Design, Synthesis and Antibacterial Evaluation of Novel Fluoroquinolone and its Derivatives

Kang-Min Wang[1], Yuan-Cheng Qin[1], Guan-Jun Cheng[1], Hua-Jun Zhu[2], Long Liang[2], Zhi-Peng Cheng[2] and Mei-Ming Luo[1,*]

[1]Key Laboratory of Green Chemistry and Technology of Ministry of Education at Sichuan University, College of Chemistry, Sichuan University, No. 29 Wangjiang Road, Chengdu 610064, Sichuan Province, P.R. China
[2]Sichuan Kelun Pharmaceutical Co. Ltd, 18, 19/F, 2/Building, Jinjiang Times Garden, 107 Jinli West Road, Chengdu 610067, P.R. China

*Corresponding author: Tel/Fax: +86 28 85462021; E-mail: luomm@scu.edu.cn

Gatifloxacin isomers, [1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(2-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid] and a series of its derivatives were designed and synthesized and evaluated for their *in vitro* antibacterial activities. Preliminary results indicated that the tested compounds **GI1**, **GI2**, **GI3** and **GI4** demonstrated excellent activity against *Staph. epidermidis*. In addition, compounds **GI1**, **GI3** and **GI4** show MIC 0.015 μg/mL against *Klebsiella peneumoniae*. It is worth noting that compound **GI2** has been found to exhibit the most prominent activity against all of the tested stains. On the basis of these promising results, some tested compounds could be selected as potential drugs candidate for further evaluation.

Keywords: : Gatifloxacin isomers, 8-Methoxyfluoroquinolones, 2-Methylpiperazine, Antibacterial activities.

INTRODUCTION

Since the discovery of norfloxacin in the early 1980s[1], fluoroquinolones (FQs) were found to have enhanced pharmacokinetics properties and potent bactericidal activity, when compared to the previous existing bactericidal drugs. However, the extensive clinical use of fluoroquinolones resulted in an increased quinolone resistance among many pathogens[2a-f]. In addition, certain adverse events (*e.g.* CNS, phototoxicity and arthropathy) became apparent, although the more serious events are rare[3a-b]. Consequently, great efforts have been devoted towards the synthesis of novel fluoroquinolones in the recent years.

The search for new fluoroquinolones to overcome growing bacterial resistance focuses largely on two approaches. One is to identify novel targets where no pre-existing resistance exists[4]. This approach, although sound in concept, has been met with little success in identifying quality lead matter due to issues associated with target validation and low hit rates from high throughput screening. The second approach is to explore existing clinically proven targets for new chemical matter or modify existing matter with limited or no cross-resistance to existing agents, eliminating the risk of target validation. This approach has been used successfully by a number of researchers to identify new series of fluoroquinolones.

The bactericidal activity of fluoroquinolones is caused by the inhibition of bacterial type-2 topoisomerase (DNA gyrase and topoisomerase IV), which is greatly influenced by the nature of the C-7substituent[5a-b]. According to structure activity relationship (SAR) analysis of fluoroquinolones, modification of the substituents at the C-7 position is the most adaptable site for chemical change and an area that could affect their potency, spectrum and safety. At the C-7, the piperazinyl groups have been utilized with optimal results. Extensively investigated substituents are 3-substituted piperazin-1-yl and 4-substituted derivatives. For example, gatifloxacin is 3-methyl piperazin-1-yl derivative. Pefloxacin, the 4-methylnorfloxacin and other 4-substituted piperazin-1-yl prodrugs of norfloxacin were prepared to improve the bioavailability of the parent[6a-c]. Furthermore, some studies have demonstrated that increasing molecular mass and bulkiness of a substituent at the C-7 position are not barriers to penetration and simply increasing the lipophilic character at C-7 increased the potent activity[7]. The antiproliferative activity of bis-quinolinium choline kinase inhibitors against the HT-29 colon cancer cell line has been improved with a higher lipophilicity of the substituents[8]. It was reported that 8-OCH₃ fluoroquinolones with N[1]-cyclopropyl substitution are much more bactericidal activity than C-8 hydrogen analogs[9]. Gatifloxacin (4th-generation, Fig. 1), having a particularly strong *in vitro* activity and broad antimicrobial spectrum, could be promising agents[10a,b]. However, dysglycemia has been noted as the life-threatening adverse effect of gatifloxacin, which led to its withdrawal from the market in the United States in 2006[11]. Despite many advances in gatifloxacin, there exsist

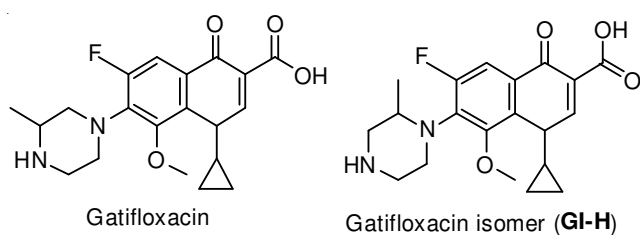continuous need for novel quinolones to overcome the limitation of gatifloxacin.


Fig. 1. Gatifloxacin and gatifloxacin isomer (GI-H)

In an effort to discover new classes of antibacterial agents, it was wisely decided to make structure modifications on gatifloxacin. Sekine and his coworkers suggested that the planar feature between the C-7 piperazinyl ring and the naphthyridine nucleus of enoxacin may affect flouroquinolone to interact with the narrow binding site of the enzyme[12,13]. Bulk group on the 7-position, might restrict the free rotation around the bond axis between the C-7 carbon and the piperazinyl N-1 nitrogen in combination with the fluorine atoms at C-6 and methoxyl at C-8 and accordingly make the dihedral angle change, thus possibly tracking the problem of bacterial resistance and dysglycemic effects. Therefore, we here first reported the synthesis of gatifloxacin isomer (**Scheme-I**) by introducing methyl group at position 2 on the piperazin-1-yl ring, which was determined by ^{1}H NMR, ^{13}C NMR, mass spectrometry analysis. As expected, it show excellent antibacterial activity, particularly against *Klebsiella pneunoniae*, when compared to gatifloxacin. Furthermore, acylation and alkylation of piperazine are important structural elements[14], present in a number of investigational and established drugs[15]. It is reported that sulfonyl fluoroquinolones are a new class of antibacterial

fluoroquinolones with especially high *in vitro* activity against Gram-positive bacteria[16]. These research results intensified our interest, herein a number of new derivatives of fluoro-quinolones were synthesized by introduction of diversified substituents, including alkyl, acyl and sulfonyl, to the nitrogen atom of the piperazine ring of the gatifloxacin isomer. Moreover, a SAR study is also explored to facilitate the further development of the fluoro-quinolones. The synthesis of ethyl ester derivative 2 has been reported earlier[17] and serverd as our key intermediate. We developed a practical and efficient synthesis as a general route to gatifloxacin isomer **GI-H** (**Scheme-I**).

2-Methylpiperazine was treated with di-*tert*-butyl dicarbonate to produce Boc-protected 2-methylpiperazine (**3**) prior to the substitution reaction to avoid the formation of eonormous quanities of gatifloxacin as byproduct and improve selection and purification of 7-C substituted gatifloxacin isomer.

Since direct nucleophilic aromatic substitution of intermediate **2** and Boc-protected 2-methylpiperazine met with no success, a new procedure have been designed, in which boron chelate-type intermediate **4**, prepared according to previously published methods[18], was treated with compound 3 to overcome the diminished reactivity induced by the 8-methoxy group and the steric hindrance induced by methyl neighbouring electrophilic nitrogen atom. The subsequent cleavage step and Boc deprotection could be combined to one-pot process. Namely, refluxing compound **5** in methanol during for 12 h to be sufficient to perform this cleavage, monitored by thin-layer chromatography, then concentrated hydrochloric acid was added and the Boc protective group of compound **6** was removed to afford the novel compound **GI-H**. The whole process is easy to workup without column chromatography purification that makes it possible to scale up.
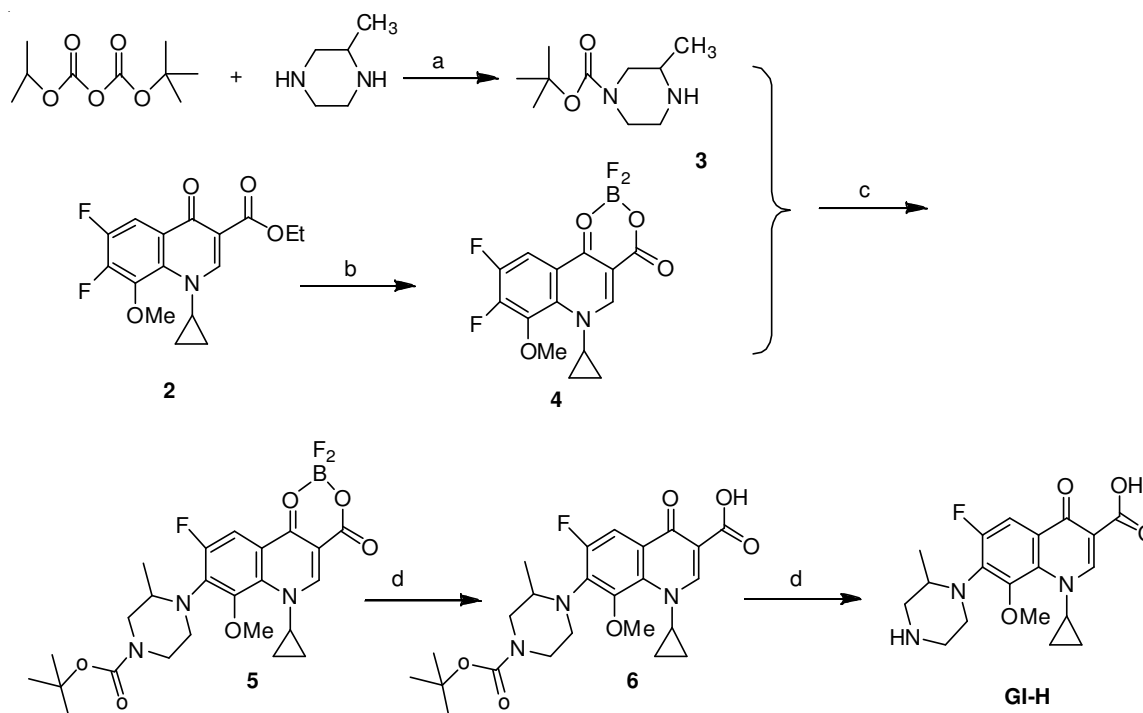
Scheme-I: Reactions and conditions: (a) di-*tert*-butyl dicarbonate (1 equiv), 2-methylpiperazine (1 equiv), CH₂Cl₂, 0 °C, 1h, 93 % for **3**; (b) BF₃-Et₂O (13 equiv), ethyl ester derivative (1 equiv), THF, reflux, 2d, 56 % for **4**; (c) boron chelate-type intermediate (1 equiv), Boc-protected 2-methyl piperazine (2.1 equiv), DMSO, 0 °C, 0.5 h, then 40 °C, 3d, 23 % for **5**; (d) CH₃OH, reflux, 12 h, then 0 °C, HCl(aq), 0.5 h, 40 % for **GI-H**

A series of novel **GI-H** derivatives (Fig. 2.) were achieved through the versatile and efficient synthetic route as following.

Preparation of N-alkyl-substituted GI: Compounds **GI-5**, **GI-7** and **GI1-3** were easily prepared by alkylation of **GI-H** by nucleophilic substitution with their corresponding halohydrocarbons (**Scheme-II**). For example, compound **GI-5** was obained by treatment of GTFXS and triethylamine with benzyl chloride in dichloromethane at room temperature.

Preparation of N-acyl and N-sulfonyl substituted GIS: Reaction of **GI-H** with a suitable acyl chloride, (**Scheme-III**) in the presence of triethylamine was performed in dichloromethane solution at room temperature, to afford derivatives **GI-8**, **GI-9**, **GI-10** and **GI-11**, respectively. Using a suitable anhydride (**Scheme-IV**) instead of acyl chloride, the acetyl derivative **GI-1** and **GI-2** was obtained. **GI-H** under the same condition was treated with the selected sulfonyl chloride, prepared from the corresponding sulfonic acid and thionyl chloride, to provide derivatives (**Scheme-IV**) **GI-3** and **GI-6**, respectively.

EXPERIMENTAL

All non-aqueous reactions were performed under an atmosphere of dry Ar (99.99 %) using oven-dried glassware and anhydrous solvents. All reagents were obtained from commercial sources and used without further purification. Melting points were determined on an XRC-1 capillary melting point apparatus and are uncorrected. Thin layer chromatography was carried out by using Merck silica gel GF254 plates. Low-resolution mass spectra were recorded on a Finnigan Surveyor MSQ instrument. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were determined on a Bruker-400 spectrometer in DMSO-d_6 or CDCl$_3$ using tetramethylsilane (TMS) as an internal standard.

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(2-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (**GI-H**).

Step-1 Boc Protection of 2-methylpiperazine: Di-*tert*-butyl dicarbonate (16.35 g, 0.075 mol) in CH$_2$Cl$_2$ (50 mL) was added to a solution of racemic 2-methylpiperazine (7.50 g,

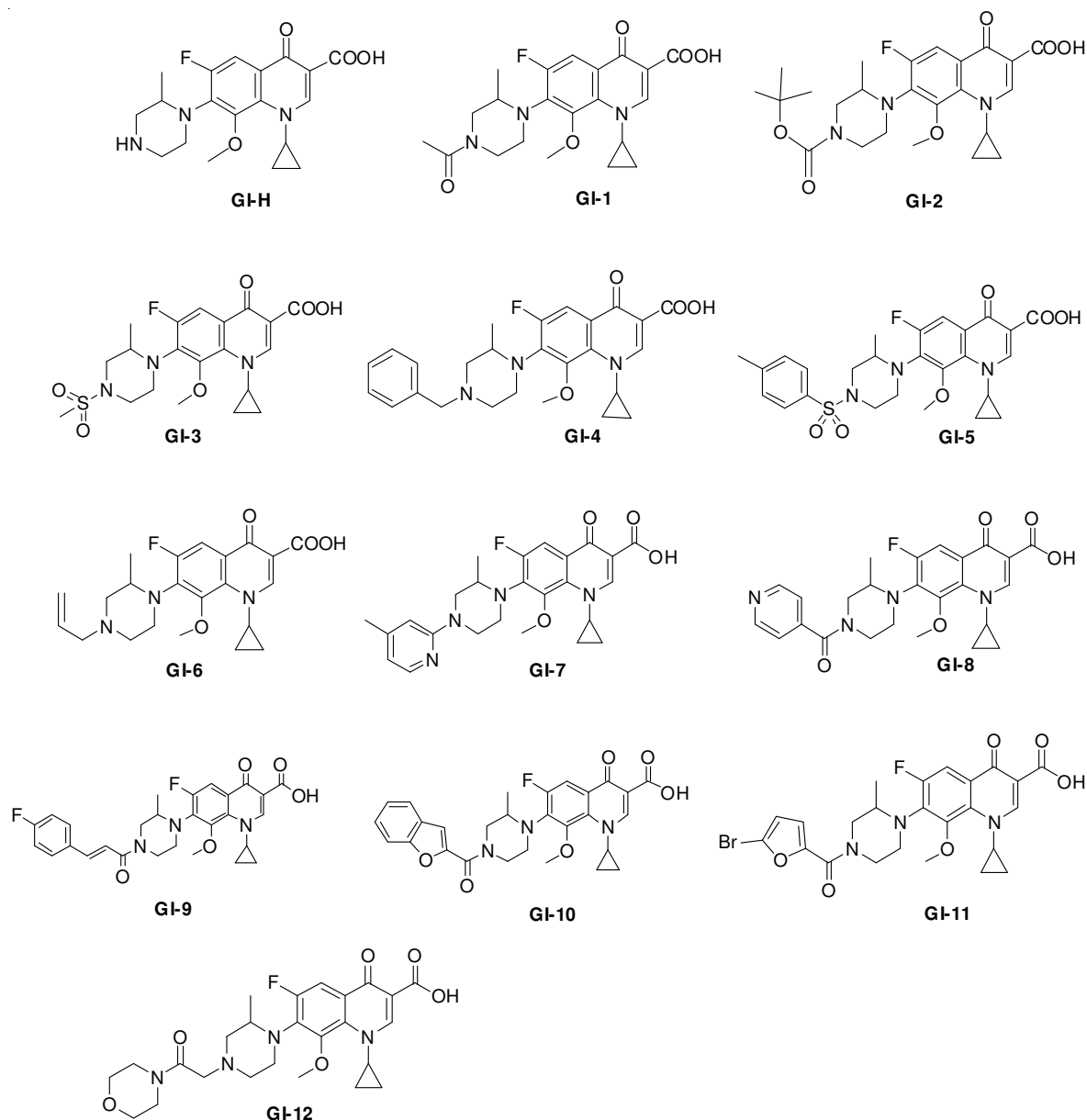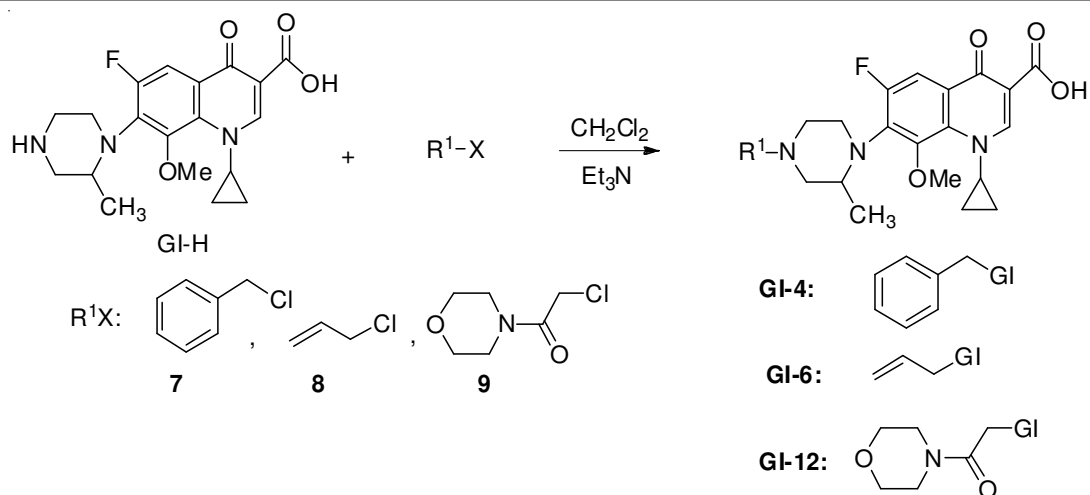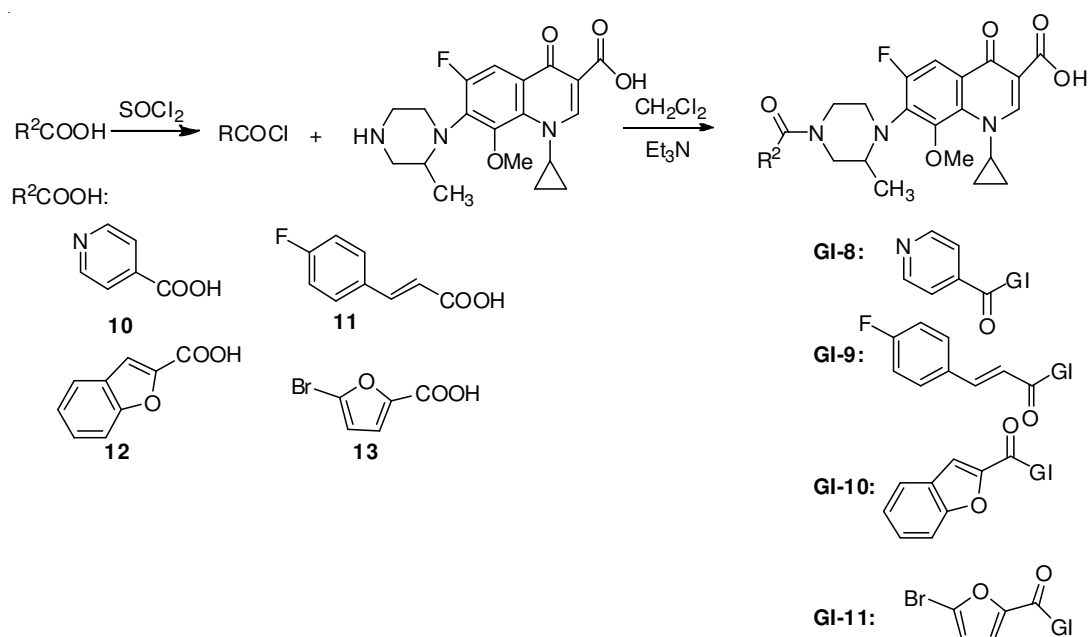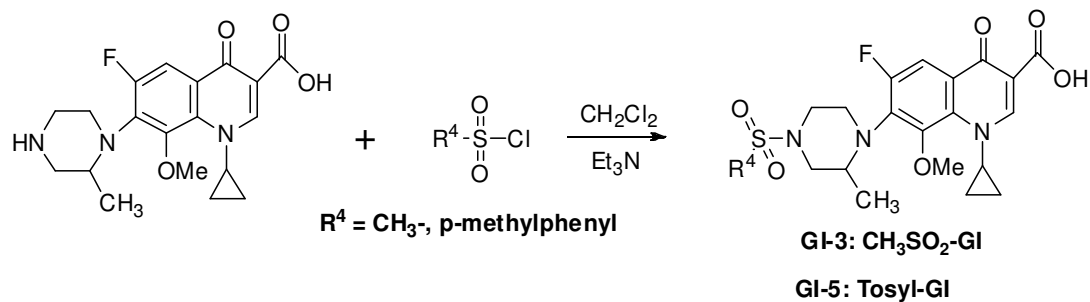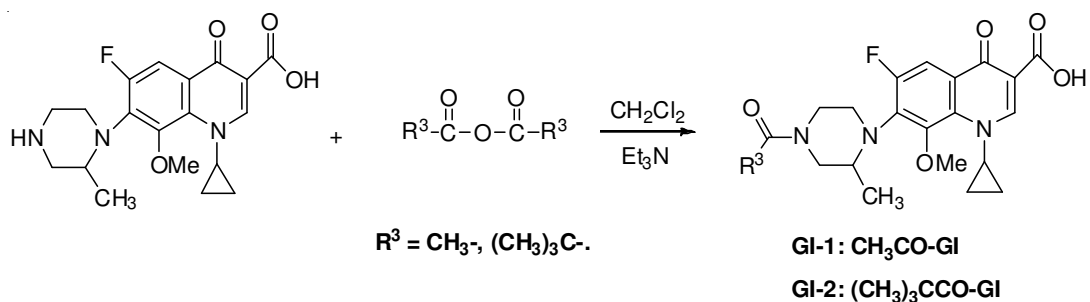Fig. 2. Structures of the novel fluoroquinolones

Scheme-II: Synthetic route of compound **GI-4**, **GI-6**, **GI-12**

Scheme-III: Synthetic route of compound **GI-8**, **GI-9**, **GI-10** and **GI-11**

R³ = CH₃-, (CH₃)₃C-.

GI-1: CH₃CO-GI

GI-2: (CH₃)₃CCO-GI

R⁴ = CH₃-, p-methylphenyl

GI-3: CH₃SO₂-GI

GI-5: Tosyl-GI

Scheme-IV: Synthetic route of compound **GI-1**, **GI-2**, **GI-3** and **GI-5**

0.075 mmol) in CH_2Cl_2 (150 mL) under cooling with salt-ice bath and the whole mixture was stirred at 0 °C for 1h. After completion of the reaction, the organic layers was washed with water (three times), dried over sodium sulfate and evaporated *in vacuo* to give 14 g (93 %) of **3**.

Step-2 Chelation: $BF_3.Et_2O$ (36 mL, 283.9 mmol) was added to a suspension of 2 (7.56 g, 23.4 mmol) in THF (150 mL). After refluxing for 48 h, the clear reaction mixture was evaporated under reduced pressure. The crude oily residue was successively washed with Et_2O, $CHCl_3$ and water affording **4** as a white solid (4.5 g, 56 %).

Step-3 Coupling: 4 (4.32 g, 12.6 mmol) was taken in dimethyl sulfoxide (DMSO), cooled to 0-5 °C. A solution of **3** (5.4 g 27 mmol) in DMSO was slowly added keeping the temperature below 5 °C. The mixture was allowed to stir at 0 °C for 0.5 h, then warmed to 40 °C for 3d. After the reaction ended (TLC monitoring), the mixture was poured into water (300 mL), forming a generous precipitate. The resulting generous suspension was then cooled at 0-5 °C and remained at that temperature for an additional hour, then filtered and dried under vacuum to obtain the crude product **5** (1.5 g, 27 %).

Step-4 deprotection: The above crude product **5** was added methanol (100 mL) and the resulting yellow suspension was heated to reflux (60-65 °C) for 12 h. The mixture was then cooled to 0 °C and 40 mL of concentrated aqueous HCl was carefully added under that temperature. The mixture was stirred for 1 h at ambient temperature and the solvent was distilled off under reduced pressure until the volume of residue reached 40 mL. The solution was washed with CH_2Cl_2 and made alkaline with saturated aqueous NaOH at 0 °C and then the pH of the solution was adjusted to 7.4 with concentrated aqueous HCl and dilute aqueous HCl. The resultant solution was extracted with chloroform (4 × 50 mL) and the combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was recrystallized from EtOH to yield crude gatifloxacin isomer **GI-H** (0.36 g, 24 %) as slightly yellowish white crystals. m.p. 163-164 °C. MS: *m/z* calcd. for $C_{19}H_{22}N_3O_4F$ [M]+: 375.1; found [M+1]+: 376.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.85 (s, 1H), 7.90 (d, $^3J_{F-H}$ = 12 Hz, 1H), 4.11-4.05 (m, 1H), 3.99 (s, 3H), 3.66-3.62 (m, 1H), 3.37-3.34 (m, 1H), 3.22-3.16 (m, 2H), 3.11-3.03 (m, 2H), 2.71 (dd, J = 11.6 Hz, 8 Hz, 1H), 1.32-1.25 (m, 1H), 1.21-1.09 (m, 2H), 1.05 (d, J = 6.4 Hz, 3H), 0.95-0.90 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.1, 166.8, 158.8 (d, $^1J_{C-F}$ = 252 Hz), 150.1, 149.0, 138.3 (d, $^3J_{C-F}$ = 12 Hz), 133.4, 124.3, 124.2, 107.8 (d, $^3J_{C-F}$ = 24 Hz), 62.5, 53.8, 53.2, 51.5, 46.6, 40.7, 16.6, 9.9, 9.0.

General procedure for the preparation of compounds **GI-4**, **GI-6** and **GI-12**. To a stirred suspension of gatifluoroxacin isomer (1 g, 2.5 mol), CH_2Cl_2 (25 mL) and triethylamine (0.84 mL, 1.8 mol) was added at room temperature. The corresponding halogenated hydrocarbons (3 mmol) and the mixture was stirred at room temperature for 6 h and then was washed with dilute aqueous HCl to adjust pH to 2-3 and finally with water. The organic layer was dried (anhydrous Na_2SO_4) and evaporated to dryness *in vacuo*. The solid crystallized from isopropanol to give the derivatives. Compound **GI-4**: m.p. 120-122 °C. MS: *m/z* calcd. for $C_{26}H_{28}N_3O_4F$ [M]+: 465.2; found [M+1]+: 466.2; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.71 (s,

1H), 8.83 (s, 1H), 7.88 (d, $^3J_{F-H}$ = 12 Hz, 1H), 7.36-7.27 (m, 5H), 4.09-4.03 (m, 1H), 3.95 (s, 3H), 3.76-3.72 (m, 1H), 3.59-3.54 (m, 2H), 3.42-3.98 (m, 1H), 3.27-3.24 (m, 1H), 2.81-2.68 (m, 2H), 2.53-2.49 (m, 1H), 2.22-2.19 (m, 1H), 1.32-1.28 (m, 1H), 1.21-1.05 (m, 5H), 0.95-0.90 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.1, 166.7, 158.8 (d, $^1J_{C-F}$ = 253 Hz), 150.3, 148.6, 138.4 (d, $^3J_{C-F}$ = 13 Hz), 137.9, 133.4, 129.1, 128.3, 127.2, 123.8, 107.7 (d, $^3J_{C-F}$ = 23 Hz), 107.6, 63.1, 62.4, 60.5, 53.8, 53.4, 53.0, 50.4, 40.7, 29.7, 17.05, 9.9, 8.9.

Compound GI-6: m.p. 158-160 °C. MS: *m/z* calcd. for $C_{22}H_{26}N_3O_4F$ [M]+: 415.1; found [M+1]+: 416.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.69 (s, 1H), 8.84 (s, 1H), 7.88 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 5.94-5.89 (m, 1H), 5.28-5.21 (m, 2H), 4.10-4.04 (m, 1H), 3.95 (s, 3H), 3.77-3.75 (m, 1H), 3.40-3.29 (m, 2H), 3.15-3.02 (m, 2H), 2.86-2.79 (m, 2H), 2.55-2.45 (m, 1H), 2.16-2.02 (m, 1H), 1.28-1.26 (m, 1H), 1.15-1.05 (m, 5H), 0.93-0.83 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 166.5, 158.3 (d, $^1J_{C-F}$ = 253 Hz), 150.4, 149.0, 138.0 (d, $^3J_{C-F}$ = 13 Hz), 133.8, 133.4, 124.1, 124.0, 119.0, 107.6 (d, $^3J_{C-F}$ = 18 Hz), 62.7, 61.6, 60.3, 53.7, 52.6, 50.5, 40.8, 29.6, 17.1, 9.9, 8.9.

Compound GI-12: m.p. 96-98 °C. MS: *m/z* calcd. for $C_{25}H_{31}N_4O_6BrF$ [M]+: 502.2; found [M+1]+: 503.2; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.66 (s, 1H), 8.84 (s, 1H), 7.90 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 4.10-4.4.06 (m, 1H), 3.96 (s, 3H), 3.77-3.63 (m, 9H), 3.50-3.40 (m, 3H), 3.27-3.24 (m, 3H), 2.86-2.83 (m, 1H), 2.71-2.65 (m, 2H), 2.34-2.31 (m, 1H), 1.31-1.28 (m, 1H), 1.21-1.11 (m, 5H), 0.95-0.90 (m, 1H). ^{13}C NMR (150 MHz, CDCl$_3$): δ = 177.0, 167.9, 166.6, 159.4, 150.1, 148.7, 138.1 (d, $^3J_{C-F}$ = 12 Hz), 133.4, 124.0, 114.3,11.9, 107.7 (d, $^3J_{C-F}$ = 13 Hz), 67.0, 62.4, 61.5, 60.3, 53.8., 53.0, 49.9, 46.3, 42.2, 40.7, 16.8, 9.9, 8.9.

General procedure for the preparation of acyl chloride: Acid (**10**, **11**, **12** and **13**) (12 mmol) was added thionyl chloride (15 mL) and the mixture was stirred and refluxed for 2 h. Redundant thionyl chloride was distilled off under reduced pressure to afford quantitative yield of the corresponding chloride which was used directly in the next step.

General procedure for the preparation of compounds **GI-1**, **GI-2**, **GI-3**, **GI-5**, **GI-7**, **GI-8**, **GI-9**, **GI-10** and **GI-11**. To a suspension of gatifluoroxacin isomer (0.7 g, 1.8 mol), CH_2Cl_2 (50 mL) and triethylamine (0.84 mL, 1.8 mol) was dropped in 15 min a solution of fresh acyl chloride prepared by above method or anhydride (1.8 mol) in CH_2Cl_2 (15 mL). The mixture was stirred at 25 °C for 0.5 h and then was washed with dilute aqueous HCl adjusting pH to 2-3 and water. The organic layer was dried (anhydrous Na_2SO_4) and evaporated to dryness *in vacuo*. The solid crystallized from 80 % EtOH to afford desired product.

Compound GI-1: m.p. 176-178 °C. MS: *m/z* calcd. for $C_{21}H_{24}N_3O_5F$ [M]+: 417.1; found [M+1]+: 418.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.60 (s, 1H), 8.83 (s, 1H), 7.90 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 4.15-3.96 (m, 2H), 3.96 (s, 3H), 3.78-3.62 (m, 3H), 3.46-3.44 (m, 2H), 3.32-3.19 (m, 2H), 2.22 (s, 3H), 1.34-1.28 (m, 1H), 1.20-1.08 (m, 5H), 0.96-0.92 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ =177.21, 166.61, 154.83 (d, $^1J_{C-F}$ = 261 Hz), 150.17, 138.03 (d, $^3J_{C-F}$ = 9Hz), 133.49, 107.96 (d, $^3J_{C-F}$ = 23 Hz), 80.09,62.5, 53.29, 40.68, 28.44, 16.00, 9.99, 9.04.

Compound GI-2: m.p. 158-160 °C. MS: *m/z* calcd. for $C_{24}H_{30}N_3O_6F$ [M]+: 475.2; found [M+1]+: 476.2; ^{1}H NMR (400

MHz, CDCl$_3$): δ = 14.64 (s, 1H), 8.84 (s, 1H), 7.90 (d, $^3J_{F-H}$ = 14.4 Hz, 1H), 4.10-4.04 (m, 1H), 3.95 (s, 3H), 3.79-3.73 (m, 1H), 3.69-3.66 (m, 2H), 3.55-3.41 (m, 2H), 3.17-3.13 (m, 2H), 1.50 (s, 9H), 1.36-1.25 (m, 1H), 1.24-1.05 (m, 5H), 0.95-0.85 (m, 2H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 169.3, 166.5, 158.2 (d, $^1J_{C-F}$ = 261 Hz), 150.25, 148.51, 137.6 (d, $^3J_{C-F}$ = 9 Hz), 133.5, 124.3, 124.2, 107.8 (d, $^3J_{C-F}$ = 23 Hz), 62.5, 52.8, 49.8, 49.2, 47.8, 47.1, 42.2, 40.7, 21.3, 21.2, 16.0, 10.0, 9.0.

Compound GI-3: m.p. 178-180 °C. MS: *m/z* calcd. for C$_{25}$H$_{25}$N$_4$O$_5$F [M]$^+$: 480.1; found [M+1]$^+$: 481.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.52 (s, 1H), 8.85 (s, 1H), 8.75 (d, J = 4 Hz, 2H), 7.93 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 7.39 (d, *J* = 4.8 Hz, 2H), 4.29-4.03 (m, 2H), 3.97 (s, 3H), 3.92-3.78 (m, 1H), 3.62-3.53 (m, 3H), 3.40-3.19 (m, 2H), 1.31-1.26 (m, 1H), 1.21-1.11 (m, 5H), 0.95-0.90 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 176.9, 168.0, 166.4, 150.3, 148.5, 143.2, 137.4 (d, $^3J_{C-F}$ = 13 Hz), 133.4, 124.4, 124.3, 107.6 (d, $^3J_{C-F}$ = 22 Hz), 62.6, 53.6, 53.7, 50.0, 48.9, 48.3, 42.7, 40.7, 30.9, 16.0, 15.8, 9.9, 9.0.

Compound GI-5: m.p. 174-176 °C. MS: *m/z* calcd. for C$_{28}$H$_{26}$N$_3$O$_5$F [M]$^+$: 519.1; found [M+1]$^+$: 520.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.60 (s, 1H), 8.85 (s, 1H), 7.92 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 7.69 (d, *J* = 8 Hz, 1H), 7.54 (d, *J* = 8 Hz, 1H), 7.44-7.40 (m, 2H), 7.34-7.30 (m, 1H), 4.29-4.03 (m, 2H), 3.98-3.82 (m, 4H), 3.82-3.70 (m, 1H), 3.59-3.56 (m, 3H), 3.32-3.30 (m, 1H), 1.32-1.30 (m, 1H), 1.21-1.11 (m, 5H), 0.95-0.92` (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 176.9, 166.4, 160.0, 159.4, 156.9, 154.6, 150.2, 148.8, 137.7 (d, $^3J_{C-F}$ = 14 Hz), 126.8, 126.6, 124.1, 124.0, 123.7, 122.3, 122.3, 112.5, 107.7 (d, $^3J_{C-F}$ = 20 Hz), 64.02, 62.6, 53.6, 49.6, 40.7, 25.3, 16.0, 9.9, 9.0.

Compound GI-8: m.p. 178-180 °C. MS: *m/z* calcd. for C$_{25}$H$_{25}$N$_4$O$_5$F [M]$^+$: 480.1; found [M+1]$^+$: 481.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.52 (s, 1H), 8.85 (s, 1H), 8.75 (d, *J* = 4 Hz, 2H), 7.93 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 7.39 (d, *J* = 4.8 Hz, 2H), 4.29-4.03 (m, 2H), 3.97 (s, 3H), 3.92-3.78 (m, 1H), 3.62-3.53 (m, 3H), 3.40-3.19 (m, 2H), 1.31-1.26 (m, 1H), 1.21-1.11 (m, 5H), 0.95-0.90 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 176.9, 168.0, 166.4, 150.3, 148.5, 143.2, 137.4 (d, $^3J_{C-F}$ = 13 Hz), 133.4, 124.4, 124.3, 107.6 (d, $^3J_{C-F}$ = 22 Hz), 62.6, 53.6, 53.7, 50.0, 48.9, 48.3, 42.7, 40.7, 30.9, 16.0, 15.8, 9.9, 9.0.

Compound GI-9: m.p. 200-202 °C. MS: *m/z* calcd. for C$_{28}$H$_{27}$N$_3$O$_5$F$_2$ [M]$^+$: 523.1; found [M+1]$^+$: 524.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.62 (s, 1H), 8.85 (s, 1H), 7.92 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 7.71 (d, *J* = 15.2 Hz, 2H), 7.54 (dd, *J* = 8.4 Hz, *J* = 5.6 Hz, 2H), 7.10 (t, 2H), 6.80 (d, *J* = 15.2 Hz, 1H), 4.29-4.04 (m, 2H), 3.97-3.82 (m, 4H), 3.82-3.70 (m, 2H), 3.53-3.25 (m, 3H), 1.32-1.26 (m, 1H), 1.21-1.11 (m, 5H), 0.95-0.92 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 176.9, 166.4, 165.5, 164.7, 162.3, 159.4, 156.9, 150.1, 148.5, 141.9, 137.4 (d, $^3J_{C-F}$ = 13 Hz),, 130.9, 129.6, 124.2, 124.1, 116.4, 116.0, 107.7 (d, $^3J_{C-F}$ = 22 Hz), 77.3, 62.6, 48.4, 46.6, 40.7, 25.3, 16.1, 10.0, 9.0.

Compound GI-10: m.p. 174-176 °C. MS: *m/z* calcd. for C$_{28}$H$_{26}$N$_3$O$_5$F [M]$^+$: 519.1; found [M+1]$^+$: 520.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.60 (s, 1H), 8.85 (s, 1H), 7.92 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 7.69 (d, *J* = 8 Hz, 1H), 7.54 (d, *J* = 8 Hz, 1H), 7.44-7.40 (m, 2H), 7.34-7.30 (m, 1H), 4.29-4.03 (m, 2H), 3.98-3.82 (m, 4H), 3.82-3.70 (m, 1H), 3.59-3.56 (m, 3H), 3.32-3.30 (m, 1H), 1.32-1.30 (m, 1H), 1.21-1.11 (m, 5H), 0.95-0.92` (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 176.9, 166.4, 160.0, 159.4, 156.9, 154.6, 150.2, 148.8, 137.7 (d, $^3J_{C-F}$ = 14 Hz), 126.8, 126.6, 124.1, 124.0, 123.7, 122.3, 122.3, 112.5, 107.7 (d, $^3J_{C-F}$ = 20 Hz), 64.02, 62.6, 53.6, 49.6, 40.7, 25.3, 16.0, 9.9, 9.0.

Compound GI-11: m.p. 176-178 °C. MS: *m/z* calcd. for C$_{24}$H$_{23}$N$_3$O$_6$BrF [M]$^+$: 547.1; found [M+1]$^+$: 548.1; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 14.58 (s, 1H), 8.85 (s, 1H), 7.92 (d, $^3J_{F-H}$ = 11.6 Hz, 1H), 7.07 (d, *J* = 3.6 Hz, 1H), 6.47 (d, *J* = 3.2 Hz, 1H), 4.21-4.17 (m, 1H), 4.10-4.4.04 (m, 2H), 3.96 (s, 3H), 3.88-3.77 (m, 2H), 3.57-3.53 (m, 2H), 3.32-3.24 (m, 1H), 1.32-1.30 (m, 1H), 1.21-1.11 (m, 5H), 0.95-0.90 (m, 1H). ^{13}C NMR (100 MHz, CDCl$_3$): δ = 177.0, 176.9, 166.4, 159.4, 158.0, 156.9, 150.2, 149.4, 148.5, 137.6 (d, $^3J_{C-F}$ = 13 Hz), 133.5, 124.3, 124.0, 119.3, 113.5, 107.8 (d, $^3J_{C-F}$ = 20Hz), 77.3, 62.5, 53.5, 49.5, 40.7, 16.0, 10.0, 9.0.

RESULTS AND DISCUSSION

Each compound was screened for *in vitro* antibacterial activity against several representative Gram-positive bacteria (*S. aureus*, *S. aureus* and *S. epidermidis*) and Gram-negative bacteria (*Escherichia coli*, *P. aeuginosa*, *K. peneumoniae*, *Acinetobacter baumannii*). The minimum inhibitory concentration values (MICs) were determined by comparison to its isomer, gatifloxacin as reference drug (Table-1)[19].

Generally, all of the synthesized compounds exhibited potent antibacterial activity. Most tested compounds possess good activity against *E. coli* and *S. epidermidis*, particularly GI-1 and GI-II. The most prominent improvement was observed against *Klebsiella peneumoniae*. The compounds **GI-H**, **GI-2** and **GI-3** show MIC 0.015 µg/mL against *Klebsiella peneumoniae*. It is worth noting that compound **GI-1** displays the most prominent activity against all of the tested stains (MIC: 0.015-16 µg/mL).

	TABLE *in vitro* ANTIBACTERIAL ACTIVITY OF GT-H AND ITS DERIVATIVES					
Strains	MIC (µg/mL)					
	S. e.	*E. coli*	P. a.	K. p.	A. b.	S. a.
GI-H	2	1	32	0.015	1	1
GI-1	0.015	2	16	0.25	0.5	0.25
GI-2	32	64	256	0.015	16	16
GI-3	6	2	8	0.015	0.5	0.5
GI-4	128	256	256	256	256	256
GI-5	32	128	256	256	256	256
GI-6	256	128	256	256	256	256
GI-7	256	256	256	256	256	256
GI-8	256	256	256	256	256	256
GI1-9	256	256	256	256	256	256
GI-10	256	256	256	256	256	256
GI-11	0.015	0.5	256	256	256	256
GI-12	4	16	256	256	256	256

S.e. = *Staphylococcus epidermidis*; P.a. = *Pseudomonas aeruginosa*;
K.p. = *Klebsiella peneumoniae*; A.b. = *Acinetobacter baumannii*;
S.a. = *Staphylococcus aureus* R866.

In order to further know an effect of varying the N-4' substitution on activity, a series of novel synthesized quinolones were investigated. An interest in an influence of N-substituent **GI-H** derivatives and unsubstituents, thus N-substituted compound **GI-1** and **GI-3** seems to have more inhibitory activity than unsubstituents **GI-H**, while better than other unsubstituents against both Gram-positive and Gram-negative bacteria. When compared to bulkier N-acyl compound **GI-2**, simple N-acyl compound **7a** display 16 to 2000-fold increase in activity against all of the tested bacteria. Furthermore, this same trend can be seen in the comparison of N-sulfonyl substituted compounds **GI-3** and **GI-4**.

Conclusion

In summary, gatifluoroxacin isomers **GI-H**, [1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(2-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid] and a series of its derivatives were designed and synthesized and evaluated for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative strains. Compounds **GI1**, **GI2**, **GI3** and **GI4** demonstrated excellent activity against both Gram-positive and Gram-negative bacteria. Compound **GI-1** has been found to exhibit the most prominent activity against all of the tested stains (MIC: 0.015-16 µg/mL).

As to SAR study among the thirteen novel synthesized quinolones, it have been demonstrated that the activity fluctuates in a broad range, depending on the substituents on the nitrogen atom of the C-7 piperazine ring.

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