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

Guo-qiang Hu, Yong Yang, Lei Yi, Guo-qiang Wang, Nan-nan Duan, Xiao-yi Wen, Tie-yao Cao, Song-qiang Xie, Wen-Long Huang

Institute of Chemistry & Biology, Henan University, Kaifeng 475001, China
Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China

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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-fluoroquino- lone 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 IC50 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. 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, whereas only a few of these have been produced by modification of the carboxylic acid group at the 3-position. 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]thiazole display anticancer activity. In addition, a heterocyclic ring system related to s-triazolo[3,4-b] [1,3,4]thiazole has been widely investigated, 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-2e according to the known procedure, 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-4c 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 POCl₃ and not in the presence of other acidic media such as concentrated sulfuric acid or polyphosphoric acid.
Table 1  Growth inhibitory activities (IC_{50} μmol/L) of compounds (1a–1r) against L1210, HL60 and CHO tumor cells in the MTT assay.

<table>
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<th>Compound</th>
<th>L1210</th>
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<th>CHO</th>
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<tr>
<td>1a</td>
<td>3.6</td>
<td>0.54</td>
<td>7.8</td>
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<tr>
<td>1b</td>
<td>8.5</td>
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<td>11.6</td>
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<td>1c</td>
<td>14.2</td>
<td>2.7</td>
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<td>18.5</td>
<td>10.6</td>
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<tr>
<td>1r</td>
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</table>

Being asymmetrical, the ^1H 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. In addition, the chemical shift for all target protons such as the 5-H and 7-piperazine-H or 8-H are in close accord with the respective molecular formulae of the free bases.

The in vitro antitumor activities of 1a–1r against murine leukemia cell line (L1210), human leukaemia cell line (HL60) and Chinese hamster ovary cell line (CHO) were evaluated using the MTT assay (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; ^1H 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 mol) 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 (1H)-one 4a: yield 64%, mp > 250 °C; ^1H 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_3); IR (KBr) ν: 3356, 2952, 1625, 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 17.65; found C 55.69, H 4.87, N 17.65.

1-Cyclopropyl-6-fluoro-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-(4-pyridyl)piperazin-1-yl-quinolin-4 (1H)-one 4b: yield 72%, mp > 250 °C; ^1H 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_3); IR (KBr) ν: 3364, 3087, 2915, 1642, 1624, 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 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 (1H)-one 4c: yield 68%, mp > 250 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ: 13.72 (s, 1H, SH), 8.84 (s, 1H, H-2), 7.72 (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), 3.55–3.28 (m, 8H, piperazine-H), 2.36 (s, 3H, CH_3), 1.38–1.17 (m, 4H, CH_2CH_3); IR (KBr) ν: 3356, 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.

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-4H-1,2,4-triazol-3-yl)-1-cyclopropyl-6-fluoro-7-piperazin-1-yl-quinolin-4 (1H)-one 5a: yield 58%, mp 246–248 °C; ^1H 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_3); IR (KBr) ν: 3442, 3008, 2867, 1630, 1616, 1457 cm^{-1}; MS m/z: 402 [M+H]^+, calcd. 401.47 [M]^+. Anal.
1H NMR (DMSO-d6, 400 MHz): δ: 13.68 (s, 1H, 5-H), 9.07 (s, 1H, 2-H, 2.81 (d, J = 13.2 Hz, 1H, 5-H), 7.8.6 (d, J = 7.2 Hz, 1H, 8-H), 5.80 (s, 2H, NH2). 4.67–4.43 (m, 2H, 2H, 2HCl), 11.36 (br, 2H, 2HCl), 9.25–9.17 (br, 2H, 2HCl), 8.72–8.76 (br, 2H, 8-H), 7.62–7.58 (m, 16H, 2H, 2HCl), 1.42–1.23 (m, 8H, 2HCl), 1.40–1.22 (m, 2H, 2HCl), 1.35–1.25 (m, 16H, 2HCl). IR (KBr): ν: 3356, 3024, 2876, 1632, 1618, 1455 cm⁻¹. MS m/z: 416 [M+H]+, calcd. 415.50 [M]+. Anal. calecd. for C20H24FN8OS: C 54.93, H 5.34, N 23.60; found C 55.17, H 5.40, N 23.07.

1.3.3. Synthetic procedures for 1,2,4-Triazolo[3,4-b]1,3,4-thiadiazole-3,6-bis-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yI)-quinolin-4(IH)-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 POCl₃ (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 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 30.0%, mp 264 °C (dec.). 1H NMR (DMSO-d6, 400 MHz) δ: 11.52 (br, 2H, 2HCl), 9.12–8.97 (br, 2H, 2HCl), 7.84–7.78 (brs, 2H, 2HCl), 7.63–7.55 (m, 2H, 2HCl), 6.46–4.47 (m, 2H, 2HCl), 3.55–3.26 (m, 16H, 2HCl), 1.20–1.40 (m, 16H, 2HCl), 1552, 1442, 1383 cm⁻¹. MS m/z: 697 [M+H]+, calcd. 696.79 [M]+. Anal. calecd. for C33H24F2N10O8S-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-(4-ethylpiperazin-1-yl)-quinolin-4(IH)-one)-6-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yI)-quinolin-4(IH)-one] dihydrochloride (1b 2HCl) derived from 5a and N-Methylproloxacin 2b: yield 32%, mp 257 °C (dec.). 1H NMR (DMSO-d6, 400 MHz) δ: 11.53 (br, 2H, 2HCl), 9.22–9.15 (br, 2H, 2HCl), 8.12–8.73 (br, 2H, 2HCl), 7.65–7.53 (br, 2H, 2HCl), 4.62–4.16 (m, 2H, 2HCl), 3.35–3.24 (m, 16H, 2HCl), 1.40–1.22 (m, 2H, 2HCl). IR (KBr): v: 3352, 3032, 2884, 1645, 1627, 1560, 1453, 1324 cm⁻¹. MS m/z: 711 [M+H]+, calcd. 712.81 [M]+; Anal. calecd. for C34H29F2N10O8S·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(IH)-one)-6-[1-cyclopropyl-6-fluoro-7-(4-ethylpiperazin-1-yI)-quinolin-4(IH)-one] dihydrochloride (1c, 2HCl) derived from 5a and Enrofloxacin 2c: yield 31%, mp 252 °C (dec.). 1H NMR (DMSO-d6, 400 MHz) δ: 11.35 (br, 2H, 2HCl), 9.22–9.13 (br, 2H, 2HCl), 7.86–7.75 (br, 2H, 2HCl), 7.64–7.57 (br, 2H, 2HCl). 4.62–4.30 (m, 2H, 2HCl), 3.57–3.35 (m, 16H, 2HCl), 2.40–2.34 (m, 5H, 2HCl). IR (KBr) 1.45–1.18 (m, 11H, 2HCl).

Design, synthesis and antitumor activity of C3/C3 bis-fluoroquonolones cross-linked with triazolothiadiazole 175
(1H-2HCl) derived from 5b and Norfloxacin 6a: yield 25%, mp 242 °C (dec.). 1H NMR (DMSO-d$_6$, 400 MHz): δ: 11.56 (br, 2H, 2HCl), 9.02–8.94 (br, 2H, 2–2H), 8.52–7.58 (brs, 2H, 2–2H), 7.72–5.38 (brs, 2H, 2–2H), 4.45–3.44 (m, 2H, 2–2H), 3.56–3.30 (m, 3H, 2 Piperazine-H), 1.45–1.22 (m, 2H, 2–2H); IR (KBr) v: 3372, 3045, 2895, 1644, 1642, 1572, 1457, 1238 cm$^{-1}$; MS m/z: 699 [M+H]$^+$, calcd. 698.80 [M$^+$]$^+$, found C 56.87, H 5.11, N 17.16. Anal. calcd. for C$_{38}$H$_{40}$F$_2$N$_{10}$O$_2$S 2HCl: C 55.71, H 4.96, N 18.15; found C 55.60, H 4.78, N 18.25.

2.1.2. Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methylpiperazin-1-yl)quinolin-4(1H)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1H)-one] dihydrochloride (1k 2HCl) derived from 5c and Ciprofloxacin 2b: yield 24%, mp 251 °C (dec.). 1H NMR (DMSO-d$_6$, 400 MHz): δ: 11.80 (br, 2H, 2HCl), 9.40–9.38 (br, 2H, 2–2H), 9.15–9.08 (br, 2H, 2–2H), 7.92–7.52 (brs, 2H, 2–2H), 7.32–6.50 (m, 2H, 2–2H), 4.60–3.56 (m, 2H, 2–2H); MS m/z: 755 [M+H]$^+$, calcd. 754.84 [M$^+$]$^+$, found C 55.92, H 4.87, N 17.83.

2.1.3. Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazin-1-yl)-quinolin-4(1H)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1H)-one] dihydrochloride (1j 2HCl) derived from 5e and N-Methylciprofloxacin 2b: yield 22%, mp 248 °C (dec.). 1H NMR (DMSO-d$_6$, 400 MHz): δ: 11.70 (br, 2H, 2HCl), 9.16–9.08 (br, 2H, 2–2H), 8.51–7.86 (br, 2H, 2–2H), 7.35–6.50 (m, 2H, 2–2H), 4.60–3.56 (m, 2H, 2–2H); MS m/z: 739 [M+H]$^+$, calcd. 738.87 [M$^+$]$^+$, found C 54.25, H 4.89, N 17.45.

1H NMR (DMSO-d$_6$, 400 MHz): δ: 11.45 (br, 2H, 2HCl), 9.24–9.15 (br, 2H, 2–2H), 8.74–7.77 (m, 2H, 2–2H), 7.38–6.53 (m, 2H, 2–2H), 4.45–3.53 (m, 2H, 2–2H); MS m/z: 753 [M+H]$^+$, calcd. 752.90 [M$^+$]$^+$, found C 54.55, H 4.89, N 17.16.

2.1.4. Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-ethyl-1-piperazin-1-yl)-quinolin-4(1H)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1H)-one] dihydrochloride (1l 2HCl) derived from 5c and Norfloxacin 6a: yield 25%, mp 238 °C (dec.). 1H NMR (DMSO-d$_6$, 400 MHz): δ: 11.64 (br, 2H, 2HCl), 9.12–8.93 (br, 2H, 2–2H), 8.22–7.86 (br, 2H, 2–2H), 7.72–6.73 (m, 2H, 2–2H), 4.60–4.53 (m, 2H, 2–2H); MS m/z: 735 [M+H]$^+$, calcd. 734.81 [M$^+$]$^+$, found C 55.45, H 4.53, N 17.46.

2.1.4. Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methyl-1-piperazin-1-yl)-quinolin-4(1H)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1H)-one] dihydrochloride (1m 2HCl) derived from 5b and Levofoxacin 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, 2–2H), 7.88 (m, 2H, 2–2H), 7.64–7.57 (m, 2H, 2–2H), 5.46–4.77 (m, 4H, OCH$_2$CH$_2$ and CH), 3.55–3.37 (m, 2H, 2–2H); IR (KBr) v: 3364, 3036, 2895, 1640, 1628, 1564, 1545, 1226 cm$^{-1}$; MS m/z (%): 727 [M+H]$^+$, calcd. 726.81 [M$^+$]$^+$, found C 55.48, H 4.64, N 17.6.

2.1.4. Triazolo[3,4-b][1,3,4]thiadiazole-3-[1-cyclopropyl-6-fluoro-7-(4-methyl-1-piperazin-1-yl)-quinolin-4(1H)-one]-6-[1-ethyl-6-fluoro-7-piperazin-1-yl-quinolin-4(1H)-one] dihydrochloride (1q 2HCl) derived from 5b and Levofoxacin 6c: yield 25%, mp 220 °C (dec.). 1H NMR
(DMSO-\textsubscript{d}6, 400 MHz) \( \delta \): 11.62 (br, 2H, 2HCl), 9.22–8.98 (br, 2H, 2 × 2H), 7.86 (d, \( J = 2.4 \) Hz, 1H, 8-H), 7.64–7.52 (br, 2H, 2 × 5H), 4.64–4.53 (m, 4H, OCH\textsubscript{2}CH\textsubscript{3} and CH\(_3\)), 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\textsubscript{2}CH\textsubscript{3} and CH\(_3\)); IR (KBr) \( v \): 3363, 3025, 2925, 1634, 1618, 1557, 1456, 1234 cm\(^{-1}\); MS 
\chem{m/z}: 741 [M+H]\(^+\), calcd. 740.84 [M+H]\(^+\). Anal. calcd. for 
\chem{C\textsubscript{37}H\textsubscript{38}F\textsubscript{2}N\textsubscript{10}O\textsubscript{3}S}: 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(1H)-one]-6-[1,8-(2,1-oxypropyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-quinolin-4(1H)-one] dihydrochloride (1r-2HCl) derived from 5c and Levofloxacin 6c: yield 20%, mp 216°C (dec.). \( ^1\)H NMR (DMSO-\textsubscript{d}6, 400 MHz) \( \delta \): 11.50 (br, 2H, 2HCl), 9.17–8.96 (br, 2H, 2 × 2H), 7.88 (d, \( J = 2.4 \) Hz, 1H, 8-H), 7.74–7.60 (br, 2H, 2 × 5H), 4.66–4.50 (m, 4H, OCH\textsubscript{2}CH\textsubscript{3} and CH\(_3\)), 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\textsubscript{2}CH\textsubscript{3} and 2 × CH\(_3\)); IR (KBr) \( v \): 3358, 3027, 2934, 1640, 1624, 1562, 1455, 1232 cm\(^{-1}\); MS 
\chem{m/z}: 755 [M+H]\(^+\), calcd. 754.87 [M+H]\(^+\). Anal. calcd. for 
\chem{C\textsubscript{40}H\textsubscript{40}F\textsubscript{2}N\textsubscript{10}O\textsubscript{3}S \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\(^5\) cells was seeded into each well of a well-96 microplate and solutions of test compounds at concentrations of 0.1, 1.0, 10.0, 30.0 and 50.0 \( \mu \text{mol}/L \) added simultaneously to triplicate wells before making the final volume up to 100 \( \mu \text{L} \). Plates were incubated at 37°C for 48 h in a humidified atmosphere (5% CO\(_2\), 95% air) after which 100 \( \mu \text{L} \) 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 \( \mu \text{L} \) 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\textsubscript{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\textsubscript{570} of the control group and T is that of the treated group. The IC\textsubscript{50} was determined from the concentration-response data.

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References