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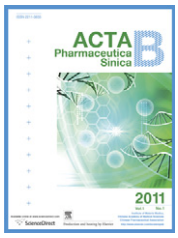

ORIGINAL ARTICLE

Design, synthesis and antitumor activity of C3/C3 bis-fluoroquonolones cross-linked with [1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole

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Abstract To contribute to the development of an efficient method for the conversion of antibacterial fluoroquinolones to antitumor fluoroquinolones, a series of C3/C3 bis-fluoroquinolone fused heterocycles cross-linked with a [1,2,4]-triazolo[3,4-*b*] [1,3,4]-thiadiazole core as a common bioisostere of two carboxylic acid groups was designed and synthesized as their hydrochloride salts. Structures were characterized by elemental analysis and spectral data and their *in vitro* antitumor activity against L1210, CHO and HL60 cell lines was screened by determination of their IC_{50} values in the methylthiazole trazolium (MTT) assay. Two compounds were highly potent against the HL60 cell line and represent promising lead compounds for future development.

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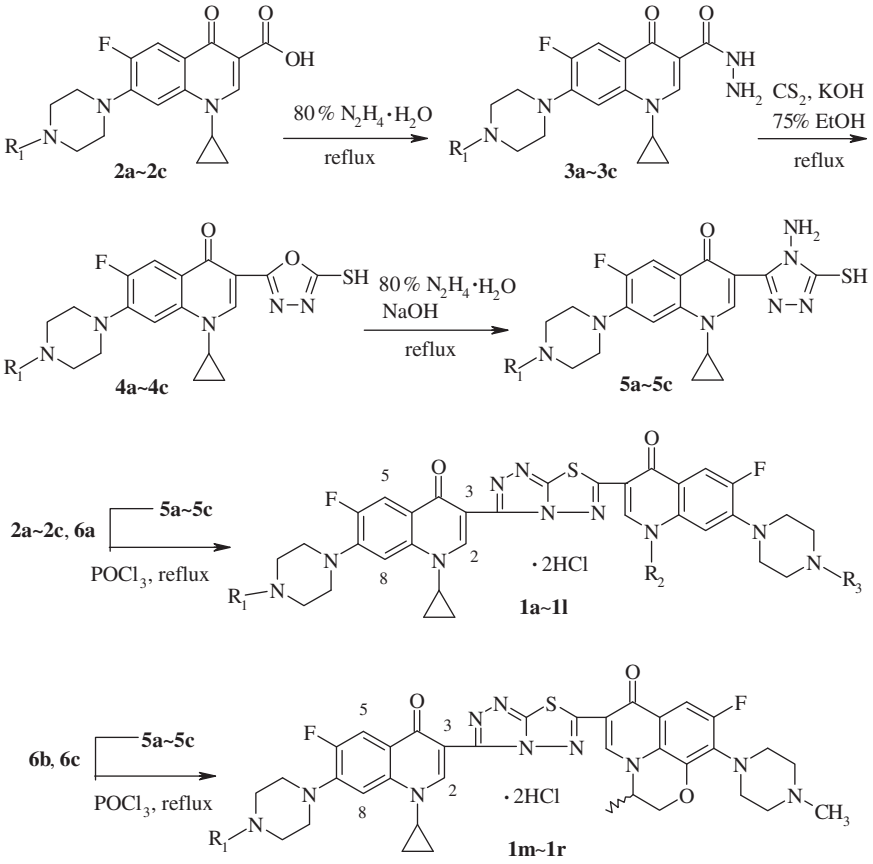
1. Introduction

Recently, the shift from antibacterial fluoroquinolones (ABFQs) to antitumor fluoroquinolones (ATFQs) based on their mechanistic similarities and sequence homologies of their target topoisomerases has received considerable attention[1,2]. Many ATFQs have been derived by structural modification of the clinical ABFQs, especially in the heterocyclic ring such as piperazine at the 7-position of the quinolone scaffold[3–5], whereas only a few of these have been produced by modification of the carboxylic acid group at the 3-position[6,7]. Unfortunately, the resultant compounds were not considered worthy of clinical evaluation due to issues with their *in vitro* toxicity, stability or bioavailability. Therefore, there remains an urgent need to develop an efficient synthetic route for ATFQs to facilitate their evaluation as therapeutic agents.

In recent reports, we have shown that it is not necessary for an ATFQ to retain the C-3 carboxylic acid group and that some ATFQs derived by isosteric replacement of the carboxylic acid group with a (fused) heterocyclic ring such as oxadiazole or *s*-triazolo[3,4-*b*] [1,3,4]thiadiazine display anticancer activity[8]. In addition, a heterocyclic ring system related to *s*-triazolo[3,4-*b*] [1,3,4]thiazole has been widely investigated[9], but the use of a fluoroquinolone scaffold as a substituent of the fused core at both the 3- and 6-positions has not been attempted. In this paper we report the synthesis from current antibacterial fluoroquinolones of five 3,6-fluoroquinolone-substituted [1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazoles in which the fused heterocyclic ring of triazolothiadiazole acts as a common bioisostere of the two carboxylic acid groups (Scheme 1).

2. Results and discussion

The intermediate ciprofloxacin hydrazide derivatives **3a**–**3c**, prepared from *N*-substituted ciprofloxacins **2a**–**2c** according to the known procedure[10], were subjected to a cyclo-condensation with carbon disulfide in the presence of excess alkali-ethanol solution to yield the oxadiazole thiols **4a**–**4c**. A convenient base-catalyzed conversion of **4a**–**4e** to the amino *s*-triazole thiols **5a**–**5c** was carried out using hydrazine hydrate. Interestingly, the condensation of **5a**–**5c** with each of the commercially available ABFQs (**2a**–**2c** and **6a**–**6c**) to produce the target compounds **1a**–**1r** was successful only in the presence of $POCl_3$ and not in the presence of other acidic media such as concentrated sulfuric acid or polyphosphoric acid.



Scheme 1 Synthetic route for the bis-fluoroquinolones cross-linked with [1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazole **1a**–**1r**. Ciprofloxacin (**2a**); *N*-Methyl ciprofloxacin (**2b**); Enrofloxacin (**2c**); Norfloxacin (**6a**); Ofloxacin (**6b**); Levofloxacin (**6c**). $R_1=R_3=H$, R_2=cyclopropyl (**1a**); $R_1=H$, R_3=methyl, R_2=cyclopropyl (**1b**); $R_1=H$, R_3=ethyl, R_2=cyclopropyl (**1c**); $R_1=R_3=H$, R_2=ethyl (**1d**); R_1=methyl, $R_3=H$, R_2=cyclopropyl (**1e**); $R_1=R_3$=methyl, R_2=cyclopropyl (**1f**); R_1=methyl, R_3=ethyl, R_2=cyclopropyl (**1g**); R_1=methyl, $R_3=H$, R_2=ethyl (**1h**); R_1=ethyl, $R_3=H$, R_2=cyclopropyl (**1i**); R_1=ethyl, R_3=methyl, R_2=cyclopropyl (**1j**); $R_1=R_3$=ethyl, R_2=cyclopropyl (**1k**); R_1=ethyl, $R_3=H$, R_2=ethyl (**1l**); *S*/*R*-(±)–$R_1=H$ (**1m**); *S*/*R*-(±)–R_1=methyl (**1n**); *S*/*R*-(±)–R_1=ethyl (**1o**); *S*-(-)–$R_1=H$ (**1p**); *S*-(-)–R_1=methyl (**1q**); *S*-(-)–R_1=ethyl (**1r**).

Table 1 Growth inhibitory activities (IC_{50} μ mol/L) of compounds (**1a**–**1r**) against L1210, HL60 and CHO tumor cells in the MTT assay.

Compound	IC_{50} (μ mol/L)		
	L1210	HL60	CHO
1a	3.6	0.54	7.8
1b	8.5	5.3	11.6
1c	14.2	2.7	15.0
1d	18.5	10.6	17.2
1e	6.2	2.7	10.5
1f	12.4	8.8	15.7
1g	16.2	4.7	17.5
1h	21.3	15.4	22.6
1i	8.6	5.0	13.7
1j	14.6	10.6	17.8
1k	18.02	7.2	19.5
1l	23.6	18.4	26.2
1m	15.6	14.2	16.8
1n	17.2	16.8	21.4
1o	18.0	16.4	23.7
1p	1.5	0.12	3.4
1q	2.7	1.8	5.2
1r	2.5	2.0	3.8

Being asymmetrical, the ^{1}H NMR spectra of compounds **1a–1r** displayed one two-proton singlet signal at δ 9.20–8.90 assignable to the 2-H of the fluoroquinolone scaffold; other protons such as the 5-H and 7-piperazine-H or 8-H are in close accord with their corresponding parent fluoroquinolone carboxylic acids[11]. In addition, the chemical shift for all target compounds **1a**–**1r** at δ 11.0–12.0 (exchangeable with D_2O) indicates the presence of dihydrochloride salts and the molecular ion peaks show a base peak in the MS spectrum corresponding to the respective molecular formulae of the free bases.

The *in vitro* antitumor activities of **1a**–**1r** against murine leukemia cell line (L1210), human leukocytoma cell line (HL60) and Chinese hamster ovary cell line (CHO) were evaluated using the MTT assay[12] (Table 1). Interestingly, the results reveal that all compounds show cytotoxicity with IC_{50} values in the range 0.12–26.2 μmol/L (Table 1). More importantly, the target compounds **1a** (derived from ciprofloxacin **2a**) and **1p** (derived from ciprofloxacin **2a** and levofloxacin **6c**) exhibit the most potent activity against HL60 cells. This preliminary indication of antitumor activity suggests that di-(1-cyclopropyl)-substituted fluoroquinolones and bis-fluoroquinolone hybrid molecules are promising lead compounds for further development.

3. Experimental procedures

3.1. Chemistry

Melting points were determined in sealed capillary tubes and are uncorrected. IR spectra were recorded on a Nicolet Impact 410 spectrometer; ^{1}H NMR spectra on a Bruker AM-400 spectrometer; mass spectra on an Esquire LC instrument and elemental analyses on a PE2400-II instrument. The materials and solvents were commercially available and used as received.

3.1.1. *General synthetic procedures for 1-cyclopropyl-6-fluoro-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-substituted piperazin-1-yl-quinolin-4 (1H)-one (**4a**–**4c**)*

To a solution of hydrazide **3a**–**3c** (58.0 mmol) in 95% EtOH (500 mL) containing KOH (5.0 g, 90 mmol), carbon disulfide was added at room temperature (7.0 g, 92 mmol) and the mixture was stirred overnight. The resultant precipitate was dissolved under refluxing conditions. After removal of the solvent under reduced pressure, a 3% aqueous NaOH solution (500 mL) was added to the residue and refluxed for 6 h. The filtrate was adjusted to pH 7.0 using concentrated HCl and the resulting precipitate was collected and recrystallized from DMF–EtOH to give yellow crystals **4a**–**4c**.

1-Cyclopropyl-6-fluoro-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-piperazin-1-yl-quinolin-4 (1*H*)-one **4a**: yield 64%, mp >250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.64 (s, 1H, SH), 8.87 (s, 1H, H-2), 7.72 (d, J=13.2 Hz, 1H, 5-H), 7.62 (d, J=7.2 Hz, 1H, 8-H), 4.63–4.52 (m, 1H, CH), 3.56–3.32 (m, 8H, piperazine-H), 1.26–1.14 (m, 4H, CH_2CH_2); IR (KBr) v: 3356, 3104, 2952, 1638, 1617, 1457 cm^{-1}; MS m/z: 388 $[M+H]^+$, calcd. 387.44 $[M]^+$. Anal. calcd. for $C_{18}H_{18}FN_5O_2$: C 55.80, H 4.68, N 18.08; found C 56.04, H 4.42, N 18.34.

1-Cyclopropyl-6-fluoro-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-(4-methylpiperazin-1-yl)-quinolin-4 (1*H*)-one **4b**: yield 72%, mp >250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.72 (s, 1H, SH), 8.88 (s, 1H, H-2), 7.76 (d, J=13.2 Hz, 1H, 5-H), 7.68 (d, J=7.2 Hz, 1H, 8-H), 4.62–4.55 (m, 1H, CH), 3.55–3.28 (m, 8H, piperazine-H), 2.36 (s, 3H, CH_3), 1.38–1.17 (m, 4H, CH_2CH_2); IR (KBr) v: 3364, 3087, 2874, 1644, 1625, 1456 cm^{-1}; MS m/z: 402 $[M+H]^+$, calcd. 401.47 $[M]^+$. Anal. calcd. for $C_{19}H_{20}FN_5O_2$: C 56.84, H 5.02, N 17.44; found C 57.11, H 4.87, N 17.65.

1-Cyclopropyl-6-fluoro-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-(4-ethylpiperazin-1-yl-quinolin-4 (1*H*)-one **4c**: yield 68%, mp >250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.68 (s, 1H, SH), 8.84 (s, 1H, H-2), 7.82 (d, J=13.2 Hz, 1H, 5-H), 7.64 (d, J=7.2 Hz, 1H, 8-H), 4.64–4.52 (m, 1H, CH), 3.53–3.25 (m, 8H, piperazine-H), 2.37 (s, 3H, CH_2), 1.42–1.15 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) v: 3357, 3084, 2915, 1642, 1624, 1455 cm^{-1}; MS m/z: 416 $[M+H]^+$, calcd. 415.49 $[M]^+$. Anal. calcd. for $C_{20}H_{22}FN_5O_2$: C 57.82, H 5.34, N 16.86; found C 57.69, H 5.13, N 17.06.

3.1.2. *General synthetic procedures for 3-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-1-cyclopropyl-6-fluoro-7-substituted piperazin-1-yl-quinolin-4 (1H)-one (**5a**–**5c**)*

A mixture of **4a**–**4c** (30.0 mmol), 85% hydrazine hydrate (50 mL) and 30% aqueous NaOH solution (5.0 g, 125 mmol) was stirred and refluxed for 12 h. After removal of the solvent under reduced pressure, the residue was dissolved in water (500 mL) and treated by the same procedure as for **4** to give the intermediate **5a**–**5c**.

3-(4-Amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4 (1*H*)-one **5a:** yield 58%, mp 246–248 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.87 (s, 1H, SH), 8.96 (s, 1H, H-2), 8.14 (d, J=13.2 Hz, 1H, 5-H), 7.85 (d, J=7.2 Hz, 1H, 8-H), 5.86 (s, 2H, NH_2), 4.66–4.50 (m, 1H, CH), 3.55–3.34 (m, 8H, piperazine-H), 1.17–1.26 (m, 4H, CH_2CH_2); IR (KBr) v: 3442, 3008, 2867, 1630, 1616, 1457 cm^{-1}; MS m/z: 402 $[M+H]^+$, calcd. 401.47 $[M]^+$. Anal.

calcd. for $C_{18}H_{20}FN_7OS$: C 53.85, H 5.02, N 24.42; found C 53.96, H 4.84, N 24.68.

3-(4-Amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-1-cyclopropyl-6-fluoro-7-(4-methyl- piperazin-1-yl)-quinolin-4 (1*H*)-one **5b**: yield 66%, mp 242-244 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.82 (s, 1H, SH), 9.05 (s, 1H, H-2), 8.21 (d, J=13.2 Hz, 1H, 5-H), 7.82 (d, J=7.2 Hz, 1H, 8-H), 5.80 (s, 2H, NH_2), 4.63–4.52 (m, 1H, CH), 3.52–3.32 (m, 8H, piperazine-H), 2.42 (s, 3H, CH_3), 1.35–1.20 (m, 4H, CH_2CH_2); IR (KBr) v: 3356, 3024, 2876, 1632, 1618, 1455 cm^{-1}; MS m/z: 416 $[M+H]^+$, calcd. 415.50 $[M]^+$. Anal. calcd. for $C_{19}H_{22}FN_7OS$: C 54.93, H 5.34, N 23.60; found C 55.17, H 5.11, N 23.85.

3-(4-Amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-1-cyclopropyl-6-fluoro-7-(4-ethyl- piperazin-1-yl)-quinolin-4 (1*H*)-one **5c**: yield 58%, mp 240–242 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.68 (s, 1H, SH), 9.07 (s, 1H, H-2), 8.16 (d, J=13.2 Hz, 1H, 5-H), 7.80 (d, J=7.2 Hz, 1H, 8-H), 5.76 (s, 2H, NH_2), 4.62–4.55 (m, 1H, CH), 3.52–3.34 (m, 8H, piperazine-H), 2.40 (s, 2H, CH_2), 1.42–1.22 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) v: 3368, 3016, 2938, 1640, 1625, 1457 cm^{-1}; MS m/z: 430 $[M+H]^+$, calcd. 429.52 $[M]^+$. Anal. calcd. for $C_{20}H_{24}FN_7OS$: C 55.93, H 5.63, N 22.83; found C 56.15, H 5.40, N 23.07.

*3.1.3. Synthetic procedures for 1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3,6-bis-[1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1H)-one] dihydrochloride (**1a · 2HCl**)*

A mixture of **5a** (1.0 g, 2.5 mmol), Ciprofloxacin **2a** (1.0 g, 3.0 mmol) and 4-dimethylpyridine (DMAP) (0.4 g, 3.0 mmol) in $POCl_3$ (10 mL) was stirred at room temperature for 6 h and then refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, poured into ice-water (100 mL) and adjusted to pH 10.0 with 30% aqueous NaOH solution. The resulting precipitate was collected by filtration and washed with water to give the crude free base **1a**, which was recrystallized from 95% EtOH (30 mL) and concentrated HCl (1.0 mL) to give a yellow solid **1a · 2HCl**: yield 32.0%, mp 264 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.52 (br, 2H, 2HCl), 9.12–8.97 (br, 2H, 2 × 2-H), 7.84–7.78 (brs, 2H, 2 × 8-H), 7.63–7.55 (brs, 2H, 2 × 5-H), 4.66–4.47 (m, 2H, 2 × CH), 3.55–3.26 (m, 16H, 2 × piperazine-H), 1.20–1.40 (m, 8H, 2 × CH_2CH_2); IR (KBr) v: 3356, 3054, 2947, 1633, 1616, 1552, 1442, 1338 cm^{-1}; MS m/z: 697 $[M+H]^+$, calcd. 696.79 $[M]^+$. Anal. calcd. for $C_{35}H_{34}F_2N_{10}O_2S \cdot 2HCl$: C 54.62, H 4.71, N 18.20; found C 54.93, H 4.57, N 18.46.

*3.1.4. Synthetic procedure for the compounds (**1b**–**1r**) · 2HCl*

By a procedure similar to that for **1a · 2HCl**, the compounds (**1b**–**1r**) · **2HCl** were prepared. 1,2,4-Triazolo[3,4-*b*] [1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1*H*)-one]-6-[1-cyclopropyl-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1b · 2HCl**) derived from **5a** and *N*-Methylciprofloxacin **2b**: yield 32%, mp 257 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.53 (br, 2H, 2HCl), 9.22–9.15 (br, 2H, 2 × 2-H), 8.12–7.83 (br, 2H, 2 × 8-H), 7.65–7.53 (br, 2H, 2 × 5-H), 4.62–5.16 (m, 2H, 2 × CH), 3.53–3.24 (m, 16H, 2 × piperazine-H), 2.40 (s, 3H, CH_3) 1.41–1.22 (m, 8H, 2 × CH_2CH_2); IR (KBr) v: 3352, 3032, 2884, 1645, 1627, 1560, 1453, 1324 cm^{-1}; MS m/z: 711 $[M+H]^+$, calcd. 712.81 $[M]^+$; Anal. calcd. for $C_{36}H_{36}F_2N_{10}O_2S \cdot 2HCl$: C 55.17, H 4.89, N 17.87; found C 55.34, H 4.68, N 18.07.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1*H*)-one]-6-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1c · 2HCl**) derived from **5a** and Enrofloxacin **2c:** yield 28%, mp 255 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.36 (br, 2H, 2HCl), 9.25–9.17 (br, 2H, 2 × 2-H), 7.88–7.72 (br, 2H, 2 × 8-H), 7.62–7.50 (br, 2H, 2 × 5-H), 4.60–4.44 (m, 2H, 2 × CH), 3.52–3.38 (m, 16H, 2 × piperazine-H), 2.34 (q, J=6.5 Hz, 2H, N-CH_2), 1.17–1.44 (m, 11H, 2 × CH_2CH_2 and CH_3); IR (KBr) v: 3368, 3027, 2876, 1642, 1608, 1562, 1457, 1226 cm^{-1}; MS m/z (%): 725 $[M+H]^+$, calcd. 724.84 $[M]^+$; Anal. calcd. for $C_{37}H_{38}F_2N_{10}O_2S \cdot 2HCl$: C 55.71, H 5.05, N 17.56; found C 55.93, H 5.30, N 17.82.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1*H*)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1d · 2HCl**) derived from **5a** and Norfloxacin **6a**: yield 34%, mp 240 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.46 (br, 2H, 2HCl), 9.04–8.87 (br, 2H, 2 × 2-H), 8.15–7.87 (br, 2H, 2 × 8-H), 7.62–7.55 (br, 2H, 2 × 5-H), 4.67–4.56 (m, 3H, N-CH_2 and CH), 3.55–3.27 (m, 16H, 2 × piperazine-H), 1.46–1.22 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) v: 3364, 3025, 2896, 1642, 1627, 1562, 1455, 1226 cm^{-1}; MS m/z: 685 $[M+H]^+$, calcd. 684.78 $[M]^+$. Anal. calcd. for $C_{34}H_{34}F_2N_{10}O_2S \cdot 2HCl$: C 53.90, H 4.79, N 18.49; found C 54.13, H 4.58, N 18.67.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one]-6-(1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1*H*)-one) dihydrochloride (**1e · 2HCl**) derived from **5b** and Ciprofloxacin **2a**, yield 27.0%, mp 256 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.42 (br, 2H, 2HCl), 9.17–8.92 (br, 2H, 2 × 2-H), 7.82–7.76 (brs, 2H, 2 × 8-H), 7.62–7.58 (brs, 2H, 2 × 5-H), 4.63–5.24 (m, 2H, 2 × CH), 3.50–3.24 (m, 16H, 2 × piperazine-H), 2.42 (s, 3H, CH_3), 1.42–1.23 (m, 8H, 2 × CH_2CH_2); IR (KBr) v: 3347, 3054, 2962, 1638, 1624, 1557, 1445 cm^{-1}; MS m/z: 711 $[M+H]^+$, calcd. 710.81 $[M]^+$. Anal. calcd. for $C_{36}H_{36}F_2N_{10}O_2S \cdot 2HCl$: C 55.17, H 4.89, N 17.87; found C 55.42, H 4.66, N 18.72.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3,6-bis-[1-cyclopropyl-6-fluoro-7-(4-methyl-piperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1f · 2HCl**) derived from **5b** and *N*-Methylciprofloxacin **2b**: yield 25%, mp 253 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.62 (br, 2H, 2HCl), 9.15–9.08 (br, 2H, 2 × 2-H), 8.14–7.80 (br, 2H, 2 × 8-H), 7.72–7.56 (br, 2H, 2 × 5-H), 4.62–4.47 (m, 2H, 2 × CH), 3.50–3.27 (m, 16H, 2 × piperazine-H), 2.37–2.44 (brs, 6H, 2 × CH_3), 1.40–1.22 (m, 8H, 2 × CH_2CH_2); IR (KBr) v: 3364, 3015, 2893, 1642, 1624, 1557, 1455, 1276 cm^{-1}; MS m/z: 725 $[M+H]^+$, calcd. 724.84 $[M]^+$. Anal. calcd. for $C_{37}H_{38}F_2N_{10}O_2S \cdot 2HCl$: C 55.71, H 5.05, N 17.56; found C 55.94, H 4.87, N 17.80.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one]-6-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1g · 2HCl**) derived from **5b** and Enrofloxacin **2c**: yield 31%, mp 252 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.35 (br, 2H, 2HCl), 9.22–9.13 (br, 2H, 2 × 2-H), 7.86–7.75(br, 2H, 2 × 8-H), 7.64–7.57 (br, 2H, 2 × 5-H), 4.62–4.50 (m, 2H, 2 × CH), 3.57–3.35 (m, 16H, 2 × piperazine-H), 2.40–2.34 (m, 5H, CH_3 and CH_2), 1.45–1.18 (m, 11H, 2 × CH_2CH_2 and CH_3); IR (KBr)

v: 3347, 3006, 2876, 1644, 1616, 1563, 1457, 1247 cm^{-1}; MS *m/z* (%): 739 $[M+H]^+$, calcd. 738.87 $[M]^+$. Anal. calcd. for $C_{38}H_{40}F_2N_{10}O_2S \cdot 2HCl$: C 56.22, H 5.21, N 17.25; found C 55.45, H 5.37, N 17.46.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1h · 2HCl**) derived from **5b** and Norfloxacin **6a**: yield 25%, mp 242 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.44 (br, 2H, 2HCl), 9.06–8.89 (br, 2H, 2 × 2-H), 8.17–7.84 (br, 2H, 2 × 8-H), 7.66–7.57 (br, 2H, 2 × 5-H), 4.60–4.57 (m, 3H, CH_2 and CH), 3.50–3.25 (m, 16H, 2 × piperazine-H), 1.44–1.20 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) *v*: 3372, 3045, 1644, 1626, 1572, 1457, 1238 cm^{-1}; MS *m/z*: 699 $[M+H]^+$, calcd. 698.80 $[M]^+$. Anal. calcd. for $C_{35}H_{36}F_2N_{10}O_2S \cdot 2HCl$: C 54.47, H 4.96, N 18.15; found C 54.68, H 4.72, N 18.36.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yl)-quinolin-4(1*H*)-one]-6-(1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1*H*)-one) dihydrochloride (**1i · 2HCl**) derived from **5c** and Ciprofloxacin **2a**: yield 24.0%, mp 251 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.62 (br, 2H, 2HCl), 9.22–8.94 (br, 2H, 2 × 2-H), 7.86–7.75 (brs, 2H, 2 × 8-H), 7.68–7.54 (brs, 2H, 2 × 5-H), 4.68–5.42 (m, 2H, 2 × CH), 3.54–3.18 (m, 16H, 2 × piperazine-H), 2.46–2.37 (brs, 5H, CH_2 and CH_3), 1.45–1.22 (m, 11H, CH_3 and 2 × CH_2CH_2); IR (KBr) *v*: 3367, 3038, 2967, 1636, 1628, 1557, 1452 cm^{-1}; MS *m/z*: 725 $[M+H]^+$, calcd. 724.84 $[M]^+$. Anal. calcd. for $C_{37}H_{38}F_2N_{10}O_2S \cdot 2HCl$: C 55.71, H 5.05, N 17.56; found C 55.92, H 4.87, N 17.83.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yl)-quinolin-4(1*H*)-one]-6-[1-cyclopropyl-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1j · 2HCl**) derived from **5c** and *N*-Methylciprofloxacin **2b**: yield 22%, mp 248 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.70 (br, 2H, 2HCl), 9.18–9.04 (br, 2H, 2 × 2-H), 8.20–7.86 (br, 2H, 2 × 8-H), 7.75–7.62 (br, 2H, 2 × 5-H), 4.68–5.36 (m, 2H, 2 × CH), 3.52–3.30 (m, 16H, 2 × piperazine-H), 2.41–2.45 (brs, 5H, CH_2 and CH_3), 1.42–1.20 (m, 11H, 2 × CH_2CH_2); IR (KBr) *v*: 3358, 3034, 2884, 1638, 1622, 1557, 1453, 1262 cm^{-1}; MS *m/z*: 739 $[M+H]^+$, calcd. 738.87 $[M]^+$. Anal. calcd. for $C_{38}H_{40}F_2N_{10}O_2S \cdot 2HCl$: C 56.22, H 5.21, N 17.25; found C 56.45, H 5.02, N 17.46.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3,6-bis-[1-cyclopropyl-6-fluoro-7-(4-ethyl-piperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1k · 2HCl**) derived from **5c** and Enrofloxacin **2c**: yield 20%, mp 246 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.42 (br, 2H, 2HCl), 9.24–9.15 (br, 2H, 2 × 2-H), 7.84–7.72 (br, 2H, 2 × 8-H), 7.68–7.52 (br, 2H, 2 × 5-H), 4.66–4.53 (m, 2H, 2 × CH), 3.55–3.34 (m, 16H, 2 × piperazine-H), 2.45–2.42 (m, 4H, 2 × CH_2), 1.46–1.17 (m, 14H, 2 × CH_2CH_2 and 2 × CH_3); IR (KBr) *v*: 3363, 3027, 2885, 1640, 1626, 1568, 1454, 1238 cm^{-1}; MS *m/z* (%): 753 $[M+H]^+$, calcd. 752.90 $[M]^+$. Anal. calcd. for $C_{39}H_{42}F_2N_{10}O_2S \cdot 2HCl$: C 56.72, H 5.37, N 16.96; found C 56.87, H 5.11, N 17.16.

1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yl)-quinolin-4(1*H*)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1l · 2HCl**) derived from **5c** and Norfloxacin **6a**: yield 23%, mp 238 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.64 (br, 2H, 2HCl), 9.12–8.93 (br, 2H, 2 × 2-H), 8.22–7.86 (br, 2H, 2 × 8-H), 7.72–7.63 (br, 2H, 2 × 5-H), 4.64–4.53 (m, 3H, CH_2 and CH), 3.56–3.27 (m, 16H, 2 × piperazine-H), 1.46–1.25 (m, 10H, CH_2CH_2 and 2 × CH_3); IR (KBr) *v*: 3366, 3035, 1628, 1617, 1557, 1456, 1228 cm^{-1}; MS *m/z*: 725 $[M+H]^+$, calcd. 724.84 $[M]^+$. Anal. calcd. for $C_{37}H_{38}F_2N_{10}O_2S \cdot 2HCl$: C 55.71, H 5.05, N 17.56; found C 55.90, H 4.88, N 17.84.

(*S*,*R*)-1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1*H*)-one]-6-[1,8-(2,1-oxypropyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1m · 2HCl**) derived from **5a** and Ofloxacin **6b**: yield 36%, mp 238–240 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.38 (br, 2H, 2HCl), 9.16–8.94 (br, 2H, 2 × 2-H), 7.86 (d, *J* = 2.4 Hz, 1H, 8-H), 7.62–7.55 (br, 2H, 2 × 5-H), 4.57–4.45 (m, 4H, OCH_2CHN and CH), 3.53–3.36 (m, 16H, 2 × piperazine-H), 2.46 (s, 3H, N-CH_3), 1.16–1.44 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) *v*: 3356, 3032, 2892, 1638, 1604, 1567, 1457, 1228 cm^{-1}; MS *m/z*: 727 $[M+H]^+$, calcd. 726.81 $[M]^+$. Anal. calcd. for $C_{36}H_{36}F_2N_{10}O_3S \cdot 2HCl$: C 54.07, H 4.79, N 17.51; found C 54.26, H 4.60, N 17.72.

(*S*,*R*)-1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methyl-piperazin-yl)-quinolin-4(1*H*)-one]-6-[1,8-(2,1-oxypropyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1n · 2HCl**) derived from **5b** and Ofloxacin **6b**: yield 31%, mp 235 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.50 (br, 2H, 2HCl), 9.18–8.97 (br, 2H, 2 × 2-H), 7.85 (d, *J* = 2.4 Hz, 1H, 8-H), 7.65–7.58 (br, 2H, 2 × 5-H), 4.62–4.50 (m, 4H, OCH_2CHN and CH), 3.56–3.34 (m, 16H, 2 × piperazine-H), 2.48–2.43 (m, 6H, 2 × CH_3), 1.46–1.20 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) *v*: 3358, 3027, 2918, 1632, 1615, 1557, 1455, 1236 cm^{-1}; MS *m/z*: 741 $[M+H]^+$, calcd. 740.84 $[M]^+$. Anal. calcd. for $C_{37}H_{38}F_2N_{10}O_3S \cdot 2HCl$: C 54.61, H 4.95, N 17.21; found C 54.82, H 4.67, N 17.46.

(*S*,*R*)-1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-ethyl-piperazin-yl)-quinolin-4(1*H*)-one]-6-[1,8-(2,1-oxypropyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1o · 2HCl**) derived from **5c** and Ofloxacin **6b**: yield 23%, mp 234 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.52 (br, 2H, 2HCl), 9.18–8.95 (br, 2H, 2 × 2-H), 7.86 (d, *J* = 2.4 Hz, 1H, 8-H), 7.72–7.56 (br, 2H, 2 × 5-H), 4.66–4.52 (m, 4H, OCH_2CHN and CH), 3.57–3.35 (m, 16H, 2 × piperazine-H), 2.46–2.40 (m, 5H, CH_2 and CH_3), 1.48–1.18 (m, 10H, CH_2CH_2 and 2 × CH_3); IR (KBr) *v*: 3366, 3025, 2936, 1636, 1622, 1558, 1455, 1228 cm^{-1}; MS *m/z*: 755 $[M+H]^+$, calcd. 754.87 $[M]^+$. Anal. calcd. for $C_{38}H_{40}F_2N_{10}O_3S \cdot 2HCl$: C 55.14, H 5.11, N 16.92; found C 55.38, H 5.34, N 17.11.

(*S*)-1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1*H*)-one]-6-[1,8-(2,1-oxypropyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1p · 2HCl**) derived from **5a** and Levofloxacin **6c**: yield 26%, mp 224–226 °C (dec.). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.26 (br, 2H, 2HCl), 9.17–8.96 (br, 2H, 2 × 2-H), 7.88 (d, *J* = 2.4 Hz, 1H, 8-H), 7.64–7.57 (br, 2H, 2 × 5-H), 4.56–4.47 (m, 4H, OCH_2CHN and CH), 3.55–3.37 (m, 16H, 2 × piperazine-H), 2.42 (s, 3H, N-CH_3), 1.18–1.46 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) *v*: 3364, 3036, 2895, 1642, 1608, 1564, 1455, 1226 cm^{-1}; MS *m/z* (%): 727 $[M+H]^+$, calcd. 726.81 $[M]^+$. Anal. calcd. for $C_{36}H_{36}F_2N_{10}O_3S \cdot 2HCl$: C 54.07, H 4.79, N 17.51; found C 54.28, H 4.64, N 17.6.

(*S*)-1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methyl- piperazin-yl)-quinolin-4(1*H*)-one]-6-[1,8-(2,1-oxypropyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1q · 2HCl**) derived from **5b** and Levofloxacin **6c**: yield 25%, mp 220 °C (dec.). ^1H NMR

(DMSO-d_6, 400 MHz) δ: 11.62 (br, 2H, 2HCl), 9.22–8.98 (br, 2H, 2 × 2-H), 7.86 (d, J=2.4 Hz, 1H, 8-H), 7.64–7.52 (br, 2H, 2 × 5-H), 4.64–4.53 (m, 4H, OCH_2CHN and CH), 3.53–3.32 (m, 16H, 2 × piperazine-H), 2.46–2.40 (m, 6H, 2 × CH_3), 1.45–1.17 (m, 7H, CH_2CH_2 and CH_3); IR (KBr) v: 3363, 3025, 2925, 1634, 1618, 1557, 1456, 1234 cm^{-1}; MS m/z: 741 $[M+H]^+$, calcd. 740.84 $[M]^+$. Anal. calcd. for $C_{37}H_{38}F_2N_{10}O_3S \cdot 2HCl$: C 54.61, H 4.95, N 17.21; found C 54.77, H 4.75, N 17.42.

(*S*)-1,2,4-Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-ethyl- piperazin-yl)-quinolin-4(1*H*)-one]-6-[1,8-(2,1-oxypropyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1*H*)-one] dihydrochloride (**1r · 2HCl**) derived from **5c** and Levofloxacin **6c**: yield 20%, mp 216 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.50 (br, 2H, 2HCl), 9.17–8.96 (br, 2H, 2 × 2-H), 7.88 (d, J=2.4 Hz, 1H, 8-H), 7.74–7.60 (br, 2H, 2 × 5-H), 4.66–4.50 (m, 4H, OCH_2CHN and CH), 3.56–3.35 (m, 16H, 2 × piperazine-H), 2.47–2.44 (m, 5H, CH_2 and CH_3), 1.48–1.20 (m, 10H, CH_2CH_2 and 2 × CH_3); IR (KBr) v: 3358, 3027, 2934, 1640, 1624, 1562, 1455, 1232 cm^{-1}; MS m/z: 755 $[M+H]^+$, calcd. 754.87 $[M]^+$. Anal. calcd. for $C_{38}H_{40}F_2N_{10}O_3S \cdot 2HCl$: C 55.14, H 5.11, N 16.92; found C 55.36, H 5.28, N 17.14.

3.2. *MTT assay*

The tumor cell lines, L1210, HL60 and CHO, were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum. Medium containing 5×10^3 cells was seeded into each well of a 96-well microplate and solutions of test compounds at concentrations of 0.1, 1.0, 10.0, 30.0 and 50.0 μmol/L added simultaneously to triplicate wells before making the final volume up to 100 μL. Plates were incubated at 37 °C for 48 h in a humidified atmosphere (5% CO_2, 95% air) after which 100 μL methylthiazole trazolium (MTT) solution (1.0 mg/mL in phosphate-buffered saline lacking calcium and magnesium) was added to each well. After a further incubation for 4 h at 37 °C, 100 μL DMSO was added to CHO cells and 10% sodium dodecylbenzene sulfonate (SDS) added to L1210 and HL60 cells to solubilize any MTT-formazan produced. The optical density (OD) of each well was measured at 570 nm (OD_{570}) with a microplate reader and the inhibition of cell growth (%) was calculated as $(1-T/C) \times 100$, where C is the mean OD_{570} of the control group and T is that of the treated group. The IC_{50} was determined from the concentration–response data.

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