Design, Synthesis, and *In Vitro* Antibacterial Activities of Propylene-tethered Gatifloxacin-isatin Hybrids

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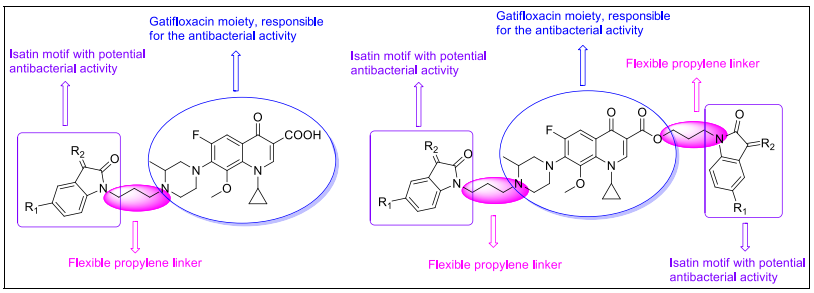

A series of novel mono-/bis-isatin-gatifloxacin hybrids **3a–f** and **4a–f** tethered through propylene were designed, synthesized, and evaluated for their *in vitro* antibacterial activities against representative Gram-positive and Gram-negative pathogens. The results indicated that all mono-isatin-gatifloxacin hybrids exhibited considerable antibacterial activities with minimum inhibitory concentration ranging from 0.06 to 4 μg/mL against the majority of the tested strains. In particular, the mono-isatin-gatifloxacin hybrid **3b** was found as potent as the parent gatifloxacin against Gram-positive organisms and could act as a starting point for further optimization.

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INTRODUCTION

Bacterial infections caused by Gram-positive and Gram-negative organisms are responsible for the majority of hospital-acquired infections and result in extensive mortality and burden on global healthcare systems [1,2]. The emergency and widespread of bacteria new virulent forms such as drug-resistant pathogens like methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis*, vancomycin-resistant *S. aureus*, and extended spectrum β-lactamase-producing *Escherichia coli* has already increased up to alarming level in the recent decades and is associated with considerable mortality [3,4]. Thus, new antibiotics are needed urgently.

Fluoroquinolones, which bearing a carboxyl group at C-3 position, a keto group at C-4 position, a fluorine atom at C-6 position, and a basic nitrogen heterocycle moiety at the C-7 position, are a family of synthetic broad spectrum antibiotics widely used in clinical practice for infections including upper and lower respiratory infections, gastrointestinal infections, gynecologic infections, sexually transmitted diseases, prostatitis, and some skin, bone, and soft tissue infections, and their value and role in the treatment of bacterial infections continue to expand [1]. Fluoroquinolones mainly act by binding two type II bacterial topoisomerase enzymes, DNA gyrase, and topoisomerase IV, and DNA gyrase is the predominately target for Gram-negative bacteria, whereas topoisomerase IV is the target for most Gram-positive bacteria [5]. The fourth generation fluoroquinolone antibiotics can act at both DNA gyrase and topoisomerase IV, so this dual action makes the strains resistant to them relatively slow [6]. Besides their typical antibacterial activities, fluoroquinolones also endow with diverse atypical biological profiles such as antimalarial [7], antitumor [8], anti-human immunodeficiency virus [9], and anti-tuberculosis [10–13] properties, which play a pivotal role in new drug discovery.

However, the resistance of pathogens to fluoroquinolone agents develops rapidly and spreads widely due to the long-term, broad, inappropriate use, and even abuse of this kind of antibiotics, making this kind of antibiotics more and more ineffective. Thus, enhancing the potency of fluoroquinolones has become increasingly urgent.

Isatin (1*H*-indole-2,3-dione) derivatives also demonstrated a broad range of biological properties such as antibacterial activity [14–19]. Therefore, incorporation of isatin into fluoroquinolones may lead to more active candidates.

As an ongoing research program and to continue our effort to develop new antibacterial agents, a series of novel propylene-tethered mono-/bis-isatin-gatifloxacin hybrids were designed, synthesized, and assessed for their *in vitro* antibacterial activities against clinically important pathogens in this study. A preliminary structure–activity relationship (SAR) study is also explored to facilitate the further design. The design strategy is illustrated in Figure 1.

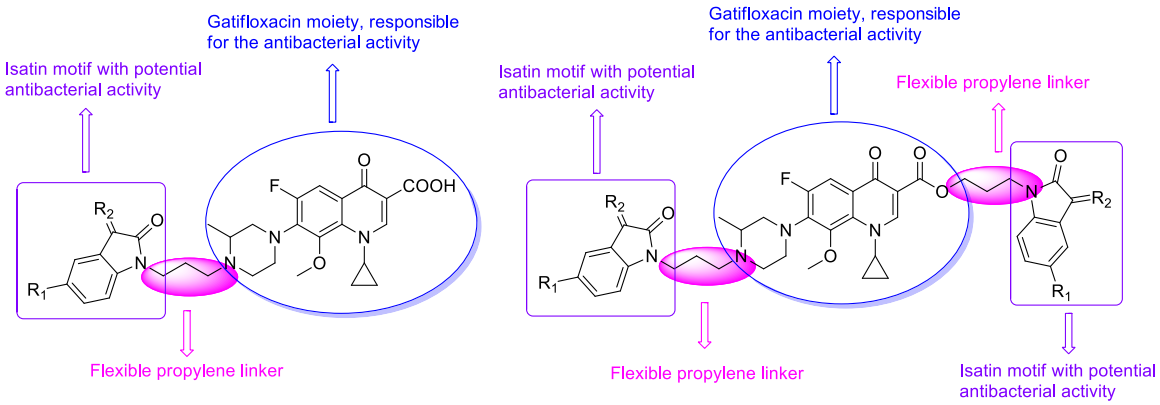

Figure 1. Illustration of design strategy. [Color figure can be viewed at wileyonlinelibrary.com]

RESULTS AND DISCUSSION

The synthetic routes for propylene-tethered mono-/bis-isatin-gatifloxacin hybrids **3a–f** and **4a–f** are depicted in Scheme 1. Alkylation of C-5 substituted isatins **1a–c** was performed with 1,3-dibromopropane to afford *N*-(3-bromopropyl) isatins **2a–c** (yield: 56–78%), which were then incorporated into gatifloxacin to provide the desired mono-isatin-gatifloxacin **3a–c** (yield: 26–33%) and bis-isatin-gatifloxacin hybrids **4a–c** (yield: 22–35%).

Scheme 1. Synthetic route for mono-/bis-isatin-gatifloxacin hybrids **3a–f** and **4a–f**.

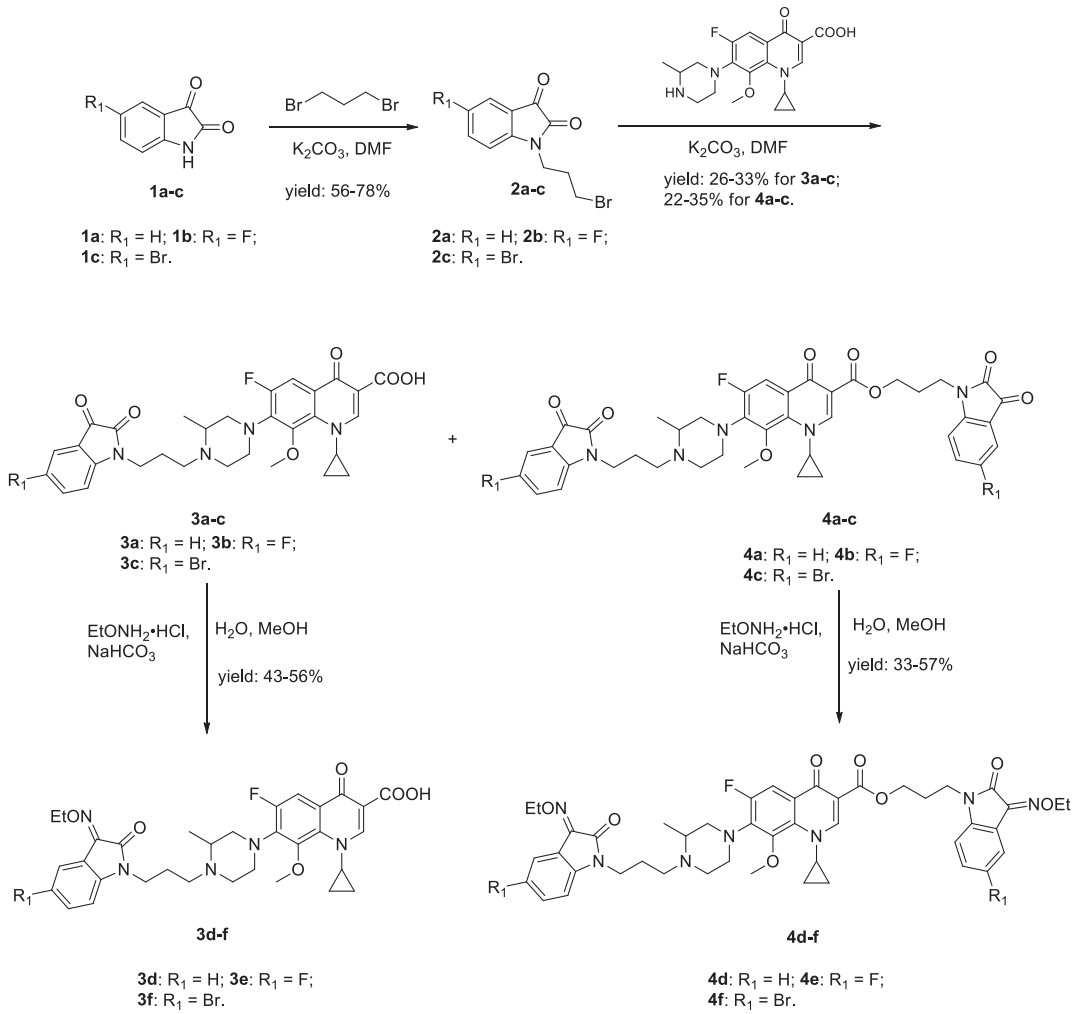

Table 1

In vitro antibacterial activity of hybrids **3a–f** and **4a–f** against Gram-positive strains.

Compound	MIC (μg/mL)							
	MSSE	MRSE	MSSA	MRSA	E.fa.1	E.fa.2	E.fm.1	E.fm.2
3a	0.50	64	0.25	0.25	1	2	32	64
3b	0.25	16	0.125	0.125	0.5	1	32	32
3c	0.25	32	0.125	0.25	1	1	32	64
3d	0.5	64	0.25	0.5	1	1	32	>64
3e	0.25	32	0.125	0.25	1	2	32	64
3f	0.5	64	0.25	0.25	2	2	64	64
4a	64	>64	>64	>64	>64	>64	>64	>64
4b	64	>64	32	64	64	>64	>64	>64
4c	>64	>64	>64	>64	>64	>64	>64	>64
4d	>64	>64	>64	>64	>64	>64	>64	>64
4e	>64	>64	>64	>64	>64	>64	>64	>64
4f	>64	>64	>64	>64	>64	>64	>64	>64
Ciprofloxacin	0.125	64	0.25	0.5	0.5	0.5	>64	>64
Gatifloxacin	0.125	32	0.125	0.25	1	0.5	16	32

E.fa.1, *Enterococcus faecalis* ATCC 29212; E.fa.2, *Enterococcus faecalis* ATCC 51299; E.fm.1, *Enterococcus faecium* ATCC 700221; E.fm.2, *Enterococcus faecium* 13-7; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus* ATCC 33591; MRSE, methicillin-resistant *Staphylococcus epidermidis* 13-3; MSSA, methicillin-sensitive *Staphylococcus aureus* ATCC 29213; MSSE, methicillin-sensitive *Staphylococcus epidermidis* ATCC 12228.

Subsequently, condensation of hybrids **3a–c** or **4a–c** with ethylhydroxylamine hydrochloride yielded other conjugates **3d–f** (yield: 43–56%) and **4d–f** (yield: 33–57%).

All propylene-tethered mono-/bis-isatin-gatifloxacin **3a–f** and **4a–f** were assessed for their *in vitro* antibacterial activity against representative Gram-positive and Gram-negative pathogens using standard techniques [4]. The minimum inhibitory concentration (MIC) is obtained from three independent experiments, defined as the concentration of the compound required to give complete inhibition of bacterial growth, and the MIC values of the hybrids **3a–f** and **4a–f** against Gram-positive and Gram-negative strains, along with those of gatifloxacin and ciprofloxacin for comparison, were listed in Tables 1 and 2, respectively.

As it can be seen from Table 1, all mono-isatin-gatifloxacin **3a–f** displayed promising activities against the majority of the tested Gram-positive strains with MIC ranging from 0.125 to 2 μg/mL. Among them, the most active hybrid **3b** was highly potent against clinically important pathogens methicillin-sensitive *S. epidermidis*, methicillin-sensitive *S. aureus*, methicillin-resistant *S. aureus*, and *Enterococcus faecalis* with MIC of 0.25, 0.125, 0.125, and 0.5 μg/mL, respectively, and was no inferior to gatifloxacin (MIC: 0.125–0.5 μg/mL).

From Table 1, it can be concluded that all mono-isatin-gatifloxacin **3a–f** showed considerable activities against most of the tested Gram-negative organisms with MIC in a range of 0.06 to 4 μg/mL but less active than the parent gatifloxacin (MIC: ≤0.03–1 μg/mL). Interestingly, the hybrid **3b** also exhibited the most active potency against the tested Gram-negative strains.

The SAR revealed that all mono-isatin-gatifloxacin hybrids were more potent than the corresponding bis-isatin-gatifloxacin analogs against both Gram-positive and Gram-negative organisms, which was in accordance with the previous study that carboxylic acid at C-3 position is essential for gyrase binding and bacterial membrane transport [1]; substituents at C-3 and C-5 positions of isatin moiety influenced the antibacterial activity significantly: introduction of ethoxyimino at C-3 position disfavored the activity; hybrids with electron-withdrawing -F and -Br at C-5 position of isatin moiety exhibited higher potency than unsubstituted analogs.

CONCLUSIONS

In summary, a series of novel propylene-tethered mono-/bis-isatin-gatifloxacin hybrids **3a–f** and **4a–f** were designed, synthesized, and assessed for their *in vitro* antibacterial activities against representative Gram-positive and Gram-negative pathogens. The results revealed that all mono-isatin-gatifloxacin hybrids endowed with considerable antibacterial activities, and hybrid **3b** was comparable with the parent gatifloxacin against Gram-positive organisms, could act as a starting point for further investigation. Moreover, the enriched SAR paves the way for further optimization.

EXPERIMENTAL

The mixture of isatin (10 mmol), 1,3-dibromopropane (30 mmol), and potassium carbonate (50 mmol) in

Table 2

In vitro antibacterial activity of hybrids **3a–f** and **4a–f** against Gram-negative strains.

	MIC (μg/mL)														
Compound	E.co. 1	E.co. 2	K.p.1	K.p.2	P.a.	A.c.	E.c.	E.a.	S.m.1	M.m.	P.r.	P.v.	P.m.	S.m.2	C.f.
3a	0.25	>64	8	2	8	4	0.5	1	2	0.25	0.125	0.5	0.5	16	0.5
3b	0.06	32	4	0.5	2	0.5	0.125	0.5	0.25	0.125	0.06	0.125	0.25	2	0.25
3c	0.125	64	4	1	2	1	0.125	0.5	0.5	0.5	0.125	0.25	0.25	4	0.125
3d	0.5	>64	8	4	16	8	0.5	2	2	1	1	2	4	16	2
3e	0.25	64	4	2	4	2	0.25	1	0.5	0.25	0.25	0.5	0.5	8	1
3f	0.5	64	4	4	8	4	0.25	2	1	0.5	1	0.5	1	4	1
4a	32	>64	>64	64	>64	32	16	64	64	64	64	>64	32	>64	64
4b	32	>64	64	32	>64	64	16	32	64	32	64	>64	16	>64	32
4c	32	>64	32	64	>64	32	32	64	64	32	64	>64	32	>64	64
4d	>64	>64	64	>64	>64	>64	64	64	>64	64	>64	>64	64	>64	64
4e	64	>64	64	64	>64	64	64	32	>64	64	>64	>64	64	>64	64
4f	>64	>64	64	64	>64	>64	64	64	>64	64	>64	>64	64	>64	64
Ciprofloxacin	≤0.03	8	0.5	≤0.03	0.25	0.5	≤0.03	≤0.03	0.06	≤0.03	≤0.03	≤0.03	≤0.03	4	≤0.03
Gatifloxacin	≤0.03	16	0.5	≤0.03	0.5	0.125	≤0.03	0.125	0.125	≤0.03	≤0.03	≤0.03	0.125	1	≤0.03

A.c., *Acinetobacter calcoacetious* ATCC 19606; C.f., *Citrobacter freundii* ATCC 43864; E.a., *Enterobacter aerogenes* ATCC 13048; E.c., *Enterobacter cloacae* ATCC 43560; E.co.1, *Escherichia coli* ATCC 25922 extended spectrum β-lactamases (−); E.co.2, *Escherichia coli* 14-11 extended spectrum β-lactamases (+); K.p.1, *Klebsiella pneumoniae* ATCC 700603 extended spectrum β-lactamases (+); K.p.2, *Klebsiella pneumonia* 7 extended spectrum β-lactamases (−); MIC, minimum inhibitory concentration; M.m., *Morganella morganii* ATCC 25830; P.a., *Pseudomonas aeruginosa* ATCC 27853; P.r., *Providentia rettgeri* ATCC 31052; P.v., *Proteus vulgaris* ATCC 29905; P.m., *Proteus mirabilis* 13-1; S.m.1, *Serratia marcescens* ATCC 21074; S.m.2, *Stenotrophomonas maltophilia* ATCC 13636.

dimethylformamide (100 mL) was stirred at room temperature for 3 days and then filtered. The filtrate was concentrated under reduced pressure, and the reside was purified by silica gel chromatography eluted with PE: EA = 2 : 1 to give *N*-(2-bromopropyl) isatin **2a–c** (yield: 56–78%) as red solids.

The mixture of intermediates **2a–c** (1.2 mmol), gatifloxacin (1 mmol), and potassium carbonate (3 mmol) in dimethylformamide (10 mL) was stirred at room temperature overnight. After filtration, the filtrate was concentrated under reduced pressure, and the residue was purified by reverse phase chromatography with formic acid as additive to afford mono-/bis-isatin-gatifloxacin hybrids **3a–c** (yield: 26–33%) and **4a–c** (yield: 22–35%).

The mixture of hybrids **3a–c** or **4a–c** (1 mmol), sodium bicarbonate (3 mmol), and ethoxyamine hydrochloride (1.2 mmol) in water (10 mL) and MeOH (50 mL) was stirred at 50°C for 12 h. After removal of the solvent, the residue was purified by reverse phase chromatography with formic acid as additive to afford mono-/bis-isatin-gatifloxacin hybrids **3d–f** (yield: 43–56%) and **4d–f** (yield: 33–57%).

1-Cyclopropyl-7-(4-(3-(2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3a). Yellow solid, yield: 26%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.95–1.09 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.99–2.06 (2H, m, –CH_2–), 2.96–3.31 (7H, m, piperazine-7H), 3.77 (3H, s, OCH_3), 3.86 (2H, t, –CH_2–), 4.00–4.02 (1H, m, cyclopropyl-CH), 4.24 (2H, t, –CH_2–), 7.07 (1H, t, Ar–H), 7.32 (1H, d, Ar–H), 7.46 (1H, d, Ar–H), 7.60–7.63 (2H, m, Ar–H), 8.46 (1H, s, C2-H). ESI-MS *m/z*: 563 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{30}H_{31}FN_4O_6$: C, 64.05; H, 5.55; N, 9.96. Found: C, 63.81; H, 5.37; N, 9.73.

1-Cyclopropyl-6-fluoro-7-(4-(3-(5-fluoro-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3b). Yellow solid, yield: 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94–1.08 (4H, m, 2 × cyclopropyl-CH_2), 1.14 (3H, d, CH_3), 1.97–2.03 (2H, m, –CH_2–), 3.00–3.34 (7H, m, piperazine-7H), 3.80 (3H, s, OCH_3), 3.88 (2H, t, –CH_2–), 3.90–3.99 (1H, m, cyclopropyl-CH), 4.23 (2H, t –CH_2–), 7.21–7.31 (2H, m, Ar–H), 7.76–7.63 (2H, m, Ar–H), 8.40 (1H, s, C2-H). ESI-MS *m/z*: 581 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{30}H_{30}F_2N_4O_6$: C, 62.06; H, 5.21; N, 9.65. Found: C, 61.89; H, 5.03; N, 9.41.

1-7-(4-(3-(5-Bromo-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c). Yellow solid, yield: 29%. ^{11}H NMR (400 MHz, DMSO-d_6) δ 0.84–0.96 (4H, m, 2 × cyclopropyl-CH_2), 1.06 (3H, d, CH_3), 1.98–2.04 (2H, m, –CH_2–), 2.90–3.31 (7H, m, piperazine-7H), 3.80 (3H, s, OCH_3), 3.89 (2H, t, –CH_2–), 3.98–4.01 (1H, m, cyclopropyl-CH), 4.18 (2H, t –CH_2–), 7.25 (1H, d, Ar–H), 7.59–7.64 (2H, m, Ar–H), 7.88 (1H, s, Ar–H), 8.38 (1H, s, C2-H). ESI-MS *m/z*: 641 $[M + H]^+$, 643 $[M + 2 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{30}H_{30}FBrN_4O_6$: C, 56.17; H, 4.71; N, 8.73. Found: C, 55.94; H, 4.49; N, 8.55.

1-Cyclopropyl-7-(4-(3-(3-(ethoxyimino)-2-oxoindolin-1-yl) propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3d). Yellow solid, yield: 49%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94–1.08 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.34 (3H, t, $NOCH_2\underline{CH_3}$), 2.00–2.05 (2H, m, –CH_2–), 2.91–3.32 (7H, m, piperazine-7H), 3.79 (3H, s, OCH_3), 3.88 (2H, t, –CH_2–), 3.99–4.01 (1H, m, cyclopropyl-CH), 4.19 (2H, t, –CH_2–), 4.42 (2H, q, $NO\underline{CH_2}CH_3$), 7.06 (1H, t, Ar–H), 7.28 (1H, d, Ar–H), 7.42–7.45 (1H, m, Ar–H), 7.63 (1H, d, Ar–H), 7.84 (1H, d, Ar–H), 8.42 (1H, s, C2-H). ESI-MS *m/z*: 606 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{36}FN_5O_6$: C, 63.46; H, 5.99; N, 11.56. Found: C, 63.17; H, 5.74; N, 11.39.

1-Cyclopropyl-7-(4-(3-(3-(ethoxyimino)-5-fluoro-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3e). Yellow solid, yield: 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94–1.09 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.34 (3H, t, $NOCH_2\underline{CH_3}$), 1.99–2.04 (2H, m, –CH_2–), 2.93–3.33 (7H, m, piperazine-7H), 3.79 (3H, s, OCH_3), 3.89 (2H, t, –CH_2–), 3.99–4.00 (1H, m, cyclopropyl-CH), 4.18 (2H, t, –CH_2–), 4.41 (2H, q, $NO\underline{CH_2}CH_3$), 7.30–7.32 (2H, m, Ar–H), 7.57–7.64 (2H, m, Ar–H), 8.41 (1H, s, C2-H). ESI-MS *m/z*: 624 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{35}F_2N_5O_6$: C, 61.63; H, 5.66; N, 11.23. Found: C, 61.39; H, 5.51; N, 11.03.

7-(4-(3-(5-Bromo-3-(ethoxyimino)-2-oxoindolin-1-yl) propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3f). Yellow solid, yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93–1.08 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.33 (3H, t, $NOCH_2\underline{CH_3}$), 1.98–2.03 (2H, m, –CH_2–), 2.88–3.31 (7H, m, piperazine-7H), 3.80 (3H, s, OCH_3), 3.89 (2H, t, –CH_2–), 3.98–4.00 (1H, m, cyclopropyl-CH), 4.19 (2H, t, –CH_2–), 4.43 (2H, q, $NO\underline{CH_2}CH_3$), 7.25 (1H, d, Ar–H), 7.58–7.64 (2H, m, Ar–H), 7.88 (1H, s, Ar–H), 8.38 (1H, s, C2-H). ESI-MS *m/z*: 684 $[M + H]^+$, 686 $[M + 2 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{32}H_{35}FBrN_5O_6$: C, 56.15; H, 5.15; N, 10.23. Found: C, 55.91; H, 5.01; N, 10.03.

3-(2,3-Dioxoindolin-1-yl)propyl 1-cyclopropyl-7-(4-(3-(2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4a). Yellow solid, yield: 22%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.96–1.08 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.82–1.86 (2H, m, –CH_2–), 2.02–2.12 (4H, m,

–CH_2–), 3.13–3.64 (7H, m, piperazine-7H), 3.76–3.88 (7H, m, OCH_3 and 2 × –CH_2–), 4.00–4.02 (1H, m, cyclopropyl-CH), 4.24 (2H, t, –CH_2–), 7.07 (1H, t, Ar–H), 7.17 (1H, t, Ar–H), 7.28–7.33 (2H, m, Ar–H), 7.46 (1H, d, Ar–H), 7.60–7.3 (4H, m, Ar–H), 8.48 (1H, s, C2-H). ESI-MS *m/z*: 750 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{41}H_{40}FN_5O_8$: C, 55.68; H, 5.38; N, 9.34. Found: C, 55.39; H, 5.11; N, 9.17.

3-(5-Fluoro-2,3-dioxoindolin-1-yl)propyl 1-cyclopropyl-6-fluoro-7-(4-(3-(5-fluoro-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4b). Yellow solid, yield: 23%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93–1.06 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.83–1.85 (2H, m, –CH_2–), 1.97–2.03 (2H, m, –CH_2–), 2.28–2.34 (2H, m, –CH_2–), 2.83–3.24 (7H, m, piperazine-7H), 3.75–3.87 (5H, m, OCH_3 and –CH_2–), 3.90 (2H, t, –CH_2–), 3.98–3.99 (1H, m, cyclopropyl-CH), 4.20 (2H, t, –CH_2–), 7.25–7.32 (4H, m, Ar–H), 7.57–7.72 (3H, m, Ar–H), 8.40 (1H, s, C2-H). ESI-MS *m/z*: 786 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{41}H_{38}F_3N_5O_8$: C, 62.67; H, 4.87; N, 8.91. Found: C, 62.41; H, 4.63; N, 8.72.

3-(5-Bromo-2,3-dioxoindolin-1-yl)propyl 7-(4-(3-(5-bromo-2,3-dioxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4c). Yellow solid, yield: 35%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92–1.04 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.82–1.85 (2H, m, –CH_2–), 1.99–2.03 (2H, m, –CH_2–), 2.27–2.32 (2H, m, –CH_2–), 2.80–3.26 (7H, m, piperazine-7H), 3.74–3.87 (5H, m, OCH_3 and –CH_2–), 3.90 (2H, t, –CH_2–), 3.90–3.92 (1H, m, cyclopropyl-CH), 4.19 (2H, t, –CH_2–), 7.20–7.37 (2H, m, Ar–H), 7.58–7.69 (3H, m, Ar–H), 7.87 (1H, s, Ar–H), 8.00 (1H, s, Ar–H), 8.36 (1H, s, C2-H). ESI-MS *m/z*: 906 $[M + H]^+$, 908 $[M + 2 + H]^+$, 910 $[M + 4 + H]^+$. Elemental *Anal*. Calcd (%) for $C_{41}H_{38}FBr_2N_5O_8$: C, 54.26; H, 4.22; N, 7.72. Found: C, 54.01; H, 3.97; N, 7.46.

3-(3-(Ethoxyimino)-2-oxoindolin-1-yl)propyl 1-cyclopropyl-7-(4-(3-(3-(ethoxyimino)-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4d). Yellow solid, yield: 33%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92–1.04 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.31–1.40 (6H, m, 2 × $NOCH_2\underline{CH_3}$), 1.81–1.84 (2H, m, –CH_2–), 2.00–2.03 (2H, m, –CH_2–), 2.31–2.33 (2H, m, –CH_2–), 2.79–3.25 (7H, m, piperazine-7H), 3.74–3.91 (7H, m, OCH_3 and 2 × –CH_2–), 3.96–3.98 (1H, m, cyclopropyl-CH), 4.18 (2H, t, –CH_2–), 4.38–4.47 (4H, m, 2 × $NO\underline{CH_2}CH_3$), 7.06–7.10 (2H, m, Ar–H), 7.20 (1H, d, Ar–H), 7.26 (1H, d, Ar–H), 7.43–7.49 (2H, m, Ar–H), 7.46 (1H, d, Ar–H), 7.84 (1H, d, Ar–H), 7.92 (1H, d, Ar–H), 8.40 (1H, s, C2-H). ESI-MS *m/z*: 836 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{45}H_{50}FN_7O_8$: C, 64.66; H, 6.03; N, 11.73. Found: C, 64.39; H, 5.78; N, 11.47.

3-(3-(Ethoxyimino)-5-fluoro-2-oxoindolin-1-yl) propyl 1-cyclopropyl-7-(4-(3-(3-(ethoxyimino)-5-fluoro-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4e). Yellow solid, yield: 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93–1.06 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.32–1.38 (6H, m, 2 × $NOCH_2\underline{CH_3}$), 1.81–1.84 (2H, m, –CH_2–), 1.99–2.04 (2H, m, –CH_2–), 2.38–2.40 (2H, m, –CH_2–), 2.85–3.27 (7H, m, piperazine-7H), 3.74–3.89 (7H, m, OCH_3 and 2 × –CH_2–), 3.92–3.94 (1H, m, cyclopropyl-CH), 4.18 (2H, t, –CH_2–), 4.40–4.50 (4H, m, 2 × $NO\underline{CH_2}CH_3$), 7.21–7.38 (4H, m, Ar–H), 7.57–7.71 (3H, m, Ar–H), 8.40 (1H, s, C2-H). ESI-MS *m/z*: 872 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{45}H_{48}F_3N_7O_8$: C, 61.99; H, 5.55; N, 11.25. Found: C, 61.71; H, 5.29; N, 11.01.

3-(5-Bromo-3-(ethoxyimino)-2-oxoindolin-1-yl)propyl 7-(4-(3-(5-bromo-3-(ethoxyimino)-2-oxoindolin-1-yl)propyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (4f). Yellow solid, yield: 41%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92–1.04 (7H, m, 2 × cyclopropyl-CH_2 and CH_3), 1.31–1.40 (6H, m, 2 × $NOCH_2\underline{CH_3}$), 1.81–1.84 (2H, m, –CH_2–), 1.99–2.03 (2H, m, –CH_2–), 2.27–2.32 (2H, m, –CH_2–), 2.80–3.22 (7H, m, piperazine-7H), 3.74–3.90 (7H, m, OCH_3 and 2 × –CH_2–), 3.92–3.94 (1H, m, cyclopropyl-CH), 4.18 (2H, t, –CH_2–), 4.40–4.51 (4H, m, 2 × $NO\underline{CH_2}CH_3$), 7.21 (1H, d, Ar–H), 7.26 (1H, d, Ar–H), 7.59 (2H, dd, Ar–H), 7.68 (1H, d, Ar–H), 7.87 (1H, s, Ar–H), 8.00 (1H, s, Ar–H), 8.36 (1H, s, C2-H). ESI-MS *m/z*: 994 $[M + H]^+$. Elemental *Anal*. Calcd (%) for $C_{45}H_{48}FBr_2N_7O_8$: C, 54.39; H, 4.87; N, 9.87. Found: C, 54.11; H, 4.59; N, 9.59.

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