Novel N-4-piperazinyl-ciprofloxacin-chalcone hybrids: Synthesis, physicochemical properties, anticancer and topoisomerase I and II inhibitory activity

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2013

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2013.08.040 · Source: PubMed

European Journal of Medicinal Chemistry 69 (2013) 427–438

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel *N*-4-piperazinyl-ciprofloxacin-chalcone hybrids: Synthesis, physicochemical properties, anticancer and topoisomerase I and II inhibitory activity

Mohamed Abdel-Aziz [a], So-Eun Park [b], Gamal El-Din A.A. Abuo-Rahma [a, *], Mohamed A. Sayed [c], Youngjoo Kwon [b]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt
[b] College of Pharmacy and Graduate School of Pharmaceutical Sciences, Global Top 5 Program, Ewha Womans University, Seoul 120-750, Republic of Korea
[c] Department of Pharmaceutics, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt

ARTICLE INFO

Article history:
Received 7 April 2013
Received in revised form
22 August 2013
Accepted 25 August 2013
Available online 12 September 2013

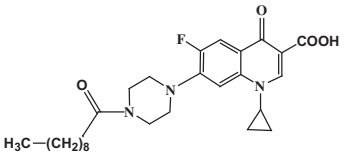
Keywords:
Anti-tumor
Ciprofloxacin
Chalcone
Topoisomerase
Hybrids

ABSTRACT

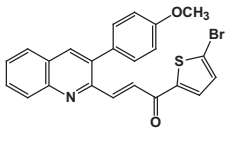
A group of novel *N*-4-piperazinyl-ciprofloxacin-chalcone hybrids was prepared. One-dose anticancer test results indicated that compounds **3a** and **3g** exhibited the highest ability to inhibit the proliferation of different cancer cell lines. Compound **3a** exhibited a broad-spectrum of anti-tumor activity without pronounced selectivity while compound **3g** revealed high selectivity toward the leukemia subpanel with selectivity ratio of 6.71 at GI_{50} level. Moreover, compounds **3e** and **3j** have shown remarkable topo II inhibitory activity compared to etoposide at 100 μM and 20 μM concentrations. Compounds **3e** and **3j** exhibited comparably potent topo I inhibitory activity at 20 μM concentration compared to camptothecin. Compounds **3e** and **3j** exhibited strong topo II inhibitory activities compared to topo I at 20 μM concentration. Studying of the solubility and partition coefficient revealed higher lipophilicity of the hybrids **3a**–**j** compared to the parent ciprofloxacin.

1. Introduction

Fluoroquinolones are known synthetic antibacterial agents which have been the subject of many research interests [1]. Recent reports have shown the importance of fluoroquinolones as anti-tumor agents [2]. DNA topoisomerases (topo) are ubiquitous enzymes that perform essential cellular functions involved in replication, recombination, and packaging and unfolding of DNA in chromatin [3]. Topo I and II inhibitors which have various structures with different side chains have been reported [4,5]. Clinically potent anticancer agents, topo inhibitors like camptothecin [6], etoposide [7] and doxorubicin [8] bind to the cleavable complex formed between topo and DNA, and keep it from going back to the original DNA. Now agents directly inhibiting topo are urgently being requested. Additionally, topo II represents the cellular target for quinolone antibacterial agents and a wide variety of anticancer drugs that was attributed to the mechanistic similarities and sequence homologies of their target topoisomerase II [9]. It is reported that ciprofloxacin inhibits mitochondrial topoisomerase II and therefore affects cellular energy metabolism. In a concentration exceeding 80 μg/mL, ciprofloxacin induces apoptosis while at 25 μg/mL concentration it inhibits proliferation of Jurkat cells without any symptoms of cell death through inhibition of mitosis [10]. Moreover, it was reported that ciprofloxacin could be used for the experimental adjunctive therapy of lung cancer [11], ciprofloxacin derivative I (Chart 1) showed potent *in vitro* anti-tumor activity [2]. Furthermore, *in vitro* evaluation of ciprofloxacin analog, trovafloxacin showed its high effectiveness in inhibition of the



Ciprofloxacin analogue I

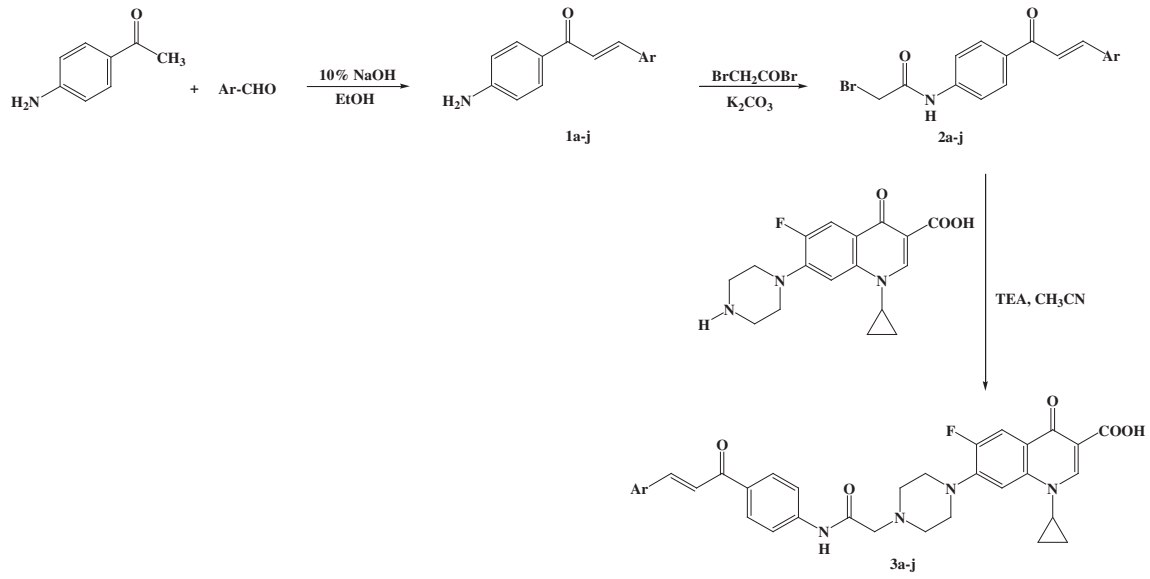


Chalcone analogue II

Chart 1. The structures of ciprofloxacin analog I and II.

* Corresponding author. Tel.: +201003069431; fax: +20862369075.
E-mail addresses: gamalaburahma@yahoo.com, gamaleldin67@hotmail.com (G.El-DinA.A. Abuo-Rahma).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.08.040



a: Ar = -C_6H_5, b: Ar = 2-Cl-C_6H_4, c: Ar = 3-Cl-C_6H_4, d: Ar = 4-Cl-C_6H_4, e: Ar = 4-OCH_3-C_6H_4, f: Ar = 3,4-di-OCH_3-C_6H_3, g: Ar = 3,4,5-tri-OCH_3-C_6H_2, h: Ar = 3-NO_2-C_6H_4, i: Ar = 2,4-di-CH_3-C_6H_3, j: Ar = 3,4-OCH_2O-C_6H_3.

Scheme 1. Synthesis of the target ciprofloxacin-chalcone hybrids **3a**–**j**.

growth of P388 murine leukemia cells [12]. It is well established that, the inhibition of topoisomerase II and physicochemical properties of fluoroquinolones is greatly affected by the nature of the C-7 substituent [13]. Studies demonstrated that the introduction of O-benzyl moiety on oxime group of *N*-(2-oxyimino) piperazinyl quinolone series changes the biological profile of piperazinyl quinolones from antibacterial to cytotoxic activity [9].

Naturally occurring and synthetic chalcone derivatives are of current interest as cytotoxic agents [14,15]. Chalcones had been reported to inhibit cancer cell proliferation, induce apoptosis in various cell types and exhibit remarkable effect against skin carcinogenesis [16]. Several mechanisms have been reported for the cytotoxic action of chalcone derivatives including; inhibiting tubulin polymerization [17], inhibition of angiogenesis, induction of apoptosis, anti-estrogenic activity and reversal of multidrug resistance or combination of these mechanisms [18]. A recent report showed that the 2-phenylquinoline/chalcone hybrid (chalcone II, Chart 1) is highly active against the growth of MDA-MB-231 cells with IC_{50} less than 0.10 μM in addition to inhibition of H1299SKBR-3, MCF-7 and SKBR-3 cells with IC_{50} of 0.71, 0.91 and 0.52 μM, respectively [19].

Several reports mentioned that the introduction of a substituent on *N*-4-piperazinyl moiety of quinolones altered physicochemical properties of quinolones that may affect the cytotoxicity of these compounds and selectivity of these hybrids toward topo I and topo II [20–23]. Based on the above mentioned studies, we report herein the synthesis of certain C-7-piperazinyl-ciprofloxacin-chalcone hybrids for the purpose of improvement of the physicochemical properties of ciprofloxacin and/or synergistic effect through combining ciprofloxacin and chalcone in one compact structure. The designed compounds were evaluated for their *in vitro* anticancer activities using different cancer cell lines in addition to evaluation of their inhibitory activity against topoisomerase I and II enzymes and DNA unwinding assay. Also, the effect of introducing the designed *N*-4-piperazinyl substituents on the physicochemical properties of ciprofloxacin has been studied.

2. Results and discussion

2.1. Chemistry

The synthesis of designed compounds is outlined in Scheme 1. Chalcone derivatives **1a**–**j** were synthesized by a base catalyzed Claisen–Schmidt condensation of 4-aminoacetophenone with different benzaldehyde derivatives [24–26]. Treatment of the chalcone derivative intermediates **1a**–**j** with bromoacetyl bromide in the presence of potassium carbonate afforded the corresponding 2-bromo-*N*-{4-[3-arylacryloyl]phenyl}acetamides **2a**–**j** in high yields. Alkylation of ciprofloxacin with the acylated chalcones **2a**–**j** in acetonitrile using triethylamine as a base gave the target ciprofloxacin-chalcone hybrids **3a**–**j** in a good yield (Scheme 1) [27]. The prepared compounds were identified by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The purity of the newly prepared compounds was checked by elemental analyses. The NMR data for compounds **3a**–**j** showed the known characteristic pattern for both the parent ciprofloxacin and chalcone derivatives in addition to the characteristic singlet of $-N-CH_2-$ moiety at δ = 4.1–4.4 ppm.

2.2. Biological investigations

2.2.1. Screening of anticancer activity

Compounds **3a**, **3d**, **3e**, **3g**, and **3j** were selected by the National Cancer Institute (NCI) according to the protocol of the Drug Evaluation Branch of the National Cancer Institute, Bethesda, USA for *in vitro* anticancer screening [28]. Primary *in vitro* one-dose anticancer assay was performed in full NCI 60 cell lines derived from nine tumor subpanels, including leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancer cell lines. The selected compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determination was made with a protein binding dye sulforhodamine B (SRB). Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the

Table 1
One-dose growth (%) of nine different cancer cell types for compounds **3a** and **3g**.

Panel	Cell line	Growth (%)	
		Compound **3a**	Compound **3g**
Leukemia	CCRF-CEM	15.18	−13.74
	HL-60(TB)	40.91	−33.71
	K-562	14.15	1.66
	MOLT-4	18.99	−1.10
	RPMI-8226	2.45	−18.77
Non-small cell lung cancer	A549/ATCC	4.12	−19.23
	HOP-62	59.41	4.38
	HOP-92	14.94	−1.75
	NCI-H226	57.39	−9.81
	NCI-H23	23.99	−35.60
	NCI-H322M	73.70	−7.05
	NCI-H460	7.12	−19.97
	NCI-H522	10.59	−71.54
Colon cancer	COLO 205	107.97	−12.83
	HCC-2998	70.06	−11.57
	HCT-116	−83.61	−88.19
	HCT-15	56.81	18.22
	HT29	11.39	−3.37
	KM12	25.29	−65.07
	SW-620	41.03	6.38
CNS cancer	SF-268	36.95	−1.11
	SF-295	15.17	−27.16
	SF-539	26.19	−15.75
	SNB-19	40.27	34.93
	SNB-75	61.80	76.59
	U251	14.91	−55.79
Melanoma	LOX IMVI	5.67	−54.74
	MALME-3M	51.04	31.44
	M14	31.85	−14.11
	MDA-MB-435	19.16	−1.52
	SK-MEL-2	53.73	30.03
	SK-MEL-28	85.67	34.04
	SK-MEL-5	13.64	−42.87
	UACC-257	61.13	30.77
	UACC-62	35.47	−23.47
Ovarian cancer	IGROV1	42.88	−1.19
	OVCAR-3	54.88	−25.75
	OVCAR-4	86.41	16.43
	OVCAR-5	82.60	16.88
	OVCAR-8	6.30	4.74
	NCI/ADR-RES	93.47	98.19
	SK-OV-3	93.58	33.28
Renal cancer	786-0	23.23	−67.76
	A498	57.71	−43.84
	ACHN	40.05	10.80
	CAKI-1	73.07	57.23
	RXF-393	41.72	−87.64
	SN12C	11.31	10.33
	TK-10	39.90	19.07
	UO-31	44.99	2.96
Prostate cancer	PC-3	55.18	21.51
	DU-145	43.12	2.17
Breast cancer	MCF-7	12.50	26.46
	MDA-MB-231/ATCC	55.15	27.55
	HS 578T	51.91	76.78
	BT-549	9.61	−86.69
	T-47D	85.08	17.55
	MDA-MB-468	33.76	−1.82

untreated control cells. Compound **3a** achieved remarkable cell growth inhibition activity against most of the tested cell lines including leukemia, non-small cell lung cancer A549/ATCC, HOP-92, NCI-H23, NCI-H460, NCI-H522, colon cancer HT29, KM12, CNS cancer SF-295, SF-539, SF-539, U251, melanoma LOX IMVI, MDA-MB-435, SK-MEL-5, ovarian cancer OVCAR-8, renal cancer 786-0, SN12C, breast cancer MCF-7, BT-549 cell lines. A complete cell death was recorded for the colon cancer cell lines HCT-116 where the growth percent was −83.61. Compound **3a** revealed moderate cell growth inhibition against leukemia HL-60 (TB), colon cancer SW-620, CNS cancer SF-268, SNB-19, melanoma M14, ovarian cancer IGROV1, renal cancer ACHN, RXF-393, TK-10, UO-31, prostate cancer DU-145, breast cancer MDA-MB-468 cell lines (Table 1).

Compound **3g** achieved remarkable cell growth inhibition activity against most of the tested cell lines (Table 1). A complete cell death was recorded for leukemia CCRF-CEM, HL-60 (TB), MOLT-4, RPMI-8226, non-small cell lung cancer A549/ATCC, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522, colon cancer COLO 205, HCC-2998, HCT-116, HT29, KM12, CNS cancer SF-268, SF-295, SF-539, U251, melanoma LOX IMVI, M14, MDA-MB-435, SK-MEL-5, UACC-62, ovarian cancer IGROV1, OVCAR-3, renal cancer 786-0, A498, RXF-393, breast cancer BT-549 and MDA-MB-468 cell lines. Compound **3g** indicated a remarkable cell growth inhibition activity against most of the tested cell lines including leukemia K-562, non-small cell lung cancer HOP-62, colon cancer HCT-15, SW-620, ovarian cancer OVCAR-4, OVCAR-5, OVCAR-8, renal cancer ACHN, SN12C, TK-10, UO-31, prostate cancer PC-3, DU-145, breast cancer MCF-7, MDA-MB-231/ATCC, T-47D cell lines. Compound **3g** revealed moderate cell growth inhibition against CNS cancer SNB-19, melanoma MALME-3M, SK-MEL-2, SK-MEL-28, UACC-257 and ovarian cancer SK-OV-3 cell lines (Table 1). The results indicated also that compound **3d** exhibited moderate cell growth inhibition against non-small cell lung cancer NCI-H522, colon cancer HCT-116 cell lines (Supplementary material), while compound **3e** revealed a remarkable cell growth inhibition activity only against colon cancer HCT-116 cell line (Supplementary material).

The obtained results indicate that compounds **3a** and **3g** exhibited the highest ability to inhibit the proliferation of different cancer cell lines (Table 1) compared to compounds **3d**, **3e** and **3j** derivatives. From the obtained results; several conclusions could be deduced, a different chalcone substituent attached to the piperazinyl moiety of ciprofloxacin might contribute to the activity of the synthesized compounds; the presence of three OCH_3 groups is preferable over the presence of one OCH_3 groups (compound **3g** has superior anticancer activity against different cancer cell lines over compound **3e**). Also the presence of an electron donating group (OCH_3) in compounds **3e** and **3g** has better anticancer activity against different cancer cell lines over the presence of an electron withdrawing group (Cl) in compound **3d**. Meanwhile, the unsubstituted derivative **3a** exhibited better anticancer activity against different cancer cell lines than the derivatives containing an electron withdrawing group (compound **3d**).

2.2.2. *In vitro five-dose full NCI 60 cell panel assay*

Compounds **3a** and **3g** were selected for advanced five-dose testing against the full panel of 60 human tumor cell lines. All the 60 cell lines representing nine tumor subpanels were incubated at five different concentrations (0.01, 0.1, 1, 10 and 100 μM). The outcomes were used to create log concentration versus % growth inhibition curves and three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition (TGI) and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h. From the results in Table 2, it is clear that compound **3a** exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels. Compound **3a** showed high activity against most of the tested cell lines with GI_{50} ranging from 0.43 to 39.6 μM (Table 2). The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines toward the test agent) (μM) by their individual subpanel MID (μM). Ratios between 3 and 6 refer to

Table 2
NCI *in vitro* testing results of compound **3a** at five-dose level in μM.

Panel	Cell line	GI_{50}			TGI Conc. per cell line	LC_{50}
		Conc. per cell line	Subpanel MID[b]	Selectivity ratio (MID[a]/MID[b])		
Leukemia	CCRF-CEM	4.09	4.30	1.27	>100	>100
	HL-60(TB)	9.44			>100	>100
	K-562	3.07			>100	>100
	MOLT-4	5.13			>100	>100
	RPMI-8226	2.52			6.73	>100
	SR	1.54			6.10	>100
Non-small cell lung cancer	A549/ATCC	2.41	3.19	1.71	ND[c]	>100
	HOP-62	4.46			>100	>100
	HOP-92	0.43			3.82	>100
	NCI-H226	6.83			>100	>100
	NCI-H23	2.48			14.3	>100
	NCI-H322M	4.04			58.70	>100
	NCI-H460	3.17			>100	>100
	NCI-H522	1.70			7.78	>100
Colon cancer	COLO 205	36.3	13.02	0.42	>100	>100
	HCC-2998	39.6			>100	>100
	HCT-116	0.66			2.68	9.55
	HCT-15	3.68			>100	>100
	HT29	2.99			10.30	>100
	KM12	4.11			>100	>100
	SW-620	3.80			>100	>100
CNS cancer	SF-268	4.13	3.17	1.72	>100	>100
	SF-295	2.11			6.25	>100
	SF-539	3.60			>100	>100
	SNB-19	3.05			>100	>100
	SNB-75	3.22			>100	>100
	U251	2.92			>100	>100
Melanoma	LOX IMVI	2.32	5.05	1.08	>100	>100
	MALME-3M	2.89			8.87	>100
	M14	5.22			>100	>100
	MDA-MB-435	2.94			>100	>100
	SK-MEL-2	2.94			11.30	>100
	SK-MEL-28	18.8			>100	>100
	SK-MEL-5	2.74			13.00	>100
	UACC-257	3.90			90.80	>100
	UACC-62	3.71			>100	>100
Ovarian cancer	IGROV1	3.67	8.89	0.61	>100	>100
	OVCAR-3	3.64			>100	>100
	OVCAR-4	9.47			>100	>100
	OVCAR-5	5.35			>100	>100
	OVCAR-8	3.07			>100	>100
	NCI/ADR-RES	9.75			>100	>100
	SK-OV-3	27.30			>100	>100
Renal cancer	786-0	4.50	2.92	1.87	>100	>100
	A498	1.28			7.37	>100
	ACHN	3.30			31.90	>100
	CAKI-1	1.76			5.32	>100
	RXF-393	2.50			>100	>100
	SN12C	3.02			>100	>100
	TK-10	4.19			>100	>100
	UO-31	2.78			45.40	>100
Prostate cancer	PC-3	3.16	3.45	1.58	>100	>100
	DU-145	3.74			>100	>100
Breast cancer	MCF-7	3.52	3.84	1.42	>100	>100
	MDA-MB-231/ATCC	1.84			5.13	>100
	HS 578T	4.99			>100	>100
	BT-549	3.56			>100	>100
	T-47D	6.66			>100	>100
	MDA-MB-468	2.45			23.20	>100
MID[a]			5.46			

[a] Average sensitivity of all cell lines in μM.
[b] Average sensitivity of all cell lines of a particular subpanel in μM.
[c] Not determined.

moderate selectivity; ratios >6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria rated nonselective [29]. In this context, compound **3a** was found to have broad-spectrum anti-tumor activity against the nine tumor subpanels tested with selectivity ratios ranging between 0.42 and 1.87 at the GI_{50} level. Compound **3a** was found to be broad-spectrum anti-tumor agent against different tested tumor subpanels with no selectivity toward the tested cell lines.

Furthermore, compound **3g** exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels and showed high activity against most of the tested cell lines with GI_{50} ranging from 0.21 to 57.6 μM (Table 3).

Table 3
NCI *in vitro* testing results of compound **3g** at five-dose level in μM.

Panel	Cell line	GI_{50}			TGI	LC_{50}
		Conc. per cell line	Subpanel MID[b]	Selectivity ratio (MID[a]/MID[b])	Conc. per cell line	
Leukemia	CCRF-CEM	0.32	0.42	6.71	ND[c]	>100
	HL-60(TB)	1.11			>100	>100
	K-562	0.27			>100	>100
	MOLT-4	0.31			ND[c]	>100
	RPMI-8226	0.29			0.77	>100
	SR	0.21			>100	>100
Non-small cell lung cancer	A549/ATCC	0.37	0.51	5.53	2.14	>100
	HOP-62	0.36			11.40	56.90
	NCI-H226	0.95			26.00	>100
	NCI-H23	0.56			2.91	14.20
	NCI-H322M	0.29			2.30	>100
	NCI-H460	0.37			3.79	>100
	NCI-H522	0.66			3.19	17.9
Colon cancer	COLO 205	1.62	0.89	3.17	15.4	>100
	HCC-2998	2.76			11.4	>100
	HCT-116	0.23			0.69	ND[c]
	HCT-15	0.45			>100	>100
	HT29	0.23			0.63	9.13
	KM12	0.53			3.17	>100
	SW-620	0.42			3.20	>100
CNS cancer	SF-268	1.06	1.82	1.55	61.20	>100
	SF-295	0.53			3.13	53.10
	SF-539	0.49			2.62	>100
	SNB-19	2.78			>100	>100
	SNB-75	5.75			>100	>100
	U251	0.33			1.29	12.90
Melanoma	LOX IMVI	0.53	1.08	2.61	2.65	>100
	MALME-3M	1.81			7.65	>100
	M14	0.77			7.16	>100
	MDA-MB-435	0.30			1.70	>100
	SK-MEL-2	1.98			5.38	43.70
	SK-MEL-28	1.10			17.00	98.20
	SK-MEL-5	1.06			4.06	>100
	UACC-257	1.42			65.40	>100
	UACC-62	0.80			3.63	27.90
Ovarian cancer	IGROV1	0.40	9.38	0.30	1.72	8.43
	OVCAR-3	0.29			0.92	9.04
	OVCAR-4	1.61			84.60	>100
	OVCAR-5	1.87			7.36	>100
	OVCAR-8	0.34			26.70	>100
	NCI/ADR-RES	57.60			>100	>100
	SK-OV-3	3.57			>100	>100
Renal cancer	786-0	0.60	0.73	3.86	2.38	7.70
	A498	0.36			1.80	5.47
	ACHN	0.45			7.09	>100
	CAKI-1	1.13			>100	>100
	RXF-393	0.34			1.06	3.47
	SN12C	0.53			12.20	>100
	TK-10	1.29			16.50	>100
	UO-31	1.17			6.80	>100
Prostate cancer	PC-3	0.56	0.61	4.62	>100	>100
	DU-145	0.67			>100	>100
Breast cancer	MCF-7	0.47	9.63	0.29	>100	>100
	MDA-MB-231/ATCC	0.51			ND[c]	>100
	HS 578T	53.8			>100	>100
	BT-549	0.30			1.17	6.98
	T-47D	2.40			>100	>100
	MDA-MB-468	0.33			1.78	40.70
MID[a]			2.82			

[a] Average sensitivity of all cell lines in μM.
[b] Average sensitivity of all cell lines of a particular subpanel in μM.
[c] Not determined.

Compound **3g** was found to have broad-spectrum anti-tumor activity against the tested nine tumor subpanels with selectivity ratios ranging between 0.29 and 6.71 at the GI_{50} level. Compound **3g** exhibited high selectivity toward the leukemia subpanel with selectivity ratio of 6.71 at GI_{50} level and moderate selectivity toward the non-small cell lung cancer, colon cancer, renal cancer and prostate cancer subpanels with selectivity ratio of 5.53, 3.17, 3.86 and 4.62 respectively, at GI_{50} level.

2.2.3. Topoisomerase I and II inhibitory activities

The conversion of supercoiled plasmid DNA to relaxed DNA by topo I and II was examined in the presence of the test compounds

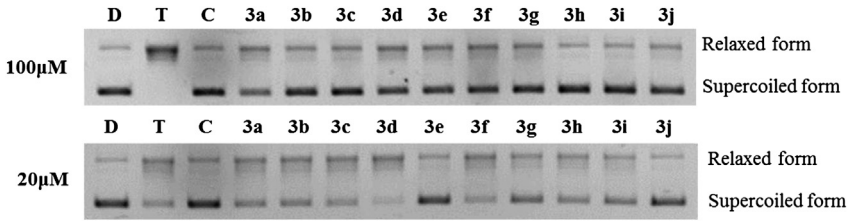


Fig. 1. Topoisomerase I inhibitory activity of compounds **3a**–**j**. The compounds were examined in a final concentration of 20 μM and 100 μM, respectively, as designated. Lane D: pBR322 only, Lane T: pBR322 + topo I, Lane C: pBR322 + topo I + camptothecin, Lanes **3a**–**j**: pBR322 + topo I + compounds in designated concentrations.

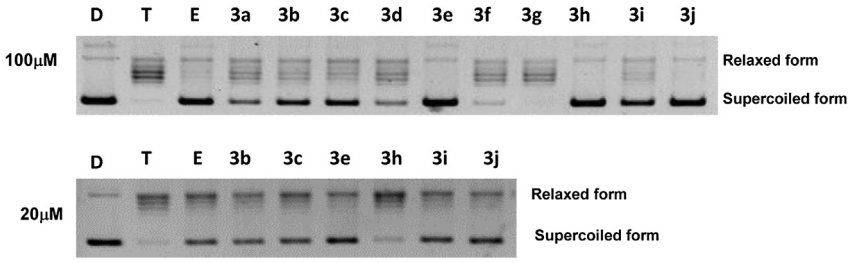


Fig. 2. Topoisomerase II inhibitory activity of compounds **3a**–**j**. Lane D: pBR322 only, Lane T: pBR322 + topo IIα, Lane E: pBR322 + topo IIα + etoposide, Lanes **3a**–**j**: pBR322 + topo IIα + compounds in designated concentrations.

3a–**j** as shown in Figs. 1 and 2. Camptothecin and etoposide, well-known topo I and II inhibitors, respectively, were used as positive controls. Inhibitory activities were evaluated both at 100 μM and 20 μm. As shown in Fig. 1, all of the tested compounds exhibited remarkable topo I inhibitory activity at 100 μM concentration. Compounds **3f**, **3h**–**j** displayed the most significant topo I inhibitory activity and they revealed stronger topo I inhibitory activity than the positive control, camptothecin (Table 4). Compounds **3a**–**e** and **3g** displayed remarkable topo I inhibitory activity compared to the positive control, camptothecin (Table 4) at 100 μM concentration. Moreover, Compounds **3e** and **3j** exhibited significant topo I inhibitory activity at 20 μM concentration compared to the positive control, camptothecin (Table 4).

The topo I inhibitory activities of the tested compounds are summarized in Fig. 1 and Table 4. The results indicated that the substituted chalcone derivatives gave good topo I inhibitory activity at 100 μM concentration than the unsubstituted one. Moreover, compound **3j** gave the best topo I inhibitory activity among the tested group at 100 μM concentration.

Table 4
Topo I and II inhibitory activities of the test compounds **3a**–**j**.

Compounds	Topo I (% inhibition)		Topo II (% inhibition)	
	100 μM	20 μM	100 μM	20 μM
3a	57.2	6.4	35.7	NA[c]
3b	70.8	4.2	66.7	21.3
3c	68.8	3.1	52.9	24.6
3d	67.5	0.4	27.7	NA[c]
3e	67.6	25.3	84.3	49.6
3f	73.2	2.5	9.0	NA[c]
3g	67.4	11.5	5.2	NA[c]
3h	90.5	11.1	82.8	8.8
3i	85.1	11.0	78.9	29.4
3j	91.3	25.9	85.7	43.8
Etoposide[a]			77.7	38.7
Camptothecin[b]	71.9	30.6		

[a] Etoposide: positive control for topo II.
[b] Camptothecin: positive control for topo I.
[c] Not applicable.

Most of the tested compounds have shown significant topo II inhibitory activity at 100 μM as summarized in Table 4. Compounds **3e** and **3j** have shown stronger topo II inhibitory activity than etoposide at 100 μM and 20 μM concentration. Compounds **3h** and **3i** displayed stronger topo II inhibitory activity than etoposide at 100 μM concentration. Compounds **3b** and **3c** exhibited moderate topo II inhibitory activity at 100 μM and 20 μM concentration as shown in Fig. 2 and Table 4. Moreover, compounds **3e**, **3h**–**j** showed both significant topo I and II inhibitory activities. The results also indicated that compounds **3e** and **3j** exhibited strong topo II inhibitory activities compared to topo I at 20 μM concentration. While compound **3e** showed remarkable topo II inhibitory activity compared to topo I at 100 μM concentration.

The results of topo II inhibitory activity listed in Table 4 indicated that the entire compounds with dioxolo moiety (compound **3j**) have shown stronger topo II inhibitory activity among the tested compounds at 100 μM concentration while compound **3e** with *p*-methoxy substituent has shown stronger topo II inhibitory activity among the tested compounds at 20 μM concentration. Moreover, increasing the number of methoxy groups in compounds **3f** and **3g** have deleterious effect on the topo II inhibitory activities of these compounds.

The obtained results indicated that introduction of the chalcones derivatives into the *N*-4-piperazinly moiety of ciprofloxacin increases dramatically the anticancer activity of the tested compounds relative to the very weak anticancer ciprofloxacin [2]. Increasing the antiproliferative activity of the tested compounds may be attributed to either synergistic effect of the chalcone derivatives and/or alteration of the physicochemical properties of ciprofloxacin. Moreover it is obvious that the type of substituent on chalcone moiety has a key role in the anticancer activity. Additionally, there is no direct correlation between the anticancer activity of the tested compounds and their inhibitory effect against topo I and topo II (c.f compound **3j** against **3g**).

2.2.4. DNA unwinding assay

DNA unwinding assay was carried out using a supercoiled pHOT1 DNA as a substrate, since unwinding of the double strand of DNA helix is a practical characteristic of intercalating drugs [30]. As

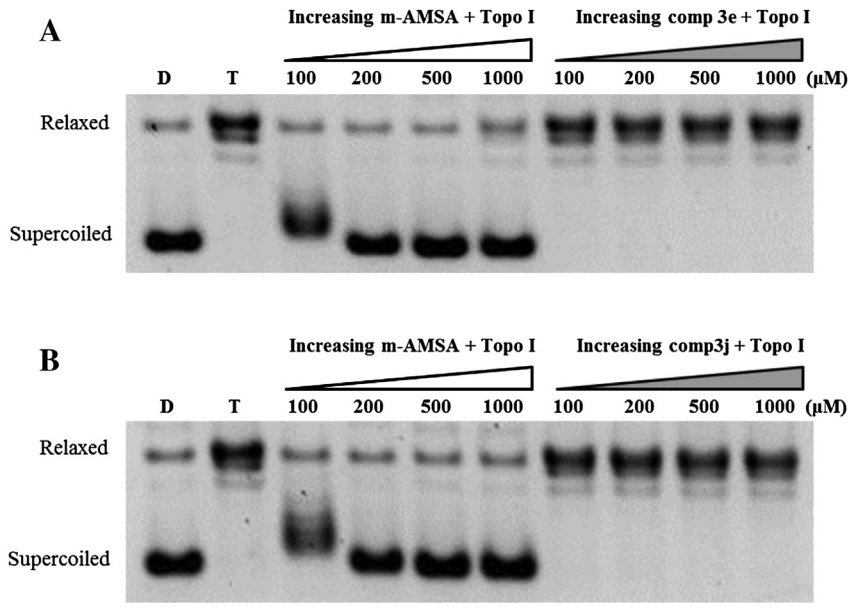


Fig. 3. DNA unwinding activity of compounds **3a** (A) and **3j** (B). The supercoiled DNA pHOT1 was incubated with excess amount of topo I at the 37 °C for 30 min followed by addition of each compound in designated concentrations and additional incubation at 37 °C for 30 min.

shown in Fig. 3, amsacrine (m-AMSA), a well-known DNA intercalator [31,32], blocked unwinding of pHOT1 DNA in the presence of excess topo I in dose-dependent manner which is consistent with result previously reported [33] while compounds **3e** and **3j** did not block DNA unwinding at high concentration up to 1000 μM treatment. Based on this result it is estimated that compounds **3e** and **3j** do not interact with DNA but interact with topoisomerases for their inhibitory activity.

2.3. Physicochemical properties

2.3.1. Solubility determination

Solubility of ciprofloxacin and the synthesized hybrids **3a**–**j** was determined at different pH's of 6.9 and 7.8 at 37 °C and the results are listed in Table 5. The pH-solubility profile of ciprofloxacin and its intrinsic solubility as a function of temperature reported by Yu et al. [34] showed that both have an endothermic heat of solution with minimum solubility at pH ~7. In agreement, Ross and Riley [35] found that the intrinsic solubility of fluoroquinolones at 25 °C ranged from 0.0297 to 2.75 mg/mL. The results of pH-solubility profiles of ciprofloxacin and hybrids **3a**–**j** showed a clear decrease in the solubility of the synthesized compounds **3a**–**j** compared to ciprofloxacin at different pH's of 6.9 and 7.8 at 37 °C. Additionally, ciprofloxacin and the synthesized hybrids **3a**–**j** showed higher solubility in pH 6.9 over that of pH 7.8. From the above mentioned results, it is obvious that the introduction of *N*-4-piperazinyl chalcone substituents decreased the water solubility of ciprofloxacin by alteration of the zwitter ionic nature of the parent ciprofloxacin that was attributed to their effect on the *N*-4 protonation ability [27].

2.3.2. Partition coefficient

The experimental partition coefficient ($\log P_{exp}$) between *n*-octanol and buffer for the newly synthesized hybrids **3a**–**j** compared to the parent ciprofloxacin was determined using traditional shake-flask method [35,36], the results are listed in Table 6. From these results, the experimental partition coefficient ($\log P_{exp}$) of ciprofloxacin was found to be −0.1432. Introduction of *N*-4-piperazinyl chalcone derivatives significantly altered the partition coefficient pattern in a range from −0.0812 to 1.4684 (Table 6). The increasing of $\log P_{exp}$ of the synthesized compounds **3a**–**j** compared to ciprofloxacin has a promising effect on increasing the lipophilicity of these compounds that may effect cell penetration.

Table 5
Solubility of ciprofloxacin compared and the synthesized hybrids **3a**–**j** in pH's of 6.9 and 7.8 at 37 °C.

Compounds	Solubility (mg/mL)	
	pH 6.9	pH 7.8
Ciprofloxacin	0.23131	0.21024
3a	0.00273	0.00253
3b	0.00245	0.00212
3c	0.03441	0.02973
3d	0.00277	0.00223
3e	0.00298	0.00242
3f	0.00075	0.00068
3g	0.00213	0.00201
3h	0.03067	0.02743
3i	0.00212	0.00207
3j	0.00266	0.00224

Table 6
Partition coefficient ($\log P_{exp}$) of ciprofloxacin compared to the synthesized hybrids **3a**–**j**.

Compounds	$\log P_{exp}$
Ciprofloxacin	−0.1432
3a	1.2934
3b	1.2912
3c	0.3021
3d	0.4705
3e	0.6842
3f	1.4593
3g	0.7532
3h	−0.0812
3i	1.4684
3j	0.5625

3. Conclusion

A group of novel *N*-4-piperazinyl-ciprofloxacin-chalcone derivatives was prepared and characterized by their spectral data. One-dose anticancer test results indicated that compounds **3a** and **3g** exhibited the highest ability to inhibit the proliferation of different cancer cell lines. *In vitro* five-dose full NCI 60 cell panel assay revealed that compound **3a** exhibited a broad-spectrum antitumor activity against the nine tumor subpanels tested without pronounced selectivity while compound **3g** revealed high selectivity toward the leukemia subpanel with selectivity ratio of 6.71 at GI_{50} level. Most of the tested compounds have shown good topo I and topo II inhibitory activity at 100 μM. Compounds **3e** and **3j** exhibited comparable topo I inhibitory activity at 20 μm concentration compared to the positive control, camptothecin. Additionally, compounds **3e** and **3j** have shown remarkable topo II inhibitory activity compared to etoposide at 100 μM and 20 μM concentrations. The topo inhibitory activity of compounds **3e** and **3j** might be an attribute of affecting to topo not binding to DNA. Both solubility and partition coefficient determination revealed increase of the lipid solubility of the prepared hybrids **3a**–**j** compared to the parent ciprofloxacin that may affect on the cell permeability of these compounds.

This preliminary study of the anti-tumor activity of the compounds **3a** and **3g** represents a novel strategy for the discovery of promising lead anticancer compounds which requires further investigations.

4. Experimental section

4.1. Chemistry

Reactions were monitored by TLC: Pre-coated plastic sheets, 0.2 mm silica gel with fluorescent indicator (Macherey–Nagel). Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. ^{1}H-NMR spectra were recorded using a Bruker Advance 300 MHz NMR spectrometer. ^{13}C-NMR spectra were recorded using a Bruker Advance (75 MHR) spectrometer. LC-MS measurements were performed on an Agilent Technologies 1100 LC/MSD trap using TMS as internal reference. Chemical shifts δ values are given in parts per million (ppm) using $CDCl_3$ (7.29), DMSO-d_6 (2.5) or DMSO-d_6 + CF_3COOH as solvents and coupling constants (J) in Hz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet; bs, broad singlet instrument. Elemental analyses were performed at the Analytical Laboratory, Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan. Chalcones **1a**–**j** are synthesized according to a reported procedure [24–26]. The compounds **2d**, **2e** and **2j** were synthesized as reported [37].

4.1.1. General procedure for the synthesis of 2-bromo-N-{4-[3-arylacryloyl]-phenyl}acetamides *2a–c, 2f–i*

To a stirred mixture of chalcones **1a**–**j** (6.30 mmol) in dichloromethane (20 mL) and potassium carbonate (1.30 mmol) in 100 mL water, cool in an ice bath, bromoacetyl bromide (1.856 g, 9.20 mmol) in 30 mL dichloromethane was added in a dropwise manner with stirring over 30 min. Stirring was continued for 2 h at 0 °C, and at r. t. overnight. The reaction mixture was extracted with dichloromethane (2 × 60 mL). The organic layer was washed with distilled water (2 × 40 mL), dried over anhydrous sodium sulfate, filtered, evaporated under vacuum and the residue was recrystallized from ethanol.

*4.1.1.1. 2-Bromo-N-(4-((E)-3-phenylacryloyl)phenyl)acetamide (**2a**).* Pale yellow powder; m.p: 157–158 °C; Yield = 1.8 g, 83.72%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.74 (s, 1H, –NH), 8.19 (d, 2H, J = 8.4 Hz, Ar–H), 7.90–7.72 (m, 6H, 5Ar–H^+ =CH), 7.51–7.44 (m, 3H, 2Ar–H^+ =CH), 4.11 (s, 2H, $-CH_2$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.56, 165.34, 143.45, 143.10, 134.73, 132.83, 130.47, 129.91, 128.86, 128.76, 120.20, 118.68, 30.28; LC-MS: m/z ($C_{17}H_{14}BrNO_2$) = 344 (M^+ + 1, 100%), 346.0 (92%).

*4.1.1.2. 2-Bromo-N-(4-((E)-3-(2-chlorophenyl)acryloyl)phenyl)acetamide (**2b**).* Yellow powder; m.p: 188–189 °C; Yield = 1.7 g, 71.73%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.81(s, 1H, –NH), 8.24–8.02 (m, 3H, 2Ar–H^+ =CH), 7.82 (d, 2H, J = 8.4 Hz, Ar–H), 7.66–7.55 (m, 2H, Ar–H), 7.52–7.44 (m, 3H, 2Ar–H^+ =CH), 4.14 (s, 2H, $-CH_2$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.34, 165.35, 143.12, 138.04, 134.28, 132.52, 132.38, 131.76, 130.05, 130.01, 129.93, 128.46, 127.57, 124.68, 118.71, 118.66, 30.26; LC-MS: m/z ($C_{17}H_{13}BrClNO_2$) = 378 (M^+ + 1, 92%), 380 (100%), 382 (30%).

*4.1.1.3. 2-Bromo-N-(4-((E)-3-(3-chlorophenyl)acryloyl)phenyl)acetamide (**2c**).* Pale yellow powder; m.p: 157–158 °C; Yield = 1.9 g, 80.20%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.76 (s, 1H, –NH), 8.22 (d, 2H, J = 8.4 Hz, Ar–H), 8.07–8.01 (m, 2H, Ar–H), 7.83–7.68 (m, 4H, 3Ar–H^+ =CH), 7.52–7.45 (m, 2H, 1Ar–H^+ =CH), 4.12 (s, 2H, $-CH_2$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.43, 165.34, 143.05, 141.68, 137.01, 136.99, 133.78, 132.67, 132.61, 130.57, 130.06, 129.95, 127.84, 127.75, 123.55, 118.65, 30.24; LC-MS: m/z ($C_{17}H_{13}BrClNO_2$) = 378 (M^+ + 1, 92%), 380.0 (100%), 382 (31%).

*4.1.1.4. 2-Bromo-N-(4-((E)-3-(3,4-dimethoxyphenyl)acryloyl)phenyl) acetamide (**2f**).* Yellow powder; m.p: 149–150 °C; Yield = 2.0 g, 79.05%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.75 (s, 1H, –NH), 8.19 (d, 2H, J = 8.6 Hz, 2H, Ar–H), 7.82–7.73 (m, 4H, 3Ar–H^+ =CH), 7.56–7.54 (m, 2H, 1Ar–H^+ =CH), 7.03 (d, 1H, J = 8.6 Hz, Ar–H), 4.12 (s, 2H, $-CH_2$), 3.89 (s, 3H, $-OCH_3$), 3.83 (s, 3H, $-OCH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.40, 165.31, 151.00, 149.05, 143.98, 142.72, 133.11, 130.26, 129.76, 129.60, 127.62, 123.82, 119.50, 118.62, 55.70, 55.60, 30.57; LC-MS: m/z ($C_{19}H_{18}BrNO_4$) = 404 (M^+ + 1, 92%), 406 (100%).

*4.1.1.5. 2-Bromo-N-(4-((E)-3-(3,4,5-trimethoxyphenyl)acryloyl) phenyl)acetamide (**2g**).* Yellow powder; m.p: 166–167 °C; Yield = 2.3 g, 84.56%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.70 (s, 1H, –NH), 8.17 (d, 2H, J = 8.6 Hz, Ar–H), 7.84–7.65 (m, 4H, 2Ar–H^+ 2CH=), 7.21 (s, 2H, Ar–H), 4.10 (s, 2H, $-CH_2$), 3.85 (s, 6H, 2-OCH_3), 3.70 (s, 3H, $-OCH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.50, 165.34, 153.08, 152.90, 144.00, 142.86, 132.97, 130.26, 129.89, 121.10, 118.63, 106.50, 60.10, 56.12, 30.27; LC-MS: m/z ($C_{20}H_{20}BrNO_5$) = 434 (M^+ + 1, 92%), 436 (100%).

*4.1.1.6. 2-Bromo-N-(4-((E)-3-(3-nitrophenyl)acryloyl)phenyl)acetamide (**2h**).* Yellow powder; m.p: 190–191 °C; Yield = 2.0 g, 81.97%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.71(s, 1H, –NH), 8.68 (s, 1H, Ar–H), 8.26–8.05 (m, 5H, Ar–H), 7.78–7.52 (m, 4H, 2Ar–H^+ –CH=CH), 4.09 (s, 2H, $-CH_2$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.31, 165.33, 148.32, 143.12, 140.78, 136.60, 134.89, 132.46, 130.20, 130.08, 124.52, 124.41, 122.80, 118.62, 30.25; LC-MS: m/z ($C_{17}H_{13}BrN_2O_4$) = 389 (M^+ + 1, 100%), 391.0 (90%).

*4.1.1.7. 2-Bromo-N-(4-((E)-3-(2,4-dimethylphenyl)acryloyl)phenyl) acetamide (**2i**).* Buff red powder; m.p: 182–183 °C; Yield = 1.90 g, 81.55%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.81(s, 1H, –NH), 8.18 (d, J = 8.4 Hz, 2H, Ar–H), 8.00–7.36 (m, 5H, 3Ar–H^+ –CH=CH), 7.18–7.09 (m, 2H, Ar–H), 4.13 (s, 2H, $-CH_2$), 2.41(s, 3H, $-CH_3$), 2.32 (s, 3H, $-CH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.59, 165.32, 142.84, 140.53, 140.15, 137.86, 132.99, 131.38, 130.53, 129.80, 127.04, 126.78, 121.66, 118.68, 30.27, 20.88, 19.20; LC-MS: m/z ($C_{19}H_{18}BrNO_2$) = 372 (M^+ + 1, 92%), 374 (100%).

*4.1.2. General procedure for synthesis of compounds **3a–j***

To a mixture of ciprofloxacin (0.662 g, 0.02 mol) and the respective chalcone derivative (0.02 mol) in acetonitrile (50 mL), TEA (0.04 M) was added. The mixture was heated under reflux for 5 h. The precipitate formed was filtered off while hot, washed with acetonitrile and dried under vacuum.

*4.1.2.1. 7-(4-(4-((E)-3-(Phenylacryloyl)phenylcarbamoyl)methyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3a**).* Pale yellow powder; m.p: 269–270 °C; Yield = 0.80 g, 67.0%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.20 (s, 1H, –NH), 8.78 (s, 1H, H-2), 8.23 (d, 2H, J = 8.4 Hz, Ar–H), 7.97–7.70 (m, 7H, 5Ar–H$^+$ =CH + H-5), 7.64 (d, 2H, J = 7 Hz, H-8), 7.56–7.47 (m, 2H, Ar–H$^+$ =CH), 4.46 (s, 2H, –CH$_2$), 4.24–3.71 (m, 9H, –*N*–CH cyclopropyl + 8H, piperazinyl-H), 1.44–1.33 (m, 2H, cyclopropyl-H), 1.24–1.22 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.35, 176.22, 165.77, 163.30, 159.16, 158.65, 158.14, 157.63, 152.50, 146.50, 143.40, 142.01, 134.59, 133.00, 132.00, 128.54, 120.63, 118.82, 116.82, 113.00, 109.19, 106.81, 60.50, 51.48, 37.96, 33.20, 7.38; LC-MS: m/z = 595.4, M + 1 (100%) Anal. Calcd. $C_{34}H_{31}FN_4O_5 \cdot 0.25H_2O$ (594.23): C, 68.16; H, 5.30; N, 9.35. Found: C, 68.22; H, 5.22; N, 9.44.

*4.1.2.2. 7-(4-((4-((E)-3-(2-Chlorophenyl)acryloyl)phenylcarbamoyl) methyl)-piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3b**).* Pale yellow powder; m.p: 248–250 °C; Yield = 0.88 g, 70.40%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.10 (s, 1H, –NH), 8.68 (s, 1H, H-2), 8.24 (d, 2H, J = 8.4 Hz, Ar–H), 8.19–8.08 (m, 4H, 4Ar–H), 7.98–7.86 (m, 3H, Ar–H$^+$ =CH + H-5), 7.65 (d, 1H, J = 9 Hz, H-8), 7.57–7.42 (m, 2H, Ar–H$^+$ =CH), 4.46 (s, 2H, –CH$_2$), 3.89–3.46 (m, 9H, –*N*–CH cyclopropyl + piperazinyl 8H), 1.45–1.38 (m, 2H, cyclopropyl-H), 1.24–1.20 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.35, 175.00, 163.29, 161.66, 159.15, 158.63, 158.12, 157.60, 152.50, 142.50, 134.40, 132.28, 131.37, 129.20, 123.00, 120.56, 118.81, 118.75, 116.75, 112.93, 109.12, 106.90, 61.10, 51.80, 38.04, 34.00, 7.40; LC-MS: m/z = 629.4, M + 1 (100%), 631.4 (32%). Anal. Calcd. $C_{34}H_{30}ClFN_4O_5$ (628.19): C, 64.91; H, 4.81; N, 8.91. Found: C, 64.63; H, 4.74; N, 8.88.

*4.1.2.3. 7-(4-((4-((E)-3-(3-Chlorophenyl)acryloyl)phenylcarbamoyl) methyl)-piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3c**).* Pale yellow powder; m.p: 290–292 °C; Yield = 0.6 g, 48%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.90 (s, 1H, –NH), 8.69 (s, 1H, H-2), 8.20 (d, 2H, J = 8.4 Hz, Ar–H), 7.95–7.60 (m, 8H, 4Ar–H, H-5 + H-8 + 2=CH), 7.46–7.44 (m, 2H, Ar–H), 4.41 (s, 2H, –CH$_2$), 3.89–3.60 (m, 8H, piperazinyl-H), 3.43–3.39 (m, 1H, –*N*–CH cyclopropyl), 1.44–1.35 (m, 2H, cyclopropyl-H), 1.24–1.19 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.35, 175.00, 165.62, 163.08, 158.47, 157.96, 157.44, 147.50, 143.48, 141.95, 140.10, 138.20, 136.93, 133.88, 130.70, 129.68, 127.63, 120.65, 119.51, 116.83, 113.02, 109.21, 107.50, 58.80, 52.10, 38.20, 34.20, 7.50; LC-MS: m/z = 629.4, M + 1 (100%), 631.4 (32%). Anal. Calcd. $C_{34}H_{30}ClFN_4O_5$ (628.19): C, 64.91; H, 4.81; N, 8.91. Found: C, 64.65; H, 4.76; N, 9.31.

*4.1.2.4. 7-(4-((4-((E)-3-(4-Chlorophenyl)acryloyl)phenylcarbamoyl) methyl)-piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3d**).* Pale yellow powder; m.p: >300 °C; Yield = 0.85 g, 68%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.09 (s, 1H, –NH), 8.70 (s, 1H, H-2), 8.25 (d, 2H, J = 8.4 Hz, Ar–H), 8.01–7.84 (m, 6H, 4Ar–H + H-5 + =CH), 7.78–7.64 (m, 2H, H-8 + =CH), 7.53 (d, 2H, Ar–H, J = 8.4 Hz, Ar–H), 4.45 (s, 2H, –CH$_2$), 3.89–3.50 (m, 9H, –*N*–CH cyclopropyl + piperazinyl-H), 1.43–1.36 (m, 2H, cyclopropyl-H), 1.25–1.20 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.35, 175.50, 165.78, 163.20, 159.15, 158.64, 158.12, 157.61, 152.10, 146.20, 142.02, 135.01, 133.57, 132.10, 130.27, 129.93, 128.76, 122.42, 120.65, 118.85, 116.83, 113.01, 109.20, 106.85, 66.33, 52.50, 38.15, 35.71, 7.40. LC-MS: m/z = 629.4, M + 1 (100%), 631.4 (32%). Anal. Calcd. $C_{34}H_{30}ClFN_4O_5$ (628.19): C, 64.91; H, 4.81; N, 8.91. Found: C, 64.65; H, 4.64; N, 9.31.

*4.1.2.5. 7-(4-((4-((E)-3-(4-Methoxyphenyl)acryloyl)phenylcarbamoyl)methyl)-piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3e**).* Pale yellow powder; m.p: 286–288 °C; Yield = 0.90 g, 72.10%; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.10 (s, 1H, –NH), 8.75 (s, 1H, H-2), 8.20 (d, 2H, J = 8.4 Hz, Ar–H), 7.87–7.75 (m, 7H, 4Ar–H, H-5 + 2=CH), 7.65 (d, 1H, J = 8 Hz, H-8), 7.02 (d, 2H, J = 8.4 Hz, Ar–H), 4.45 (s, 2H, –CH$_2$), 3.87 (s, 3H, –OCH$_3$), 3.85–3.52 (m, 9H, –*N*–CH cyclopropyl-H, piperazinyl-H), 1.42–1.37 (m, 2H, cyclopropyl-H), 1.25–1.22 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.34, 175.20, 165.80, 161.25, 159.15, 158.63, 158.11, 157.60, 143.51, 141.30, 139.40, 133.46, 130.80, 129.56, 127.18, 120.54, 118.77, 116.73, 114.09, 112.92, 109.11, 106.86, 59.50, 55.50, 51.51, 38.03, 29.91, 7.80; LC-MS: m/z = 625.5, M + 1 (100%) Anal. Calcd. for $C_{35}H_{33}FN_4O_6 \cdot 0.25H_2O$ (624.24): C, 66.81; H, 5.37; N, 8.91. Found: C, 66.61; H, 5.15; N, 8.83.

*4.1.2.6. 7-(4-((4-((E)-3-(3,4-Dimethoxyphenyl)acryloyl)phenylcarbamoyl)methyl)-piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3f**).* Yellow powder; m.p: 236–237 °C; Yield = 0.77 g, 59.20%; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 14.92 (s, 1H, –COOH), 9.28 (s, 1H, –NH), 8.64 (s, 1H, H-2), 7.99 (d, 2H, J = 8.4 Hz, Ar–H), 7.89 (d, 1H, J_{HF} = 12.8 Hz, H-5), 7.75–7.68 (m, 3H, 2Ar–H$^+$ =CH), 7.39–7.15 (m, 4H, 2Ar–H, H-8, =CH), 6.89 (d, 1H, J = 8.4 Hz, Ar–H), 4.00 (s, 2H, –CH$_2$), 3.97 (s, 3H, –OCH$_3$), 3.92 (s, 3H, –OCH$_3$), 3.57–3.29 (m, 5H, –*N*–CH cyclopropyl-H + piperazinyl-H), 3.10–2.85 (m, 4H, piperazinyl-H), 2.91–2.68 (m, 4H, piperazinyl-H), 1.43–1.37 (m, 2H, cyclopropyl-H), 1.22–1.17 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6), 188.77, 175.60, 166.80, 161.25, 159.40, 158.10, 157.60, 155.23, 151.5, 147.31, 145.45, 144.40, 141.41, 138.98, 134.16, 129.80, 127.82, 124.60, 123.50, 119.20, 118.88, 112.60, 107.94, 104.95, 62.01, 57.50, 56.02, 53.18, 49.81, 35.37, 8.20; LC-MS: m/z = 655.4, M + 1 (100%); Anal. Calcd. for $C_{36}H_{35}FN_4O_7$ (654.25): C, 66.04; H, 5.39; N, 8.56. Found: C, 65.76; H, 5.23; N, 8.44.

*4.1.2.7. 7-(4-((4-((E)-3-(3,4,5-Trimethoxyphenyl)acryloyl)phenylcarbamoyl)- methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3g**).* Pale yellow powder; m.p: 271–273 °C. Yield = 0.72 g (53.0%); ^{1}H-NMR (300 MHz, DMSO-d_6 + CF_3COOH) δ 11.10 (s, 1H, –NH), 8.71 (s, 1H, H-2), 8.26 (d, 2H, J = 8.4 Hz, Ar–H), 8.00–7.95 (m, 4H, 2Ar–H + H-5 + =CH), 7.75–7.65 (m, 2H, H-8 + =CH), 7.24 (s, 2H, Ar–H), 4.44 (s, 2H, –CH$_2$), 3.90 (s, 6H, 2-OCH$_3$), 3.76 (s, 3H, –OCH$_3$), 3.85–3.50 (m, 8H, piperazinyl-H), 3.25–3.10 (m, 1H, –*N*–CH cyclopropyl), 1.42–1.37 (m, 2H, cyclopropyl-H), 1.25–1.19 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.60, 175.40, 165.80, 161.30, 159.14, 158.63, 158.12, 157.61, 153.05, 149.20, 147.50, 142.20, 136.10, 132.00, 130.30, 120.66, 118.84, 116.84, 113.03, 109.21, 106.86, 106.31, 79.00, 55.87, 55.20, 53.20, 52.50, 34.50, 8.30. LC-MS: m/z = 685.5, M + 1 (100%); Anal. Calcd. for $C_{37}H_{37}FN_4O_8 \cdot 0.25H_2O$ (684.3): C, 64.48; H, 5.48; N, 8.13. Found: C, 64.43; H, 5.36; N, 8.15.

*4.1.2.8. 7-(4-((4-((E)-3-(3-Nitrophenyl)acryloyl)phenylcarbamoyl) methyl)-piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3h**).* Pale yellow powder; m.p: >300 °C; Yield = 0.88 g, 70.40%; ^{1}H-NMR (300 MHz, DMSO-d_6 + CF_3COOH) δ 11.13 (s, 1H, –NH), 8.79 (s, 1H, H-2), 8.71 (s, 1H, Ar–H), 8.39–8.17 (m, 5H, 4Ar–H$^+$ =CH), 8.01 (d, 1H, J = 13 Hz, H-5),

7.91–7.70 (m, 4H, 2Ar–H + H-8 + =CH), 7.65 (d, 1H, J = 8.4 Hz, Ar–H), 4.44 (s, 2H, $-CH_2$), 3.92–3.39 (m, 9H, –*N*–CH cyclopropyl-H + 8 piperazinyl-H), 1.42–1.32 (m, 2H, cyclopropyl-H), 1.23–1.20 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6), 187.34, 175.50, 167.00, 165.20, 159.15, 158.64, 158.13, 157.61, 142.50, 141.30, 140.70, 139.30, 138.60, 135.00, 130.20, 126.10, 124.48, 120.71, 118.87, 116.89, 113.07, 112.50, 109.25, 106.84, 61.20, 51.51, 46.30, 34.50, 9.10; LC-MS: m/z = 640.50, M + 1 (100%) Anal. Calcd. for $C_{34}H_{30}FN_5O_7.H_2O$ (639.63): C, 62.09; H, 4.90; N, 10.65. Found: C, 61.93; H, 4.52; N, 10.55.

*4.1.2.9. 7-(4-((4-((E)-3-(2,4-Dimethylphenyl)acryloyl)phenylcarbamoyl)-methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3i**).* Pale yellow powder; m.p: 270–272 °C; Yield = 0.85 g, 68.50%; ^{1}H-NMR (300 MHz, DMSO-d_6 + CF_3COOH) δ 11.14 (s, 1H, –NH), 8.60 (s, 1H, H-2), 8.13 (d, 2H, J = 8.4 Hz, Ar–H), 7.96–7.71 (m, 7H, 3Ar–H + H-5 + H-8 + 2=CH), 7.03 (d, 2H, J = 6.0 Hz, Ar–H), 4.37 (s, 2H, $-CH_2$), 3.96–3.64 (m, 9H, –*N*–CH cyclopropyl-H, 8 piperazinyl-H), 2.38 (s, 3H, $-CH_3$), 2.32 (s, 3H, $-CH_3$), 1.47–1.29 (m, 2H, cyclopropyl-H), 1.24–1.14 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.34, 174.90, 165.78, 162.50, 159.15, 158.64, 158.13, 157.61, 142.50, 140.70, 139.30, 138.60, 128.50, 126.10, 122.50, 120.61, 118.80, 118.20, 116.80, 114.90, 112.98, 109.17, 106.60, 105.40, 61.20, 51.51, 46.30, 34.50, 28.91, 28.60, 9.10. LC-MS: m/z = 623.4, M + 1 (100%) Anal. Calcd. for $C_{36}H_{35}FN_4O_5 \cdot 0.5H_2O$ (622.26): C, 68.45; H, 5.74; N, 8.87. Found: C, 68.34; H, 5.45; N, 8.81.

*4.1.2.10. 7-(4-((4-((E)-3-(Benzo[d][1,3]dioxol-5-yl)acryloyl)phenylcarbamoyl)-methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3j**).* Pale yellow powder; m.p: 296–298 °C; Yield = 1.0 g, 78.40%; ^{1}H-NMR (300 MHz, DMSO-d_6 + CF_3COOH) δ 11.10 (s, 1H, –NH), 8.73 (s, 1H, H-2), 8.18 (d, 2H, J = 8.4 Hz, Ar–H), 8.02–7.53 (m, 6H, 3Ar–H, H-5 + 2=CH), 7.28 (d, 1H, J = 8.4 Hz, H-8), 6.94 (d, 2H, J = 8.4 Hz, Ar–H), 6.10 (s, 2H, $-OCH_2O$), 4.42 (s, 2H, $-CH_2$), 4.05–3.40 (m, 9H, –*N*–CH cyclopropyl-H + 8 piperazinyl-H), 1.44–1.37 (m, 2H, cyclopropyl-H), 1.24–1.19 (m, 2H, cyclopropyl-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 187.35, 176.22, 165.62, 163.13, 158.50, 157.90, 156.50, 152.50, 149.46, 148.09, 146.50, 143.40, 141.73, 138.99, 134.50, 129.55, 129.26, 120.76, 119.15, 116.94, 113.12, 109.31, 106.81, 101.45, 64.8, 62.50, 51.48, 46.30, 35.50, 7.38. LC-MS: m/z = 639.40, M + 1 (100%) Anal. Calcd. $C_{34}H_{30}ClFN_4O_5 \cdot H_2O$ (638.22): C, 64.02; H, 5.07; N, 8.53. Found: C, 63.80; H, 4.64; N, 8.38.

4.2. Biological assays

4.2.1. Anticancer activity

The methodology of the NCI anticancer screening has been described in detail elsewhere (http://www.dtp.nci.nih.gov) [27]. Briefly, the primary anticancer assay was performed at approximately 60 human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, SRB. Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compound were tested *in vitro* against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. Using the seven absorbance measurements [time zero (Tz), control growth in the absence of drug (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as: [(Ti − Tz)/(C − Tz)] − 100 for concentrations for which Ti > Tz, and [(Ti − Tz)/Tz] − 100 for concentrations for which Ti < Tz. Three-dose response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from [(Ti − Tz)/(C − Tz)] − 100 = 50, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in TGI was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from [(Ti − Tz)/Tz] − 100 = −50. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The $logGI_{50}$, logTGI, and $logLC_{50}$ were then determined, defined as the mean of the logs of the individual GI_{50}, TGI, and LC_{50} values. The lowest values are obtained with the most sensitive cell lines. Compound having $logGI_{50}$ values −4 and <−4 was declared to be active.

4.2.2. Topo I topo II inhibitory activity

DNA topo I inhibition assay was determined following the previously reported method [38]. The prepared compounds were dissolved in DMSO at 20 mM as stock solution. The activity of DNA topo I was determined by assessing the relaxation of supercoiled DNA pBR322. The mixture of 100 ng of plasmid pBR322 DNA and 0.4 units of recombinant human DNA topo I (TopoGEN INC., USA) was incubated without and with the prepared compounds at 37 °C for 30 min in the relaxation buffer (10 mM Tris–HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine, 5% glycerol). The reaction in the final volume of 10 mL was terminated by adding 2.5 mL of the stop solution containing 5% sarcosyl, 0.0025% bromophenol blue, and 25% glycerol. DNA samples were then electrophoresed on a 1% agarose gel at 15 V for 7 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 mg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation). DNA topo II inhibitory activity of compounds was measured as follows [39]. The mixture of 200 ng of supercoiled pBR322 plasmid DNA and 1 unit of human DNA topo IIα (TopoGEN INC., USA) was incubated without and with the prepared compounds in the assay buffer (10 mM Tris–HCl (pH 7.9) containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, and 15 mg/mL bovine serum albumin) for 30 min at 30 °C. The reaction in a final volume of 20 mL was terminated by the addition of 3 mL of 7 mM EDTA. Reaction products were analyzed on 1% agarose gel at 25 V for 4 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 mg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated using AlphaImager™ (Alpha Innotech Corporation). The added amount of DMSO for each reaction mixture was same in both of enzyme only and enzyme with designated compound for topo I and II assays. Etoposide, and camptothecin were purchased from Sigma and used as positive controls.

4.2.3. DNA unwinding assay

One hundred ng of pHOT1 plasmid DNA (TopoGEN INC., USA) was relaxed by incubation with 3 units of human DNA topo I (TopoGEN INC., USA) in topo I reaction buffer (10 mM Tris–HCl pH 7.9) for 30 min at 37 °C. Then variable amounts of the testing compounds were added and a total 10 μL of reaction mixture was continued for additional incubation for 30 min at 37 °C. The reaction was terminated by the addition of 3 μL of 7 mM EDTA. Reaction products were analyzed on 1% agarose gel at 15 V for 15 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated using AlphaImager™ (Alpha Innotech Corporation). m-AMSA was used as a positive control.

4.3. Physicochemical properties

4.3.1. Solubility determination

The aqueous solubility of ciprofloxacin and their hybrids **3a**–**j** in a buffer of pH's of 6.9 and 7.8, at 37 °C was measured. A known excess (10 mg) of the prepared compounds **3a**–**j** or ciprofloxacin was placed into suitable stopper sealed Erlenmeyer flasks containing fixed volume of buffer of pH's of 6.9 and 7.8. Flasks were equilibrated for 48 h, in a temperature controlled shaking water bath maintained at 37 °C and shaked at rate of 100 rpm. Preliminary experiments indicated that after 48 h all solutions were saturated. After the equilibrium was reached, the excess solid was allowed to settle down and filtered. Aliquots of the filtrate properly diluted with the corresponding solvents to obtain absorbance in the linear calibration range for each system and the composition of the liquid phase was determined by UV spectroscopy at corresponding wave length, λ_{max} of 272 nm. All the experiment results were an average of at least three agreeing independent measurements.

4.3.2. Determination of partition coefficient

The experimental partition coefficient (P_{exp}) between *n*-octanol and phosphate buffer was determined by a slight modification of the method earlier described by Vazquez et al. [35,40]. Briefly, 100 μL of a stock solution (0.2 mg/mL) was diluted with 1.9 mL of appropriate phosphate buffer solution (pH 7.4) and mixed with 2 mL of octanol (the organic and aqueous phase was mutually saturated), the vials were protected from light by wrapping in aluminum foil. The two phases were vortexed for 3 min and agitated for 5 h in a shaking water bath at 25 + 0.1 °C. After equilibration, the octanol phase was removed with a Pasteur pipette and both phases were assayed spectrophotometrically (λ_{max} = 272 nm) to determine drug concentration. The partition coefficient was calculated as the ratio between molar concentration in *n*-octanol and aqueous phase. The total concentration in both phases was measured by spectrophotometry and the experimental partition coefficients ($\log P_{exp}$) was calculated by the following equation: $\log P\ o/w$ = log[C] octanol/[C] aqueous.

All partition coefficient determinations were made in triplicate. Spectronic Genesys, connected to an IBM computer loaded with the Winspec application software (Milton Roy, USA) and Jenway 6505 (Jenway LTD., UK) ultraviolet–visible spectrophotometers with matched 1 cm quartz cells were used throughout this study for all measurements.

Acknowledgment

Authors thank the Development Therapeutics Program of the National Cancer Institute, Bethesda, MD, USA, for *in vitro* evaluation of anticancer activity. This work was funded by the Faculty of Pharmacy, Minia University, Minia, Egypt (Chemistry, Physicochemical properties) and by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0002646) (for topoisomerase I & II inhibitory activities measurement).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.08.040.

References

[1] H. Koga, A. Itoh, S. Murayama, S. Suzue, T. Irikura, Structure-activity relationships of antibacterial 6,7- and 7,8-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids, J. Med. Chem. 23 (1980) 1358–1363.
[2] J. Azéma, B. Guidetti, J. Dewelle, B. Le Calve, T. Mijatovic, A. Korolyov, J. Vaysse, M. Malet-Martino, R. Martino, R. Kiss, 7-((4-Substituted)piperazin-1-yl) derivatives of ciprofloxacin: synthesis and in vitro biological evaluation as potential antitumor agents, Bioorg. Med. Chem. 17 (2009) 5396–5407.
[3] J.C. Wang, DNA topoisomerases, Annu. Rev. Biochem. 65 (1996) 635–692.
[4] S. Matsui, W. Endo, C. Wrzosek, K. Haridas, P. Seetharamulu, F. Hausheer, Y. Rustum, Characterisation of a synergistic interaction between a thymidylate synthase inhibitor, ZD1694, and a novel lipophilic topoisomerase I inhibitor karenitecin, BNP1100: mechanisms and clinical implications, Eur. J. Cancer 35 (1999) 984–993.
[5] S. Alper, T. Arpaci, E. Aki, I. Yalcin, Some new bi- and ter-benzimidazole derivatives as topoisomerase I inhibitors, IL Farmaco 58 (2003) 497–507.
[6] Y.H. Hsiang, R. Hertzberg, S. Hecht, L.F. Liu, Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I, J. Biol. Chem. 260 (1985) 14873–14878.
[7] K.C. Chow, T.L. MacDonald, W.E. Ross, DNA binding by epipodophyllotoxins and *N*-acyl anthracyclines: implications for mechanism of topoisomerase II inhibition, Mol. Pharmacol. 34 (1988) 467–473.
[8] K.M. Tewey, T.C. Rowe, L. Yang, B.D. Halligan, L.F. Liu, Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II, Science 226 (1984) 466–468.
[9] S. Rajabalian, A. Foroumadi, A. Hafiee, S. Emami, Functionalized *N*(2-oxyiminoethyl)piperazinyl quinolones as new cytotoxic agents, J. Pharm. Pharm. Sci. 10 (2007) 153–158.
[10] R. Koziel, J. Szczepanowska, A. Magalska, K. Piwocka, J. Duszynski, K. Zablocki, Ciprofloxacin inhibits proliferation and promotes generation of aneuploidy in Jurkat cells, J. Physiol. Pharmacol. 61 (2010) 233–239.
[11] T. Kloskowski, N. Gurtowska, M. Nowak, R. Joachimiak, A. Bajek, J. Olkowska, T. Drewa, The influence of ciprofloxacin on viability of A549, HepG2, A375.S2, B16 and C6 cell lines in vitro, Acta Pol. Pharm. 68 (2011) 859–865.
[12] H. Thadepalli, F. Salem, S.K. Chuah, S. Gollapudi, Antitumor activity of trovafloxacin in an animal model, In Vivo 19 (2005) 269–276.
[13] J.M. Domagala, L.D. Hanna, C.L. Heifetz, M.P. Hutt, T.F. Mich, J.P. Sanchez, M. Solomon, New structure-activity relationships of the quinolone antibacterials using the target enzyme. The development and application of a DNA gyrase assay, J. Med. Chem. 29 (1986) 394–404.
[14] S.J. Won, C.T. Liu, L.T. Tsao, J.R. Weng, H.H. Ko, J.P. Wang, C.N. Lin, Synthetic chalcones as potential anti-inflammatory and cancer chemopreventive agents, Eur. J. Med. Chem. 40 (2005) 103–112.
[15] G. Achanta, A. Modzelewska, L. Feng, S.R. Khan, P. Huang, A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome, Mol. Pharmacol. 70 (2006) 426–433.
[16] N.J. Lawrence, R.P. Patterson, L.L. Ooi, D. Cook, S. Ducki, Effects of alpha-substitutions on structure and biological activity of anticancer chalcones, Bioorg. Med. Chem. Lett. 16 (2006) 5844–5848.
[17] R.J. Anto, K. Sukumaran, G. Kuttan, M.N. Rao, V. Subbaraju, R. Kuttan, Anticancer and antioxidant activity of synthetic chalcones and related compounds, Cancer Lett. 97 (1995) 33–37.
[18] S. Ducki, The development of chalcones as promising anticancer agents, IDrugs 10 (2007) 42–46.
[19] C.H. Tseng, Y.L. Chen, C.Y. Hsu, T.C. Chen, C.M. Cheng, H.C. Tso, Y.J. Lu, C.C. Tzeng, Synthesis and antiproliferative evaluation of 3-phenylquinolinylchalcone derivatives against non-small cell lung cancers and breast cancers, Eur. J. Med. Chem. 59 (2013) 274–282.
[20] P. Yogeeswari, D. Sriram, R. Kavya, S. Tiwari, Synthesis and in-vitro cytotoxicity evaluation of gatifloxacin Mannich bases, Biomed. Pharmacother. 59 (2005) 501–510.
[21] M.J. Nieto, F.L. Alovero, R.H. Manzo, M.R. Mazzieri, Benzenesulfonamide analogs of fluoroquinolones. Antibacterial activity and QSAR studies, Eur. J. Med. Chem. 40 (2005) 361–369.

[22] T.D. Gootz, P.R. Mcguirk, M.S. Moynihan, S.L. Haskell, Placement of alkyl substituents on the C-7 piperazine ring of fluoroquinolones: dramatic differential effects on mammalian topoisomerase II and DNA gyrase, Antimicrob. Agents Chemother. 38 (1994) 130–133.
[23] F.L. Alovero, X.S. Pan, J.E. Morris, R.H. Manzo, L.M. Fisher, Engineering the specificity of antibacterial fluoroquinolones: benzenesulfonamide modifications at C-7 of ciprofloxacin change its primary target in *Streptococcus pneumoniae* from topoisomerase IV to gyrase, Antimicrob. Agents Chemother. 44 (2000) 320–325.
[24] Y. Fengping, P. Yangping, S. Gonghua, L. Jizong, Solid phase synthesis of amino-chalcones, J. Chem. Res. 2005 (2005) 311–312.
[25] K. Nakaya, K. Funabiki, K. Shibata, H. Muramatsu, M.M. Matsul, Fluorescent α,β-unsaturated carbonyl compounds and 2-methoxypyridines. Their application to a quantitative analysis of carnitines, Bull. Chem. Soc. Jpn. 69 (1996) 2961–2966.
[26] F.L. Ansari, S. Umbreen, L. Hussain, T. Makhmoor, S.A. Nawaz, M.A. Lodhi, S.N. Khan, F. Shaheen, M.I. Choudhary, M.I. Atta-ur-Rahman, Syntheses and biological activities of chalcone and 1,5-benzothiazepine derivatives: promising new free-radical scavengers, and esterase, urease, and alpha-glucosidase inhibitors, Chem. Biodivers. 2 (2005) 487–496.
[27] G.A. Abuo-Rahma, H.A. Sarhan, G.F.M. Gad, Design, synthesis, antibacterial activity and physicochemical parameters of novel *N*-4-piperazinyl derivatives of norfloxacin, Bioorg. Med. Chem. 17 (2009) 3879–3886.
[28] The methodology of the NCI anticancer screening has been described in detail elsewhere. http://www.dtp.nci.nih.gov.
[29] S.A. Rostom, Synthesis and in vitro antitumor evaluation of some indeno[1,2-c]pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems, Bioorg. Med. Chem. 14 (2006) 6475–6485.
[30] B.A.D. Neto, A.A.M. Lapis, Recent developments in the chemistry of deoxyribonucleic acid (DNA) intercalators: principles, design, synthesis, applications and trends, Molecules 14 (2009) 1725–1746.
[31] J.L. Nitiss, Targeting DNA topoisomerase II in cancer chemotherapy, Nat. Rev. Cancer 9 (2009) 338–350.
[32] J.L. Nitiss, DNA topoisomerase II and its growing repertoire of biological functions, Nat. Rev. Cancer 9 (2009) 327–337.
[33] J.R. Vance, K.F. Bastow, Inhibition of DNA topoisomerase II catalytic activity by the antiviral agents 7-chloro-1,3-dihydroxyacridone and 1,3,7-trihydroxyacridone, Biochem. Pharmacol. 58 (1999) 703–708.
[34] X. Yu, G. Zipp, G. Ray Davison, The effect of temperature and pH on the solubility of quinolone compounds: estimation of heat of fusion, Pharm. Res. 11 (1994) 522–527.
[35] D.L. Ross, C.M. Riley, Aqueous solubilities of some variously substituted quinolone antimicrobials, Int. J. Pharm. 63 (1990) 237–250.
[36] S.A. Breda, A.F. Jimenez-Kairuz, R.H. Manzo, M.E. Olivera, solubility behavior and biopharmaceutical classification of novel high-solubility ciprofloxacin and norfloxacin pharmaceutical derivatives, Int. J. Pharm. 371 (2009) 106–113.
[37] G.A. Abuo-Rahma, M. Abdel-Aziz, M. Mourad, H. Farag, Synthesis, anti-inflammatory activity and ulcerogenic liability of novel nitric oxide donating/chalcone hybrids, Bioorg. Med. Chem. 20 (2012) 195–206.
[38] M. Fukuda, K. Nishio, F. Kanzawa, H. Ogasawara, T. Ishida, H. Arioka, K. Bojanowski, M. Oka, N. Saijo, Synergism between cisplatin and topoisomerase I inhibitors, NB-506 and SN-38, in human small cell lung cancer cells, Cancer Res. 56 (1996) 789–793.
[39] D.H. Kang, J.S. Kim, M.J. Jung, E.S. Lee, Y. Jahng, Y. Kwon, Y. Na, New insight for fluoroquinophenoxazine derivatives as possibly new potent topoisomerase I inhibitor, Bioorg. Med. Chem. Lett. 18 (2008) 1520–1524.
[40] J.L. Vazquez, S. Merino, O. Domeinech, M. Berlanga, M. Vinas, M.T. Montero, J. Hernandez-Borrell, Determination of the partition coefficients of a homologous series of ciprofloxacin: influence of the *N*-4 piperazinyl alkylation on the antimicrobial activity, Int. J. Pharm. 220 (2001) 53–62.