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GATIFLOXACIN-1,2,3-TRIAZOLE-ISATIN HYBRIDS TETHERED THROUGH METHYLENE AND ACETYL AND THEIR ANTIBACTERIAL ACTIVITIES

Hua GUO* and Quan-Ping DIAO

School of Chemistry and Life Science, Anshan Normal University

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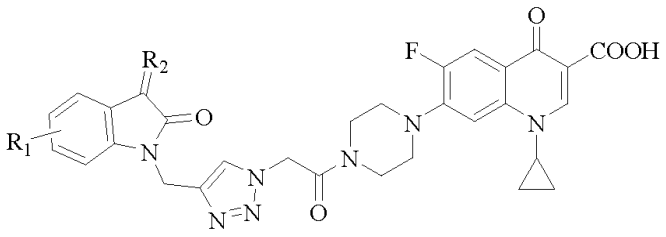
In this work, a series of gatifloxacin-1,2,3-triazole-isatin hybrids tethered with methylene and acetyl (**8a-n**) were designed and synthesized, and the antibacterial activity profiles together with cytotoxicity were also investigated. The preliminary results indicated that all of the hybrids showed promising activity against a panel of Gram-positive and Gram-negative bacteria with MIC values in a range of ≤0.03 to 64 μg/mL. The cytotoxicity results demonstrated all hybrids displayed acceptable cytotoxicity towards VERO cells. Among these 14 hybrids, **8b** and **8g** with low cytotoxicity were no inferior to the parent gatifloxacin against Gram-positive and Gram-negative pathogens. Furthermore, we discussed the structure-activity relationship and structure-cytotoxicity relationship so as to point out the direction for further rationale design and modification of this series of hybrids.



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INTRODUCTION

Gram-positive and Gram-negative pathogens usually bring out the infections, even cause the death if there is no effective treatment.[1,2] In the last several decades, antibiotics such as fluoroquinolones are used commonly to battle with bacterial infections, but the emergency and widely spread of drug-resistant bacteria make pathogens less and less susceptible to the antibiotics used currently.[3,4] Drug-resistant bacteria have already been a knotty problem in the world healthcare system as evidenced by that roughly 700,000 drug-resistant pathogens related deaths occur around the world annually, and the deaths may increase to 10 million in the middle of this century if drug-resistant bacteria could not get effective control.[5,6] Therefore, it is urgent to develop antibacterial agents which are more effective and sensitive to both drug-susceptible and drug-resistant organisms.

Gatifloxacin, a fourth generation of fluoroquinolone, which could inhibit the bacterial enzymes DNA gyrase and topoisomerase IV, possesses excellent broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens. However, gatifloxacin has not been used for the treatment of bacterial infections due to the significant side effects including dysglycemia.[7,8] In recent years, various gatifloxacin derivatives were prepared and the antimicrobial activities were tested in order to obtain the compounds with the illustrious antibacterial activity and the reduced toxicity.[7–9] Among them, the alkyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **1** showed promising antibacterial activity and low cytotoxicity. Further study revealed that the linker between fluoroquinolone and 1,2,3-triazole moieties, as well as the carbon spacer between 1,2,3-triazole and isatin moiety influenced the activity and cytotoxicity significantly. The linker ethylene was more favorable than propylene between

* Corresponding author: m13704127672@163.com

fluoroquinolone and 1,2,3-triazole motifs, suggesting the shorter linker was preferred.[10-18] Moreover, *N*-acylated fluoroquinolone derivatives showed enhanced antibacterial activity and reduced cytotoxicity when compared with the parent, indicating acetyl moiety might be an excellent linker.[19–21]

In this study, methylene group was inserted as a linker between isatin and 1,2,3-triazole motifs, and acetyl moiety was introduced a linker between gatifloxacin and 1,2,3-triazole moieties, respectively. All the synthesized gatifloxacin-1,2,3-triazole-isatin hybrids tethered with methylene and acetyl were screened for their *in vitro* antibacterial activity against both drug-sensitive and drug-resistant pathogens, as well as the cytotoxicity towards VERO cells. Our primary objective was to identify the optimal linkers between isatin and 1,2,3-triazole motifs as well as between gatifloxacin and 1,2,3-triazole moieties so as to facilitate the development of the drug candidates with higher efficiency and lower toxicity. The illustration of the design strategy is depicted in **Figure 1**.

RESULTS AND DISCUSSION

The synthetic route for methylene and acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n** was described in **Scheme 1**. Isatin/5-fluoroisatin/5-methylisatin/7-fluoroisatin/5,7-dichloroisatin **1a-e** were alkylated with propargyl bromide in presence of potassium carbonate (K_2CO_3), providing *N*-propargyl isatin intermediates **2a-e.**[22,23] Then, intermediates **3a-i** were obtained by the condensation of *N*-propargyl isatins **2a-e** with the alkoxyamine hydrochlorides. Later, 2-bromoacetic acid **4** was treated with sodium azide, giving 2-azidoacetic acid **5**, which was then reacted with dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) to get succinimidyl ester **6.**[24] Condensation of gatifloxacin with succinimidyl ester **6** in presence of *N*,*N*-diisopropylethylamine (DIPEA) yielded 2-azidoacetyl gatifloxacin **7**. Finally, cyclization of *N*-propargyl isatin intermediates **2a-e** or **3a-i** with 2-azidoacetyl gatifloxacin **7** in presence of copper acetate ($Cu(OAc)_2$) provided the desired acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n**.[18]

The chemical structures and yields of acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n** were listed in **Table 1**. From **Table 1**, it can be seen that the yields of the hybrids except **8e** (yield: 22%) were in a range of 43% to 79%, and the low yield for **8e** might be attributed to the electron deficiency (two chlorine atoms) which reduced the reactivity of propargyl at N-1 position of isatin moiety.

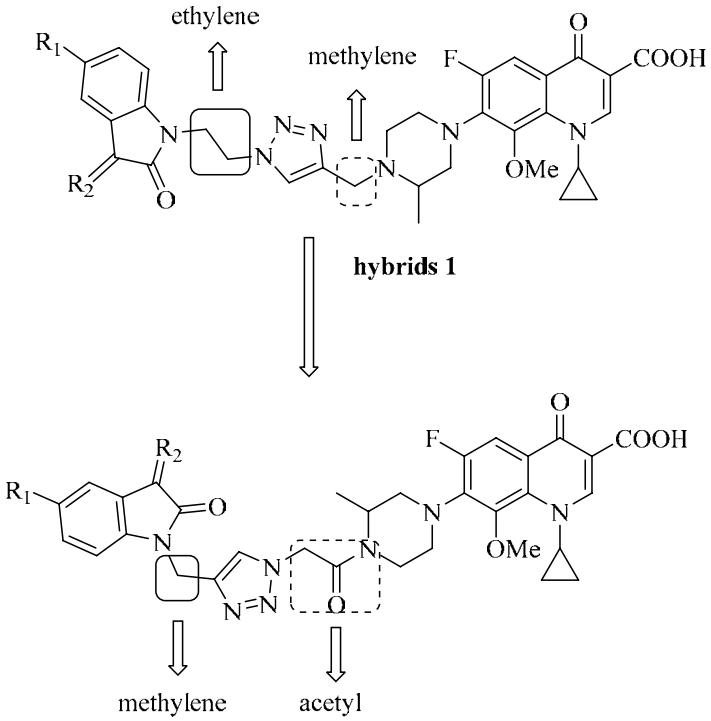
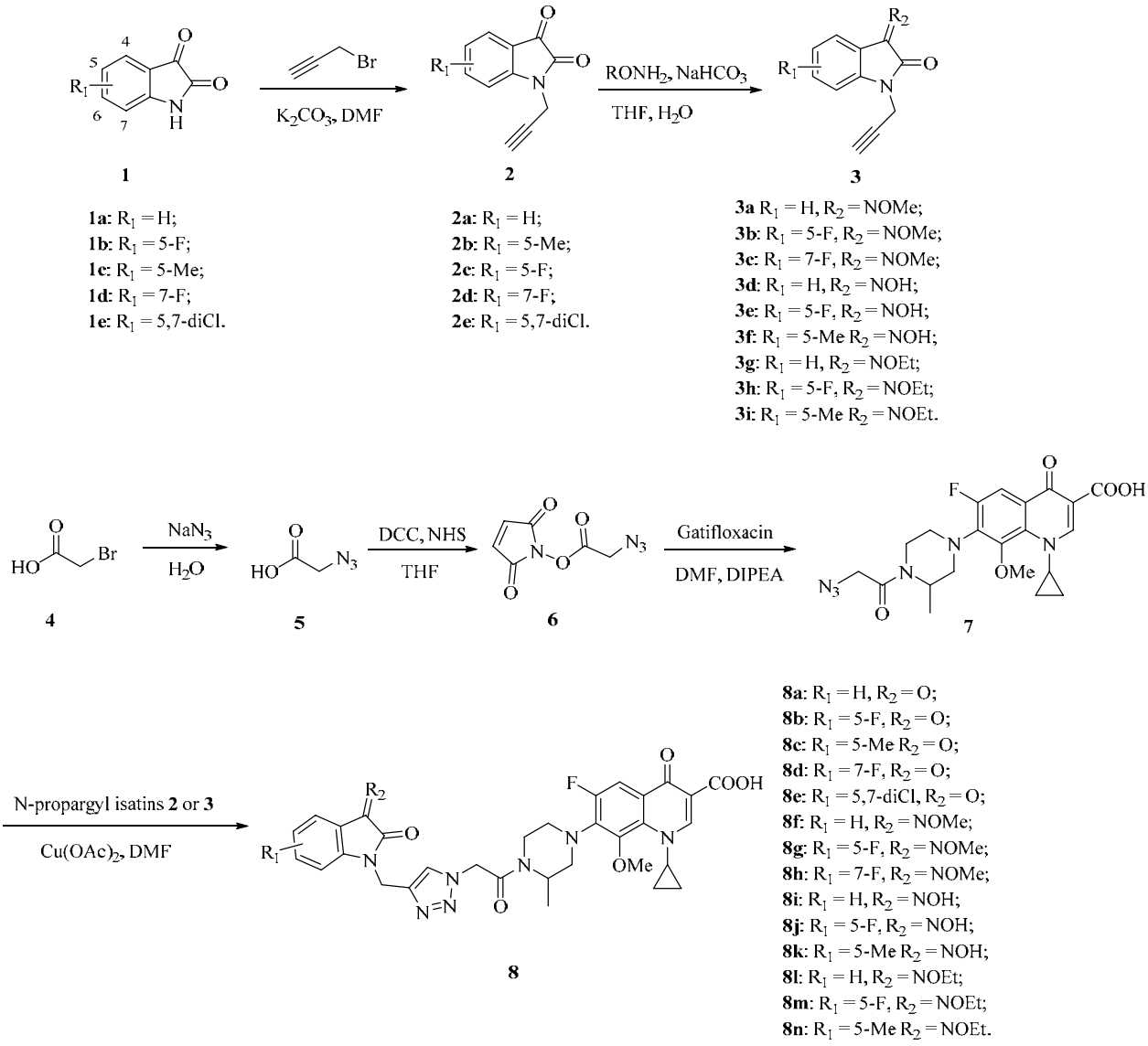


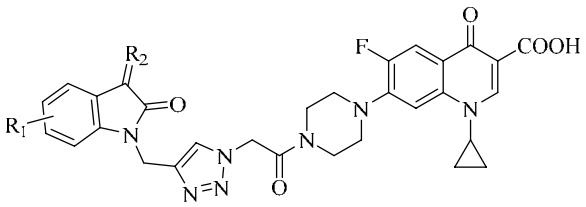
Fig. 1 – The design strategy of methylene and acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids.



Scheme 1 – Synthesis of methylene and acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n**.

Table 1

Chemical structures and yields of gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n**



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Compd.	R_1	R_2	Yield (%)
8a	H	O	63%
8b	5-F	O	71%
8c	5-Me	O	59%
8d	7-F	O	43%
8e	5,7-diCl	O	22%
8f	H	NOMe	79%
8g	5-F	NOMe	68%

Table 1 (continued)

8h	7-F	NOMe	57%
8i	H	NOH	47%
8j	5-F	NOH	56%
8k	5-Me	NOH	69%
8l	H	NOEt	51%
8m	5-F	NOEt	65%
8n	5-Me	NOEt	69%

Table 2

In vitro activity of gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n** against Gram-positive strains

Compd.	**MIC (μg/mL)**								
	S.a.	**MSSA**	**MRSA**	**S.e.**	**MSSE**	**MRSE**	**S.p.**	**E.fa**	**E.fm**
8a	0.06	1	2	0.5	1	4	0.5	1	16
8b	0.12	0.5	1	1	0.5	2	0.5	2	32
8c	0.12	0.25	1	0.5	1	2	0.25	1	16
8d	1	4	8	4	8	18	2	8	64
8e	0.25	2	4	1	2	8	1	8	32
8f	0.25	1	1	0.5	1	1	0.25	1	16
8g	0.06	0.12	1	0.25	0.5	1	0.12	2	8
8h	0.5	1	1	0.5	4	4	4	8	16
8i	0.25	0.5	1	0.5	1	1	0.5	1	16
8j	0.5	1	1	0.5	2	4	0.5	2	16
8k	0.25	1	0.5	0.25	1	2	1	2	16
8l	0.5	2	4	1	2	2	2	4	32
8m	0.5	1	4	2	2	4	4	16	32
8n	0.25	1	2	1	1	2	2	8	16
gatifloxacin	0.12	0.25	1	0.12	0.12	0.5	0.25	1	16
vancomycin	1	1	1	0.5	0.5	1	0.25	4	1

Abbreviations: S.a., *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; S.e., *S. epidermidis*; MSSE, methicillin-sensitive *S. epidermidis*; MRSE, methicillin-resistant *S. epidermidis*; S.p., *S. pneumoniae*; E.fa., *E. faecalis*; E.fm., *E. faecium*.

Table 3

In vitro activity of gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n** against Gram-negative strains

Compd.	**MIC (μg/mL)**									
	E.co.1	**E.co.2**	**K.p.1**	**K.p.2**	**P.a.**	**A.c.**	**E.c.**	**E.a.**	**S.m.**	**C.f.**
8a	0.25	0.5	0.03	1	1	2	0.12	0.12	4	2
8b	0.12	0.12	≤0.03	0.5	1	2	≤0.03	0.12	4	1
8c	0.5	0.5	0.06	0.5	2	1	≤0.03	0.25	8	2
8d	1	1	2	0.5	16	8	0.5	4	16	8
8e	0.5	1	0.5	1	4	2	1	0.5	16	4
8f	0.5	0.5	≤0.03	1	2	1	0.06	0.12	16	2
8g	0.5	0.5	0.03	1	4	2	0.25	0.25	8	4
8h	1	2	0.25	2	2	1	0.5	1	16	8
8i	4	4	0.5	0.25	4	2	1	1	32	8
8j	2	2	0.5	0.25	2	2	1	0.5	8	16
8k	2	4	0.25	0.5	2	4	0.5	1	8	16
8l	1	0.5	0.12	1	0.5	2	0.12	0.25	8	1
8m	1	0.5	0.25	1	2	4	0.25	0.5	8	2
8n	2	1	0.12	2	1	4	0.12	0.5	4	4
gatifloxacin	0.12	0.12	0.06	0.5	0.5	1	≤0.03	0.25	8	1
vancomycin	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

Abbreviations: E.co.1, *Escherichia coli* ESBLs(-); E.co.2, *Escherichia coli* ESBLs(+); K.p.1, *Klebsiella pneumoniae* ESBLs(+); K.p.2, *Klebsiella pneumonia* ESBLs(-); P.a., *Pseudomonas aeruginosa*; A.c., *Acinetobactercal coacetious*; E.c., *Enterobacter cloacae*; E.a., *Enterobacter aerogenes*; S.m., *S. maltophilia*; C.f., *C. freundii*; ESBLs(+): Extended spectrum beta-lactamases (ESBLs).

The antibacterial activity of the methylene and acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n** against a panel of Gram-positive and Gram-negative bacteria including drug-resistant

strains was investigated, and the minimum inhibitory concentration (MIC) values were presented in **Table 2** and **3**.

It can be seen from **Table 2**, all hybrids **8a-n** exhibited promising activity against *S. aureus*, MSSA, MRSA, *S. epidermidis*, MSSE, MRSE, *S. pneumoniae*, *E. faecalis*, and *E. faecium* with MIC values ranging from 0.06 to 32 μg/mL, and some of them were comparable to or better than the parent gatifloxacin (MIC: 0.12-16 μg/mL) and reference vancomycin (MIC: 0.25-4 μg/mL) against certain strains. The structure-activity relationship (SAR) revealed that introduction of methyl group at C-5 position of isatin moiety could enhance the activity greatly, while fluoro has little influence on the activity. Shift the fluoro to C-7 position or incorporation of chloro at both C-5 and C-7 positions was harmful to the activity as evidenced by that hybrids **8d** and **8e** showed the lowest activity in the series, suggesting that introduction of substituents at C-7 position of isatin motif may interfere the interaction between the hybrid molecular and action target. Replacement of ketone by methyloxime (R_2 position) at C-3 position of isatin motif was favorable to the activity, while ethyloxime and oxime could not increase the activity, and the relative contribution order was methyloxime > ketone ≈ oxime > ethyloxime. The most active hybrid **8g** (MIC: 0.06-8 μg/mL) was no inferior to the parent gatifloxacin (MIC: 0.06-8 μg/mL) against the tested Gram-positive pathogens and was 2-16 times more potent than gatifloxacin and vancomycin against *S. aureus*, MSSA, and *S. pneumoniae* strains.

From the data presented in **Table 3**, it can be concluded that all hybrids **8a-n** (MIC: ≤0.03-32 μg/mL) showed considerable activity against *E. coli* ESBLs(-), *E. coli* ESBLs(+), *K. pneumoniae* ESBLs(+), *K. pneumonia* ESBLs(-), *A. coacetious*, *E. cloacae*, *E. aerogenes*, *S. maltophilia*; and *C. freundii*. The antibacterial activity of **8a-g** was no inferior to the parent gatifloxacin (MIC: ≤0.03-8 μg/mL) and was far more potent than vancomycin (MIC: >128 μg/mL) against all tested Gram-negative bacteria. The SAR revealed that replacement of ketone at C-3 position of isatin fragment by methyloxime, ethyloxime and oxime was disfavorable to the activity, and the contribution order was ketone ≥ methyloxime > ethyloxime > oxime. Hybrids with fluoro at C-5 position exhibited higher activity, while hybrids with 5-methyl, 7-fluoro and 5,7-dichloro showed lower activity when compared to unsubstituted analogs, suggesting the substituents at C-5 and C-7 positions have great influence on the interaction between the hybrid molecular and bonding sites. Among them, the most potent hybrid **8b** (MIC: ≤0.03-4 μg/mL) was comparable to or superior to the parent gatifloxacin against the tested Gram-positive strains.

Table 4

In vitro cytotoxicity of methylene and acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids 8a-**n** towards VERO cells

Compd.	CC_{50}(μg/mL)
8a	32
8b	32
8c	128
8d	16
8e	64
8f	64
8g	16
8h	32
8i	128
8j	16
8k	32
8l	128
8m	16
8n	64
gatifloxacin	>128

From **Table 4**, all hybrids **8a-n** also displayed acceptable cytotoxicity towards VERO cells with half-cytotoxic concentration (CC_{50}) in a range of 16 to 128 μg/mL, but they were more toxic than the parent gatifloxacin (CC_{50}: >128 μg/mL). The structure-cytotoxicity relationship suggested that incorporation of oxime, ethyloxime or methyloxime at C-3 position or introduction of either electron-donating or electron-withdrawing groups at C-5 or C-7 position of isatin motif could increase the cytotoxicity when compared with unsubstituted analogs.

Among them, hybrids **8b** (CC_{50}: 32 μg/mL) and **8g** (CC_{50}: 16 μg/mL) with highest activity against Gram-positive and Gram-negative pathogens respectively, also demonstrated acceptable cytotoxicity towards VERO cells. Thus, these two hybrids could serve as lead compounds for further investigations.

EXPERIMENTAL

1. Synthesis

To a suspension of gatifloxacin (50 mmol) in *N,N*-dimethylformamide (DMF, 500 mL), NHS ester **6** (60 mmol), DIPEA (100 mL) in tetrahydrofuran (THF, 100 mL) was added during a period of 10 min under nitrogen atmosphere at 0 ^{o}C. The mixture was stirred at room temperature overnight, and then the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography eluted with dichloromethane (DCM) : methanol (MeOH) =10:1 to give the gatifloxacin intermediate **7**.

To a mixture of gatifloxacin derivative **7** (6 mmol) and isatin intermediates **2** or **3** (8 mmol) in DMF (60 mL), $Cu(OAc)_2$ (1 mmol) was added. The mixture was stirred at 40 ^{o}C for 8 h, and the filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by reverse phase column with formic acid as additive to give the desired products **8**.

1.1. *1-cyclopropyl-7-(4-(2-(4-((2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8a**)

Yellow solid, yield: 63%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03-1.43 (7H, m, cyclopropyl-4H and -CH_3), 3.15-3.56 (5H, m, piperazinyl-5H), 3.74-4.26 (6H, m, piperazinyl-2H, cyclopropyl-1H and -OCH_3), 5.01 (2H, s, -CH_2- linker), 5.53-5.65 (2H, m, -CH_2- linker), 7.15 (1H, t, *J* = 8.0 Hz, Ar-H), 7.20 (1H, d, *J* = 8.0 Hz, Ar-H), 7.59 (1H, d, *J* = 8.0 Hz, Ar-H), 7.66 (1H, t, *J* = 8.0 Hz, Ar-H), 7.80 (1H, d, *J* = 8.0 Hz, Ar-H), 8.11 (1H, s, Ar-H), 8.72 (1H, s, Ar-H), 14.92 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{32}H_{31}FN_7O_7$ $[M+H]^+$: 644.22635; Found: 644.22407.

1.2. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((5-fluoro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8b**)

Yellow solid, yield: 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01-1.42 (7H, m, cyclopropyl-4H and -CH_3), 3.17-3.55 (5H, m, piperazinyl-5H), 3.73-4.26 (6H, m, piperazinyl-2H, cyclopropyl-1H and -OCH_3), 5.01 (2H, s, -CH_2- linker), 5.43-5.65 (2H, m, -CH_2- linker), 7.22 (1H, d, *J* = 4.0 Hz, Ar-H), 7.48-7.56 (2H, m, Ar-H), 7.76 (1H, d, *J* = 12.0 Hz, Ar-H), 8.11 (1H, s, Ar-H), 8.71 (1H, s, Ar-H), 14.94 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{32}H_{30}F_2N_7O_7$ $[M+H]^+$: 662.21693; Found: 662.21518.

1.3. *1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(2-(4-((5-methyl-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8c**)

Yellow solid, yield: 71%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01-1.52 (7H, m, cyclopropyl-4H and -CH_3), 2.32 (3H, s, -CH_3), 3.26-3.55 (6H, m, piperazinyl-6H), 3.71-3.75 (4H, m, piperazinyl-1H, and -OCH_3), 4.01-4.02 (1H, m, cyclopropyl-1H), 5.02 (2H, s, -CH_2- linker), 5.22-5.39 (2H, m, -CH_2- linker), 7.16 (1H, d, *J* = 8.0 Hz, Ar-H), 7.38-7.40 (2H, m, Ar-H), 7.83-7.86 (2H, m, Ar-H), 8.82 (1H, s, Ar-H).

1.4. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((7-fluoro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8d**)

Yellow solid, yield: 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01-1.38 (7H, m, cyclopropyl-4H and -CH_3), 3.30-3.57 (4H, m, piperazinyl-4H), 3.76 (3H, s, -OCH_3), 4.03-4.04 (1H, m, cyclopropyl-1H), 4.47-4.82 (3H, m, piperazinyl-3H), 5.23 (2H, s, -CH_2- linker), 5.32-5.39 (2H, m, -CH_2- linker), 7.12 (1H, t, *J* = 4.0 Hz, Ar-H), 7.34-7.39 (1H, m, Ar-H), 7.44 (1H, d, *J* = 8.0 Hz, Ar-H), 7.87-7.92 (2H, m, Ar-H), 8.82 (1H, s, Ar-H), 14.82 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{32}H_{30}F_2N_7O_7$ $[M+H]^+$: 662.21693; Found: 662.21447.

1.5. *1-cyclopropyl-7-(4-(2-(4-((5,7-dichloro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8e**)

Yellow solid, yield: 22%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02-1.42 (7H, m, cyclopropyl-4H and -CH_3), 3.14-3.55 (6H, m, piperazinyl-6H), 3.73-3.81 (4H, m, piperazinyl-1H and -OCH_3), 4.15-4.16 (1H, m, cyclopropyl-1H), 5.29 (2H, s, -CH_2- linker), 5.43-5.69 (2H, m, -CH_2- linker), 7.20 (1H, d, *J* = 8.0 Hz, Ar-H), 7.64 (1H, d, *J* = 12.0 Hz, Ar-H), 7.76 (1H, d, *J* = 12.0 Hz, Ar-H), 8.12 (1H, s, Ar-H), 8.72 (1H, s, Ar-H), 14.91 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{32}H_{29}Cl_2FN_7O_7$ $[M+H]^+$: 712.14841; Found: 712.14639.

1.6. *1-cyclopropyl-6-fluoro-8-methoxy-7-(4-(2-(4-((3-(methoxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8f**)

Yellow solid, yield: 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01-1.49 (7H, m, cyclopropyl-4H and -CH_3), 3.29-3.55 (5H, m, piperazinyl-5H), 3.74-3.76 (4H, m, piperazinyl-1H and -OCH_3), 4.02-4.03 (1H, m, cyclopropyl-1H), 4.27-4.46 (4H, m, piperazinyl-1H and -$NOCH_3$), 5.09 (2H, s, -CH_2- linker), 5.19-5.32 (2H, m, -CH_2- linker), 7.06 (1H, t, *J* = 8.0 Hz, Ar-H), 7.20 (1H, d, *J* = 8.0 Hz, Ar-H), 7.36 (1H, d, *J* = 8.0 Hz, Ar-H), 7.81 (1H, d, *J* = 8.0 Hz, Ar-H), 7.90-7.95 (2H, m, Ar-H), 8.84 (1H, s, Ar-H). HRMS-ESI: m/z Calcd. for $C_{33}H_{34}FN_8O_7$ $[M+H]^+$: 673.25290; Found: 673.24989.

1.7. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((5-fluoro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8g**)

Yellow solid, yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02-1.38 (7H, m, cyclopropyl-4H and -CH_3), 3.29-3.54 (5H, m, piperazinyl-5H), 3.73-3.77 (4H, m, piperazinyl-1H and -OCH_3), 4.02-4.03 (1H, m, cyclopropyl-1H), 4.28-4.32 (4H, m, piperazinyl-1H and -$NOCH_3$), 5.08 (2H, s, -CH_2- linker), 5.20-5.32 (2H, m, -CH_2- linker), 7.08-7.20 (2H, m, Ar-H), 7.68 (1H, d, *J* = 4.0 Hz, Ar-H), 7.82 (1H, s, Ar-H), 7.92 (1H, d, *J* = 12.0 Hz, Ar-H), 8.85 (1H, s, Ar-H), 14.65 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{33}H_{33}F_2N_8O_7$ $[M+H]^+$: 691.24348; Found: 691.24071.

1.8. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((7-fluoro-3-(methoxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8h**)

Yellow solid, yield: 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.82-1.43 (7H, m, cyclopropyl-4H and -CH_3), 3.13-3.24 (5H, m, piperazinyl-5H), 3.70-3.79 (4H, m, piperazinyl-1H and

-OCH_3), 3.98-3.99 (1H, m, cyclopropyl-1H), 4.19-4.26 (4H, m, piperazinyl-1H and -$NOCH_3$), 5.09 (2H, s, -CH_2- linker), 5.40-5.63 (2H, m, -CH_2- linker), 7.10-7.15 (1H, m, Ar-H), 7.38 (1H, t, *J* = 8.0 Hz, Ar-H), 7.66 (1H, d, *J* = 12.0 Hz, Ar-H), 7.78 (1H, t, *J* = 8.0 Hz, Ar-H), 8.02 (1H, s, Ar-H), 8.64 (1H, s, Ar-H). HRMS-ESI: m/z Calcd. for $C_{33}H_{33}F_2N_8O_7$ $[M+H]^+$: 691.24348; Found: 691.24052.

1.9. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((3-(hydroxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8i**)

Yellow solid, yield: 47%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.00-1.42 (7H, m, cyclopropyl-4H and -CH_3), 3.16-3.55 (5H, m, piperazinyl-5H), 3.73-3.81 (4H, m, piperazinyl-1H and -OCH_3), 4.15-4.25 (2H, m, piperazinyl-1H and cyclopropyl-1H), 5.03 (2H, s, -CH_2- linker), 5.76 (2H, s, -CH_2- linker), 7.09 (1H, t, *J* = 8.0 Hz, Ar-H), 7.18 (1H, d, *J* = 8.0 Hz, Ar-H), 7.42 (1H, t, *J* = 8.0 Hz, Ar-H), 7.76 (1H, d, *J* = 12.0 Hz, Ar-H), 8.02 (1H, s, Ar-H), 8.32 (1H, s, Ar-H), 8.74 (1H, s, Ar-H). HRMS-ESI: m/z Calcd. for $C_{32}H_{32}FN_8O_7$ $[M+H]^+$: 659.23725; Found: 659.23402.

1.10. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((5-fluoro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8j**)

Yellow solid, yield: 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02-1.47 (7H, m, cyclopropyl-4H and -CH_3), 3.28-3.57 (5H, m, piperazinyl-5H), 3.73-3.75 (4H, m, piperazinyl-1H and -OCH_3), 4.02-4.04 (1H, m, cyclopropyl-1H), 4.14-4.16 (1H, m, piperazinyl-1H), 5.05 (2H, d, *J* = 12.0 Hz, -CH_2- linker), 5.21-5.35 (2H, m, -CH_2- linker), 6.97-7.14 (3H, m, Ar-H), 7.79-7.95 (2H, m, Ar-H), 8.85 (1H, s, Ar-H), 14.68 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{32}H_{31}F_2N_8O_7$ $[M+H]^+$: 677.22783; Found: 677.22512.

1.11. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((3-(hydroxyimino)-5-methyl-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8k**)

Yellow solid, yield: 69%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.03-1.41 (7H, m, cyclopropyl-4H and -CH_3), 2.26 (3H, s, -CH_3), 3.17-3.55 (6H, m, piperazinyl-6H), 3.73 (3H, s, -OCH_3), 4.16-4.18 (2H, m, piperazinyl-1H and cyclopropyl-1H), 5.00 (2H, s, -CH_2- linker), 5.38-5.67 (2H, m, -CH_2- linker), 7.04 (1H, d, *J* = 8.0 Hz, Ar-H), 7.24 (1H, d, *J* = 4.0 Hz, Ar-H), 7.80 (1H, d, *J* = 12.0 Hz, Ar-H), 8.00 (1H, s, Ar-H), 8.73 (1H, s, Ar-H), 14.92 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{33}H_{34}FN_8O_7$ $[M+H]^+$: 673.25290; Found: 673.25076.

1.12. *1-cyclopropyl-7-(4-(2-(4-((3-(ethoxyimino)-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8l**)

Yellow solid, yield: 51%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20-1.41 (10H, m, $NOCH_2\underline{CH_3}$, cyclopropyl-4H and -CH_3), 3.34-3.42 (4H, m, piperazinyl-4H), 3.70-3.82 (6H, m, -OCH_3 and piperazinyl-3H), 4.45-4.58 (3H, m, $NO\underline{CH_2}CH_3$ and cyclopropyl-1H), 5.02 (2H, s, -CH_2- linker), 5.52 (2H, s, -CH_2- linker), 7.10 (1H, t, *J* = 8.0 Hz, Ar-H), 7.19 (1H, d, *J* = 4.0 Hz, Ar-H), 7.46 (1H, d, *J* = 8.0 Hz, Ar-H), 7.90-7.95 (2H, m, Ar-H), 8.03 (1H, s, Ar-H), 8.68 (1H, s, Ar-H). HRMS-ESI: m/z Calcd. for $C_{34}H_{36}FN_8O_7$ $[M+H]^+$: 687.26855; Found: 687.26346.

1.13. *1-cyclopropyl-6-fluoro-7-(4-(2-(4-((5-fluoro-2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8m**)

Yellow solid, yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00-1.47 (10H, m, $NOCH_2\underline{CH_3}$, cyclopropyl-4H and -CH_3), 3.27-3.52 (5H, m, piperazinyl-5H), 3.73-3.75 (4H, m, piperazinyl-1H and -OCH_3), 4.03-4.05 (1H, m, cyclopropyl-1H), 4.45-4.56 (3H, m, $NO\underline{CH_2}CH_3$ and piperazinyl-1H), 5.24 (2H, d, *J* = 12.0 Hz, -CH_2- linker), 5.30-5.36 (2H, m, -CH_2- linker), 6.99-7.14 (2H, m, Ar-H), 7.19-7.88 (3H, m, Ar-H), 8.85 (1H, s, Ar-H), 14.69 (1H, brs, COOH). HRMS-ESI: m/z Calcd. for $C_{34}H_{35}F_2N_8O_7$ $[M+H]^+$: 705.25913; Found: 705.25686.

1.14. *1-cyclopropyl-7-(4-(2-(4-((3-(ethoxyimino)-5-methyl-2-oxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (**8n**)

Yellow solid, yield: 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28-1.50 (10H, m, $NOCH_2\underline{CH_3}$, cyclopropyl-4H and -CH_3), 2.27 (3H, s, -CH_3), 3.28-3.55 (5H, m, piperazinyl-5H), 3.73-3.75 (4H, m, piperazinyl-1H and -OCH_3), 4.01-4.03 (1H, m, cyclopropyl-1H), 4.50-4.60 (3H, m, $NO\underline{CH_2}CH_3$ and piperazinyl-1H), 5.08 (2H, s, -CH_2- linker), 5.17-5.32 (2H, m, -CH_2- linker), 7.06 (1H, d, *J* = 8.0 Hz, Ar-H), 7.20 (1H, d, *J* = 8.0 Hz, Ar-H), 7.79 (2H, s, Ar-H), 7.94 (1H, d, *J* = 12.0 Hz, Ar-H), 8.86 (1H, s, Ar-H). HRMS-ESI: m/z Calcd. for $C_{35}H_{38}FN_8O_7$ $[M+H]^+$: 701.28420; Found: 701.28039.

2. MIC determination

The antibacterial activity of all hybrids against representative Gram-positive and Gram-negative strains were tested by means of standard two-fold serial dilution method using agar media.[25] Petri dishes were incubated with 10^4 colony-forming units (cfu) and incubated at 35 °C for 18-24 h.

3. Cytotoxicity

The cytotoxicity (CC_{50}) of the methylene and acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n**, together with the parent gatifloxacin were examined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in a mammalian VERO cells.[26] The CC_{50} values were calculated by Bliss analyses.

CONCLUSIONS

In conclusion, fourteen methylene and acetyl tethered gatifloxacin-1,2,3-triazole-isatin hybrids **8a-n** were designed, synthesized and examined for their *in vitro* antibacterial activity against a panel of Gram-positive and Gram-negative pathogens as well as cytotoxicity towards VERO cells. The synthesized hybrids showed promising *in vitro* activity against both drug-sensitive and drug-resistant organisms, and acceptable cytotoxicity towards VERO. Among them, hybrids **8b** and **8g** with highest activity against Gram-positive and Gram-negative pathogens respectively, also demonstrated acceptable cytotoxicity towards VERO cells.

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