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RESEARCH ARTICLE

Synthesis and potential antitumor activity of 7-(4-substituted piperazin-1-yl)-4-oxoquinolines based on ciprofloxacin and norfloxacin scaffolds: *in silico* studies

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Abstract

The potential antitumor activities of a series of 7-(4-substituted piperazin-1-yl)fluoroquinolone derivatives (**1**–**14a,b**) using ciprofloxacin and norfloxacin as scaffolds are described. These compounds exhibit potent and broad spectrum antitumor activities using 60 human cell lines in addition to the inherent antibacterial activity. Compounds **1a**, **2a**, **3b**, **6b** and **7a** were found to be the most potent, while **2b**, **5b**, and **6a** were found to have an average activity. The results of this study demonstrated that compounds **1a**, **2a**, **3b**, **6b** and **7a** (mean GI_{50}; 2.63–3.09 μM) are nearly 7-fold more potent compared with the positive control 5-fluorouracil (mean GI_{50}; 22.60 μM). More interestingly, compounds **1a**, **2a**, **3b**, **6b** and **7a** have an almost antitumor activity similar to gefitinib (mean GI_{50}; 3.24 μM) and are nearly 2-fold more potent compared to erlotinib (mean GI_{50}; 7.29 μM). *In silico* study and ADME-Tox prediction methodology were used to study the antitumor activity of the most active compounds and to identify the structural features required for antitumor activity.

Keywords

ADME-T prediction, antitumor activity, fluoroquinolones, *in silico* study, synthesis

History

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Introduction

Cancer is continuing to be a major health problem worldwide to treat and is the leading cause of human death[1–4]. The development of novel anticancer agents remains an important and a challenge goal in medicinal chemistry and therefore, developing new effective anticancer drugs is an important strategy in cancer treatment[3,4]. The antibacterial fluoroquinolones have been found to be one of the fastest growing groups of drugs in recent years[5–8]. Quinolones are known for their antibacterial and antitumor activities through alteration of the normal functions of bacterial gyrase, and are found to be a topoisomerase II inhibitor in humans[9–18]. Ciprofloxacin (Figure 1), a commonly used broad-spectrum fluoroquinolone antibiotic, has shown anticancer activity in several cancer cell lines[19,20]. Other fluoroquinolone derivatives such as levofloxacin and ofloxacin have also been shown to inhibit the growth of cell bladder cancer cell lines (Figure 1)[21]. Voreloxin is a quinolone derivative that shows potent cytotoxicity towards eukaryotic cancer cell lines without antibacterial activity (Figure 1)[22,23]. Voreloxin inhibits topoisomerase II and intercalates DNA and it is currently being evaluated in a Phase 2 clinical trial for ovarian cancer[22,23].

Recently we reported the synthesis and antibacterial activity of bulky arylsulfonylfluoroquinolones based on ciprofloxacin and norfloxacin scaffolds[6]. It was found that compounds carrying dimethoxy or dichloro substituents exhibited excellent antibacterial activity compared with ciprofloxacin as a reference drug. From the detailed analysis of the results of our studies, we conclude that antibacterial activity of these compounds significantly depends on the electronic effect of methoxy and chloro groups.

We thus initiated a screening program to search for novel utility of 7-(4-substituted piperazin-1-yl)fluoroquinolones, such as 7-(4-arylsulfonylpiperazin-1-yl)fluoroquinolones, 7-(4-((phenylsulfonyl)carbamoyl)piperazin-1-yl)-fluoroquinolones and 7-(4-alkylpiperazin-1-yl)fluoroquinolones, as potential antitumor agents in addition to the reported antibacterial activity.[6,24] To the best of our knowledge, there are no reports concerning antitumor activity of bulky arylsulfonylfluoroquinolones were reported.

In this context, the present work describes the investigation of the antitumor properties of 7-(4-arylulfonylpiperazin-1-yl)fluoroquinolones (**1**–**7ab**), 7-(4-alkylpiperazin-1-yl)fluoroquinolones (**8**–**13ab**) and 7-(4-((phenylsulfonyl)carbamoyl)piperazin-1-yl)-fluoroquinolones (**14ab**) based on ciprofloxacin and norfloxacin scaffolds and the achievement of a better antitumor profile at lower concentrations using 60 human cancer cell lines and

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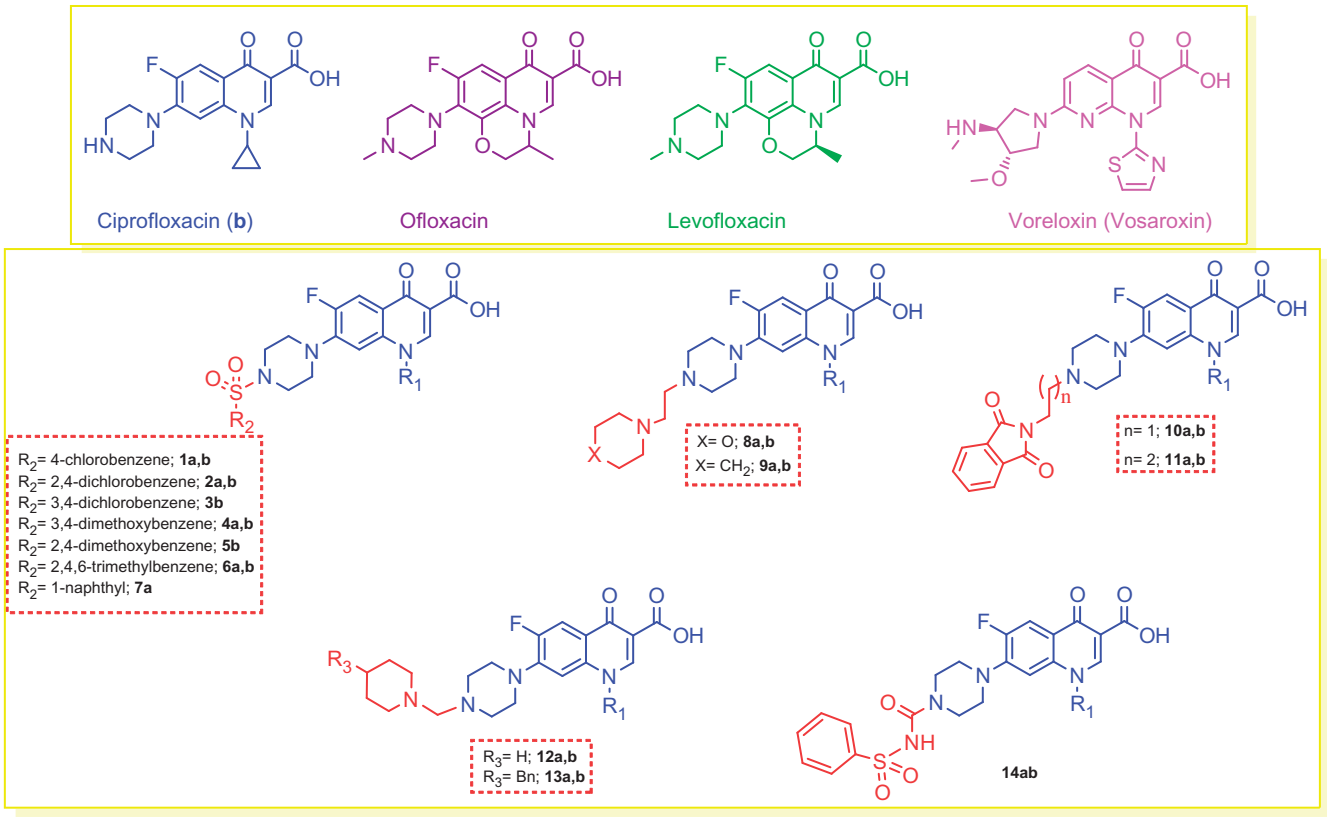


Figure 1. Reported antitumor quinolones and designed *N*-piperazinyl fluoroquinolones **1–14ab**.

keeping the inherent antibacterial activity. Moreover, *in silico* study and ADME-T prediction were used to identify the structural features required for the antitumor properties of the designed compounds. The rationale for testing of these 7-(4-substituted piperazin-1-yl)fluoroquinolones as antitumor agents was the following: (i) delineate the structure-activity relationship (SAR) for the antitumor activity of the arylsulfonylfluoroquinolones with compounds incorporating ciprofloxacin and norfloxacin scaffolds; (ii) investigate and compare the antitumor activity of 4-arylsulfonyl, 4-alkylpiperazinyl and 4-phenylsulfonylcarbamoyl derivatives; (iii) compare the efficacy of the mono and dichloro of arylsulfonylfluoroquinolones versus the methoxy derivatives for the inhibitory power against various tumor cell lines, in compounds incorporating the same scaffold. Furthermore, derivatives incorporating the bulkier trimethylbenzenesulfonyl moieties were also included in the study, in order to explore as much chemical space as possible.

Materials and methods

Chemistry

Melting points (uncorrected) were recorded on Barnstead 9100 Electrothermal melting apparatus at the Pharmaceutical Chemistry Department, King Saud University, Riyadh, Saudi Arabia. IR spectra were recorded on a FT-IR Perkin-Elmer spectrometer at Research Center, King Saud University, Riyadh, Saudi Arabia. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on Bruker 700 MHz or 500 MHz spectrometer using CD_3OD, $CDCl_3$ and DMSO-d6 as solvents at Research Center, King Saud University, Riyadh, Saudi Arabia. The chemical shifts are expressed in δ ppm using TMS as internal standard. Mass spectra were recorded on a Clarus 600 GC/MS (Middletown, CT) and Varian, TQ 320 GC/MS/MS mass spectrometers (West Sussex, UK) at Research Center, King Saud University, Riyadh, Saudi Arabia. Elemental analysis was carried out for C, H and N, at Research Center, King Saud University, Riyadh, Saudi Arabia. Solvent evaporation was performed under reduced pressure using Buchan Rotatory Evaporator at the Pharmaceutical Chemistry Department, King Saud University, Riyadh, Saudi Arabia. Thin layer chromatography was performed on precoated (0.25 mm) silica gel GF254 plates (E. Merck, Darmstadt, Germany), compounds were detected with 254 nm UV lamp. Silica gel (60–230 mesh) was employed for routine column chromatography separations. Compounds 7-arylsulfonyl-piperazin-1-ylfluoroquinolones (**1–7a,b**) were prepared according to the reported procedure[6,24].

*General method for synthesis of ciprofloxacin and norfloxacin containing 2-(morpholin-4-yl)ethyl and 2-(piperidin-1-yl)ethyl fragments (**8, 9ab**)*

A mixture of norfloxacin (**a**) or ciprofloxacin (**b**) (1 mmol) and K_2CO_3 (1.1 mmol) was stirred in DMF (10 mL) at room temperature for 20 min. To the resulted mixture, the 1-(2-chloroethyl)piperidine or 4-(2-chloroethyl)morpholine (1.1 mmol) in DMF (5 mL) was added dropwise over a period of 10 min. The reaction mixture was further stirred at room temperature for 24 h. The separated solid was then filtered, washed with cold water, dried and crystallized from the appropriate solvent.

*1-Ethyl-6-fluoro-4-oxo-7-(4-(2-(morpholin-1-yl)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**8a**)*

White powder, 52% yield; mp 217–219 °C ($MeOH/CH_2Cl_2$); IR (KBr) ν max/cm^{-1}: 3440 (OH), 1719 (C=O), 1641 (C=O). ^{1}HNMR ($CDCl_3$): δ 1.54–1.58 (3H, t, $J = 7.5$ Hz), 2.54-2.63 (8H, m), 2.75–2.76 (4H, d, $J = 2.5$ Hz), 3.35–3.36 (4H, d, $J = 3.5$ Hz), 3.75–3.82 (4H, d, $J = 5.0$ Hz), 4.31–4.36 (2H, q, $J = 7.5$ Hz), 6.88–6.89 (1H, d, $J = 6.5$ Hz), 8.02–8.08 (1H, dd, $J = 12.5$, 7.5 Hz), 8.67–8.68 (1H, d, $J = 6.0$ Hz), 15.29 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ 14.44, 49.81, 49.85, 53.23, 54.15, 55.49, 56.34, 66.93,

103.72, 108.32, 112.81, 120.52, 137.11, 146.07, 147.08, 152.52, 154.52, 167.23, 176.97; $C_{22}H_{29}FN_4O_4$ m/z: 432.5 (34.6%). Anal. Calcd: C, 61.10; H, 6.76; N, 12.95. Founded: C, 61.31; H, 6.74; N, 12.84.

*1-Cyclopropyl-6-fluoro-7-(4-(2-morpholinoethyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic (**8b**)*

White powder, 66% yield; mp 145–147 °C (MeOH/CH_2Cl_2); IR(KBr) ν max/cm^{-1}: 3441 (OH), 1733 (C=O), 1628 (C=O). ^{1}HNMR ($CDCl_3$): δ 1.16 (2H, s), 1.35–1.36 (2H, d, $J = 6.0$ Hz), 2.61 (4H, s), 2.80–2.82 (2H, t, $J = 7.5$ Hz), 3.24 (2H, s), 3.30 (2H, s), 3.45 (1H, s), 3.63 (2H, s), 3.74 (4H, s), 3.78 (2H, s), 4.46–4.47 (2H, d, $J = 6.0$ Hz), 7.28 (1H, s), 8.15 (1H, s), 8.56 (1H, s); ^{13}CNMR ($CDCl_3$): δ 8.29, 35.34, 39.69, 45.33, 49.28, 50.82, 63.43, 105.50, 108.27, 112.59, 120.57, 138.96, 145.36, 147.64, 160.80, 166.78, 177.07; $C_{23}H_{29}FN_4O_4$ m/z: 444.2 (5.5%). Anal. Calcd: C, 62.15; H, 6.58; N, 12.60. Founded: C, 62.24; H, 6.52; N, 12.40.

*1-Ethyl-6-fluoro-4-oxo-7-(4-(2-(piperidin-1-yl)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**9a**)*

Yellow powder, 69% yield; mp 259–260 °C (MeOH/CH_2Cl_2); IR(KBr) ν max/cm^{-1}: 3440 (OH), 1730 (C=O), 1636 (C=O). ^{1}HNMR ($CDCl_3$/CD_3OD): δ 1.56–1.59 (3H, t, $J = 7.0$ Hz), 1.71 (2H, s), 1.94 (4H, s), 2.79 (4H, s), 2.88 (2H, s), 3.30–3.33 (6H, d, $J = 13.5$ Hz), 3.43 (4H, s), 4.51–4.52 (2H, d, $J = 7.0$ Hz), 7.08–7.09 (1H, d, $J = 6.5$ Hz), 7.90–7.93 (1H, d, $J = 13.5$ Hz), 8.80 (1H, s); ^{13}CNMR ($CDCl_3$/CD_3OD): δ 15.24, 22.89, 24.12, 50.85, 51.13, 53.51, 53.95, 54.43, 54.93, 106.24, 108.66, 113.00, 121.35, 138.88, 147.43, 149.22, 154.01, 169.72, 178.22; $C_{23}H_{31}FN_4O_3$ m/z: 430.6 (28.7%). Anal. Calcd: C, 64.17; H, 7.26; N, 13.01. Founded: C, 64.28; H, 6.17; N, 13.09.

*1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(2-(piperidin-1-yl)ethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**9b**)*

Yellow powder, 73% yield; mp 265–266 °C (MeOH/CH_2Cl_2); IR(KBr) ν max/cm^{-1}: 3443 (OH), 1737 (C=O), 1640 (C=O). ^{1}HNMR ($CDCl_3$/CD_3OD): δ 1.23 (2H, s), 1.44–1.45 (2H, d, $J = 6.0$ Hz), 1.71 (2H, s), 1.92 (4H, s), 2.80 (4H, s), 2.87 (2H, s), 3.28–3.29 (6H, dd, $J = 5.5$, 7.0 Hz), 3.45 (4H, s), 3.76 (1H, s), 7.54–7.55 (1H, d, $J = 7.0$ Hz), 7.86–7.89 (1H, d, $J = 13.0$ Hz), 8.77 (1H, s); ^{13}CNMR ($CDCl_3$/CD_3OD): δ 8.85, 22.96, 24.24, 37.01, 50.71, 53.62, 53.95, 54.53, 54.94, 107.00, 108.33, 112.63, 112.83, 140.79, 149.20, 169.61; $C_{24}H_{31}FN_4O_3$ m/z: 442.7 (5.6%). Anal. Calcd: C, 65.14; H, 7.06; N, 12.66. Founded: C, 65.35; H, 7.15; N, 12.23.

*General method for synthesis of norfloxacin and ciprofloxacin containing phthalimide fragments (**10**, **11ab**)*

A mixture of norfloxacin (**a**) or ciprofloxacin (**b**) (1.0 mmol) and K_2CO_3 (1.1 mmol) was stirred in DMF (10 mL) at room temperature for 20 min. To the resulted mixture, the 2-(2-bromoethyl)isoindoline-1,3-dione or 2-(3-bromopropyl)isoindoline-1,3-dione (1.1 mmol) in DMF (5 mL) was added drop-wise over a period of 10 min. The reaction mixture was further stirred at room temperature for 24 h. The separated solid was then filtered, washed with cold water, dried and crystallized from the appropriate solvent.

*7-(4-(3-(1,3-Dioxoisoindolin-2-yl)ethyl)piperazin-1-yl)-1-ethyl-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**10a**)*

White powder, 64% yield; mp 250–252 °C (CH_2Cl_2); IR(KBr) ν max/cm^{-1}: 3441 (OH), 1731 (C=O), 1719 (C=O), 1637 (C=O). ^{1}HNMR ($CDCl_3$): δ 1.24–1.30 (3H, t, $J = 13.5$ Hz), 2.51 (1H, s), 2.65 (5H, s), 3.26 (4H, s), 3.77 (2H, s), 4.51–4.64 (2H, q, $J = 6.5$ Hz), 7.15–7.16 (1H, d, $J = 6.5$ Hz), 7.85–7.92 (5H, m), 8.94 (1H, s), 15.36 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ 14.44, 28.16, 34.93, 36.89, 39.28, 53.64, 55.62, 62.72, 103.89, 107.99, 112.54, 123.38, 128.99, 131.29, 132.04, 134.10, 134.30, 147.27, 152.52, 167.17, 168.14, 168.56, 176.96; $C_{26}H_{25}FN_4O_5$ m/z: 492.5 (7.7%). Anal. Calcd: C, 63.41; H, 5.12; N, 11.38. Founded: C, 63.29; H, 5.22; N, 11.56.

*1-Cyclopropyl-7-(4-(2-(1,3-dioxoisoindolin-2-yl)ethyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**10b**)*

White powder, 61% yield; mp 189–192 °C (CH_2Cl_2). ^{1}HNMR ($CDCl_3$): δ 1.16–1.18 (2H, d, $J = 5.5$ Hz), 1.34–1.36 (2H, d, $J = 6.0$ Hz), 2.43 (2H, s), 3.36 (4H, s), 3.63 (6H, m), 3.98 (1H, s), 6.66 (1H, s), 7.48–7.50 (4H, d, $J = 6.0$ Hz), 8.15–8.16 (1H, d, $J = 12.5$ Hz), 8.41 (1H, s), 15.12 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ 8.16, 35.03, 37.79, 45.08, 49.12, 55.51, 57.01, 63.32, 103.88, 109.07, 112.78, 123.01, 133.38, 134.40, 135.53, 149.65, 151.50, 165.19, 167.00, 168.82, 174.77; $C_{27}H_{25}FN_4O_5$ m/z: 504.5 (15.6%). Anal. Calcd: C, 64.28; H, 4.99; N, 11.11. Founded: C, 64.38; H, 5.10; N, 10.99.

*7-(4-(3-(1,3-Dioxoisoindolin-2-yl)propyl)piperazin-1-yl)-1-ethyl-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**11a**)*

White powder, 63% yield; mp 205-206 °C (Hexane/CH_2Cl_2). ^{1}HNMR ($CDCl_3$): δ 1.59–1.61 (3H, t, $J = 7.0$ Hz), 1.92–1.94 (2H, t, $J = 6.5$ Hz), 2.54 (2H, s), 2.65 (4H, s), 3.23 (4H, s), 3.82–3.84 (2H, t, $J = 7.0$ Hz), 4.33-4.34 (2H, d, $J = 7.0$ Hz), 6.77–6.78 (1H, d, $J = 6.5$ Hz), 7.72–7.74 (2H, dd, $J = 5.0$, 8.0 Hz), 7.86–7.88 (2H, dd, $J = 5.5$, 8.5 Hz), 8.01-8.04 (1H, d, $J = 13.0$ Hz), 8.68 (1H, s), 15.14 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ14.48, 25.20, 36.42, 49.76, 52.65, 52.68, 103.63, 108.33, 112.62, 120.49, 123.19, 132.27, 133.96, 137.09, 146.06, 147.09, 152.50, 154.50, 167.27, 168.52, 176.99; $C_{27}H_{27}FN_4O_5$ m/z: 506.5 (6.4%). Anal. Calcd: C, 64.02; H, 5.37; N, 11.06. Founded: C, 64.11; H, 5.35; N, 11.09.

*1-Cyclopropyl-7-(4-(3-(1,3-dioxoisoindolin-2-yl)propyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**11b**)*

White powder, 59% yield; mp 210–212 °C (Hexane/CH_2Cl_2); IR(KBr) ν max/cm^{-1}: 3437 (OH), 1726 (C=O), 1711 (C=O), 1627 (C=O).^{1}HNMR ($CDCl_3$): δ 1.31–1.33 (4H, t, $J = 5.5$ Hz), 1.84–1.93 (2H, m), 2.14–2.16 (2H, t, $J = 6.0$ Hz), 3.60–3.62 (2H, t, $J = 6.0$ Hz), 3.78-4.01 (7H, m), 4.29–4.31 (2H, t, $J = 6.0$ Hz), 7.16–7.17 (1H, d, $J = 6.5$ Hz), 7.77–7.92 (5H, m), 8.68 (1H, s), 15.10 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ 8.13, 27.70, 31.33, 34.56, 36.49, 49.74, 52.79, 55.82, 59.06, 61.59, 104.71, 109.83, 112.95, 123.15, 132.09, 133.93, 137.94, 144.44, 148.32, 152.27, 164.98, 166.31, 168.48, 172.99; $C_{28}H_{27}FN_4O_5$ m/z: 518.8 (9.7%). Anal. Calcd: C, 64.86; H, 5.25; N, 10.80. Founded: C, 64.76; H, 5.20; N, 10.89.

*General method for synthesis of ciprofloxacin and norfloxacin containing 4-(piperidin-1-ylmethyl) fragments (**12**, **13ab**)*

To a mixture of norfloxacin (**a**) or ciprofloxacin (**b**) (1.0 mmol) and the piperidine or 4-benzylpiperidine (1.0 mmol) in methanol (10 mL), 1 mL of formaline (37%) was added. The reaction mixture was heated overnight at a reflux temperature. The reaction mixture was cooled, the solvent was removed under vacuum, the solid obtained washed with water, dried and re-crystallised from an appropriate solvent.

*1-Ethyl-6-fluoro-4-oxo-7-(4-(piperidin-1-ylmethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**12a**)*

Yellow powder, 74% yield; mp >300 °C (MeOH); IR(KBr) ν max/cm^{-1}: 3438 (OH), 1728 (C=O), 1629 (C=O). ^{1}HNMR ($CDCl_3$): δ 1.14-1.15 (9H, d, $J = 7.0$ Hz), 1.52-1.53 (3H, d, $J = 7.0$ Hz), 2.72 (1H, s), 2.84 (3H, s), 3.28 (4H, s), 3.47–3.49 (1H, d, $J = 7.0$ Hz), 3.64–3.65 (1H, d, $J = 6.5$ Hz), 4.07 (1H, s), 4.25–4.27 (2H, t, $J = 7.0$ Hz), 6.76–6.77 (1H, d, $J = 6.5$ Hz), 7.94–7.96 (1H, d, $J = 13.5$ Hz), 8.59 (1H, s), 15.05 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ 14.45, 18.45, 30.94, 49.20, 49.96, 51.07, 58.45, 63.43, 64.40, 88.07, 103.77, 108.31, 112.82, 120.39, 137.136, 146.28, 147.06, 152.53, 154.53, 167.26, 176.98; $C_{22}H_{29}FN_4O_3$ m/z: 416.9 (2.5%). Anal. Calcd: C, 63.44; H, 7.02; N, 13.45. Founded: C, 63.79; H, 6.81; N, 13.47.

*1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(piperidin-1-ylmethyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**12b**)*

Yellow powder, 79% yield; mp 256–258 °C (MeOH). ^{1}HNMR ($CDCl_3$): δ 1.14–1.18 (6H, m), 1.31–1.33 (4H, d, $J = 6.0$ Hz), 2.09 (2H, s), 2.72 (2H, s), 2.84 (4H, s), 3.30 (4H, s), 3.46–3.50 (2H, q, $J = 7.0$ Hz), 4.07 (1H, s), 7.28–7.29 (1H, d, $J = 6.5$ Hz), 7.83–7.87 (1H, dd, $J = 8.5$, 12.5 Hz), 8.62–8.64 (1H, d, $J = 6.5$ Hz), 14.96 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ 8.21, 15.28, 18.44, 30.93, 35.31, 49.21, 49.88, 51.09, 58.39, 64.39, 88.09, 104.82, 107.96, 112.34, 119.53, 139.08, 145.97, 147.31, 152.66, 154.66, 166.96, 177.01; $C_{23}H_{29}FN_4O_3$ m/z: 428.6 (8.3%). Anal. Calcd: C, 64.47; H, 6.82; N, 13.08. Founded: C, 64.57; H, 6.82; N, 13.14.

*7-(4-((4-Benzylpiperidin-1-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13a**)*

White powder, 89% yield; mp 285–287 °C (MeOH); IR(KBr) ν max/cm^{-1}: 3440 (OH), 1736 (C=O), 1641 (C=O). ^{1}HNMR ($CDCl_3$): δ 1.14-1.17 (8H, d, $J = 7.0$ Hz), 1.51–1.52 (4H, d, $J = 7.0$ Hz), 2.71 (3H, s), 2.86 (3H, s), 3.26 (4H, s), 3.46–3.48 (1H, d, $J = 7.0$ Hz), 4.09 (1H, s), 4.24–4.26 (2H, t, $J = 7.0$ Hz), 6.77–6.79 (1H, d, $J = 6.5$ Hz), 7.20–7.26 (5H, m), 7.90–7.94 (1H, dd, $J = 8.5$, 5.0 Hz), 8.56 (1H, s), 15.06 (1H, s, br); ^{13}CNMR ($CDCl_3$): δ 14.44, 29.69, 49.20, 49.75, 49.95, 51.05, 58.42, 64.39, 88.07, 103.77, 108.25, 112.57, 112.75, 120.32, 120.38, 128.14, 129.12, 137.13, 146.19, 146.27, 147.04, 152.52, 154.52, 167.25, 176.93; $C_{29}H_{35}FN_4O_3$ m/z: 506.4 (6.1%). Anal. Calcd: C, 68.75; H, 6.96; N, 11.06. Founded: C, 68.91; H, 7.02; N, 11.21.

*7-(4-((4-Benzylpiperidin-1-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13b**)*

Yellow powder, 86% yield; mp 260–262 °C (MeOH). ^{1}HNMR ($CDCl_3$): δ 1.03–1.22 (6H, m), 1.31 (2H, s), 1.55–1.57 (2H, d, $J = 6.0$ Hz), 2.23–2.39 (1H, m), 2.45–2.47 (2H, d, $J = 7.0$ Hz), 2.65 (1H, s), 2.72 (1H, s), 2.76–2.91 (4H, m), 3.27–3.31 (4H, d, $J = 15.0$ Hz), 3.48–3.48 (2H, d, $J = 3.5$ Hz), 4.11–4.22 (1H, m), 7.059 (2H, s), 7.09–7.10 (1H, d, $J = 6.0$ Hz), 7.18–7.19 (2H, d, $J = 6.5$ Hz), 7.25–7.27 (1H, d, $J = 7.0$ Hz), 7.80–7.85 (1H, m), 8.60–8.61 (1H, $J = 4.5$ Hz); ^{13}CNMR ($CDCl_3$): δ 7.18, 30.70, 31.24, 34.30, 36.67, 37.33, 42.24, 48.97, 50.07, 51.21, 80.09, 103.72, 106.86, 111.18, 118.45, 124.74, 127.12, 128.08, 138.05, 139.61, 145.03, 146.27, 151.63, 153.63, 165.98, 166.05, 175.94; $C_{30}H_{35}FN_4O_3$ m/z: 518.6 (5.5%). Anal. Calcd: C, 69.48; H, 6.80; N, 10.80. Founded: C, 69.61; H, 6.84; N, 10.57.

*General method for synthesis of ciprofloxacin and norfloxacin containing 4-(phenylsulfonyl)carbamoyl fragments (**14ab**)*

Benzenesulfonyl isocyanate (1.83 g, 1.34 ml, 0.01 mol) was added dropwise to a stirred solution of norfloxacin (**a**) or ciprofloxacin (**b**) (0.01 mol) and triethylamine (1.0 mL, 0.01 mol) in dry toluene (50 mL). The reaction mixture was heated under reflux for 3 h, then evaporated *in vacuo* and the obtained residue was triturated with ice water, filtered, dried and crystallized from methanol to afford *N*-(phenylsulfonyl)carbamoyl derivatives.

*1-Ethyl-6-fluoro-4-oxo-7-(4-((phenylsulfonyl)carbamoyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**14a**)*

Yellow powder, 81% yield; mp 255–257 °C (MeOH); IR(KBr) ν max/cm^{-1}: 3446 (OH), 3260 (NH), 1718 (C=O), 1684 (C=O), 1628 (C=O). ^{1}HNMR ($CDCl_3$/TFA): δ 1.36–1.42 (3H, dd, $J = 6.5$, 8.0 Hz), 3.66 (4H, s), 3.85 (4H, s), 4.73 (2H, s), 7.28 (3H, s), 7.48–7.51 (1H, d, $J = 14.5$ Hz), 7.93–7.94 (1H, d, $J = 6.0$ Hz), 8.08–8.22 (2H, t, $J = 14.5$ Hz), 9.13 (1H, s); ^{13}CNMR ($CDCl_3$/TFA): δ 13.49, 44.03, 46.20, 46.75, 52.29, 63.43, 104.81, 115.67, 116.12, 126.31, 127.45, 129.36, 133.32, 138.52, 141.03, 142.02, 146.56, 159.93, 160.27, 169.46, 170.99; $C_{23}H_{23}FN_4O_6S$ m/z: 502.4 (5.7%). Anal. Calcd: C, 54.97; H, 4.61; N, 11.15. Founded: C, 55.17; H, 4.60; N, 11.02.

*1-Cyclopropyl-6-fluoro-4-oxo-7-(4-((phenylsulfonyl)carbamoyl)-piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**14b**)*

Yellow powder, 78% yield; mp 185–187 °C (MeOH); IR(KBr) ν max/cm^{-1}: 3446 (OH), 3264 (NH), 1718 (C=O), 1682 (C=O), 1628 (C=O). ^{1}HNMR ($CDCl_3$/TFA): δ 1.30 (2H, s), 1.51–1.53 (2H, d, $J = 6.0$ Hz), 3.51 (4H, s), 3.57 (4H, s), 3.79 (1H, s), 7.28 (2H, s), 7.44–7.46 (1H, dd, $J = 2.0$, 8.5 Hz), 7.52–7.54 (1H, d, $J = 6.0$ Hz), 7.59–7.60 (1H, d, $J = 1.5$ Hz), 8.00–8.03 (2H, t, $J = 3.0$ Hz), 8.97 (1H, s); ^{13}CNMR ($CDCl_3$/TFA): δ 8.29, 36.84, 45.40, 49.57, 105.39, 106.18, 112.18, 115.94, 127.64, 132.98, 133.24, 134.05, 139.83, 140.27, 146.75, 148.38, 153.18, 155.21, 159.06, 169.89, 174.51; $C_{24}H_{23}FN_4O_6S$ m/z: 515.0 (4.6%). Anal. Calcd: C, 56.02; H, 4.51; N, 10.89. Founded: C, 55.84; H, 4.51; N, 11.00.

Antitumor screening

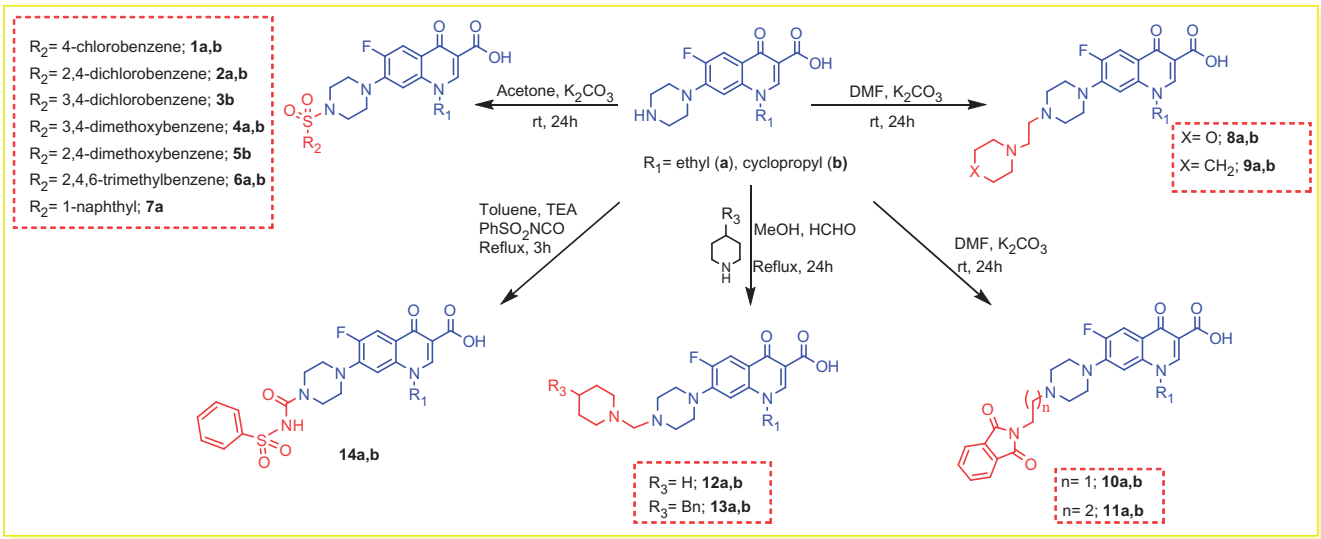
A primary anticancer assay was performed for an approximately 60 human tumor cell line panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD[25].

Three dose response parameters were calculated for each compound including GI_{50} (the drug concentration resulting in a 50% lower net protein increase in the treated cells measured by SRB staining), TGI (the drug concentration resulting in total growth inhibition), and LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment compared to that at the beginning). Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less for the maximum or minimum concentration tested. The lowest values are obtained with the most sensitive cell lines. The compounds having $GI_{50} \leq 100$ μM were declared to be active[25].

Results and discussion

Chemistry

Scheme 1 outlines the synthetic pathway used to obtain compounds (**1–14a,b**). Compounds **1–7a,b** were prepared according to our previous report by the reaction of norfloxacin (**a**) or ciprofloxacin (**b**) with the appropriate arylsulfonyl chloride in acetone in the presence of K_2CO_3 at room temperature for 24 h[6]. On the other hand, compounds **8–11a,b** were prepared by the



Scheme 1. Synthesis of 7-(4-substituted piperazin-1-yl)-4-oxoquinolines (**1–14a,b**).

reaction of alkyl halides with norfloxacin (**a**) or ciprofloxacin (**b**) in DMF containing anhydrous K_2CO_3. Mannich bases **12, 13a,b** were prepared (74–89% yield) by heating at reflux temperature of norfloxacin (**a**) or ciprofloxacin (**b**) and piperidine derivatives as secondary amine with excess formaldehyde in methanol. Moreover, compounds **14a,b** were also prepared in a better yield (79–85%) through the reaction of norfloxacin (**a**) or ciprofloxacin (**b**) with benzenesulfonyl isocyanate in toluene (Scheme 1). The structural formulae and the purity of the synthesized compounds were checked by thin layer chromatography and spectral analysis. The NMR spectra were identified by their chemical shifts, multiplicities and coupling constants. In general, ^{1}H NMR spectra showed the characteristic chemical shifts for the fluoroquinolone nucleus.

Antitumor activity

The designed compounds **1**–**14a,b** in addition to the main scaffold ciprofloxacin (**b**) were tested for their *in vitro* antitumor activity. Sixteen compounds and ciprofloxacin (**b**) were selected by National Cancer Institute, Bethesda, MD (Table 1) on the basis of degree of the structure variation and computer modelling techniques for evaluation of their antitumor activity[25]. The selected compounds were subjected to *in vitro* antitumor assay against tumor cells in a full panel of 60-cell lines taken from nine different organs (lung, colon, breast, ovary, blood, kidney, skin, prostate and brain). The compounds were grouped into three series, including 7-(4-arenesulfonylpiperazin-1-yl)fluoroquinolone (**1**–**7a,b**), 7-(4-alkylpiperazin-1-yl)fluoroquinolones (**8b**, **10b**, **11b** and **12b**) and 7-(4-((phenylsulfonyl)carbamoyl)piperazin-1-yl)-fluoroquinolone (**14b**) (Table 1, Figure S1, Supplementary file), which were first evaluated at a single dose concentration of 10 μM, the percentages of growth inhibitions over the 60 tested cell lines were determined and the results were compared with 5-fluorouracil (5-Flu)[26], gefitinib (*Iressa*^{TM})[27–29] and erlotinib (*Tarceva*^{TM})[30] as reference drugs. In the screening methodology, each cell line was inoculated and incubated for 24–47 h. Molecules were then added at a single concentration and the culture was incubated for further 48 h. End point determination were made with a protein binding dye, sulforhodamine (SRB)[25]. Results for each tested molecule were reported as the percentage growth of the treated cells comparing to the untreated control cells. The screening results and percentages of growth inhibitions over sensitive cell lines are shown in Tables 1 and 2.

The screening results of the selected molecules at 10 μM concentration showed that 7-(4-arylsulfonylpiperazin-1-yl)fluoroquinolones (**1–7a,b**) are more active compared with 7-(4-alkylpiperazin-1-yl)fluoroquinolones (**8b**, **10b**, **11b** and **12b**) and 7-(4-((phenylsulfonyl)carbamoyl)piperazin-1-yl)-fluoroquinolone (**14b**) in addition to the parent ciprofloxacin (**b**) scaffold as indicated by the number of sensitive cell lines and cytotoxic effect (Table 1, Supporting Information Figure S1).

The synthesized 7-(4-arylsulfonylpiperazin-1-yl)fluoroquinolones (**1–7a,b**) displayed significant activity in the *in vitro* screening on the tested cell lines in 10 μM concentration with positive cytotoxic effect (PCE) of 6/58 ~ 58/58. (Tables 1 and 2, Figure S1, Supporting Information). These molecules showed a cytotoxic effect on the most of the cancer cell lines especially compounds **1a**, **2a**, **3b**, **6b** and **7a** with mean growth inhibition percentages (MGI%) of 75.5%, 94.1%, 89.2%, 43.8% and 93.7% respectively. Arylsulfonylpiperazinyl fluoroquinolones (**2b**, **5b** and **6a**) showed moderate activity with MGI% of 17.3%, 7.3% and 18.3% respectively. Derivatives based on norfloxacin (**a**) scaffold were more active compared with the corresponding ciprofloxacin (**b**) derivatives as indicated by mean growth inhibition percentages (MGI% = 75.5, **1a**; 3.5, **1b**; 94.1, **2a** and 17.3, **2b**). Moreover the dimethoxy derivatives of arylsulfonylpiperazinyl fluoroquinolones (MGI% = 2.3, **4a**; 1.8, **4b** and 7.3, **5b**) were less active than the corresponding dichloro derivatives of 7-arenesulfonyl-piperazinyl fluoroquinolones (MGI% = 94.1, **2a**; 17.3, **2b** and 89.2, **3b**) emphasizing the importance of chloro fragment which may attributed to lipophilic character and electronic effect of the chloro derivatives. Replacement of the methoxy group with trimethyl substituent lead to improvement of the antitumor activity such as compounds **6a** and **6b** with mean growth inhibition percentages (MGI%) of 18.3% and 43.8% respectively. On the other hand, introducing 1-naphthalenesulfonyl moiety in compound **7a** led to sharp increase of the antitumor activity (MGI% = 93.7) compared with parent ciprofloxacin (**b**) and molecules containing trimethylbenzenesulfonyl moiety such as compounds **6a** and **6b** with mean growth inhibition percentages (MGI%) of −1.4, 18.3 and 43.8% respectively (Table 1, Figure S1, Supporting Information).

By investigating the variation in selectivity and broad spectrum of the tested compounds over the full panel of cell lines, it was revealed that nearly all of arylsulfonylpiperazinyl fluoroquinolones (**1–7a,b**) (Tables 1 and 2) showed significant inhibition for the most cell lines used in this assay (leukemia,

Table 1. Antitumor activity of the designed 7-(4-substituted piperazin-1-yl)fluoroquinolones at 10 μM concentration.

Compd No	60 cell lines assay in one dose 10.0 mM concentration			
	MGI%	PCE	RGI%	Most sensitive cell lines
1a	75.59	56/56	>100.0 to 31.3	*Leukemia* (CCRF-CEM, HL-60 (TB), K-562, MOLT-4, PRMI-8226), *NSC Lung Cancer* (A549/ATCC, NCI-H226, HOP-92, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), *Colon Cancer* (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), *CNS Cancer* (SF-268, SF-295, SF-539, SNB-19,SNB-75, U251), *Melanoma* (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62), *Ovarian Cancer* (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), *Renal Cancer* (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), *Prostate Cancer* (PC-3, DU-145), *Breast Cancer* (MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T-47D)
1b	3.5	7/53	59.75 to 10.12	*Melanoma* (LOX IMVI, M14), *Renal Cancer* (A498, CAKI-1, UO-31), *Prostate Cancer* (PC-3), *Breast Cancer* (T-47D)
2a	94.17	56/58	>100.0 to 25.22	*Leukemia* (CCRF-CEM, HL-60(TB), K-562, MOLT-4, PRMI-8226, SR), *NSC Lung Cancer* (A549/ATCC, HOP-62, NCI-H226, HOP-92, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), *Colon Cancer* (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), *CNS Cancer* (SF-268, SF-295, SF-539, SNB-19,SNB-75, U251), *Melanoma* (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-5, UACC-257, UACC-62), *Ovarian Cancer* (IGROV1, OVCAR-3, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), *Renal Cancer* (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), *Prostate Cancer* (PC-3, DU-145), *Breast Cancer* (MCF7, MDA-MB-231/ATCC, BT-549, T-47D, MDA-MB-468)
2b	17.39	30/58	79.75 to 11.78	*Leukemia* (CCRF-CEM, HL-60(TB), MOLT-4, PRMI-8226, SR), *NSC Lung Cancer* (HOP-62, NCI-H226, NCI-H23, NCI-H322M), *Colon Cancer* (COLO 205, HCT-116, HCT-15), *CNS Cancer* (SF-268, SNB-75), *Melanoma* (LOX IMVI, SK-MEL-28, SK-MEL-5, UACC-62), *Ovarian Cancer* (OVCAR-4), *Renal Cancer* (A498, ACHN, CAKI-1, RXF 393, SN12C, UO-31), *Prostate Cancer* (PC-3), *Breast Cancer* (MCF7, BT-549, T-47D, MDA-MB-468)
3b	89.25	58/58	>100.0 to 46.49	*Leukemia* (CCRF-CEM, HL-60(TB), K-562, MOLT-4, PRMI-8226, SR), *NSC Lung Cancer* (A549/ATCC, HOP-62, NCI-H226, HOP-92, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), *Colon Cancer* (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), *CNS Cancer* (SF-268, SF-295, SF-539, SNB-19,SNB-75, U251), *Melanoma* (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62), *Ovarian Cancer* (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), *Renal Cancer* (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), *Prostate Cancer* (PC-3, DU-145), *Breast Cancer* (MCF7, MDA-MB-231/ATCC, BT-549, T-47D, MDA-MB-468)
4a	2.33	9/58	55.93 to 10.04	*Leukemia* (MOLT-4, SR), *CNS Cancer* (SF-268, SNB-75), *Melanoma* (LOX IMVI, M14), *Renal Cancer* (UO-31), *Prostate Cancer* (PC-3), *Breast Cancer* (T-47D)
4b	1.84	6/58	35.09 to 10.43	*NSC Lung Cancer* (HOP-62, HOP-92), *CNS Cancer* (SNB-75), *Melanoma* (LOX IMVI), *Renal Cancer* (UO-31), *Breast Cancer* (T-47D)
5b	7.33	19/58	46.83 to 10.48	*Leukemia* (CCRF-CEM, HL-60(TB), K-562, MOLT-4, PRMI-8226, SR), *NSC Lung Cancer* (HOP-62, NCI-H226), *CNS Cancer* (SF-268, SF-539, SNB-19, SNB-75), *Melanoma* (LOX IMVI, SK-MEL-5, UACC-62), *Renal Cancer* (A498, UO-31), *Breast Cancer* (T-47D, MDA-MB-468)
6a	18.31	46/58	66.90 to 10.12	*Leukemia* (CCRF-CEM, HL-60(TB), K-562, MOLT-4, PRMI-8226, SR), *NSC Lung Cancer* (A549/ATCC, HOP-62, NCI-H226, HOP-92, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), *Colon Cancer* (HCC-2998, HCT-116, HCT-15, KM12), *CNS Cancer* (SF-268, SF-295, SF-539, SNB-19,SNB-75, U251), *Melanoma* (LOX IMVI, M14, MDA-MB-435, SK-MEL-2, SK-MEL-5, UACC-62), *Ovarian Cancer* (IGROV1, OVCAR-3, OVCAR-5, OVCAR-8, NCI/ADR-RES), *Renal Cancer* (A498, ACHN, CAKI-1, RXF 393, SN12C, UO-31), *Prostate Cancer* (PC-3), *Breast Cancer* (MDA-MB-231/ATCC, BT-549, T-47D, MDA-MB-468)
6b	43.83	56/58	91.28 to 13.08	*Leukemia* (CCRF-CEM, HL-60(TB), K-562, MOLT-4, PRMI-8226, SR), *NSC Lung Cancer* (A549/ATCC, HOP-62, NCI-H226, HOP-92, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), *Colon Cancer* (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), *CNS Cancer* (SF-268, SF-295, SF-539, SNB-19,SNB-75, U251), *Melanoma* (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62), *Ovarian Cancer* (IGROV1, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), *Renal Cancer* (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), *Prostate Cancer* (PC-3, DU-145), *Breast Cancer* (MCF7, MDA-MB-231/ATCC, BT-549, T-47D, MDA-MB-468)
7a	93.76	58/58	>100.0 to 40.83	*Leukemia* (CCRF-CEM, HL-60(TB), K-562, MOLT-4, PRMI-8226, SR), *NSC Lung Cancer* (A549/ATCC, HOP-62, NCI-H226, HOP-92, NCI-H23, NCI-H322M, NCI-H460, NCI-H522), *Colon Cancer* (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620), *CNS Cancer* (SF-268, SF-295, SF-539, SNB-19,SNB-75,

(*continued*)

Compd No	60 cell lines assay in one dose 10.0 mM concentration			
	MGI%	PCE	RGI%	Most sensitive cell lines
				U251), *Melanoma* (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62), *Ovarian Cancer* (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), *Renal Cancer* (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, TK-10, UO-31), *Prostate Cancer* (PC-3, DU-145), *Breast Cancer* (MCF7, MDA-MB-231/ATCC, BT-549, T-47D, MDA-MB-468)
Ciprofloxacin (**b**)	−1.41	2/57	28.85 to 13.76	*Renal Cancer* (UO-31, A498)
8b	−2.20	5/57	24.51 to 10.05	*NSC Lung Cancer* (HOP-92, NCI-H522), *Melanoma* (MALME-3M), *Renal Cancer* (UO-31), *Prostate Cancer* (PC-3)
10b	−2.69	3/58	36.83 to 13.18	*CNS Cancer (*SNB-75*), Renal Cancer* (UO-31), *Breast Cancer* (MCF7)
11b	−4.16	3/57	38.76 to 13.64	*Leukemia* (CCRF-CEM, PRMI-8226), *Renal Cancer* (UO-31)
12b	−4.72	2/57	32.52 to 13.02	*Leukemia* (SR), *Renal Cancer* (UO-31)
14b	−2.46	4/57	26.16 to 10.00	*Leukemia* (MOLT-4, SR), *Renal Cancer* (UO-31, A498)

MGI%: Mean growth inhibition percentage. RGI%: Range of growth inhibition percentage.
PCE: Positive cytotoxic effect which is ratio between number of cell lines with percentage growth inhibition from10 to >100 and total number of cell lines.

non-small cell lung (NSCLC), colon, CNS, melanoma, ovarian, renal and breast cancer cell lines) with growth inhibition reached to >100% (Tables 1 and 2), while alkylpiperazinyl fluoroquinolones (**8b**, **10b**, **11b** and **12b**)**,** phenylsulfonylcarbamoylpiperazinyl fluoroquinolone (**14b**) and ciprofloxacin (**b**) (Table 1, Figure S1, Supporting Information) are mainly active against renal cell lines (A498 and UO-31) with growth inhibition reached to 36%. It is clear that the agreement of the compounds under investigation in the inhibition of renal cell lines could be correlated to a similar inhibitory mechanism related to the common structural feature in ciprofloxacin core, while the selectivity of arylsulfonylpiperazinyl fluoroquinolones (**1–7a,b**) (Tables 1 and 2, Figure S1, Supporting Information) over other cell lines is probably caused by the differences in the hydrocarbon skeleton around the core structure (ciprofloxacin scaffold).

Moreover, among the 7-arenesulfonyl-piperazin-1-ylfluoroquinolones (**1–7a,b**), compounds **1a**, **2a**, **2b**, **3b**, **6a**, **6b** and **7a** showed broad spectrum and significant inhibition for cancer cells (58 cell lines; Tables 1 and 2, Figure S1, Supporting Information) and possessed a considerable cytotoxic activity against cell lines of leukemia (GI% = 60.9 – >100), non-small cell lung (GI% = 53.0 – >100), colon (GI% = 52.3 – >100), CNS (GI% = 53.5 – >100), melanoma (GI% = 51.5 – >100), ovarian (GI% = 57.7 – >100), renal (GI% = 56.3 – >100), prostate (GI% = 68.3 – 98.4) and breast (GI% = 58.8 – >100) cancer cells. This potential inhibition at the mentioned concentration indicates a high potency for the compounds **1a**, **2a**, **2b**, **3b**, **6a**, **6b** and **7a** with a strong lethal effect over cancer cells.

Regarding the activity toward individual cell lines (Table 2); compounds **1a**, **2a**, **3b**, **6b** and **7a** showed selective activity against leukemia cell lines such as CCRF-CEM (GI % values of >100, 97.2, 83.0, 62.6 and 87.9, respectively), HL-60 (GI % values of 91.0, >100, >100, 91.2 and >100, respectively), and PRMI-8226 (GI % values of 86.5, 90.9, 95.1, 77.6 and 93.5, respectively). Non-small cell lung; NCI-H23 cell line proved to be selectively sensitive to **1a**, **2a**, **3b**, **6b** and **7a** with GI% values of >100, >100, 98.2, 42.6 and 99.9, respectively. In addition, compounds **1a**, **2a** and **7a** proved to have equal susceptibility to the HOP-92 cell line with GI% value of >100. Concerning colon cancer; compounds **2a**, **3b** and **7a** showed GI% values of >100 with colon COLO 205 cancer cells. On the other hand, compounds **1a**, **2a**, **3b**, **6b** and **7a** verified sensitivity with GI% values of 92.1, 98.1, 99.7, 71.7 and 96.0 to colon HT-29 cancer cells. Respecting melanoma; compounds **1a**, **2a**, **3b**, **6b** and **7a** are active against LOX IMVI cell lines with GI% values of 80.1, >100, 98.7, 70.3 and >100, respectively. Pertaining to renal cancer; compounds **1a**, **2a**, **3b** and **7a** were active against A498 cell line with GI% values of >100, >100, 86.2 and >100, respectively. Renal ACHN and UO-31 cell lines are sensitive to compounds **2a** and **7a** with GI% value of >100. Relating to breast cancer; MCF7, MDA-MB-231/ATCC, T-47D and MDA-MB-468 cell lines possess convinced response to compounds **1a**, **2a**, **3b**, **6b** and **7a** with GI% range of 46.0 – >100. On the other hand, ovarian IGROV1, OVCAR-3, OVCAR-5 and NCI/ADR-RES cell lines are receptive to compound **1a**, **2a**, **3b**, **6b** and **7a** with GI% range of 40.5 – >100.

Finally, compounds **1a**, **2a**, **3b**, **6b** and **7a** were selected in advanced assay against a panel of approximately 60 tumor cell lines at 10-fold dilution of five concentration (100, 10, 1, 0.1, and 0.01 μM) and the results were compared with 5-fluorouracil (5-Flu), gefitinib (*Iressa*™) and erlotinib (*Tarceva*™) as reference drugs (Tables 3 and 4; Figure S2, Supporting Information; http://dtp.nci.nih.gov/dtpstandard/dwindex/index.jsp). Based on the cytotoxicity assays, three antitumor activity dose-response parameters were calculated for tested molecules against each cell lines: GI_{50}, molar concentration of the compound that inhibits 50% net cell growth; TGI, molar concentration of the compound leading to total inhibition; and LC_{50}, molar concentration of the compounds leading to 50% net cell death. Furthermore mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for the tested molecules.

The screening data analysis indicated that compounds **1a**, **2a**, **3b**, **6b** and **7a** possessed potent *in vitro* antitumor activity, with GI_{50} values across the 60 cell lines ranging from 1.22 to 5.37 μM (Tables 3 and 4; Figure S2, Supporting Information). Mean GI_{50} of compounds **1a**, **2a**, **3b**, **6b** and **7a** in comparison with 5-fluorouracil (5-Flu), gefitinib (*Iressa*™) and erlotinib (*Tarceva*™) as standard antitumor drugs are given in Table 3 and Figure S2, Supporting Information.

Compounds **1a**, **2a**, **3b**, **6b** and **7a** (Tables 3 and 4) exhibited remarkable growth inhibitory activity pattern against leukemia (GI_{50} = 2.89, 3.07, 3.02, 2.57 and 3.48 μM), non-small cell lung cancer (GI_{50} = 2.87, 2.49, 3.07, 2.88 and 3.04 μM), colon cancer (GI_{50} = 3.38, 3.15, 3.79, 3.46 and 3.39 μM), CNS (GI_{50} = 3.57, 3.03, 3.68, 2.95 and 3.09 μM), breast cancer (GI_{50} = 2.95, 2.51,

Table 2. Percentage of growth inhibition of the most active fluoroquinolones against individual cell lines.

	% Growth Inhibition (GI %)							
Subpanel tumor cell lines	**1a**	**2a**	**2b**	**3b**	**6a**	**6b**	**7a**	5-Flu
Leukemia								
CCRF-CEM	>100	97.22	18.97	83.11	18.2	62.67	87.9	57.1
HL-60(TB)	91.04	>100	13.13	>100	26.95	91.28	>100	47.9
K-562	78.59	89.06	–	83.06	20.11	36.45	84.58	42.3
MOLT-4	76.39	98.2	24.07	96.56	24.12	38.96	91.59	43.1
PRMI-8226	86.57	90.93	21.26	95.14	35.93	77.65	93.53	41.4
SR	–	>100	17.66	>100	35.52	60.94	97.85	24.8
Non-small cell lung cancer								
A549/ATCC	84.43	97.95	–	93.98	21.96	79.7	96.22	34.2
HOP-62	–	80.5	11.78	84.8	28.48	42.73	84.87	47.8
NCI-H226	39.86	86.87	39.72	58.6	23.12	28.19	78.11	69.5
HOP-92	>100	>100	–	87.8	12.64	53.06	>100	50.6
NCI-H23	>100	>100	16.79	98.24	22.28	42.63	99.94	39.0
NCI-H322M	54.08	>100	12.84	78.73	16.46	25.29	>100	59.5
NCI-H460	94.08	89.1	–	94.23	17.22	60.54	91.96	13.0
NCI-H522	91.61	>100	–	>100	13.46	32.68	>100	58.0
Colon cancer								
COLO 205	87.87	>100	11.94	>100	–	42.44	>100	40.2
HCC-2998	75.54	69.37	–	75.16	17.51	52.34	68.42	>100
HCT-116	95.2	93.63	13.45	96.05	20.85	67.81	94.55	17.8
HCT-15	84.34	92.42	16.18	93.76	28.47	44.94	88.9	26.5
HT29	92.14	98.13	–	99.74	–	71.74	96.04	27.1
KM12	95.7	90.6	–	85.43	12.79	39.33	88.28	40.7
SW-620	76.04	83.65	–	79.29	–	20.17	77.19	50.1
CNS cancer								
SF-268	77.28	75.44	16.35	80.36	16.29	43.33	83.61	59.0
SF-295	96.06	>100	–	>100	51.60	66.75	92.32	69.1
SF-539	78.3	91.42	–	88.63	20.08	53.5	82.56	>100
SNB-19	79.39	73.77	–	75.33	10.12	33.73	71.16	65.9
SNB-75	58.16	25.22	70.20	86.8	29.22	63.38	85.92	65.9
U251	87.97	87.91	–	87.56	16.65	80.32	85.77	50.3
Melanoma								
LOX IMVI	80.12	>100	38.57	98.79	38.72	70.39	>100	30.4
MALME-3M	76.25	>100	–	>100	–	40.29	>100	58.2
M14	81.1	84.12	–	81.47	24.44	36.45	82.14	–
MDA-MB-435	74.9	87.23	–	87.01	10.88	23.91	88.61	36.6
SK-MEL-2	56.19	>100	–	>100	11.31	–	91.18	95.5
SK-MEL-28	55.42	–	49.08	51.5	–	30.87	40.83	–
SK-MEL-5	73.4	>100	79.75	97.94	20.46	45.06	>100	33.7
UACC-257	70.99	>100	–	91.13	–	13.08	85.98	19.5
UACC-62	78.52	83.85	14.58	70.38	24.14	23.40	68.78	39.7
Ovarian cancer								
IGROV1	90.63	92.5	–	70.7	20.62	64.31	86.31	51.2
OVCAR-3	71.75	92.11	–	90.13	10.76	–	>100	47.4
OVCAR-4	62.33	–	35.94	79.24	–	24.39	76.15	59.4
OVCAR-5	48.09	92.08	–	87.52	11.41	57.79	84.54	44.3
OVCAR-8	87.42	94.16	–	94.52	18.51	40.52	91.57	–
NCI/ADR-RES	31.3	90.66	–	46.49	15.18	30.14	78.65	47.6
SK-OV-3	59.23	98.3	–	73.19	–	18.39	84.38	77.5
Renal cancer								
786-0	76.35	81.27	–	72.2	–	24.42	75.73	48.7
A498	>100	>100	23.58	86.25	21.15	37.03	>100	>100
ACHN	71.35	>100	14.87	95.05	24.24	48.7	>100	39.3
CAKI-1	56.36	95.25	12.12	96.94	10.66	32.91	99.06	39.4
RXF 393	86.6	72.06	67.74	70.95	11.11	48.66	70.98	34.3
SN12C	88.39	89.73	13.79	89.63	11.46	86.08	88.09	54.0
TK-10	46.4	87.63	–	66.81	–	13.54	74.01	66.9
UO-31	48.92	>100	40.04	89.98	66.90	83.86	>100	41.3
Prostate cancer								
PC-3	74.74	98.4	18.91	83.79	38.94	32.08	92.18	58.2
DU-145	68.32	88.44	–	82.81	–	28.56	81.12	35.5
Breast cancer								
MCF7	85.71	10.75	82.38	86.55	–	46.0	85.4	11.5
MDA-MB-231/ATCC	58.88	74.46	–	63.36	16.33	15.37	76.08	78.1
HS 578T	81.1	–	–	–	–	–	–	>100
BT-549	87.41	>100	13.68	>100	26.26	22.67	>100	37.8
T-47D	58.92	98.3	26.38	83.7	25.94	37.05	94.71	56.7
MDA-MB-468	–	>100	65.11	>100	19.73	34.85	95.21	–

Table 3. Average antitumor activity of compounds **1a**, **2a**, **3b**, **6b** and **7a** against tumor cell lines from nine different organs at 10-fold dilution of five concentrations; median growth inhibitory (GI_{50}, μM), total growth inhibitory (TGI, μM) and median lethal (LC_{50}, μM).

		Subpanel tumor cell lines									
Compound	Activity	Leukemia	NSC lung cancer	Colon cancer	CNS cancer	Melanoma	Ovarian cancer	Renal cancer	Prostate cancer	Breast cancer	MG-MID[a]
1a	**GI_{50}**	**2.89**	**2.87**	**3.38**	**3.57**	**2.56**	**3.16**	**2.93**	**3.21**	**2.95**	**2.95**
	TGI	80.96	53.38	73.15	70.26	29.36	54.29	54.00	54.11	54.63	29.51
	LC_{50}	c	c	95.24	c	89.65	c	c	c	c	95.49
2a	**GI_{50}**	**3.07**	**2.49**	**3.15**	**3.03**	**2.30**	**2.96**	**2.73**	**2.62**	**2.51**	**2.63**
	TGI	62.54	19.61	72.89	34.84	6.36	39.54	23.97	8.72	8.44	14.12
	LC_{50}	c	73.15	77.21	77.16	39.48	76.67	82.47	c	88.06	57.54
3b	**GI_{50}**	**3.02**	**3.07**	**3.79**	**3.68**	**2.54**	**3.39**	**3.19**	**3.29**	**3.04**	**3.09**
	TGI	62.55	84.28	87.41	c	52.41	88.90	87.07	c	81.37	60.25
	LC_{50}	c	c	c	c	87.08	c	c	c	c	95.49
6b	**GI_{50}**	**2.57**	**2.88**	**3.46**	**2.95**	**22.31**	**2.89**	**2.78**	**3.40**	**2.85**	**3.01**
	TGI	76.70	c	c	c	c	c	c	c	76.89	89.12
	LC_{50}	c	c	c	c	c	c	c	c	c	c
7a	**GI_{50}**	**3.48**	**3.04**	**3.39**	**3.09**	**3.20**	**3.07**	**3.50**	**3.71**	**2.81**	**3.09**
	TGI	63.22	46.59	67.85	63.40	28.33	62.35	63.09	c	38.49	29.51
	LC_{50}	c	c	86.79	c	99.66	c	c	c	c	95.49
5-Flu[b,c]	**GI_{50}**	**15.1**	**c**	**8.4**	**72.1**	**70.6**	**61.4**	**45.6**	**22.7**	**76.4**	**22.60**
	TGI	c	c	c	c	c	c	c	c	c	c
	LC_{50}	c	c	c	c	c	c	c	c	c	c
Gefitinib[c] *(Iressa)*	**GI_{50}**	**3.54**	**7.79**	**7.01**	**8.13**	**5.27**	**6.62**	**2.66**	**1.65**	**7.80**	**3.24**
	TGI	12.64	28.67	31.16	21.98	14.24	28.31	16.15	22.53	24.34	19.3
	LC_{50}	39.64	65.05	66.38	49.2	36.11	70.79	42.22	75.05	65.47	49.3
Erlotinib[c] *(Tarceva)*	**GI_{50}**	**23.66**	**12.09**	**51.68**	**16.98**	**25.34**	**5.50**	**2.46**	**20.90**	**24.72**	**7.29**
	TGI	96.57	73.76	100	82.10	80.09	76.66	42.59	100	70.53	64.3
	LC_{50}	100	97.71	100	100	93.98	97.06	89.15	100	96.57	95.1

[a]Full panel mean-graph midpoint (μM). c: Compounds showed values >100 μM.
[b]5-Fluorouracil as reference drug.
[c]http://dtp.nci.nih.gov/dtpstandard/dwindex/index.jsp.

3.04, 2.85 and 2.81 μM), ovarian cancer (GI_{50} = 3.16, 2.96, 3.39, 2,89 and 3.07 μM), melanoma cancer (GI_{50} = 2.56, 2.30, 2.54, 22.31 and 3.20 μM), prostate cancer (GI_{50} = 3.21, 2.62, 3.29, 3.40 and 3.71 μM) and renal cancer (GI_{50} = 2.93, 2.73, 3.19, 2.78 and 3.50 μM), respectively. Compounds **1a**, **2a**, **3b**, **6b** and **7a** (mean $GI_{50;}$ 2.63–3.09 μM) are nearly seven-fold more potent compared with the positive control 5-Flu (mean $GI_{50;}$ 22.60 μM). More interestingly, compounds **1a**, **2a**, **3b**, **6b** and **7a** have an almost antitumor activity similar to gefitinib (mean $GI_{50;}$ 3.24 μM) and are nearly 2-fold more potent compared to erlotinib (mean $GI_{50;}$ 7.29 μM).

The structure correlation study revealed that (1) potent antitumor activity of tested compounds depended on the presence of a combination of quinolone scaffold and arylsulfonyl fragment in one molecule; such as arylsulfonylquinolones (**1**–**7ab**) (7.3–94.1 MGI%) were more active in comparison with alkylated quinolones **8b**, **10b** and **11b** (−4.1 MGI%); (2) attachment of a halogen atom to arylsulfonyl derivative such as compounds **1**, **2ab** allowed sharp increase of activity (17.3–94.1 MGI%) in comparison with ciprofloxacin scaffold (−1.4 MGI%); (3) introduction of a methoxy group at the arylsulfonyl fragment such as compounds **4a**, **4b** and **5b** led to the decrease of activity; (4) the substituent in the arylsulfonyl fragment proved crucial and manipulated the antitumor activity; accordingly the introduction of an electron-withdrawing moiety, such as chloro fragment (compounds **1a**, **2a**, **2b** and **3b**), improved the antitumor activity in comparison with electron-donor moiety, such as methoxy group (compounds **4a**, **4b** and **5b**), and methyl group (compounds **6a** and **6b**); (5) replacement of the steric bulky trimethylbenzenesulfonyl substituent (compounds **6a** and **6b**; MGI% = 18–43) with 1-naphthylsulfonyl moiety (compound **7a**; MGI% = 93) led to improvement of the antitumor activity; (6) it is clear also that the compounds contain norfloxacin scaffold is more active in comparison with that contain ciprofloxacin scaffold such as compounds **1a** and **1b** (MGI% = 75.5 and 3.5, respectively).

In silico *studies*

Lipinski's rule of five (the effect of lipophilic and steric parameters)[31–33]. As a part of our study; the compliance of compounds to the Lipinski's rule of five was evaluated[31]. In addition, the polar surface area (PSA) of the compounds was also calculated (Table 5), since it is another key property that has been linked to drug bioavailability, where passively absorbed compounds with a PSA > 140 $Å^2$ are thought to have low oral bioavailability[32]. The results disclosed in Table 5 show that all of the synthesized compounds comply with these rules. Hence; theoretically, all of these compounds should present good passive oral absorption and differences in their bioactivity cannot be attributed to this property.

The introduction of electron withdrawal/donating groups incorporating the arylsulfonyl fragment and the variation of the substituents on the *N*-piperazine moiety of the ciprofloxacin and norfloxacin scaffolds have allowed us to evaluate the influence of lipophilicity and steric parameters at the pharmacophoric part of the molecules. Table 5 gathers physicochemical properties of some selected compounds such as ClogP (lipophilic factors), molar refractometry and polar surface area (steric factors) for each compound, determined by using iLab2 online program (https://ilab.acdlabs.com/iLab2/index.php). Although molar refractometry (MR) does not exert a significant effect on activity in tested compounds, an increase in potency was observed in compounds **1a**, **2a**, **3b**, **6b** and **7a** with MR values of 120–132 cm^3/mol. Regarding lipophilicity (logP); from the data

Table 4. Influence of compounds **1a**, **2a**, **3b**, **6b** and **7a** on the growth of the individual tumor cell lines; median growth inhibitory (GI_{50}, μM).

	GI_{50} (μM)				
Subpanel tumor cell lines	**1a**	**2a**	**3b**	**6b**	**7a**
Leukemia					
CCRF-CEM	2.93	3.03	2.61	2.80	3.67
HL-60(TB)	2.18	2.22	2.04	NT	2.58
K-562	2.91	4.00	3.92	NT	3.93
MOLT-4	3.83	3.55	4.00	NT	4.46
PRMI-8226	3.08	2.55	2.53	2.34	2.79
Non-small cell lung cancer					
A549/ATCC	2.81	2.89	3.08	2.23	2.87
HOP-62	4.47	3.08	4.77	3.65	4.6
NCI-H226	3.02	2.60	3.58	1.23	3.71
HOP-92	NT	1.22	1.54	1.52	1.25
NCI-H23	2.67	2.33	2.90	3.41	3.13
NCI-H322M	NT	2.86	3.24	5.19	2.6
NCI-H460	3.16	3.13	3.66	3.04	3.43
NCI-H522	1.73	1.81	1.99	2.80	2.78
Colon cancer					
COLO 205	2.62	2.44	3.50	3.40	2.02
HCC-2998	4.10	2.29	4.47	5.37	4.26
HCT-116	3.18	3.56	3.86	2.98	3.88
HCT-15	3.62	2.82	3.30	2.55	3.15
HT29	2.64	3.63	3.77	3.15	3.95
KM12	4.07	3.42	3.62	3.38	2.96
SW-620	3.43	3.91	4.05	3.40	3.57
CNS cancer					
SF-268	3.85	3.86	4.77	3.96	4.39
SF-539	4.12	1.92	3.39	3.14	2.43
SNB-19	3.97	3.07	3.25	2.56	3.47
SNB-75	2.60	NT	NT	2.23	1.89
U251	3.10	3.30	3.33	2.89	3.29
Melanoma					
LOX IMVI	2.40	1.48	1.44	1.42	1.63
MALME-3M	1.99	1.95	2.30	NT	2.83
M14	2.85	3.24	4.06	4.69	4.37
MDA-MB-435	2.47	1.97	2.97	NT	3.13
SK-MEL-2	2.57	2.75	3.16	NT	3.55
SK-MEL-28	4.88	3.21	2.67	NT	6.09
SK-MEL-5	1.80	1.87	2.04	3.03	2.24
UACC-257	2.16	2.65	2.48	>100	2.72
UACC-62	2.02	1.61	1.75	2.43	2.25
Ovarian cancer					
IGROV1	3.14	2.33	2.73	2.66	2.47
OVCAR-3	3.51	2.54	3.90	3.17	3.69
OVCAR-4	2.20	2.58	2.28	2.37	2.60
OVCAR-5	4.25	3.43	4.14	NT	3.38
OVCAR-8	2.80	3.30	3.33	2.87	3.09
NCI/ADR-RES	2.98	3.28	4.01	3.29	3.20
SK-OV-3	3.28	3.00	NT	3.02	3.11
Renal cancer					
786-0	3.77	3.19	4.02	4.08	4.50
A498	1.81	2.99	2.91	2.37	2.74
ACHN	3.09	2.62	3.10	2.46	2.88
CAKI-1	2.75	2.01	2.71	1.80	2.33
RXF 393	3.32	2.08	2.98	2.41	3.19
SN12C	2.92	2.77	2.97	2.61	2.59
TK-10	3.73	4.02	4.31	4.57	7.60
UO-31	2.07	2.20	2.58	1.96	2.21
Prostate cancer					
PC-3	2.31	2.43	2.46	NT	2.66
DU-145	4.11	2.81	4.31	3.40	3.97
Breast cancer					
MCF7	2.97	2.95	2.84	2.59	2.72
MDA-MB-231/ATCC	2.51	1.88	2.14	2.03	1.91
HS 578T	4.52	2.53	4.26	2.40	2.68
BT-549	2.44	2.64	2.98	3.34	3.7
T-47D	3.20	3.11	3.77	4.81	3.35
MDA-MB-468	2.10	1.98	2.25	1.97	2.52

NT: Not tested.

gathered in Table 5, there is a clear influence of lipophilicity on antitumor activity compared to polar surface area for all compounds. The optimal lipophilicity for the most active compounds was found to lie in the range of 2.14∼2.87 (Table 5).

ADME-Tox evaluation[34,35]. To estimate the prospect of designed compounds as antitumor agents compared with the reported antitumor agents ciprofloxacin (**b**) and 5-Flu; their drug-likeness were calculated according to absorption, distribution, metabolism, elimination, toxicity (ADME-T) program, and defined human intestinal absorption (HIA) model[34,35]. It was predicted that the examined compounds could be transported across the intestinal epithelium, and they can cross the blood–brain barrier and are of medium aqueous soluble. The values of HIA, BBB crossing and solubility prediction for all compounds are presented in Table 5 using iLab2 online program (https://ilab.acdlabs.com/iLab2/index.php). In general, all compounds presented some advantages when compared to ciprofloxacin and 5-Flu. No marked differences, in human oral bioavailability (>70%), human intestinal absorption (100%), human jejunum permeability and health effects in rodent toxicity profiles, were observed among the compounds. However, the absorption related parameters call for attention, since the promising compounds were calculated to be at least as soluble as the reported compounds, and are predicted to have oral bioavailability and absorption significantly higher than that of the reported antitumor agents ciprofloxacin (**b**) and 5-Flu. Accordingly; it can be deduced from these results that the pharmacokinetic profile of the designed compounds is affected and modified by the presence of arylsulfonyl moiety connected to 7-piperazinyl moiety of fluoroquinolone scaffolds.

Toxicities, drug score and drug-likeness profiles. Currently there are many approaches that assess a compound drug-likeness based on topological descriptors, fingerprints of molecular drug-likeness structure keys or other properties[36,37]. In the Osiris program (http://www.organic-chemistry.org/prog/peo) the occurrence frequency of each fragment is determined within the collection created by shredding 3300 traded drugs as well as 15 000 commercially available chemicals (Fluka) yielding a complete list of all available fragments[36,37]. In this work, we used the Osiris program for calculating the fragment based drug-likeness of the active compounds also comparing them with voreloxin, ciprofloxacin, levofloxacin, 5-Flu, erlotinib and gefitinib (Figure 2). Interestingly, the derivatives **1a**, **2a**, **2b**, **3b**, **5b** and **7a** presented better drug-likeness values (from 2.53 to 4.72) than ciprofloxacin, 5-Flu, erlotinib and gefitinib (2.07, −4.5, −6.73 and −2.62 respectively) and possessed similar results to levofloxacin and voreloxin (5.77 and 4.71 respectively). In this study we also verified the drug-score[37] as the theoretical data showed that compounds **1a**, **2a**, **2b**, **3b**, **5b**, **6a**, **6b** and **7a** presented values once again higher than 5-Flu, erlotinib and gefitinib (Figure 2). Moreover, we used the Osiris program to predict the overall toxicity of the most active derivatives as it may point to the presence of some fragments generally responsible for the irritant, mutagenic, tumorigenic, or reproductive effects in these molecules[36,37]. Interestingly, all of the active derivatives presented a low *in silico* toxicity risk profile, better than 5-Flu and similar to voreloxin, ciprofloxacin, levofloxacin, erlotinib and gefitinib (Figure 2). These theoretical data reinforced the cytotoxicity experimental data described in this work pointing these compounds as lead compounds for further study.

Conclusion

Sixteen different quinolones based on ciprofloxacin and norfloxacin scaffolds were evaluated for their antitumor activity,

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Table 5. The predicted ADME-Tox and calculated Lipinski's rule of five of the tested compounds and reported antitumor agents.

ADME-Tox	**1a**	**2a**	**2b**	**3b**	**4b**	**5b**	**6a**	**6b**	**7a**	**10b**	**13b**	Cip[a]	5-Flu[b]
Solubility (LogS)	–4.18	–4.91	–5.38	–5.38	–3.94	–3.94	–4.47	–4.94	–5.05	–4.20	–4.42	–3.32	–1.76
F (%)[c]	>70% (0.77)	>70% (0.77)	>70% (0.77)	>70% (0.77)	>70% (0.71)	>70% (0.71)	>70% (0.77)	>70% (0.77)	>70% (0.53)	30–70% (0.32)	<30% (0.47)	>70% (0.93)	>70% (0.92)
HIA (%)[d]	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	75%	71% (69%)	98%
Pe (cm/s)[e]	6.1×10^{-4}	6.4×10^{-4}	7.0×10^{-4}	7.0×10^{-4}	2.9×10^{-4}	6.6×10^{-4}	6.9×10^{-4}	6.9×10^{-4}	6.2×10^{-4}	2.6×10^{-4}	0.40×10^{-4}	0.09×10^{-4}	1.5×10^{-4}
LogBB[f] (LogPS)[g]	–1.14 (–3.3)	–1.15 (–3.1)	–0.68 (–2.2)	–0.75 (–2.2)	–0.68 (–4.1)	–0.76 (–3.1)	0.19 (–2.5)	–0.74 (–2.4)	–1.32 (–3.2)	–0.26 (–3.7)	–1.45 (–4.4)	–0.13 (–3.9)	–0.05 (–3.0)
LD_{50} mouse (mg kg^{-1}, oral)	1700	1500	1300	1300	1000	1200	1400	1300	1200	1300	6100	3500 (5000)	860
LD_{50} mouse (mg kg^{-1}, intraperitoneal)	640	650	890	890	790	820	700	580	510	400	1100	930 (1165)	500
LD_{50} mouse (mg kg^{-1}, intravenous)	120	180	140	140	140	130	99	96	110	92	360	120 (122)	120
LD_{50} mouse (mg kg^{-1}, subcutaneous)	2200	2000	1700	1700	1800	1900	1300	1100	1200	490	4200	1400	400
Ames test	0.11	0.15	0.29	0.29	0.25	0.36	0.09	0.22	0.18	0.76	0.38	0.77	0.7
MR (cm^3/mol)[h]	120.5 ± 0.4	125.4 ± 0.4	127.9 ± 0.4	127.9 ± 0.4	130.9 ± 0.4	130.9 ± 0.4	129.6 ± 0.4	132.13 ± 0.4	133.2 ± 0.4	128.8 ± 0.3	126.7 ± 0.4	83.3 ± 0.3	25.9 ± 0.4
LogP[i]	2.14	2.75	2.87	2.87	1.52	1.52	2.57	2.69	2.73	0.69	1.76	–1.53	–0.84
TPSA ($Å^2$)[j]	106.6	106.6	106.6	106.6	125.1	125.1	106.6	106.6	106.6	101.5	135.7	72.9	58.2
MW[k]	493.9	528.3	540.3	540.3	531.5	531.5	501.5	513.5	509.5	506.5	514.5	331.3	130.0
nON[l]	8	8	8	8	10	10	8	8	8	9	10	6	4
nOHNH[m]	1	1	1	1	1	1	1	1	1	1	2	2	2
nRotB[n]	5	5	5	5	7	7	5	5	5	6	5	3	0
Nviolations[o]	0	1	1	1	1	1	1	1	1	1	1	0	0

[a]Ciprofloxacin, [b]5-Flurouracil, [c]Human oral bioavailability (Probability), [d]Human intestinal absorption, [e]Permeability (Human Jejunum), [f]Blood Brain Barrier, [g]Rate of brain penetration, [h]Molar refractometry, [i]Calculated lipophilicity, [j]Total polar surface area, [k]Molecular weigh, [l]No. of hydrogen bond acceptor, [m]No. of hydrogen bond donor, [n]No. of Rotatable Bonds, [o]No. of violation from Lipinski's rule of five.

Compd No.	Toxicity effects			
	M	T	I	R
1a	**Low**	**Low**	**Low**	**Low**
2a	**Low**	**Low**	**Low**	**Low**
2b	**Low**	**Low**	**Low**	**Low**
3b	**Low**	**Low**	**Low**	**Low**
5b	**Low**	**Low**	**Low**	**Low**
6a	**Low**	**Low**	**Low**	**Low**
6b	**Low**	**Low**	**Low**	**Low**
7a	**Low**	**Low**	**Low**	**Low**
Ciprofloxacin	**Low**	**Low**	**Low**	**Low**
Levofloxacin	**Low**	**Low**	**Low**	**Low**
Voreloxin	**Low**	**Low**	**Low**	**Low**
5-Flu	**High**	**High**	**High**	**High**
Erlotinib	**Low**	**Low**	**Low**	**Low**
Gefitinib	**Low**	**Low**	**Low**	**Low**

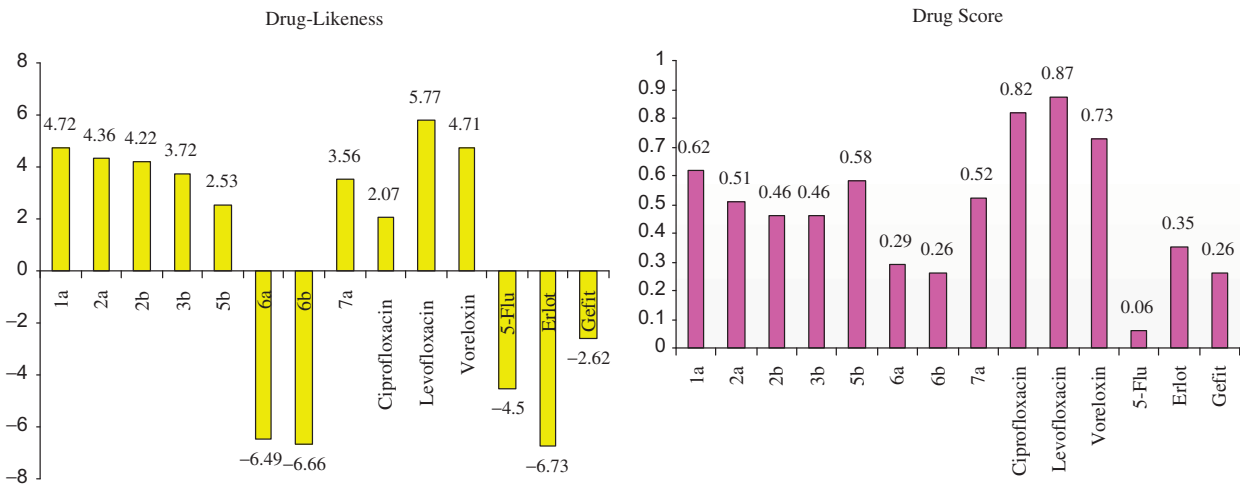
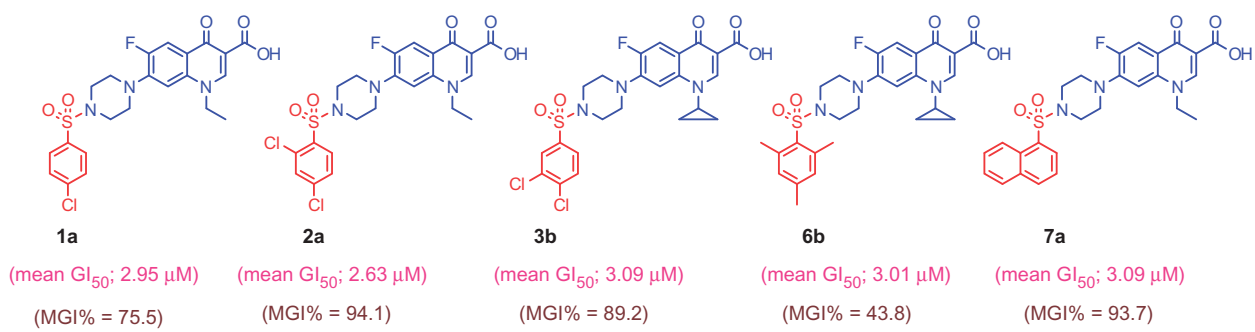


Figure 2. *In silico* toxicity risks (upper panel), drug-Likeness (lower left panel) and drug-Score (lower right panel) of the reported and the most active antitumor fluoroquinolone derivatives compared with reference drugs 5-Flu, Erlotinib and Gefitinib (M, mutagenic; T, tumorigenic; I, irritant; R, reproductive).



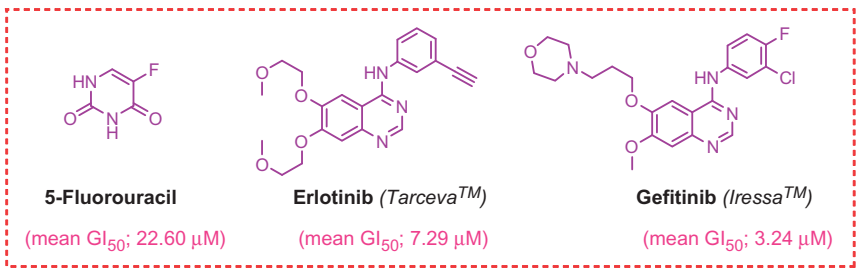


Figure 3. Outcomes of GI_{50} (µM) of compounds **1a**, **2a**, **3b**, **6b**, **7a** and reference drugs 5-Flu, Erlotinib and Gefitinib using tumor cell lines from nine different.

where most of the tested compounds exhibited significant antitumor activity. Compounds **1a**, **2a**, **3b**, **6b** and **7a** were possessed the most potent broad-spectrum antitumor activities (mean $GI_{50;}$ 2.63-3.09 μM) and were nearly seven-fold (Figure 3) more potent compared with the positive control 5-Flu (mean $GI_{50;}$ 22.60 μM). More interestingly, compounds **1a**, **2a**, **3b**, **6b** and **7a** (Figure 3) have an almost antitumor activity similar to gefitinib (mean $GI_{50;}$ 3.24 μM) and are nearly 2-fold more potent compared to erlotinib (mean $GI_{50;}$ 7.29 μM). The results of this study demonstrated that the electronic effect in the arylsulfonyl fragment proved crucial and manipulated the antitumor activity. The designed 7-substituted 1-piperazinyl fluoroquinolones are compatible with Lipinski's rule of five (molecular weight, ClogP, hydrogen bond-donating and accepting capabilities). *In vitro* antitumor activity, together with *in silico* studies, revealed that compounds **1a**, **2a**, **3b**, **6b** and **7a** could be considered as promising leads for further development of more potent antitumor agents.

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Declaration of interest

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Supplementary material available online

Supplementary Figures S1 and S2