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REGULAR ARTICLE

Anti-proliferative activity, molecular modeling studies and interaction with calf thymus DNA of novel ciprofloxacin analogues

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Abstract. In our pursuit to expand new potential anticancer leads, a series of eighteen novel 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substituted piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid analogues have been synthesized, characterized and evaluated anti-proliferative activity against five human cancer cell lines such as A549 (lung cancer), Mia Paca (pancreatic cancer), HeLa (cervical cancer), MDA MB-231 (breast cancer), MCF-7 (breast cancer) and normal embryonic kidney cell line (HEK) were carried out using MTT assay. Few of the synthesized analogues exhibited potent anticancer activity against the cancer cell lines at a lower concentration. The synthesized compounds showed the less toxic effect on normal human embryonic kidney cell line (HEK) compared with doxorubicin. Noticeably, compound **3o** exhibited potent activity against all five cancer cell lines compared with ciprofloxacin. Further study exposed that compound **3o** could competently intercalate into calf thymus DNA to form **3o**-DNA complex which might block DNA replication to apply anti-proliferative activity. Docking simulation studies supported by molecular interactions with DNA type II topoisomerase. These derivates can become lead structures for the development of potential anticancer drugs.

Keywords. Ciprofloxacin; anti-proliferative activity; docking studies; DNA binding.

1. Introduction

Cancer is a group of diseases; it can cause different signs in the human body where it is located. In recent times it has been declared as the second reason for death in the United States.[1] Fluoroquinolones (FQs) represent a family of broad-spectrum antibacterial agents, which inhibit the bacterial DNA gyrase and type II DNA topoisomerase in mammalian cells.

Transitional cell carcinoma growth was inhibited by FQ derivatives like ofloxacin and levofloxacin.[2] In particular, ciprofloxacin (**CP**) had low side effects, one of the broad-spectrum FQ antibiotics and known to exhibit antiproliferative,[3] and apoptotic activities in numerous cancer cell lines such as prostate cancer cell lines (HRPC and PC-3),[4] transitional cell carcinoma cell lines (MBT-2 and T24),[5] colon carcinoma cell lines (CC-531, SW-403 and HT-29) and lung cancer cell lines (A549).[6,7]

Also, these are known to inhibit proliferation of jurkat cells without any symptoms of cell death through inhibition of mitosis.[8] **CP-**induced significant morphological alterations in non-small-cell lung cancer cell line (NCI-H460),[9] and exhibited a cytotoxic effect against A549 cells (lung cancer),[10] ovarian cancer cell line (CHO AA8) and murine glioma cell line (GL26).[11,12] **CP** after oral administration might reach higher concentration in urine than in serum and exhibit potential anticancer

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activity against bladder cancer cell lines (HTB9) and also known to exhibit cell cycle arrest at the S/G_2-M checkpoints.[13] **CP** prevents topoisomerase II, which leads to cell death by apoptosis in malignant cells but not in normal cells.[14]

FQ derivatives possess anticancer activity; further, several reports cited that introduction of a substituent on *N*-4 piperazinyl moiety of FQ, alters the physicochemical properties and enhances the activity.[15–22] Lipophilicity of camptothecin was enhanced due to C-7 substitution and it is lead to the discovery of phase II clinical trial agent gimatecan.[23] Lipophilicity of the substituent in bis-quinolinium compounds improved their activity against colon cancer cell line (HT-29).[24] Based on these studies, we recently reported 1-cyclopropyl-6-fluoro-4-oxo-7-(4-substituted piperazine-1-yl)-1,4-dihydroquinoline-3-carboxylic acid derivatives as antiproliferative agents.[25]

These fruitful anticancer results have attracted us to explore various **CP** derivatives. Pigeon *et al*., synthesized aniline or acetanilide hooked 2-ferrocenyl-1,1-diphenyl-but-l-ene derivatives and demonstrated that aniline or acetanilide group enhanced the anticancer activity of the molecule when evaluated against breast cancer cells.[26] Hence, we envisaged synthesizing C7-piperazinyl **CP** acetanilide hybrids anticipating enhanced physicochemical properties of **CP** and/or synergistic effect through combining **CP** and acetanilide in one compact structure. In this study, we concerted on the synthesis, anti-proliferative evaluation and DNA binding. The synthesized compounds (**3a–r**) were evaluated for their *in vitro* anticancer activity on five human cancer cell lines. There is a curiosity in discovering the binding of molecules with DNA for the rational design and construction of efficient drugs. In the current study, we evaluated the DNA-binding interactions of newly synthesized **CP** derivative by using fluorescence spectroscopic technique.

2. Experimental

2.1 *Materials and methods*

Ciprofloxacin, CtDNA and dried solvents were purchased from Sigma Aldrich, USA and used as received. All reactions were monitored by analytical Thin Layer Chromatography (TLC) performed on E-Merck 0.25 mm pre-coated silica gel glass plates (60 F254). Visualization of the spots on TLC plates was achieved by exposure to UV light (254 nm). Column chromatography was performed using silica gel (Acme, 100–200 mesh). Melting points were determined using Stuart SMP30 system and are uncorrected. IR spectra were recorded as KBr pellets on Jasco FTIR-4200 spectrometer. The UV spectral studies were performed on a spectrophotometer (JASCO model V-650). The fluorescence spectra performed on a spectrofluorometer (JASCO model FP-6300). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C), in $CDCl_3$. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane ($\delta = 0.0$) as an internal standard and coupling constants (J) in Hertz. Low-resolution mass spectra (ESI-MS) were recorded on Shimadzu.

2.2 *Synthesis*

2.2a *Synthesis of 2-chloro-N-(substituted phenyl) acetamide (**2a–r**)*: 2-Chloroacetyl chloride (24 mmol) was slowly added dropwise to a mixture of various anilines (20 mmol) and Et_3N (24 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for an additional 20 h. After the solvent was removed under reduced pressure, the residue was washed with ice water, and the precipitate was separated by filtration. The crude product was purified by crystallization using a mixture of ether/hexane (**2a–r**). Spectral data of *2-chloro-N-(4-chlorophenyl) acetamide* (***2a***): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.26 (s, 2H), 7.47 (d, 2H, J = 8.7 Hz), 7.72 (d, 2H J = 8.7 Hz), 9.05 (s, 1H), ^{13}C NMR (100.61 MHz, $CDCl_3$) 165.46, 136.43, 133. 129.12, 120.42, 49.16. ESI-MS (m/z): calcd. for $C_8H_7Cl_2NO$ 202.99, found 203.23$[M + H]^+$.

2.2b *Synthesis of title compounds (**3a–r**)*: To a solution of **CP** (0.6036 mmol) in dry DMF (2 mL), triethylamine (1.8108 mmol) and potassium iodide (0.0603 mmol) were added at RT under N_2 atmosphere. To the resultant mixture, **2a** (0.6036 mmol) was added and heated at 125 °C. After the reaction was complete, as indicated by TLC, DMF was evaporated in vacuo. The obtained residue was diluted with 20 mL of water. The compound was extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resultant crude was purified by column chromatography [CH_2Cl_2/MeOH (1–10%)] to get the title compounds (**3a–r**).

2.2b1 7-(4-(2-(4-Chlorophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3a***) IR (KBr): ~3465 cm^{-1}(O-H stretch), ~3285 cm^{-1} (N-H stretch of amide), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1590 cm^{-1} (N-H bending of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1035 cm^{-1} (C-F stretch), ~820 cm^{-1} (C-H out-of-plane bending of para disubstituted benzene), ~770 cm^{-1} (C- Cl stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.29–7.31

(d, 2H, J = 8.7 Hz), 7.37–7.39 (d, 1H, J_{H-F} = 7.0 Hz), 7.54–7.56 (d, 2H, J = 8.7 Hz), 7.99–8.02 (d, 1H, J_{H-F} = 12.7 Hz), 8.74 (s, 1H), 9.05 (s, 1H), 14.97 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 136.61, 133.31, 129.62, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}ClFN_4O_4$ 498.14, found 499.23 $[M + H]^+$.

2.2b2 7-(4-(2-(3-Chlorophenylamino)-2-oxoethyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydro quinoline-3-carboxylicacid (***3b***) IR (KBr): ~3470 cm^{-1} (O-H stretch), ~3280 cm^{-1} (N-H stretch of amide), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1580 cm^{-1} (N-H bending of amide), ~1425 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1030 cm^{-1} (C-F stretch), ~770 cm^{-1} (C-H out-of-plane bending of meta disubstituted benzene), ~750 cm^{-1} (C-Cl stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.32 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.28 (s, 2H), 3.40 (m, 4H), 3.60 (tt, 1H, J = 7.2 Hz, J = 4.0 Hz), 7.23–7.25 (d, 1H, J = 8.6 Hz), 7.33 (t, 1H), 7.40 (d, 1H, J_{H-F} = 7.5 Hz), 7.45 (d, 1H, J = 8.6 Hz), 7.85 (s, 1H), 7.99 (d, 1H, J_{H-F} = 13.2 Hz), 8.75 (s, 1H), 9.15 (s, 1H), 15.01 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 136.61, 134.21, 133.31, 129.62, 127.90, 122.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. For $C_{25}H_{24}ClFN_4O_4$ 498.14, found 499.28 $[M + H]^+$.

2.2b3 7-(4-(2-(2-Chlorophenylamino)-2-oxoethyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylicacid (***3c***) IR (KBr): ~3455 cm^{-1} (O-H stretch), ~3275 cm^{-1} (N-H stretch of amide), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1675cm^{-1} (C=O stretch of amide), ~1590 cm^{-1} (N-H bending of amide), ~1410 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1030 cm^{-1} (C-F stretch), ~740 cm^{-1} (C-H out-of-plane bending of ortho disubstituted benzene), ~760 cm^{-1} (C-Cl stretch). ^{1}H NMR (400 MHz, CDCl3) δ 1.22 (t, 2H, J = 7.2 Hz), 1.32 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.28 (s, 2H), 3.4 (m, 4H), 3.6 (tt, 1H, J = 7.2 Hz, J = 4.0 Hz), 7.35 (t, 1H), 7.43 (t, 1H), 7.4 (d, 1H, J_{H-F} = 7.5 Hz), 7.55 (d, 1H, J = 8.6 Hz), 7.75 (d, 1H, J = 8.6 Hz), 7.99 (d, 1H, J_{H-F} = 13.2 Hz), 8.75 (s, 1H), 9.15 (s, 1H), 15.01 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 136.61, 134.21, 131.31, 126.42, 122.49, 121.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 112.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}ClFN_4O_4$ 498.14, found 499.32 $[M + H]^+$.

2.2b4 1-Cyclopropyl-6-fluoro-7-(4-(2-(3-methoxypheny lamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroq uinoline-3-carboxylicacid (***3d***) IR (KBr): ~3470 cm^{-1} (O-H stretch), ~3285 cm^{-1} (N-H stretch of amide), ~2975 cm^{-1} (alkene and aromatic C-H stretch), ~2880 cm^{-1} (cyclopropyl C-H stretch), ~1715 cm^{-1} (C=O stretch of acid carbonyl group), ~1665 cm^{-1} (C=O stretch of amide), ~1540 cm^{-1} (N-H bending of amide), ~1405 cm^{-1} (C-O stretch of carbonyl group), ~1270 cm^{-1} (O-H bending), ~1150 cm^{-1} (C-O stretch of -OCH_3), ~1045 cm^{-1} (C-F stretch), ~770 cm^{-1} (C-H out-of-plane bending of meta disubstituted benzene). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, 2H, J = 7.2 Hz), 1.42 (t, 2H, J = 6.7 Hz), 2.88 (m, 4H), 3.26 (s, 2H), 3.43 (m, 4H), 3.56 (tt, 1H, J = 7.2 Hz, J = 4.0 Hz), 3.83 (s, 3H), 6.71 (d, 1H, J = 8.3 Hz), 7.06 (d, 1H, J = 9.0 Hz), 7.24 (d, 1H, J_{H-F} = 8.3 Hz), 7.34 (t, 1H), 7.4 (d, 1H, J = 7.5 Hz), 8.02 (d, 1H, J_{H-F} = 12.8 Hz), 8.77 (s, 1H), 9.00 (s, 1H), 14.96 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 139.61, 134.21, 129.62, 127.9, 122.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 116.7, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 54.8, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{27}FN_4O_5$ 494.19, found 495.29 $[M + H]^+$.

2.2b5 1-Cyclopropyl-6-fluoro-7-(4-(2-(4-methoxypheny lamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroq uinoline-3-carboxylicacid (***3e***) IR (KBr): ~3460 cm^{-1} (O-H stretch), ~3280 cm^{-1} (N-H stretch of amide), ~2975 cm^{-1} (alkene and aromatic C-H stretch), ~2880 cm^{-1} (cyclopropyl C-H stretch), ~1715 cm^{-1} (C=O stretch of acid carbonyl group), ~1665 cm^{-1} (C=O stretch of amide), ~1540 cm^{-1} (N-H bending of amide), ~1400 cm^{-1} (C-O stretch of carbonyl group), ~1270 cm^{-1} (O-H bending), ~1160 cm^{-1} (C-O stretch of -OCH_3), ~1040 cm^{-1} (C-F stretch), ~830 cm^{-1} (C-H out-of-plane bending of para disubstituted benzene). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.88 (m, 4H), 3.26 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 3.8 (s, 3H), 6.89 (d, 2H, J = 8.3 Hz), 7.39 (d, 1H, J_{H-F} = 7.5 Hz), 7.5 (d, 2H, J = 9.0 Hz), 8.02 (d, 1H, J_{H-F} = 12.8 Hz), 8.74 (s, 1H), 8.89 (s, 1H), 14.97 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.62 (d, J_{C-F} = 2.2 Hz), 167.36, 166.63, 153.31 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 139.65, 134.61, 132.34, 122.62, 119.91 (d, J_{C-F} = 8.1 Hz), 115.67, 111.57 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 55.18, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{27}FN_4O_5$ 494.19, found 495.23 $[M + H]^+$.

2.2b6 7-(4-(2-(3-Chloro-4-fluorophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3f***) IR (KBr): ~3470 cm^{-1} (O-H stretch), ~3280 cm^{-1} (N-H stretch of amide), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1590 cm^{-1} (N-H bending of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1025 cm^{-1} (C-F stretch), ~1130 cm^{-1} (C-F stretch), ~750 cm^{-1} (C-Cl stretch).^{1}H NMR (400 MHz, DMSO-D_6) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.19 (t, 1H), 7.54 (d, 1H), 7.89 (s,1H), 7.93 (d, 1H, J_{H-F} = 12.7 Hz), 8.06 (s, 1H), 8.72 (s, 1H), 9.08 (s, 1H), 15.15 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 148.95, 134.61, 133.21, 124.32, 123.42, 120.09, 119.91 (d, J_{C-F} = 8.1 Hz), 113.56, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{23}ClF_2N_4O_4$ 516.13, found 517.32 $[M + H]^+$.

2.2b7 7-(4-(2-(2-Bromophenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylicacid (***3g***) IR (KBr): ~3460 cm^{-1} (O-H stretch), ~3280 cm^{-1} (N-H stretch of amide), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1590 cm^{-1} (N-H bending of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1035 cm^{-1} (C-F stretch), ~750 cm^{-1} (C-H out-of-plane bending of ortho disubstituted benzene), ~560 cm^{-1} (C-Br stretch).^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.1 (t, 1H), 7.31 (t, 1H), 7.4 (d, 1H, J_{H-F} = 7.0 Hz), 7.71 (d, 1H, J = 8.9 Hz), 7.86 (d, 1H, J = 8.9 Hz), 8.02 (d, 1H, J_{H-F} = 12.7 Hz), 8.74 (s, 1H), 9.07 (s, 1H), 14.97 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.05, 134.61, 132.31, 131.65, 127.62, 125.42, 119.91 (d, J_{C-F} = 8.1 Hz), 117.23, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}BrFN_4O_4$ 542.09, found 543.23 $[M + H]^+$.

2.2b8 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(phenylamino) ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylicacid (***3h***) IR (KBr): ~3465 cm^{-1} (O-H stretch), ~3285 cm^{-1} (N-H stretch of amide), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1590 cm^{-1} (N-H bending of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1035 cm^{-1} (C-F stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.38 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.13 (t, 1H), 7.33 (t, 2H), 7.37 (d, 1H, J_{H-F} = 7.0 Hz), 7.59 (d, 2H, J = 7.6 Hz), 8.01 (d, 1H, J_{H-F} = 12.7 Hz), 8.72 (s, 1H), 9.02 (s, 1H), 14.96 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.50, 134.51, 129.02, 128.42, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 117.83, 111.97 (d, J_{C-F} = 24.14 Hz), 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{25}FN_4O_4$ 464.18, found 464.29 $[M + H]^+$.

2.2b9 1-Cyclopropyl-6-fluoro-7-(4-(2-(methyl(phenyl) amino)-2-oxoethyl) piperazin-1-yl)-4-oxo-1,4-dihydro quinoline-3-carboxylicacid (***3i***) IR (KBr): ~3475 cm^{-1} (O-H stretch), ~2975 cm^{-1} (alkene and aromatic C-H stretch), ~2885 cm^{-1} (cyclopropyl C-H stretch), ~1720 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1035cm^{-1} (C-F stretch).^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.49 (s, 3H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.27 (d, 2H, J = 7.6 Hz), 7.39 (d, 1H, J_{H-F} = 7.0 Hz), 7.43 (t, 1H), 7.53 (t, 2H), 8.01 (d, 1H, J_{H-F} = 12.7 Hz), 8.72 (s, 1H), 14.96 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.50, 134.51, 129.82, 127.42, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 117.83, 111.97 (d, J_{C-F} = 24.14 Hz), 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 34.67, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{27}FN_4O_4$ 478.2, found 479.36 $[M + H]^+$.

2.2b10 1-Cyclopropyl-6-fluoro-7-(4-(2-(3-nitropheny lamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3j***) IR (KBr): ~3470 cm^{-1} (O-H stretch), ~3285 cm^{-1} (N-H stretch of amide), ~2975 cm^{-1} (alkene and aromatic C-H stretch), ~2880 cm^{-1} (cyclopropyl C-H stretch), ~1715 cm^{-1} (C=O stretch of acid carbonyl group), ~1665 cm^{-1} (C=O stretch of amide), ~1540 cm^{-1} (N-H bending of amide), ~1520 cm^{-1} (N-O asymmetric stretch), ~1405 cm^{-1} (C-O stretch of carbonyl group), ~1360 cm^{-1} (N-O symmetric stretch), ~1270 cm^{-1} (O-H bending), ~1150 cm^{-1} (C-O stretch of -OCH_3), ~1045 cm^{-1} (C-F stretch), ~780 cm^{-1} (C-H out-of-plane bending of meta disubstituted benzene). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.32 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.28 (s, 2H), 3.40 (m, 4H), 3.60 (tt, 1H, J = 7.2 Hz, J = 4.0 Hz), 7.15 (d, 1H, J = 8.6 Hz), 7.33 (t, 1H), 7.4 (d, 1H, J_{H-F} = 7.5 Hz), 7.55 (d, 1H, J = 8.6 Hz), 7.85 (s, 1H), 7.99 (d, 1H, J_{H-F} = 13.2 Hz), 8.75 (s, 1H), 9.15 (s, 1H), 15.01 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d,

J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 137.61, 135.21, 133.31, 128.62, 126.60, 122.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}FN_5O_6$ 509.18, found 510.32 $[M + H]^+$.

2.2b11 1-Cyclopropyl-7-(4-(2-(ethyl(phenyl)amino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3k***) IR (KBr): ~3475 cm^{-1} (O-H stretch), ~3000 cm^{-1} (alkane C-H stretch), ~2900 cm^{-1} (alkene and aromatic C-H stretch), ~2885 cm^{-1} (cyclopropyl C-H stretch), ~1720 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1035 cm^{-1} (C-F stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 1.35 (t, 3H), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 3.86 (q, 2H), 7.27 (d, 2H, J = 7.6 Hz), 7.39 (d, 1H, J_{H-F} = 7.0 Hz), 7.43 (t, 1H), 7.53 (t,2H), 8.01 (d, 1H, J_{H-F} = 12.7 Hz), 8.72 (s, 1H), 14.96 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.50, 134.51, 129.82, 127.42, 122.45, 119.91 (d, J_{C-F} = 8.1 Hz), 117.83, 111.97 (d, J_{C-F} = 24.14 Hz), 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 34.67, 13.56, 8.12. ESI-MS (m/z): calcd. for $C_{27}H_{29}FN_4O_4$ 492.20, found 493.36 $[M + H]^+$.

2.2b12 1-Cyclopropyl-6-fluoro-7-(4-(2-(4-nitrophenylamino)-2-oxoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3l***) IR (KBr): ~3470 cm^{-1}(O-H stretch), ~3285 cm^{-1} (N-H stretch of amide), ~2975 cm^{-1} (alkene and aromatic C-H stretch), ~2880 cm^{-1} (cyclopropyl C-H stretch), ~1715 cm^{-1} (C=O stretch of acid carbonyl group), ~1665 cm^{-1} (C=O stretch of amide), ~1540 cm^{-1} (N-H bending of amide), ~1530 cm^{-1} (N-O asymmetric stretch), ~1405 cm^{-1} (C-O stretch of carbonyl group), ~1350 cm^{-1} (N-O symmetric stretch), ~1270 cm^{-1} (O-H bending), ~1150 cm^{-1} (C-O stretch of -OCH_3), ~1045 cm^{-1} (C-F stretch), ~830 cm^{-1} (C-H out-of-plane bending of para disubstituted benzene). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.31 (d, 2H, J = 8.7 Hz), 7.39 (d, 1H, J_{H-F} = 7.0 Hz), 7.56 (d, 2H, J = 8.7 Hz), 8.02 (d, 1H, J_{H-F} = 12.7 Hz), 8.74 (s, 1H), 9.05 (s, 1H), 14.97 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.95, 136.61, 133.31, 129.62, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{24}FN_5O_6$ 501.16, found 502.28 $[M + H]^+$.

2.2b13 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-oxo-2-(3-(trifluoromethyl) phenylamino) ethyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (***3m***) IR (KBr): ~3470 cm^{-1}(O-H stretch), ~3280 cm^{-1} (N-H stretch of amide), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1590 cm^{-1} (N-H bending of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1210 cm^{-1} (C-F stretch), ~1030 cm^{-1} (C-F stretch), ~770 cm^{-1} (C-H out-of-plane bending of meta disubstituted benzene). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.32 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.28 (s, 2H), 3.40 (m, 4H), 3.60 (tt, 1H, J = 7.2 Hz, J = 4.0 Hz), 7.15 (d, 1H, J = 8.6 Hz), 7.33 (t, 1H), 7.4 (d, 1H, J_{H-F} = 7.5 Hz), 7.55 (d, 1H, J = 8.6 Hz), 7.85 (s, 1H), 7.99 (d, 1H, J_{H-F} = 13.2 Hz), 8.75 (s, 1H), 9.15 (s, 1H), 15.01 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 137.61, 135.21, 133.31, 128.62, 126.60, 125.34, 122.91, 120.42, 119.91 (d, J_{C-F} = 8.1 Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{24}F_4N_4O_4$ 532.18, found 533.32 $[M + H]^+$.

2.2b14 7-(4-(2-(3-Chloro-2-methylphenylamino)-2-oxoethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3n***) IR (KBr): ~3465 cm^{-1} (O-H stretch), ~3285 cm^{-1} (N-H stretch of amide), ~3000 cm^{-1} (alkane C-H stretch), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1} (cyclopropyl C-H stretch), ~1725 cm^{-1} (C=O stretch of acid carbonyl group), ~1670 cm^{-1} (C=O stretch of amide), ~1590 cm^{-1} (N-H bending of amide), ~1420 cm^{-1} (C-O stretch of carbonyl group), ~1285 cm^{-1} (O-H bending), ~1035 cm^{-1} (C-F stretch), ~740 cm^{-1} (C-Cl stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.32 (t, 2H, J = 6.7 Hz), 2.21 (s, 3H), 2.89 (m, 4H), 3.28 (s, 2H), 3.4 (m, 4H), 3.6 (tt, 1H, J = 7.2 Hz, J = 4.0 Hz), 7.16 (d, 1H, J = 8.6 Hz), 7.23 (t, 1H), 7.4 (d, 1H, J_{H-F} = 7.5 Hz), 7.45 (d, 1H, J = 8.6 Hz), 7.99 (d, 1H, J_{H-F} = 13.2 Hz), 8.75 (s, 1H), 9.15 (s, 1H), 15.01 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 137.61, 135.21, 134.31, 132.24, 127.62, 124.6, 119.91 (d, J_{C-F} = 8.1 Hz), 115.67, 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 13.56, 8.12. ESI-MS (m/z): calcd. for $C_{26}H_{26}ClFN_4O_4$ 512.16, found 512.28, $[M + H]^+$.

2.2b15 1-Cyclopropyl-7-(4-(2-(2,4-dimethylphenylamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3o***) IR (KBr): ~3465 cm^{-1} (O-H stretch), ~3285 cm^{-1} (N-H stretch of amide), ~3000 cm^{-1} (alkane C-H stretch), ~2970 cm^{-1} (alkene and aromatic C-H stretch), ~2890 cm^{-1}

(Cyclopropyl C-H stretch), ∼1725 cm^{-1} (C=O stretch of acid carbonyl group), ∼1670 cm^{-1} (C=O stretch of amide), ∼1590 cm^{-1} (N-H bending of amide), ∼1420 cm^{-1} (C-O stretch of carbonyl group), ∼1285 cm^{-1} (O-H bending), ∼1035 cm^{-1} (C-F stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.39 (t, 2H, J = 6.7 Hz), 2.29 (s, 6H), 2.91 (m, 4H), 3.29 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.00 (s, 1H), 7.04 (d, 1H, J = 8.6 Hz), 7.37 (d,1H, J_{H-F} = 7.5 Hz), 7.91 (d, 1H, J_{H-F} = 12.7 Hz), 7.98 (d, 1H, J = 8.6 Hz), 8.7 (s, 1H), 9.03 (s, 1H), 14.95 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.99 (d, J_{C-F} = 2.2 Hz), 167.37, 166.87, 154.87 (d, J_{C-F} = 249.3 Hz), 152.35, 147.44, 145.47 (d, J_{C-F} = 10.3 Hz), 139.03, 134.4, 132.95, 131.08, 127.51, 121.5, 120.01 (d, J_{C-F} = 8.1 Hz), 112.61 (d, J_{C-F} = 24.14 Hz), 108.1, 104.93 (d, J_{C-F} = 3.7 Hz), 62.07, 53.22, 50.04, 35.31, 20.85, 17.83, 8.24. ESI-MS (m/z): calcd. for $C_{27}H_{29}FN_4O_4$ 492.21, found 493.32 $[M + H]^+$.

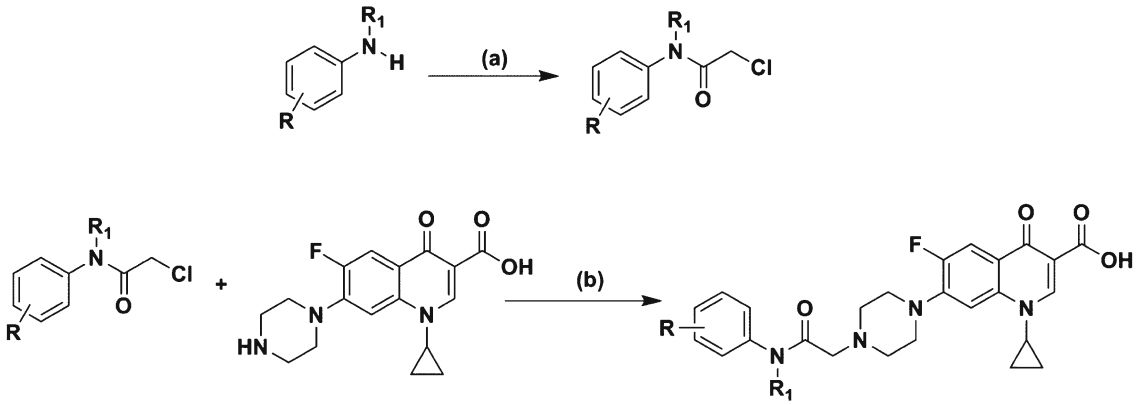
2.2b16 1-Cyclopropyl-7-(4-(2-(2,5-dimethylpheny lamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3p***) IR (KBr): ∼3465 cm^{-1} (O-H stretch), ∼3285 cm^{-1} (N-H stretch of amide), ∼3000 cm^{-1} (alkane C-H stretch), ∼2970 cm^{-1} (alkene and aromatic C-H stretch), ∼2890 cm^{-1} (cyclopropyl C-H stretch), ∼1725 cm^{-1} (C=O stretch of acid carbonyl group), ∼1670 cm^{-1} (C=O stretch of amide), ∼1590 cm^{-1} (N-H bending of amide), ∼1420 cm^{-1} (C-O stretch of carbonyl group), ∼1285 cm^{-1} (O-H bending), ∼1035 cm^{-1} (C-F stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.12 (s, 3H), 2.34 (s, 3H), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 6.83 (d,1H, J = 8.6 Hz), 7.07 (d, 1H, J = 8.6 Hz), 7.29 (s,1H), 7.93 (d, 1H, J_{H-F} = 12.7 Hz), 8.06 (s, 1H), 8.72 (s, 1H), 9.8 (s, 1H), 15.15 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 177.02 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 154.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 139.03, 136.87, 135.36, 130.19, 125.56, 123.32, 121.42, 119.94 (d, J_{C-F} = 8.1 Hz), 112.62 (d, J_{C-F} = 24.14 Hz), 108.12, 104.93 (d, J_{C-F} = 3.7 Hz), 62.13, 53.21, 50.05, 35.32, 21.24, 17.47, 8.24. ESI-MS (m/z): calcd. for $C_{27}H_{29}FN_4O_4$ 492.21, found 493.34 $[M + H]^+$.

2.2b17 1-Cyclopropyl-7-(4-(2-(2,6-diethylpheny lamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3q***) IR (KBr): ∼3465 cm^{-1} (O-H stretch), ∼3285 cm^{-1} (N-H stretch of amide), ∼3000 cm^{-1} (alkane C-H stretch), ∼2970 cm^{-1} (alkene and aromatic C-H stretch), ∼2890 cm^{-1} (cyclopropyl C-H stretch), ∼1725 cm^{-1} (C=O stretch of acid carbonyl group), ∼1670 cm^{-1} (C=O stretch of amide), ∼1590 cm^{-1} (N-H bending of amide), ∼1420 cm^{-1} (C-O stretch of carbonyl group), ∼1285 cm^{-1} (O-H bending), ∼1230 cm^{-1} (C-C stretch of ethyl), ∼1035 cm^{-1} (C-F stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 1.35 (t, 6H), 2.6 (q, 4H), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.11 (d,2H, J = 8.6 Hz), 7.37 (t, 1H), 7.93 (d, 1H, J_{H-F} = 12.7 Hz), 8.06 (s, 1H), 8.72 (s, 1H), 9.8 (s, 1H), 15.15 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.98 (d, J_{C-F} = 2.2 Hz), 168.69, 166.9, 154.89 (d, J_{C-F} = 249.3 Hz), 147.44, 145.57 (d, J_{C-F} = 10.3 Hz), 141.08, 139.01, 132.24, 128.06, 126.52, 120.01 (d, J_{C-F} = 8.1 Hz), 112.31 (d, J_{C-F} = 24.14 Hz), 108.03, 104.96 (d, J_{C-F} = 3.7 Hz), 61.69, 53.55, 49.95, 35.33, 25.11, 14.57, 8.24. ESI-MS (m/z): calcd. for $C_{29}H_{33}FN_4O_4$ 520.24, found 521.34 $[M + H]^+$.

2.2b18 1-Cyclopropyl-7-(4-(2-(3,4-dichloropheny lamino)-2-oxoethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (***3r***) IR (KBr): ∼3465 cm^{-1} (O-H stretch), ∼3285 cm^{-1} (N-H stretch of amide), ∼2970 cm^{-1} (alkene and aromatic C-H stretch), ∼2890 cm^{-1} (cyclopropyl C-H stretch), ∼1725 cm^{-1} (C=O stretch of acid carbonyl group), ∼1670 cm^{-1} (C=O stretch of amide), ∼1590 cm^{-1} (N-H bending of amide), ∼1420 cm^{-1} (C-O stretch of carbonyl group), ∼1285 cm^{-1} (O-H bending), ∼1035 cm^{-1} (C-F stretch), ∼780 cm^{-1} (C-Cl stretch). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 2H, J = 7.2 Hz), 1.28 (t, 2H, J = 6.7 Hz), 2.89 (m, 4H), 3.27 (s, 2H), 3.44 (m, 4H), 3.56 (tt, 1H, J = 7.0 Hz, J = 4.0 Hz), 7.54 (d, 1H, J = 8.8 Hz), 7.64 (d, 1H, J = 8.8 Hz),7.90 (s, 1H), 7.93 (d, 1H, J_{H-F} = 12.7 Hz), 8.06 (s, 1H), 8.72 (s, 1H), 9.8 (s, 1H), 15.15 (s, 1H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ 176.82 (d, J_{C-F} = 2.2 Hz), 167.56, 166.43, 153.21 (d, J_{C-F} = 249.3 Hz), 147.23, 145.12 (d, J_{C-F} = 10.3 Hz), 138.05, 134.61, 131.21, 130.32, 129.42, 124.09, 121.23, 119.91 (d, J_{C-F} = 8.1Hz), 111.97 (d, J_{C-F} = 24.14 Hz), 107.12, 104.89 (d, J_{C-F} = 3.7 Hz), 60.67, 51.12, 49.16, 35.58, 8.12. ESI-MS (m/z): calcd. for $C_{25}H_{23}Cl_2FN_4O_4$ 532.10, found 533.22 $[M + H]^+$.

2.3 *Cell culture and cell proliferation assay*

Cell viability was determined by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Human lung cancer cell line (A549), human cervical cancer cell line (HeLa), Human Breast carcinoma cell lines MDA MB-231, MCF7, Human Pancreatic Cancer line MiaPaca-2 and Human Embryonic Kidney Cell lines (HEK) were employed in the current study. Cells (1×10^4 cells/well) were seeded to 96-well culture plate and cultured with or without different concentrations of compounds for 48 h in a final volume of 200 µL. After treatment, the medium was removed and 10 µL of MTT (10 mg/mL in PBS) was added to the fresh medium. After 2 h incubation at 37 °C, 100 µL extraction buffer was added to each well and plates were agitated for 1 min. The optical density (O.D) was read at 570 using microplate reader (Multimode Varioskan Flash Instrument-Thermo Scientific Ltd). Per cent inhibition of proliferation was calculated as a fraction of control (without compound). All the experiments were carried out in triplicates. The results were represented as a



Reagents and conditions: (a) 2-chloroacetyl chloride, Et_3N, CH_2Cl_2, 0-25^{0}C, 20 h (b) Et_3N, KI, DMF, 125^{0}C

Scheme 1. Synthesis of ciprofloxacin derivatives (**3 a–r**).

percentage of cytotoxicity/viability. From the percentage of cytotoxicity, the IC_{50} values are calculated.

2.4 *Molecular docking studies*

Further, the molecular docking studies of **3a–r** were performed using human topo-II isomerase being the target enzyme of **CP** using Schrödinger suite 2013. Crystal co-ordinates for DNA topo-II isomerase was taken from the Protein Data Bank (PDB ID: 4G0U). The multi-step Schrödinger's protein preparation tool (PPrep) has been used for the final preparation of receptor model. Hydrogen was added to the model automatically via the maestro interface. PPrep neutralizes side chains and residues which are not involving in salt bridges. This step is then followed by restrained minimization using the OPLS 2005 force field to RMSD of 0.3 A^0. The 2D structure of **3a–r** were sketched and converted to 3D using maestro interface. Ligands were prepared for docking using Ligprep, a module of Schrodinger. A total of 10 conformations were generated for all the compounds. Grid box was generated with coordinates of X:26.2195; Y:99.8115; Z:32.8506 by considering co-crystal ligand *i.e.*, amsacrine. Docking studies were performed using GLIDE, a module of Schrödinger.

2.5 *DNA binding*

2.5a *UV- Visible measurement*: The DNA binding experiments were carried in Tris–HCl buffer solution (5 mM, pH 7.4) using the compound solution in DMSO. In UV–visible measurements, a constant concentration of compound **3o** was treated with different concentrations of the CtDNA. The DNA solutions of equivalent concentrations were measure as reference solutions in the experiment. The absorbance (A) was recorded after successive additions of various concentrations of CtDNA. An equal amount of CtDNA was added to the compound solution and the reference solution while measuring the spectra to eliminate the absorbance of the CtDNA itself.

2.5b *Fluorescence measurements*: The fluorescence spectral titrations were performed on a spectrofluorometer (JASCO model FP-6300). The fluorescence emission spectra were measured at 300 K over a wavelength range of 520–740 nm with an exciting wavelength at 500 nm. Before measuring fluorescence spectra, all solutions were stirred and allowed to equilibrate for 5 min. For correct background fluorescence blank of Tris–HCl buffer was subtracted.

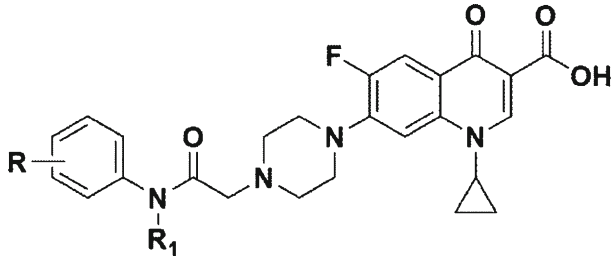
3. Results and Discussion

3.1 *Chemistry*

In the current study, we synthesized a total of eighteen (**3a–r**) 7-(substituted piperazin-1-yl) derivatives of **CP** in a two-step process. The first step includes preparation of 2-chloro-*N*-(substitutedphenyl) acetamides (**2a–r**) by coupling substituted anilines with chloroacetyl chloride.[27] In the second step, 2-chloro-*N*-(substituted phenyl) acetamides were coupled with **CP** yielding the title compounds (Scheme 1 and Table 1).

In general ^{1}H NMR of all the title compounds displayed two triplets in the range 1.10–1.39 ppm and a multiplet in the range 3.56–3.6 ppm corresponding to the protons of cyclopropyl ring. Two multiplets of piperazine protons resonated in the range 2.88–3.44 ppm. Two sharp doublets resonated in the range 7.2–8.02 ppm due to C-5 and C-8 protons of the FQ moiety. The C-2 protons of FQ showed a sharp singlet in the range 8.64–8.77 ppm. One of the singlet peak due to the proton of amide in the range of 8.7–9.15 ppm. Broad peak owing to the proton of carboxylic acid functional group in the

Table 1. Synthesized compounds: structure, m.p, yield and docking score (**3a**–**r**).



Entry	R	R_1	M.p (°C)	Yield (%)	Docking Score (SP)
3a	4-Cl	H H	222–224	73	−7.963
3b	3-Cl	H	249–251	67	−7.743
3c	2-Cl	H	289–290	63	−7.78
3d	3-OCH_3	H	219–221	77	−7.694
3e	4-OCH_3	H	208–210	67	−7.95
3f	3-Cl-4-F	H	245–246	64	−7.848
3g	2-Br	H	284–285	65	−7.892
3h	H	H	259–261	82	−7.831
3i	H	CH_3	189–190	85	−7.206
3j	3-NO_2	H	265–266	82	−7.521
3k	H	CH_2CH_3	180–182	64	−5.382
3l	4-NO_2	H	257–258	60	−7.924
3m	3-CF_3	H	222–223	65	−8.048
3n	3-Cl-2-CH_3	H	268–269	63	−7.847
3o	2,4-diCH_3	H	256–258	65	−7.446
3p	2,5-diCH_3	H	244–245	62	−7.68
3q	2,6-diC_2H_5	H	261–262	67	−7.459
3r	3,4-diCl	H	214–216	71	−7.729
Ciprofloxacin					−7.577

range 14.96–15.15 ppm. The acetyl link protons showed multiplet in the range 3.26–3.3. Further, the structure of the title compounds was substantiated from ^{13}C NMR and ESI-MS respectively.

3.2 *Antiproliferative activity*

The antiproliferative activity of **3a**–**r** on human cancer cell lines such as lung cancer (A-549), pancreatic cancer (Mia Paca-2), cervical cancer (HeLa), metastatic breast cancer (MDA MB-231), breast cancer (MCF-7) cell lines along with a non-cancerous human embryonic kidney cell line (HEK) was carried using MTT assay,[28] and the results are tabulated in Table 2. **CP** and doxorubicin are used as reference standards.

All the compounds **3a**–**r** showed significant growth inhibition on A549 cell line with IC_{50} values ranging from 11.69 ± 0.26 to 15.27 ± 1.68 μM. Substitution of chloro, methoxy at various positions (**3a**, **3b**, **3c** and **3d**) demonstrated lowest IC_{50} and better growth inhibition whereas compounds bearing electron withdrawing groups (**3j** and **3l**) like nitro at meta and para position exhibited moderate activity against A549 (lung cancer) cell line. Substitution with 2,4-dimethylphenyl at C7 position (**3o**) exhibited promising anticancer activity against Miapaca, HeLa, MDA MB-231 cancer cell lines. Nevertheless, **3o** was found to be potent than **CP** against MCF7 cell line with IC_{50} value 26.45 μM.

From the IC_{50} values, it is clear that most of the active compounds exhibited less cytotoxicity towards the normal embryonic kidney cell line (HEK) compared to their anti-cancer potential against tested cancer cell lines, which justifies the role of the novel synthesized compounds as anti-cancer agents.

3.3 *Molecular docking studies*

Further, the molecular docking studies of **3a–r** were performed using human topo-II isomerase being the target enzyme of **CP**. Docking scores by standard precision (Glide-SP) and shown in Table 1. Amino acid interaction pattern of a few active compounds **3d, 3o** and **3n** are shown in Figure S11 (Supplementary Information) along with amsacrine (topoisomerase inhibitor) as standard. Amsacrine has shown docking score of −5.86.

Table 2. IC_{50} values (μM) for compounds (**3a–r**) in five human cancer cell lines (A549, MiaPaca, HeLa, MDA MB-231, MCF-7) as well as normal cell line HEK.

Entry	A549[a]	MiaPaca[b]	HeLa[c]	MDA MB 231[d]	MCF7[d]	HEK[e]
3a	13.29 ± 0.44	45.4 ± 0.52	>100	34.3 ± 0.41	>100	72.41 ± 1.69
3b	11.69 ± 0.26	37.02 ± 0.47	76.3 ± 0.76	32.79 ± 0.4	>100	73.63 ± 1.08
3c	11.71 ± 0.18	35.57 ± 0.48	91.95 ± 0.2	49.77 ± 0.42	>100	>100
3d	12.64 ± 0.37	70.23 ± 1.69	49.08 ± 0.88	30.76 ± 0.82	>100	>100
3e	12.72 ± 0.83	36.92 ± 0.74	51.77 ± 0.48	>100	86.37 ± 0.61	>100
3f	12.36 ± 0.35	33.22 ± 0.32	>100	62.85 ± 1.05	76.8 ± 0.89	>100
3g	13.1 ± 1.78	41.14 ± 0.96	77.89 ± 1.13	>100	>100	64.54 ± 0.66
3h	13.83 ± 1.61	59.22 ± 0.33	79.76 ± 1.76	78.08 ± 0.31	>100	>100
3i	13.25 ± 0.27	59.35 ± 1.66	75.18 ± 0.92	>100	>100	>100
3j	12.99 ± 0.67	69.01 ± 0.99	60.23 ± 0.57	41.53 ± 0.57	>100	>100
3k	12.71 ± 0.85	30.04 ± 0.9	83.02 ± 1.65	42.05 ± 1.25	>100	>100
3l	13.84 ± 0.94	40.55 ± 1.94	78.57 ± 1	44.93 ± 0.22	75.55 ± 1.25	>100
3m	15.27 ± 1.68	40.25 ± 1.94	77.54 ± 0.26	54.47 ± 0.62	>100	>100
3n	13.74 ± 0.84	29.12 ± 0.34	61.57 ± 0.52	33.44 ± 0.44	>100	>100
3o	14.21 ± 0.66	26.6 ± 0.05	29.28 ± 0.11	25.34 ± 0.25	26.45 ± 0.33	>100
3p	14.09 ± 0.89	48.94 ± 0.77	68.62 ± 0.25	45.65 ± 0.86	>100	76.58 ± 1.67
3q	13.88 ± 0.09	35.39 ± 0.22	>100	70.62 ± 1.47	63.92 ± 0.35	>100
3r	13.32 ± 0.17	36.93 ± 0.31	85.09 ± 0.46	84.34 ± 2.69	>100	>100
CP	19.31 ± 0.58	21.62 ± 0.28	65.82 ± 0.74	25.47 ± 0.37	>100	96.72 ± 1.23
Doxo	4.45 ± 0.02	4.25 ± 0.03	4.29 ± 0.006	4.16 ± 0.01	4.92 ± 0.01	64.58 ± 1.92

IC_{50} is concentration at which 50% of cells undergo cytotoxic cell death due to compound treatment.
IC_{50} values are indicated as the mean ± SD of three independent experiments.
CP = Ciprofloxacin, Doxo = Doxorubicin.
[a]Lung cancer, [b]pancreatic cancer, [c]cervical cancer, [d]breast cancer, [e]normal embryonic kidney cells.

3.4 *DNA binding*

3.4a *UV- Visible spectra*: We explore the absorbance spectra of **3o**-CtDNA interaction with UV-Visible spectra. We observed compound **3o** peak was at near 220nm. Although on the following addition of CtDNA to compound **3o**, the compound absorbance was slowly decreasing, that means hypochromic effect (Figure S12, Supplementary Information). This hypochromic result is owing to the overlap of electron cloud of the compound **3o** with the CtDNA base pairs and it is a feature of an intercalating binding mode.[29–32] The intrinsic binding constant (Kb) was found out from following equation.[33]

$$[\mathrm{DNA}]/|\varepsilon_a - \varepsilon_f| = [\mathrm{DNA}]/|\varepsilon_b - \varepsilon_f| + 1/\mathrm{K_b}|\varepsilon_b - \varepsilon_f \quad (1)$$

Here [DNA] represents the concentration of DNA in base pairs, ε_a is apparent extinction coefficient, ε_f is the extinction coefficient of the compound in the absence of DNA and ε_b is extinction coefficient of compound when fully bound to DNA.[34,35] K_b is the equilibrium binding constant (in M–1) of compound binding to DNA.

K_b was calculated by the ratio of slope to intercept (Figure S13, Supplementary Information). The K_b for compound **3o** is $9.312 \times 10^4 M^{-1}$. These outcome point toward that compound **3o** binding strength is good through the intercalate mode.

3.4b *Fluorescence spectra*: The synthesized compound can replace EB from EB-DNA, the fluorescence of the solution will be quenched owing to the free EB molecules are readily quenched by the adjacent water molecules.[36,37] The quenching fluorescence of EB-CtDNA by the compound (**3o**) is shown in Figure S14 (Supplementary Information).

The compound **3o** had no fluorescence, so the compound binding with CtDNA can't be predicted directly by emission spectra. The spectroscopic changes of EB-CtDNA are often utilized to study the interaction between CtDNA and newly synthesized molecule.[38–40]

The quenching of EB-CtDNA by the synthesized compound **3o** is in good agreement with the linear Stern–Volmer equation, which gives additional evidence that **3o** binds to DNA.

$$\frac{I_0}{I} = 1 + K_{sv}[Q] \quad (2)$$

In the above equation I_0 is the emission intensity in the absence of quencher, I is the emission intensity in the presence of quencher, K_{sv} is the Stern–Volmer quenching constant, and [Q] is the quencher concentration.

The linear relationship of I_0/I versus [Q] recommend that the quenching result for this system is a static type, means non-fluorescence complex is formed between compound **3o** and CtDNA. Stern–Volmer quenching constant (K_{sv}) is given by the ratio of the slope to the intercept (Figure S15, Supplementary Information), value of K_{sv} is $5.1 \times 10^4 M^{-1}$. This statistics obviously indicate the interaction of **3o** with CtDNA.

4. Conclusion

In summary, we conclude that electron donating substitution affects the anti-proliferative activity of **CP** derivatives. These results reveal the importance of chloro, methoxy, methyl substituents at dissimilar positions and further modification on the **CP** derivatives could lead to the synthesis of a promising aspirant to develop potential anti-proliferative agent. Many of the synthesized compounds do not exhibit toxic effect on normal human embryonic kidney cell line (HEK) compared with doxorubicin. DNA-binding properties of the synthesized compounds investigated by fluorescence clearly denote that the compound can bind to DNA through intercalation mode.

Supplementary Information (SI)

Spectral data for the characterization of compounds are given in the supplementary information. Supplementary Information is available at www.ias.ac.in/chemsci.

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