J.X. Wang, J. Cao, S.J. Li, M.L. Liu, H.Y. Guo, Chin. J. Antibiot. (Chin.) 11 (2008) 674–677.
[15] A.N. Jain, J. Med. Chem. 46 (2003) 499–511.
[16] SYBYL Molecular Modeling Software, second ed., vol. X1, Tripos Inc.: St, Louis, MO, 2011.
[17] P.A. Holt, J.B. Chaires, J.O. Trent, J. Chem. Inf. Model. 48 (2008) 1602–1615.
[18] MICs were Determined as Described by the NCCLS (see National Committee for Clinical Laboratory Standards. 2001. Performance standards for antimicrobial susceptibility testing: 11th informational supplement. vol. 21, M100–S11. National Committee for Clinical Laboratory Standards, Wayne, PA). The MIC was defined as the lowest concentration of each compound resulting in inhibition of visible growth of bacteria after incubation at 37 °C for 18–24 h.
[19] Y. Chai, Z.L. Wan, B. Wang, M.L. Liu, H.Y. Guo, Eur. J. Med. Chem. 44 (2009) 4063–4069.