HETEROCYCLES, Vol. 71, No. 10, 2007, pp. 2155 - 2172. © The Japan Institute of Heterocyclic Chemistry
Received, 2nd May, 2007, Accepted, 18th July, 2007, Published online, 20th July, 2007. COM-07-11097

HETEROCYCLES [*h*]-FUSED ONTO 4-OXOQUINOLINE-3-CARBOXYLIC ACID, III.[1] FACILE SYNTHESIS AND ANTITUMOR ACTIVITY OF MODEL HETEROCYCLES [*a*]-FUSED ONTO PYRIDO[2,3-*f*]QUINOXALINE-3-CARBOXYLIC ACIDS

Mohammad Y. Abu Shuheil,[a] Mona R. Hassuneh,[b] Yusuf M. Al-Hiari,[c] Ali M. Qaisi, [c] and Mustafa M. El-Abadelah [*a]

[a]Department of Chemistry, Faculty of Science, The University of Jordan, Amman, Jordan

[b]Department of Biological Sciences, Faculty of Science, The University of Jordan, Amman, Jordan

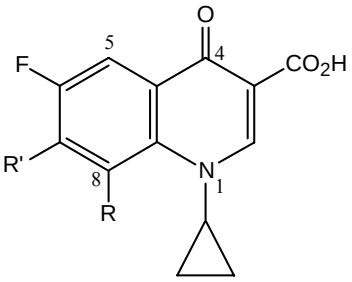
[c]Department of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman, Jordan

* E-mail: mustelab@ju.edu.jo; Fax: 00962 6 534 8932

Abstract–Direct interaction between 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid and each of (*S*)-proline, (2*S*,4*R*)-4-hydroxyproline and (*S*)–1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid in hot aqueous ethanolic $NaHCO_3$ yielded the corresponding optically pure *N*-(4-oxoquinolin-7-yl)-*α*-amino acids. The latter derivatives underwent reductive lactamization upon treatment with $Na_2S_2O_4$ in aqueous ethanol to afford moderate yields of the respective pyrido[2,3-*f*]quinoxaline-3-carboxylic acid [fused]- to tetrahydropyrrolo[1,2-*a*]-, tetrahydrohydroxylpyrrolo[1,2-*a*]-and tetrahydroisoquinolino[2,3-*a*]heterocyclics **10-12**, respectively. The antitumor activity against four human tumor cell lines showed that **10-12** displayed high levels of cytotoxicity as compared with Cisplatin. Interestingly, these compounds were more potent against breast carcinoma cell lines (MCF-7 and T-47D) than the lymphoid origin tumor cell lines (Jurkat and BHL-89). In particular, the (*S*)-proline derivative **10** exhibited preferential cytotoxicity to adherent cells (IC_{50} = 0.5 μM), indicative of better potential in blocking the growth of solid tumors rather than the disseminated ones.

INTRODUCTION

Synthetic fluoroquinolones (e.g., ciprofloxacin **1**)[2] represent a successful achievement towards the design and development of potent antiinfectious drugs,[2,3] while some related derivatives, such as **2**[4] and **3**,[5] (Chart 1) exhibit antitumor activity.[4-6] On the other hand, many biological properties and technical applications have been reported for compounds containing the quinoxalinone system,[7] and recently, a number of simple and heterocyclic-fused quinoxalinones, exemplified by **4**[8] and **5**,[9] have become interesting compounds for study as antitumor agents.[8-10]



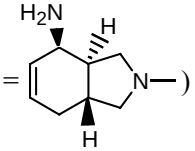
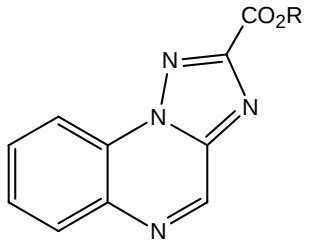
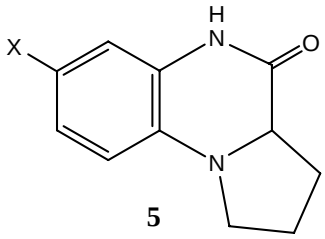
1 (R = H; R' = *N*-piperazinyl)
2 (R = F; R' = *p*-(OH) C_6H_4-)
3 (R = F; R = )

Chart 1



4



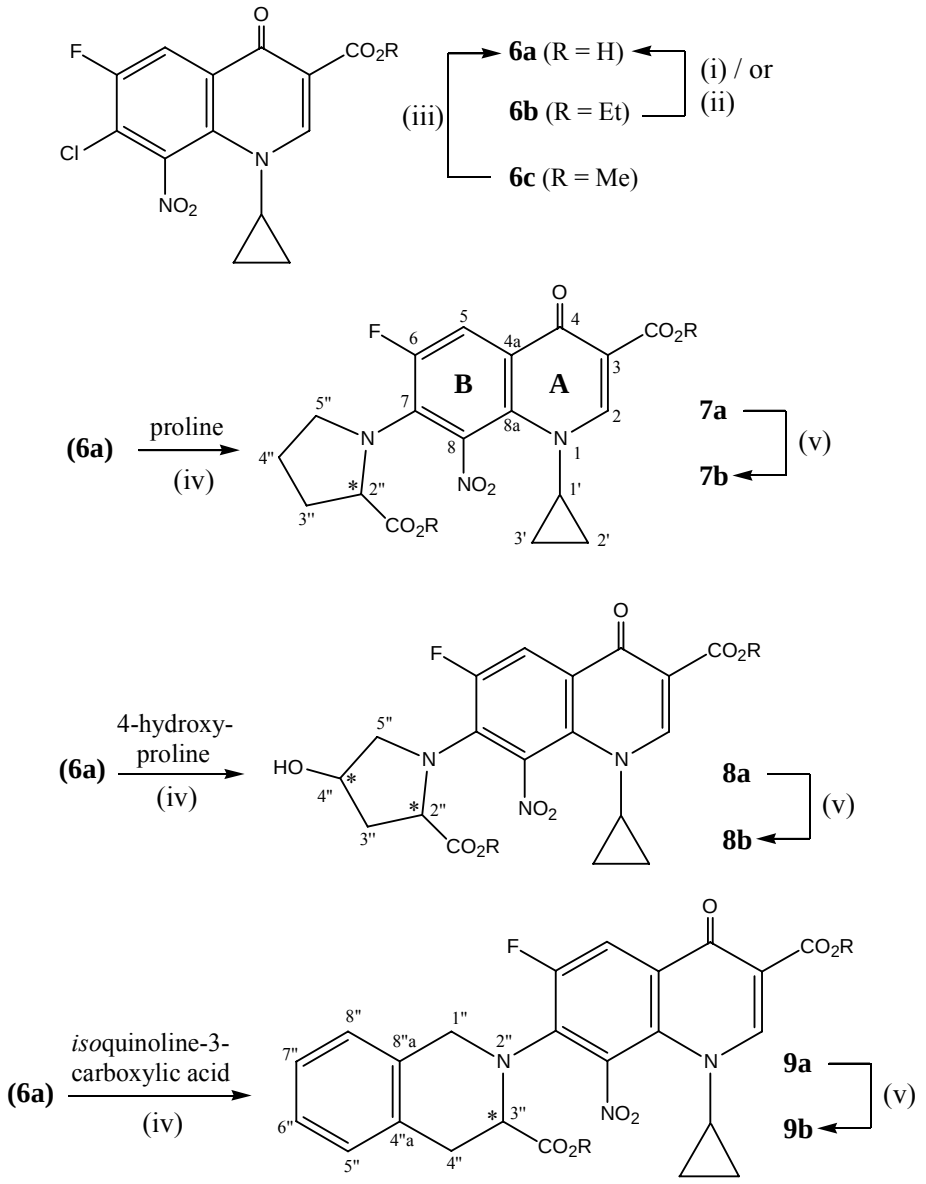
5

Owing to the potential biological interest in these heterocyclic compounds, the present work aims at the synthesis and characterization of model heterocyclic-fused pyrido[2,3-*f*]quinoxaline-3-carboxylic acids incorporating both fluoroquinolone and 3,4-dihydroquinoxalinone chemotypes. Such hybrid heterocyclic systems (**10-12 /** Scheme 2) might exhibit interesting bio-properties such as antimicrobial and / or antitumor activity.

RESULTS AND DISCUSSION

SYNTHESIS

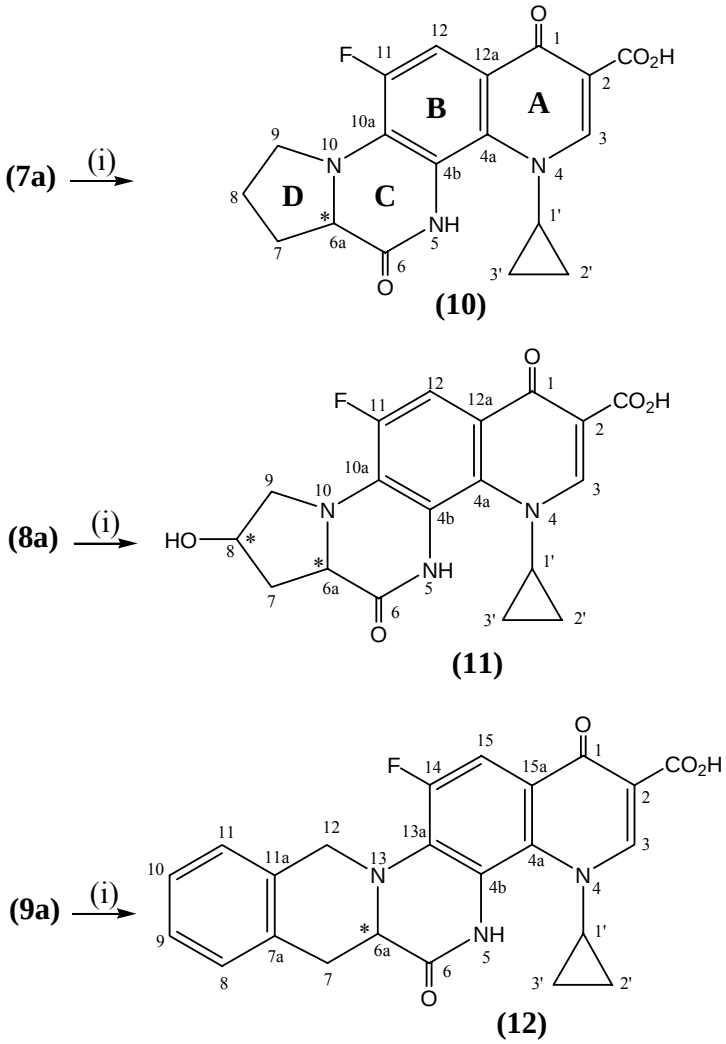
The synthon 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6a**), required in this study, is obtained by acid-catalysed hydrolysis of the corresponding ethyl ester (**6b**) using 12 % HCl [method (i)] or 50 % H_2SO_4 [method (ii)] as detailed in the Experimental part. Compared to the reported procedure[11] using AcOH and H_2SO_4, method (ii) afforded higher yield and improved purity of **6a**. The ethyl ester (**6b**) is prepared by following the procedure reported for the methyl ester analog (**6c**),[11, 12] and shows identical properties to that prepared by another route.[13] Direct interaction of **6a** with (*S*)-proline, (2*S*,4*R*)-4-hydroxyproline, or (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid in aqueous ethanol containing sodium hydrogencarbonate has been conducted at 65-85 °C for 1-2 days and worked-



Scheme 1. (i) 4*N* HCl / Δ, 24 h ; (ii) 50 % H_2SO_4 / Δ, 2 h compounds **7-9**
(**a**: R = H; **b**: R = Me)
(iii) AcOH + H_2O + H_2SO_4(8 : 7 : 1, v/v/v) / Δ, 2 h
(iv) $NaHCO_3$, 50 % aq. EtOH / Δ, then 3*N* HCl
(v) CH_2N_2 in Et_2O / 5-10 ^oC

up as detailed in the experimental section. This reaction follows an S_N -Ar (addition-elimination) path and is facilitated by the presence of the C(8)-nitro group to yield the corresponding chiral *N*-(4-oxoquinolin-7-yl)-α-amino acids (**7a-9a**) that were also converted to the respective methyl esters (**7b-9b**) upon treatment with diazomethane ethereal solution (Scheme 1). Reduction of **7a-9a** with sodium dithionite in aqueous potassium carbonate converts the 8-nitro group to an amino group, and is followed by spontaneous lactamization to afford good yields of the corresponding target products (**10-12** / Scheme 2). This preparative approach toward **10-12**, entails the construction of 3,4-dihydropyrazinone moiety onto an 8-nitro-4-oxoquinoline skeleton bearing the appropriate cyclic α-imino acid at C-7 (compounds **7a-9a**

/ Scheme 1) and is analogous to the methodology reported for the preparation of heterocyclic [*c*]-fused 3,4-dihydroquinoxalin-2-ones starting from *N*-(2-nitrophenyl)-cyclic imino acids. [9]



Scheme 2. (i) $Na_2S_2O_4$, aq. K_2CO_3 / 0-3 °C, then 3*N* HCl

SPECTRAL DATA

The spectral (IR, MS, NMR) and microanalytical data for the new compounds **7-12** are in conformity with the assigned structures, and are given in the Experimental part. Thus, the mass spectra of **7a-9a** (FAB), **7b-9b** (EI) and **10-12** (TOF ES$^+$) display the correct ions $[M]^+$, $[M+H]^+$ and $[M+Na]^+$ respectively, and for which the measured high resolution (HRMS) data are in good agreement with the calculated values. The ^{1}H- and ^{13}C- signal assignments are based on DEPT and 2D (COSY, HMQC, HMBC) experiments wherein the associated spectra showed correlations that helped in assigning the various signals to the different carbons and their attached / neighboring hydrogens. For compounds **10-12**, long-range correlations are observed between H-3 and each of C-4a, C-1', C-1 and C(2)-*C*O_2H as well as between H-12 and each of C-4a, C-1 and C-10a (in **10**, **11**), H-15 and each of C-4a, C-1 and C-13a (in **12**). Corresponding long-range correlations are also observed in **7-9** between H-2, H-5 and their neighbor

carbons. Those skeletal carbons of the fluorinated benzenoid ring (**B**) are recognizable through their characteristic signal-doublets arising from coupling with the fluorine atom, while through space $^{13}C\ldots..^{19}F$ coupling is noticeable for the stereogenic α –*CH carbon in **7-9**, and for the proximal methylene carbons (C-9 / C-12) in ring (**D**) of the respective cyclized products **10**, **11** / and **12**. It turned out that H-5 in **7-9**, which resonates at *ca.* 8.1 ppm (d, $^3J_{H\text{-}F} \approx 13$ Hz), shows sizable upfield shift in the corresponding annulated products **10-12** ($\delta \approx 7.6$ ppm). Similarly, the α –*CH proton (H-6a) is more shielded in **10-12** as compared to the respective uncyclized precursors (**7-9**). Yet, the cyclopropyl methine proton (H-1'), which resonates at *ca.* 3.6 ppm in **7-9**, experiences downfield shift (to about 4.4 ppm) in the annulated products **10-12**, probably due to the deshielding effect caused by the proximal lone pair at the pyrazinoid nitrogen (N5 / ring **C**).

OPTICAL PURITY DETERMINATION

The reaction conditions employed in the preparation of compounds **7-9** are fairly mild such that the stereocenter of the (*S*)-α-amino acid moiety is presumed to be unaffected, and hence the chiral products **7-9** are expected to be optically pure. This has been ascertained from optishift ^{1}H-NMR spectroscopic measurements in which we compared the behaviour of (*S*)-proline [(*S*)-**7b**] and racemic proline [(±)-**7b**] methyl ester derivatives in $CDCl_3$ in presence of the optically active tris 3-[heptafluoropropyl hydroxymethylene-(*d*)-camphorato] europium (III), [(+)- Eu $(hfc)_3$], as the chiral lanthanide shift reagent (LSR method).[14] One obvious criterion for optical purity was the α-methoxycarbonyl protons' signal at 3.60 ppm. This singlet of (±)-**7b** was resolved into two diastereotopic singlets (at 3.57 and 3.59 ppm) of equal integrated peak areas after the addition of (+)-Eu $(hfc)_3$ at molar ratio of [LSR] / [substrate] ≤ 0.27. No such splitting was observed for the case of (*S*)-**7b** in presence of (+)-Eu $(hfc)_3$ up to 0.54 molar ratio (≤ 3.59). These results indicate that (*S*)-**7b** is almost optically pure (ee ≥ 96 %), and infer that the *N*-arylation reaction of chiral α-amino acids with **6a** proceeds with no detectable racemization. These findings are in agreement with recently reported optishift ^{1}H-NMR experiments on the related *N*-(2-nitrophenyl)proline methyl ester using (+)-Eu $(hfc)_3$.[9]

The eventual chirality of the cyclic α-imino acid moieties in **7-9** is conceivably preserved during the mild reduction conditions with sodium dithionite, and the resulting cyclized products **10-12** are expected to be almost optically pure. This follows from the fact that reductive cyclization of *N*-(2-nitrophenyl)-(*S*)-proline with sodium dithionite has been reported to proceed without noticeable racemization of the resultant tetrahydropyrrolo[1,2-*a*]quinoxalin-4-one.[9]

ANTITUMOR ACTIVITY

Growth inhibitory effects of the novel compounds **10-12** were assessed by incubating the various cell

lines in the presence or absence of serial doubling dilutions of the test compounds. The data demonstrated that **10**-**12** significantly inhibited the growth of the lymphoid origin cell lines, Jurkat and BHl-89, in a dose dependant manner. The inhibitory effect diminished around the concentration 3 μM as shown in Figure 1. Interestingly, all compounds exhibited a slight increase of toxicity on tumor cells of lymphoid origin at lower concentrations (Figure 1).

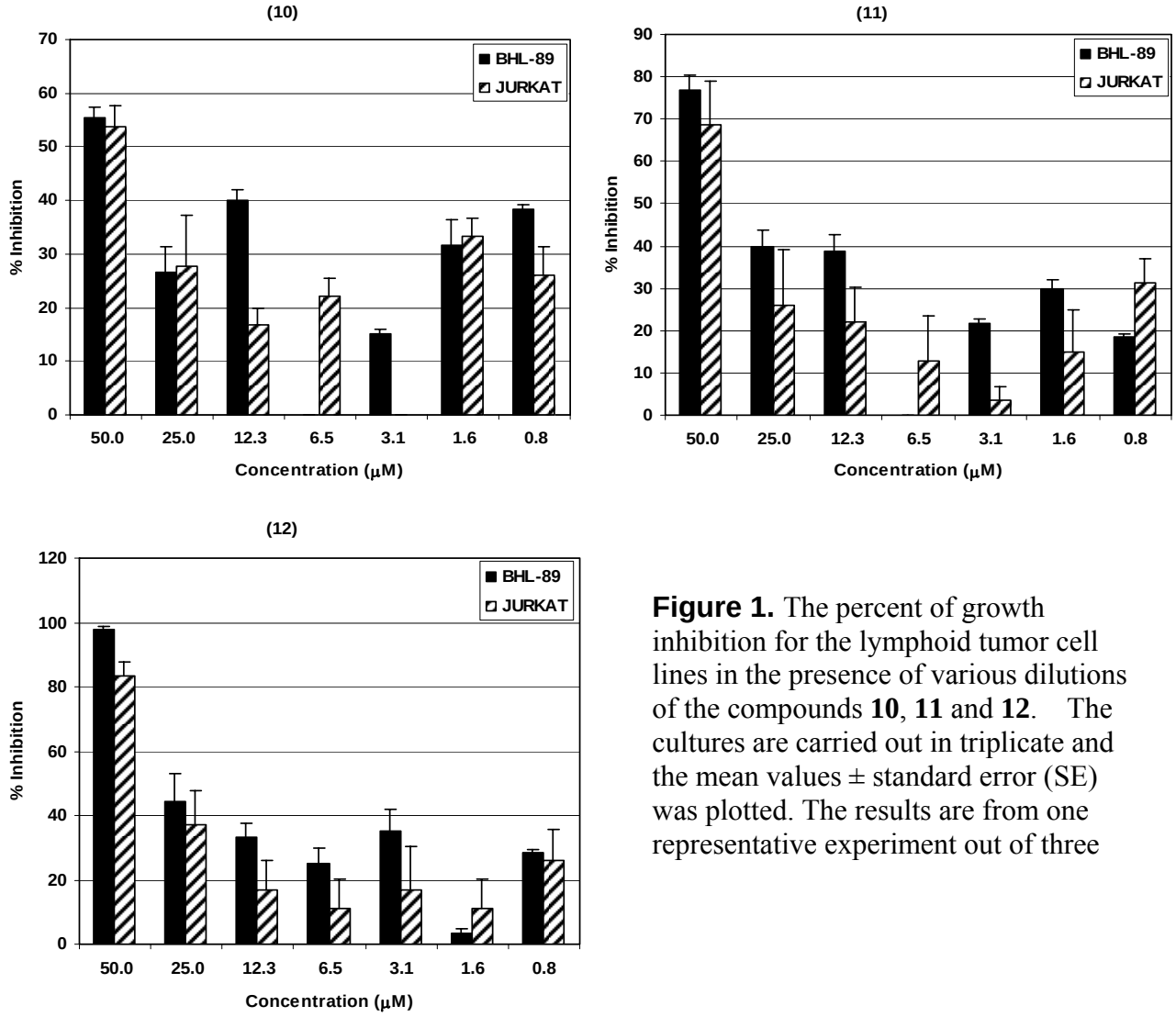


Figure 1. The percent of growth inhibition for the lymphoid tumor cell lines in the presence of various dilutions of the compounds **10**, **11** and **12**. The cultures are carried out in triplicate and the mean values ± standard error (SE) was plotted. The results are from one representative experiment out of three

This pattern was confirmed by multiple repetitions of the experiment. As for breast cancer cell lines, although we noticed a marked inhibition by all compounds, yet the pattern of inhibition was different for each of the cell lines (Figure 2). Compound **12** was significantly potent against MCF-7 showing up to 69 % inhibition at 0.8 μM concentration, whereas the same compound resulted in about 30 % inhibition for T-47D cell line at concentrations below 25 μM. On the other hand, compounds **10** and **11** were more potent inhibitors of T-47D cell line than of MCF-7.

Compounds **10**-**12** exhibited differential potency on the four cell lines, for which the IC_{50} values are shown in Table 1. The three compounds were more potent on the breast ductal carcinoma cell lines than

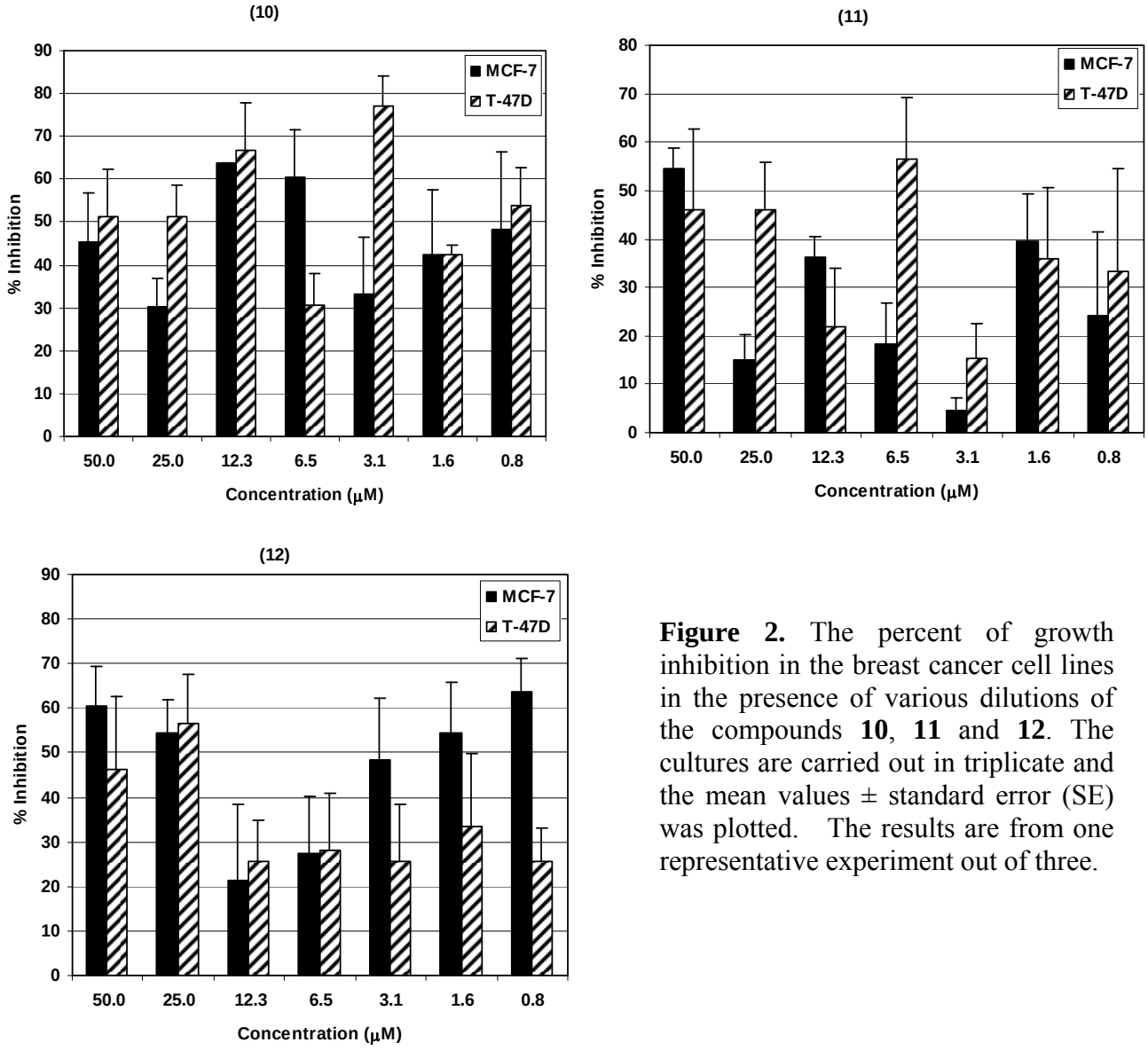


Figure 2. The percent of growth inhibition in the breast cancer cell lines in the presence of various dilutions of the compounds **10**, **11** and **12**. The cultures are carried out in triplicate and the mean values ± standard error (SE) was plotted. The results are from one representative experiment out of three.

on the lymphoid origin tumor cell lines. The IC_{50} for the B cell lymphoma BHL-89 were 20.8, 21.4 and 18.0 μM for compounds **10, 11** and **12**, respectively. Alternatively, for the T cell leukemia cell line (Jurkat) the IC_{50} values were relatively higher indicating lower potency. Moreover, the three compounds showed notably lower IC_{50} values when used on the breast ductal carcinoma cell lines T-47D and MCF-7, within the range 0.5-26.3 μM as summarized in Table 1.

Compounds **10**-**12** showed high cytotoxicity on cell lines of lymphoid origins but only in relatively high concentrations. Interestingly, all compound demonstrated a trend of increased cytotoxicity at lower concentration which warrants further investigations. Similar phenomenon is seen with other cytotoxic compounds whereby toxicity at high concentration was lower than at low concentration.[15,16] In such situations, the mechanism and antitumor effect are different from that seen at high concentrations. [15]

The IC_{50} for the three compounds was relatively higher in the Jurkat cell line than the rest of the cell lines. Jurkat cells have been shown to be supersensitive to topoisomerase inhibitor- mediated apoptosis.[17] Thus,

the lower efficacy of these compounds towards Jurkat tumor cells suggests that the tested compound might mediate cell death through topoisomerase independant pathway unlike their ancestral molecules. Moreover, the higher efficacy against breast cancer (adherent) cell lines as evidenced by the lower IC_{50} values, would suggest that these compounds are more effective on adherent cells. It has been shown that cells adherent to plastic, as in the case of (MCF-7 and T-47D) that express β1 integrins, are resistant to cytotoxic drugs that induce cell death by DNA cross-linking and topoisomerase II inhibitors.[18] Such preferential cytotoxicity to adherent cells by these compounds might indicate better potential in blocking the growth of solid tumors rather than the disseminated ones. Such tumors are usually more resistant to chemotherapeutics and usually require cocktail of multi-drugs in order to be effectively inhibited. Most importantly are the high levels of cytotoxicity achieved by these compounds as compared with other commercially available chemotherapeutic drugs such as cisplatin. For instance, the IC_{50} of cisplatin on various tumor cell lines ranged from 2.5 to 30 μM.[19] The tested compounds achieved 50 % inhibition or higher at concentrations of 1.6 and 0.8 μM as shown in Figures 1 and 2.

Table 1. The IC_{50} values for compounds **10-12** on the different tumor cell lines. The values were calculated through non linear regression; (R^2) correlation coefficient for the data obtained for each of the compounds is indicated.

Cell Lines	**(10)**	**(11)**	**(12)**
Lymphoid Origin			
BHL-89	20.8 μM (R^2 = 1)	21.4 μM (R^2 = 0.9)	18.0 μM (R^2 = 0.9)
Jurkat	44.9 μM (R^2 = 0.9)	37.5 μM (R^2 = 1)	26.5 μM (R^2 = 0.9)
Breast Carcinoma			
T-47D	4.1 μM (R^2 = 1)	6.1 μM (R^2 = 1)	12.5 μM (R^2 = 0.9)
MCF-7	0.5 μM (R^2 = 1)	26.3 μM (R^2 = 0.9)	10.5 μM (R^2 = 1)

The fact that 100 % cytotoxicity was not achieved for the tested compounds, suggests that these compounds might work at different, narrow or at even lower ranges of concentration. Thus, future studies are needed to assess the pharmacokinetics and biopharmaceutics of these compounds. In the current study we have introduced a group of new compounds exhibiting excellent potential as antitumor therapeutic agents. Further investigations are needed to assess the cytotoxic activity of **10-12** on a wider array of tumor cell lines. Also, the deciphering of the mechanism of their action as well as their *in vivo* effectiveness as antitumor agents is yet to be determined.

EXPERIMENTAL

The secondary *α*-amino acids: (*S*)-proline, trans(2*S*, 4*R*)-4-hydroxyproline, and (*S*)-isoquinoline-3-carboxylic acid, employed in this study, are biochemical grades (Acros) and were used as received. 2,4-Dichloro-5-fluoro-3-nitrobenzoic acid was purchased from Acros. Tris-[3-(heptafluoropropylhydroxy methylene)-(*d*)-camphorato]europium (III), [(+)-Eu (hfc)$_3$] was purchased from Aldrich. Melting points (uncorrected) were determined on a Gallenkamp electrothermal melting temperature apparatus. Optical rotations were measured on a Perkin-Elmer 141 polarimeter with a micro cell (100 mm path length, 1 mL), at 22 ^{o}C as solutions in $CHCl_3$ (*c*~ 0.6-1) for the esters and in DMSO (*c*~ 0.6-1), or in 5 % aqueous K_2CO_3 (*c*~ 0.6-1) for the acids. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DPX-300 instrument with Me_4Si as internal reference. EIMS spectra were obtained using a Finnigan MAT TSQ-70 spectrometer at 70 eV; ion source temperature = 200 ^{o}C. High resolution MS-ESI data were obtained with Bruker Bio TOF III. IR spectra were recorded as KBr discs on a Nicolet Impact-400 FT-IR spectrophotometer. Microanalyses were preformed at the Microanalytical Laboratory – Medicinal Chemistry division, Faculty of Pharmacy, Jordan University, Amman.

Cell lines: The breast carcinoma cell lines (MCF-7 and T-47D) and the lymphocytic tumor cell line (Jurkat) were kindly provided by Dr. M. Nagarkatti at the Medical College of Virginia Commonwealth University, USA. The B lymphoma cell line (BHL-89) was purchased from the European Cell Culture Collection (ECCC), UK. All tumor cell lines were maintained in complete RPMI-1640 medium supplemented with 10 % fetal bovine serum and 2 mM L-glutamine, 40 μg / mL gentamycine and 10 mM HEPES buffer at pH 7.2 and grown in tissue culture flasks at 37°C, 5 % CO_2, 95 % relative humidity.

Ethyl 7-Chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6b)

The title synthon, required in this work, is prepared from 2,4-dichloro-5-fluoro-3- nitrobenzoic acid, ethyl 3-(*N*,*N*-dimethylamino)acrylate and cyclopropyl amine by following the stepwise synthetic procedures reported for the methyl ester analog, [11, 12] [mp 175-176 ^{o}C (decomp.); Lit., [13] 174-176 ^{o}C (decomp.)].

7-Chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a)

This compound is prepared by acid-catalyzed hydrolysis of the corresponding ethyl ester **6b** according to the following procedures:

Mehtod (i): A vigorously stirred suspension of **6b** (5.3g, 15 mmol) in 12 % aqueous HCl (200 mL) and EtOH (60 mL) was heated at 80-85 °C under reflux. Progress of the ester hydrolysis was monitored by TLC and was completed within 24-30 h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (500 g) and the resulting heavy faint yellow precipitate was collected, washed with cold water (3 x 20 mL), dried and recrystallized from $CHCl_3$ / EtOH. Yield 4.5 g (91 %), mp 256 – 257 ^{o}C (decomp.)

[Lit.,[13] 261 °C (decomp.)]. *Anal*. Calcd for $C_{13}H_8ClFN_2O_5$: C, 47.80; H, 2.47; N, 8.58. Found: C, 47.68; H, 2.41; N, 8.36; IR (KBr): *v* 3438, 3080, 3049, 2900, 1721, 1604, 1579, 1548, 1493, 1338, 1258 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 1.02, 1.16(2m, 4H, H_2-2′ / H_2-3′), 3.71(m, 1H, H-1′), 8.45(d, $^3J_{H\text{-}F}$ = 8 Hz, 1H, H-5), 8.78(s, 1H, H-2), 13.70(s, 1H, CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.2 (C-2′/ C-3′). 39.5(C-1′), 109.7(C-3), 115.3(d, $^2J_{C\text{-}F}$ = 23 Hz, C-5), 122.7(d, $^2J_{C\text{-}F}$ = 23.6 Hz, C-7), 128.3 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C-4a), 131.9(d, $^4J_{C\text{-}F}$ = 2.3 Hz, C-8a), 141.1(d, $^3J_{C\text{-}F}$ = 1.6 Hz, C-8), 153.4(C-2), 154.5(d, $^1J_{C\text{-}F}$ = 250 Hz, C-6), 164.8(CO_2H), 175.4(d, $^4J_{C\text{-}F}$ = 2.2 Hz, C-4).

Method (ii): A vigorously stirred suspension of **6b** (5.3 g, 15 mmol) in 50 % H_2SO_4 (50 mL) and EtOH (50 mL) was heated at 80-85 °C under reflux. Progress of the ester hydrolysis was monitored by TLC and was completed within 2-3 h. Thereafter, the reaction mixture was worked up as described in method (a) above and recrystallized from $CHCl_3$ / EtOH. Yield 4.55 g (93 %), mp 256 – 257 °C (decomp.).

(*S*)-7-(2-Carboxypyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ((*S*)- 7a)

A stirred mixture of (*S*)-proline (1.1 g, 9.6 mmol), 7-chloro-1-cyclopropyl-6-fluoro-8- nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6a)** (1.0 g, 3.1 mmol) and $NaHCO_3$ (1.5 g, 18 mmol) in 50 % aqueous EtOH (140 mL) was heated at 60-65 °C for 20-24 h under reflux. The mixture slowly developed a light yellow color that changed into bright yellow, then into clear orange solution. The progress of the reaction was monitored by TLC, and was completed within 20-24 h. The orange solution was extracted with CH_2Cl_2 (50 mL) and the aqueous layer was separated, acidified with 3*N* HCl to pH 6.5 and re-extracted with CH_2Cl_2 (50 mL). The aqueous layer was again separated, cooled and re-acidified with 3*N* HCl to pH 3-4, whereby the title compound was precipitated as yellowish solid which was collected by suction filtration, washed with cold water (2 x 10 mL), dried and recrystallised from *i*-PrOH. Yield 1.1 g (87 %), mp 192 – 193 °C (decomp.); $[\alpha]_D$ -302.1° (5 % aq. Na_2CO_3, c~1). *Anal*. Calcd for $C_{18}H_{16}FN_3O_7$: C, 53.34; H, 3.98; N, 10.37. Found: C, 53.57; H, 4.00; N, 10.34; IR (KBr): *v* 3480, 3160, 3074, 2980, 2871, 1745, 1734, 1694, 1608, 1532, 1447, 1370, 1319 cm^{-1}; MS(FAB): *m/z* (% rel.int.): 406[100, $(M+H)^+$/calcd. for $C_{18}H_{16}FN_3O_7$ 405(M)]; MS(EI): 385(2, M-HF), 360(4), 343(3), 327(11), 299(6), 283(92), 268(100), 254(39), 244(56), 227(19), 218(97), 203(21), 189(22), 172(22), 133(20), 107(14); ^1H NMR (300 MHz, DMSO-d_6): δ 0.97, 1.02(2m, 4H, H_2-2′/ H_2-3′), 1.88(m, 2H, H_2-4″), 1.93(m, 1H, H_A-3″), 2.32(m, 1H, H_B-3″), 3.21(m, 1H, H_A-5″), 3.52 (m, 1H, H_B-5″), 3.68 (m, 1H, H-1′), 4.46 (dd, *J* = 6.6, 4.7 Hz, 1H, H-2″), 8.10 (d, $^3J_{H\text{-}F}$ = 13.1 Hz, 1H, H-5), 8.71 (s, 1H, H-2), 12.77 (br s, 1H, C(2″)-CO_2H), 14.29 (br s, 1H, C(3)-CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 10.4, 11.4(C-2′/ C-3′), 25.4(C-4″), 30.6(C-3″), 39.7(C-1′), 53.4(C-5″), 63.9(d, $J_{C\text{-}F}$ = 8.6 Hz, C-2″), 108.8(C-3), 113.6(d, $^2J_{C\text{-}F}$ = 23.6 Hz, C-5), 121.4(d, $^3J_{C\text{-}F}$ = 7.9 Hz, C-4a), 133.0 (C-8a), 136.4(d, $^3J_{C\text{-}F}$ = 5.9 Hz, C-8), 138.4(d, $^2J_{C\text{-}F}$

= 15.2 Hz, C-7), 152.7(C-2), 155.4(d, $^{1}J_{C\text{-}F}$ = 251 Hz, C-6), 165.3(C(3)-*C*O_2H), 173.2(d, C(2′′)-*C*O_2H), 175.7(d, $^{4}J_{C\text{-}F}$ = 2.3 Hz, C-4).

(*S*)- Methyl 1-cyclopropyl-6-fluoro-7-[2-(methoxycarbonyl)pyrrolidin-1-yl]-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate ((*S*)- 7b)

To a fine powder of (*S*)-**7a** (0.41 g, 1.0 mmol) suspended in cold Et_2O (20 mL), was added portionwise a cold fresh diazomethane ethereal solution until evolution of nitrogen has ceased. The reaction mixture was kept at 5-10 °C for 20 min, and the solvent was then evaporated at rt whereby the title compound was obtained as yellow solid. Yield 0.39 g (91 %), mp 135 – 136 °C (decomp.); $[\alpha]_D$ +230.1° ($CHCl_3$, *c*~1). *Anal*. Calcd for $C_{20}H_{20}FN_3O_7$: C, 55.43; H, 4.65; N, 9.70. Found: C, 55.66; H, 4.87; N, 9.94;IR (KBr): *v* 3004, 2978, 2843, 1753, 1702, 1651, 1617, 1540, 1456, 1336, 1277, 1243, 1202,1191, 1132 cm^{-1}; MS (EI) : *m/z* (% rel. int.): 433(2, M^+), 416(2), 374(100), 343(1), 327(18), 295(7), 258(7), 185(4), 167(4), 149(25), 111(6); MS (TOF ES $^+$): *m/z* 456 $(M+ Na)^+$, HRMS: calcd for $C_{20}H_{20}FN_3O_7Na$ 456.1183, found 456.1177; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01, 1.07(2m, 4H, H_2-2′/ H_2-3′), 2.02 (m, 2H, H_2-4′′), 2.16(m, 1H, H_A-3′′), 2.30(m, 1H, H_B-3′′), 3.13(m, 1H, H_A-5′′), 3.56 (m, 1H, H_B-5′′ overlapped with C*H*$_3$ signal), 3.59(s, 3H, C(3)-CO_2CH_3), 3.62 (m, 1H, H-1′), 3.88(s, 3H, C(2′′)-CO_2CH_3), 4.31 (dd, *J* = 8.2 Hz, 8.3 Hz, 1H, H-2′′), 8.24 (d, $^{3}J_{H\text{-}F}$ = 11.9 Hz, 1H, H-5), 8.60 (s, 1H, H-2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.6, 11.2(C-2′/ C-3′), 25.5(C-4′′), 30.0(C-3′′), 37.8(C-1′), 52.1(C(2′′)- CO_2CH_3), 52.4(C(3)-CO_2CH_3), 54.9(C-5′′), 64.0(d, $J_{C\text{-}F}$ = 7.0 Hz, C-2′′), 111.1(C-3), 115.8(d, $^{2}J_{C\text{-}F}$ = 23.0 Hz, C-5), 127.7 (d, $^{3}J_{C\text{-}F}$ = 6.9 Hz, C-4a) 130.9((d, $^{4}J_{C\text{-}F}$ = 2.0 Hz, C-8a), 136.9(d, $^{2}J_{C\text{-}F}$ = 16.3 Hz, C-7), 141.3(d, $^{3}J_{C\text{-}F}$ = 4.2 Hz, C-8), 151.7(C-2), 156.9(d, $^{1}J_{C\text{-}F}$ = 252 Hz, C-6), 165.2(C(3)-*C*O_2Me), 171.4(d, $^{4}J_{C\text{-}F}$ = 1.8 Hz, C-4), 172.7(C(2′′)-*C*O_2Me).

(±)-7-(2-Carboxypyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ((±)-7a)

This compound was prepared from (±)-proline (1.1 g, 9.6 mmol), and **6a** (1.0 g, 3.1 mmol) following the same procedure and experimental conditions noted above for (*S*)-**7a**. Yield 1.0g (80 %), mp 180 – 182 °C (decomp.); *Anal*. Calcd for $C_{18}H_{16}FN_3O_7$ C, 53.34; H, 3.98; N, 10.37. Found C, 53.12; H, 3.93; N, 10.20; MS(FAB): *m/z* (% rel.int.): 406[100, $(M+H)^+$/calcd for $C_{18}H_{16}FN_3O_7$ 405(M)]; Other spectral data are identical to that of its enantiomer (*S*)- **7a**.

(±)-Methyl 1-cyclopropyl-6-fluoro-7-[2-(methoxycarbonyl)pyrrolidin-1-yl]-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate ((±)-7b)

This compound was prepared *via* the reaction of (±)-**7a** (0.23 g, 0.57 mmol) with diazomethane ethereal solution following a similar procedure noted above for (*S*)- **7b**. Yield 0.22 g (89 %), mp 175 – 176 °C

(decomp.). *Anal*. Calcd for $C_{20}H_{20}FN_3O_7$: C, 55.43; H, 4.65; N, 9.70. Found: C, 55.08; H, 4.68; N, 9.90; MS(EI): *m/z* (%): 433 (M^+), HRMS: calcd for $C_{20}H_{20}FN_3O_7$: 433.12848, found 433.13116; Other spectral data are identical to that of its enantiomer (*S*)- **7b**.

(2″*S*,4″*R*)-7-(2″-Carboxypyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4″-hydroxy-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ((2″*S*, 4″*R*)- 8a)

A stirred mixture of (2*S*,4*R*)-4-hydroxyproline (1.2 g, 9.2 mmol), **6a** (1.0 g, 3.1 mmol) and $NaHCO_3$ (1.5 g, 18 mmol) in 50 % aqueous EtOH (140 mL) was heated at 60-65 °C for 20-24 h. Work-up of the resulting reaction mixture as described for **7a** above, produced the title compound as pale yellow solid which was recrystallized from *i*-PrOH. Yield 1.15g (89 %), mp 206 – 207 °C (decomp.); $[\alpha]_D$ -399.7° (5 % aq. $NaHCO_3$, c~1). *Anal*. Calcd for $C_{18}H_{16}FN_3O_8$: C, 51.31; H, 3.83; N, 9.97. Found: C, 50.98; H, 3.86; N, 9.75; IR (KBr): *v* 3636, 3545, 3487, 3082, 2957, 1738, 1716, 1617, 1534, 1452, 1377, 1311, 1212, 1162, 1079 cm^{-1} ; MS(FAB): *m/z* (% rel. int.): 422 [100, $(M+H)^+$ / calcd for $C_{18}H_{16}FN_3O_8$ 421 (M)]; ^1H NMR (300 MHz, DMSO-d_6): δ 0.88, 1.03(2m, 4H, H_2-2′/ H_2-3′), 1.98(m, 1H, H_A-3″), 2.21(m, 1H, H_B-3″), 3.01(m, 1H, H_A-5″), 3.68(m, 1H, H_B-5″), 3.67(m, H, H-1′), 4.35(br m, 1H, H-4″), 4.80(m, 1H, H-2″), 5.15(br s, C(4″)-OH), 8.09(d, $^3J_{H\text{-}F}$ = 13.8 Hz, 1H, H-5), 8.71(s, 1H, H-2), 12.83(br s, 1H, C(2″)-CO_2H), 14.44(br s, 1H, C(3)-CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 10.0, 11.8(C-2′/ C-3′), 38.9(C-3″), 39.8(C-1′), 60.5(C-5″), 62.1(d, $J_{C\text{-}F}$ = 10.8 Hz, C-2″), 68.8(C-4″), 108.6(C-3), 113.1(d, $^2J_{C\text{-}F}$ = 23.8 Hz, C-5), 119.8(d, $^3J_{C\text{-}F}$ = 8.0 Hz, C-4a), 133.4 (C-8a), 134.0(d, $^3J_{C\text{-}F}$ = 5.0 Hz, C-8), 138.4(d, $^2J_{C\text{-}F}$ = 14.5 Hz, C-7), 152.5(C-2), 154.6(d, $^1J_{C\text{-}F}$ = 251.6 Hz, C-6), 165.6(C(3)-CO_2H), 173.3(C(2″)-CO_2H), 175.6(d, $^4J_{C\text{-}F}$ = 2.1 Hz, C-4).

(2″*S*,4″*R*)-Methyl 1-cyclopropyl-6-fluoro-4″-hydroxy-7-[2″-(methoxycarbonyl)pyrrolidin-1-yl]-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate ((2″*S*, 4″*R*)-8b)

This compound was prepared *via* the reaction of (2″*S*,4″*R*) –**8a** (0.42 g, 1.0 mmol) with diazomethane ethereal solution following the procedure noted above for **7b**. The product was recrystallized from benzene / pet. ether (bp 40-60 °C). Yield 0.30 g (67 %), mp 105 – 106 °C (decomp. / the crystalls changed color at 90 °C); $[\alpha]_D$ +68.6° ($CHCl_3$, *c*~1). *Anal*. Calcd for $C_{20}H_{20}FN_3O_8$: C, 53.45; H, 4.49; N, 9.35. Found: C, 53.30; H, 4.54; N, 9.31; IR (KBr): *v* 3422, 3092, 3006, 2950, 1734, 1703, 1616, 1542, 1462, 1314, 1240, 1190, 1159, 1104, 1024 cm^{-1}; MS(EI): *m/z* (% rel. int.): 449 [3, (M^+)], HRMS: calcd for $C_{20}H_{20}FN_3O_8$ 449.12339, found 449.12381; ^1H NMR (300 MHz, $CDCl_3$): δ 1.01, 1.09(2m, 4H, H_2-2′/ H_2-3′), 2.27(m, 1H, H_A-3″), 2.36(m, 1H, H_B-3″), 3.16(d, *J* = 10.1 Hz, 1H, H_A-5″), 3.60(s, 4H, C(2″)-CO_2CH_3 and H-1′/ superimposed), 3.82(dd, *J* = 10.1 Hz, 3.5 Hz, 1H, H_B-5″), 3.89(s, 3H, C(3)-CO_2CH_3), 4.55(br m, 1H, H-4″), 4.74(m, 1H, H-2″), 8.18(d, $^3J_{H\text{-}F}$ = 12.3 Hz, 1H, H-5), 8.58(s, 1H,

H-2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.8, 10.9(C-2′/ C-3′), 37.9(C-1′), 38.8(C-3″), 52.3(C(3)-CO_2CH_3), 52.4(C(2″)-CO_2CH_3), 61.6(d, $J_{C\text{-}F}$ = 8.6 Hz, C-2″), 61.8(C-5″), 70.5(C-4″), 111.1(C-3), 115.6(d, $^2J_{C\text{-}F}$ = 23.2 Hz, C-5), 126.7(d, $^3J_{C\text{-}F}$ = 7.1 Hz, C-4a), 131.3 (d, $^4J_{C\text{-}F}$ = 2 Hz,C-8a), 136.1(d, $^2J_{C\text{-}F}$ = 15.8 Hz, C-7), 139.4(d, $^3J_{C\text{-}F}$ = 4.8 Hz, C-8), 151.8(C-2), 156.2(d, $^1J_{C\text{-}F}$ = 252 Hz, C-6), 165.2(C(3)-CO_2Me), 171.3(d, $^4J_{C\text{-}F}$ = 1.8 Hz, C-4), 172.2(C(2″)-CO_2Me).

(*S*)-7-(3-Carboxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ((*S*)- 9a)

A stirred mixture of (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (2.0 g, 9.4 mmol), **6a** (1.0 g, 3.1 mmol) and $NaHCO_3$ (2.1 g, 25 mmol) in 50 % aqueous EtOH (140 mL) was heated at 75-80 °C for 48 h. Work-up of the resulting reaction mixture as described for **7a** above, produced the title compound as pale yellow solid which was purified by coloumn chromatography (Si gel, eluting with $CHCl_3$ then with MeOH (97 : 3 v/v) and recrystallized from MeOHl. Yield 0.65 g (45 %); comparable yield of the title compound (*S*)-**9a** was likewise obtained when (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid was employed (in placement of its hydrochloride salt); mp 217 – 218 ^{o}C (decomp.); $[\alpha]_D$ + 151.2^o (DMSO, c~1). *Anal*. Calcd for $C_{23}H_{18}FN_3O_7$: C, 59.10; H, 3.88; N, 8.99. Found : C, 59.03; H, 3.92; N, 8.90;IR (KBr): ν 3432, 3105, 3061, 2950, 1734, 1691, 1604, 1542, 1512, 1444, 1320, 1252 cm^{-1}; MS (TOF, ES$^+$): *m/z* 468 $(M + H)^+$, HRMS: calcd for $C_{23}H_{19}FN_3O_7$ 468.1207, found 468.1202; *m/z* 490 $(M + Na)^+$, HRMS: calcd for $C_{23}H_{18}FN_3O_7Na$ 490.1026, found 490.1021; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.93, 1.05(2m, 2H) and 1.12(m, 2H) (H_2-2′ / H_2-3′), 3.01(dd, J = 15.8 Hz, 5.5 Hz, 1H, H_A-4″), 3.14(dd, J = 15.8 Hz, 4.8 Hz, 1H, H_B-4″), 3.68 (m, 1H, H-1′), 4.25(d, J = 14.9 Hz, 1H, H_A-1″), 4.39(br dd, J = 4.8 Hz, 5.5 Hz, 1H, H-3″), 4.62(d, J = 14.9 Hz, 1H, H_B-1″), 7.08(m, 1H) and 7.17(m, 3H) (H-5″, H-6″, H-7″, H-8″), 8.28 (d, $^3J_{H\text{-}F}$ = 11.3 Hz, 1H, H-5), 8.77(s, 1H, H-2), 12.89 (br s, 1H, C(3″)-CO_2H), 14.08 (br s, 1H, C(3)-CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 10.8, 11.4(C-2′/ C-3′), 32.3(C-4″), 39.1(C-1′), 52.5(br, C-1″), 61.2(d, $J_{C\text{-}F}$ = 3.8 Hz, C-3″), 109.0(C-3), 114.8(d, $^2J_{C\text{-}F}$ = 23.2 Hz, C-5), 125.6(d, $^3J_{C\text{-}F}$ = 7.7 Hz, C-4a), 126.1, 126.5, 127.0, 129.1(C-5″, C-6″, C-7″, C-8″), 132.2(d, $^4J_{C\text{-}F}$ = 2 Hz, C-8a), 132.6, 133.4(C-4″a, C-8″a), 139.4(d, $^2J_{C\text{-}F}$ = 17.5 Hz, C-7), 141.5(d, $^3J_{C\text{-}F}$ = 4.8 Hz, C-8), 153.1(C-2), 154.5(d, $^1J_{C\text{-}F}$ = 213 Hz, C-6), 165.1(C(3)-CO_2H), 172.4(C(3″)-CO_2H), 175.9(d, $^4J_{C\text{-}F}$ = 2.3 Hz, C-4).

(*S*)-Methyl 7-(3-Carboxy-1,2,3,4-tetrahydroisoquinolin-2-yl)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate ((*S*)-9b)

This compound was prepared *via* the reaction of (*S*)-**9a** (0.28 g, 0.6 mmol) with diazomethane ethereal solution following the procedure noted above for **7b**. Yield 0.19 g (64 %), mp 120 – 122 ^{o}C (decomp.;

softens at 75 °C); $[\alpha]_D$ +134.7° ($CHCl_3$, *c*~1). *Anal*. Calcd for $C_{25}H_{22}FN_3O_7$: C, 60.60; H, 4.48; N, 8.48. Found : C, 60.81; H, 4.51; N, 8.61; IR (KBr): *v* 3469, 3073, 3024, 2956, 2926, 1740, 1703, 1647, 1610, 1549, 1462, 1239, 1202, 1172 cm^{-1}; MS (EI) : *m/z* (% rel. int.): 495(3, M^+), 478(6), 461(12), 436(100), 402(77), 390(49), 304(12), 263(10), 214(9), 167(17), 149(69), 130(30), 115(20); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89(m, 1H) and 1.08(m, 3H) (H_2-2′ / H_2-3′), 3.14(dd, *J*= 16.0 Hz, 6.1 Hz, 1H, H_A-4″), 3.24(dd, *J* = 16.0 Hz, 4.8 Hz, 1H, H_B-4″), 3.60(s, 3H, C(3″)-CO_2CH_3), 3.62 (m, 1H, H-1′), 3.91(s, 3H, C(3)-CO_2CH_3), 4.20(d, *J*= 16.0 Hz, 1H, H_A-1″), 4.42(dd, *J*= 6.1 Hz, 4.8 Hz, 1H, H-3″), 4.65(d, *J*= 16.0 Hz, 1H, H_B-1″), 7.01(m, 1H) and 7.18(m, 3H) (H-5″, H-6″, H-7″, H-8″), 8.30 (d, $^3J_{H\text{-}F}$ = 11.5 Hz, 1H, H-5), 8.63(s, 1H, H-2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.4, 11.6(C-2′/ C-3′), 32.1(C-4″), 37.6(C-1′), 52.2(C(3″)-CO_2CH_3), 52.4(C(3)-CO_2CH_3), 52.5(C-1″), 60.7(d, $J_{C\text{-}F}$ = 3.8 Hz, C-3″), 111.2(C-3), 116.3(d, $^2J_{C\text{-}F}$ = 22.8 Hz, C-5), 125.8, 126.4, 126.8 and 128.7(C-5″, C-6″, C-7″, C-8″), 129.0(d, $^3J_{C\text{-}F}$ = 6.1 Hz, C-4a), 130.8(d, $^4J_{C\text{-}F}$ = 1.8 Hz, C-8a), 131.9, 132.8(C-4″a, C-8″a), 137.5(d, $^2J_{C\text{-}F}$ = 17.3 Hz, C-7), 141.4(d, $^3J_{C\text{-}F}$ = 4.0 Hz, C-8), 151.9(C-2), 157.5(d, $^1J_{C\text{-}F}$ = 253 Hz, C-6), 165.2(C(3)-CO_2Me), 171.3(d, $^4J_{C\text{-}F}$ = 2.3 Hz, C-4), 171.5(C(3″)-CO_2Me).

(*S*)- 4-Cyclopropyl-11-fluoro-1,6-dioxo-1,4,5,6,6a,7,8,9-octahydropyrido[2,3-*f*]pyrrolo[1,2-*a*]-quinoxaline- 2-carboxylic acid ((*S*)- 10)

To a stirred and cooled (0-3 °C) solution of (*S*)-**7a** (0.41 g, 1.0 mmol) and of K_2CO_3 (0.96 g, 7.0 mmol) in 20 mL water, was added dropwise a solution of sodium dithionite (0.87 g, 5.0 mmol) in 5 mL water. The reaction mixture was worked-up immediately after completion of the addition of sodium dithionite, whereby the pH of the solution was adjusted to about 4. The precipitated product was filtered, washed with water, air-dried, recrystallized twice from MeOH / $CHCl_3$ (2 :1, v /v), and was further purified by preparative TLC chromatography. Yield 0.19 g (54 %), mp >300 °C (darkens at 290 °C); $[\alpha]_D$ - 309.3° (5 % aq. K_2CO_3, *c*~1). *Anal*. Calcd for $C_{18}H_{16}FN_3O_4$: C, 60.50; H, 4.51; N, 11.76. Found: C, 60.23; H, 4.34; N, 11.51; IR (KBr): *v* 3396, 3290, 3072, 2982, 2885, 1736, 1677, 1626, 1506, 1438, 1319, 1064 cm^{-1} ; MS (TOF ES $^+$): *m/z* 380 (M+ Na)$^+$, HRMS: calcd for $C_{18}H_{16}FN_3O_4Na$ 380.1023, found 380.1017; MS(EI): *m/z* (% rel. int.): 357(71), 339(7), 313(100), 284(20), 256(51), 228(30), 216(10), 202(9), 147(6), 107(3); ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83(m, 2H) and 1.08, 1.28(2m, 2H) (H_2-2′ / H_2-3′), 1.78(m, 1H, H_A-8), 2.01(m, 1H, H_B-8), 2.14(m, 1H, H_A-7), 2.40(m, 1H, H_B-7), 3.78(m, 2H, H_2-9), 3.87(dd, *J* = 7.9 Hz, 8.2 Hz, 1H, H-6a), 4.43(m, 1H, H-1′), 7.60(d, $^3J_{H\text{-}F}$ = 12.3 Hz, 1H, H-12), 8.66(s, 1H, H-3), 10.49(s, 1H, lactam N(5)-H), 15.04(br s, 1H, CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 8.5, 11.8(C-2′/ C-3′), 24.9(C-8), 25.3(C-7), 39.1(C-1′), 51.1(d, $J_{C\text{-}F}$ = 8.0 Hz, C-9), 59.1(C-6a), 106.1(d, $^2J_{C\text{-}F}$ = 21.3 Hz, C-12), 107.5(C-2), 117.9(d, $^3J_{C\text{-}F}$ = 7.3 Hz, C-4b), 118.0(d, $^3J_{C\text{-}F}$ = 7.6 Hz, C-12a), 129.8(C-4a), 131.7 (d,$^2J_{C\text{-}F}$ = 15.2 Hz, C-10a), 150.4(d, $^1J_{C\text{-}F}$ = 244 Hz, C-11), 151.2(C-3), 164.6(C-6), 166.2(CO_2H),

176.5(d, $^4J_{C\text{-}F}$ = 3.2 Hz, C-1).

(±)-4-Cyclopropyl-11-fluoro-1,6-dioxo-1,4,5,6,6a,7,8,9-octahydropyrido[2,3-*f*]pyrrolo[1,2-*a*]-quinoxaline-2-carboxylic acid ((±)-10)

This compound was prepared by reduction of (±)-**7a** (0.6 g, 1.5 mmol) with sodium dithionite following the same procedure described above for the preparation of (*S*)-**7**. The reduction time was 5 min following the addition of sodium dithionite. Yield 0.21 g (40 %), mp >300 °C (darkens at 290 °C). *Anal*. Calcd for $C_{18}H_{16}FN_3O_4$: C, 60.50; H, 4.51; N, 11.76. Found: C, 60.15; H, 4.38; N, 11.40; MS(EI): *m/z* (% rel. int.): 357 [71, (M^+)], HRMS: calcd for $C_{18}H_{16}FN_3O_4$ 357.11245, found 357.11062; Other spectral data are identical to that of its enantiomer (*S*)- **10**.

(6a*S*,8*R*)-4-Cyclopropyl-11-fluoro-8-hydroxy-1,6-dioxo-1,4,5,6,6a,7,8,9-octahydropyrido[2,3-*f*]-pyrrolo[1,2-*a*]quinoxaline-2-carboxylic acid ((6a*S*, 8*R*)- 11)

This compound was prepared by reduction of (2′′*S*, 4′′*R*)-**8a** (0.42 g, 1.0 mmol) with sodium dithionite following the procedure and experimental conditions described above for the preparation of (*S*)-**10**. The product was recrystallized twice from MeOH / $CHCl_3$ (2 : 1, v/v). Yield 0.17 g (46 %), mp 280– 290 °C (decomp.); $[\alpha]_D$ -341.5° (DMSO, *c*~1). *Anal*. Calcd for $C_{18}H_{16}FN_3O_5$: C, 57.91; H, 4.32; N, 11.26. Found : C, 57.63; H, 4.35; N, 11.55; IR (KBr): *v* 3430, 3081, 3029, 2953, 2865, 1721, 1694, 1617, 1523, 1438, 1396, 1328, 1192, 1098 cm^{-1}; MS(EI): *m/z* (% rel.int.): 373 (16, M^+), 329(100), 309(38), 272(28), 257(36), 228(30), 216(15), 203(13), 187(11), 174(8), 147(7), 134(6); HRMS: calcd for $C_{18}H_{16}FN_3O_5$ 373.10736, found 373.10828; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83(m, 2H) and 1.07, 1.27(2m, 2H)(H_2-2′ / H_2-3′), 2.08(m, 1H, H_A-7), 2.34(m, 1H, H_B-7), 3.77(m, 1H, H_A-9), 3.90(m, 1H, H_B-9), 4.09(dd, *J* = 7.2 Hz, 7.4 Hz, 1H, H-6a), 4.33(br m, 1H, H-8), 4.41(br m, 1H, H-1′), 5.26(br s, 1H, O-H), 7.61(d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, H-12), 8.66(s, 1H, H-3), 10.46(s, 1H, lactam N(5)-H), 15.06(s, 1H, CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 8.4, 11.9(C-2′/ C-3′), 35.0(C-7), 39.0(C-1′), 58.2(C-6a), 58.5(d, $J_{C\text{-}F}$ = 8.7 Hz, C-9), 69.1(C-8), 106.3(d, $^2J_{C\text{-}F}$ = 21.7 Hz, C-12), 107.3(C-2), 117.6(d, $^3J_{C\text{-}F}$ = 7.6 Hz, C-4b), 117.8(d, $^3J_{C\text{-}F}$ = 7.8 Hz, C-12a), 129.8(C-4a), 131.5 (d,$^2J_{C\text{-}F}$ = 14.6 Hz, C-10a), 150.0(d, $^1J_{C\text{-}F}$ = 244 Hz, C-11), 151.2(C-3), 165.1(C-6), 166.2(CO_2H), 176.5(d, $^4J_{C\text{-}F}$ = 3 Hz, C-1).

(*S*)-4-Cyclopropyl-14-fluoro-1,6-dioxo-1,4,5,6,6a,7-hexahydro-12*H*-isoquinolino[2,3-*a*]pyrido[2,3-*f*]-quinoxaline-2-carboxylic acid ((*S*)-12)

This compound was prepared by reduction of (*S*)-**9a** (0.47 g, 1.0 mmol) with sodium dithionite following the same procedure and experimental conditions described above for the preparation of (*S*)-**10**. The product was first recrystallized from $CHCl_3$: pet. ether, and further purified on TLC plates (Silica gel, eluting with $CHCl_3$: MeOH : HCO_2H mixture (90 : 9 : 1, v / v / v). Yield 0.22 g (53 %), mp > 300 °C

(darkens at 280 ^{o}C); $[\alpha]_D$ -225.2^{o} (DMSO, *c*~1). *Anal*. Calcd for $C_{23}H_{18}FN_3O_4$: C, 65.87; H, 4.33; N, 10.02. Found : C, 65.66; H, 4.41; N, 10.12; IR (KBr): *v* 3457, 3209, 3061, 1721, 1684, 1616, 1592, 1518, 1443, 1314 cm^{-1}; MS (EI): *m/z* (% rel. int.): 419(29, M^+), 375(26), 332(3), 318(6), 258(4), 243(7), 188(3), 130(2), 115(6), 104(100); HRMS : calcd for $C_{23}H_{18}FN_3O_4$ 419.12810, found 419.13129; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86(m, 2H) and 1.05, 1.26(2m, 2H) (H_2-2′ / H_2-3′), 3.15(dd, *J* = 15.6 Hz, 6.4 Hz, 1H, H_A-7), 3.41(dd, *J* = 15.6 Hz, 4.6 Hz, 1H, H_B-7), 4.11(dd, *J* = 4.6 Hz, 6.4 Hz, 1H, H-6a), 4.42(m, 1H, H-1′), 4.65(d, *J* = 14.9 Hz,1H, H_A-12), 4.76(d, *J* = 14.9 Hz, 1H, H_B-12), 7.20(m, 3H) and 7.29(m, 1H) (H-8, H-9, H-10, H-11), 7.70(d, $^3J_{H\text{-}F}$ = 12.6 Hz, 1H, H-15), 8.71(s, 1H, H-3), 10.55(s, 1H, N(5)-H), 14.95(s, 1H, CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 9.1, 11.2(C-2′/ C-3′), 26.6(C-7), 39.2(C-1′), 49.8(d, $J_{C\text{-}F}$ = 10.7 Hz, C-12), 55.2(C-6a), 106.7(d, $^2J_{C\text{-}F}$ = 22.5 Hz, C-15), 107.6(C-2), 119.8(d, $^3J_{C\text{-}F}$ = 6.9 Hz, C-15a), 119.9(d, $^3J_{C\text{-}F}$ = 6.2 Hz, C-4b), 126.1, 126.8, 127.8, 128.9(C-8, C-9, C-10, C-11), 130.0(C-4a), 132.9(d, $^2J_{C\text{-}F}$ = 13.4 Hz, C-13a), 134.0, 135.1(C-7a, C-11a), 151.6(C-3), 151.6(d, $^1J_{C\text{-}F}$ = 245 Hz, C-14), 165.3(C-6), 166.0(CO_2H), 176.6(d, $^4J_{C\text{-}F}$ = 2.8 Hz, C-1).

IN VITRO CYTOTOXICITY

For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 10,000 to 50,000 cells / well, depending on the doubling time of individual cell lines. Model compounds **10-12** were prepared by dissolving each compound in DMSO and consequently diluted in complete RPMI-1640 media to make up stock solutions at 100 μM. The four cell lines were grown in the presence or absence of doubling dilutions of each of the test compounds ranging from 50-0.8 μM. The medium used to dissolve the highest concentration of each compound, was used as a vehicle control. The cell survival after 24, 48 and 72 h was determined using trypan blue exclusion as well as the colorimetric MTT assays. The percent of inhibition of the tumor cell lines growth after 72 h is calculated according to the formula [(Vehicle control growth–Experimental growth)/ Vehicle control growth] x 100.

STATISTICAL ANALYSIS

The average of percent of inhibition of cell growth ± standard error, caused by the treatment with the various compounds' concentrations, was compared using Student's *t* test; *p* values < 0.05 were considered to be statistically significant. The concentration of compounds **10-12** that reduced cell proliferation by 50 % (IC_{50}) as compared with controls was calculated by nonlinear regression fit of the mean values of the data obtained in triplicate experiments.

ACKNOWLEDGEMENTS

We wish to thank Hamdi Mango Center for Scientific Research-University of Jordan, Amman, Jordan for financial support.

REFERENCES

1. Part II : M. H. Al-Huniti, J. A. Zahra, and M. M. El-Abadelah, *Heterocycles* , 2007, **71**, 411.
2. (a) R. Wise, J. M. Andrews, and L. J. Edwards, *Antimicrob. Agents Chemother*., 1983, **23**, 559. (b) D. Felmingham, M. D. O'Hare, M. J. Robbins, R. A. Wall, A. H. Williams, A. W. Cremer, G. L. Ridgeway, and R. N. Gruneberg, *Drugs Exp. Clin. Res.*, 1985, **11**, 317. (c) F. Maurer and K. Grohe, *Ger. Offen*., 1986, **3**, **435**, **392** (*Chem. Abstr*.**,** 1986, **105**, 97158e). (d) U. Petersen, S. Bartel, K.-D. Bremm, T. Himmler, A. Krebs, and T. Schenke, *Bull. Soc. Chim. Belg.*, 1996, **105**, 683.
3. See for example: (a) T. Okada, K. Ezumi, M. Yamakawa, H. Sato, T. Tsuji, T. Tsushima, K. Motokawa, and Y. Komatsu, *Chem. Pharm. Bull.*, 1993, **41**, 126. (b) K. Grohe in, Quinolone Antibacterials, Springer-Verlag, Berlin, Heidelberg, 1998, pp. 13-62. (c) Q. Li, L. A. Mitscher, and L. L. Shen, *Med. Res. Rev*., 2000, **20**, 231. (d) G. G. Zhanel, K. Ennis, L. Vercaigne, A. Walkty, A. S. Gin, J. Embil, H. Smith, and D. J. Hoban, *Drugs*, 2002, **62**, 13. (e) A. D. Da Silva, M. V. De Almeida, M. V. N. De Souza, and M. R. C. Couri, *Curr. Med. Chem.*, 2003, **10**, 21. (f) M. Daneshtalab, Topics in Heterocyclic Chemistry, Vol. **2**, Heterocyclic Antitumor Antibiotics, Springer-Verlag, Berlin / Heidelberg, 2005, pp. 153-173.
4. (a) H. S. Elsea, P. R. McGuirk, T. D. Gootz, M. Moynihan, and N. Osheroff, *Antimicrob. Agents Chemother.*, 1993, **37**, 2179. (b) J. R. Spitzner, I. K. Chung, T. D. Gootz, P. R. McGuirk, and M. T. Muller, *Mol. Pharmacol.*, 1995, **48**, 238. (c) K. Riesbeck and A. Forsgren, *Antimicrob. Agents Chemother.*, 1995, **39**, 476. (d) K. D. Bromberg, A. B. Burgin, and N. Osheroff, *Biochemistry*, 2003, **42**, 3393.
5. (a) T. Yoshinari, E. Mano, H. Arakawa, M. Kurama, T. Iguchi, S. Nakagawa, N. Tanaka, and A. Okura, *Jpn. J. Cancer Res.*, 1993, **84**, 800. (b) A. Okura, T. Yoshinari, H. Arakawa, S. Nakagawa, E. Mano, and R. Ushijima, *PCT Int. Appl.*, 1992, **WO 9212146** (*Chem. Abstr.*, 1993, **118,** 147465).
6. (a) H. Arakawa, E. Mano, N. Hakoda, T. Yoshinari, S. Nakagawa, and A. Okura, *Anti-Cancer Drug Des.*, 1996, **11**, 221. (b) S. H. Elsea, M. Westergaard, D. A. Burden, J.-P. Lomenick, and N. Osheroff, *Biochemistry*, 1997, **36**, 2919. (c) A. M. Kamat, J. I. DeHaven, and D. L. Lamm, *Urology*, 1999, **54**, 56. (d) C. Nishijima, K. Kawada, K. Ohara, T. Shinomiya, S. Fukuda, A. Nakamura, T. Sawai, and M. Ikekita, *Res. Comm. Biochem. Cell Mol. Biol.*, 2002, **6**, 21. (e) D. Huang, K. Okada, C. Komori, E. Itoi, and T. Suzuki, *Cancer Sci.*, 2004, **95**, 845.
7. (a) G. W. H. Cheeseman and R. F. Cookson, Condensed Pyrazines, in: The Chemistry of Heterocyclic Compounds, Vol. **35**, ed. by A. Weissberger and E. C. Taylor, John Wiley & Sons, New York, 1979. (b) A. E. A. Porter, Pyrazines and their benzo derivatives in, Comprehensive Heterocyclic Chemistry, Vol. **3**, ed. by A. J. Boulton and A. McKillop, Pergamon Press, Oxford, 1984, pp 157-197, and references cited therein.

8. (a) M. Loriga, M. Fiore, P. Sanna, and G. Paglietti, *Farmaco*, 1996, **51**, 559. (b) G. Rodrigo, A. E. Robinsohn, M. E. Hedrera, M. Kogan, S. M. Sicardi, and B. M. Fernandez, *Trends Heterocycl. Chem.*, 2002, **8**, 137.
9. R. Chicharro, S. de Castro, R. L. Reino, and J. V. Aran, *Eur. J. Org. Chem.*, 2003, 2314.
10. M. Alvarez, M. Salas, and J. A. Joule, *Heterocycles*, 1991, **32**, 759.
11. (a) K. Grohe and H. Heitzer, *Liebigs Ann. Chem.*, 1987, 29. (b) U. Petersen, K. Grohe, T. Schenke, H. Hagemann, H. J. Zeiler, and K. G. Metzger, *Ger. Offen*., 1987, **3 601 567** (*Chem. Abstr.*, 1987, **107**, 236747).
12. R. M. Pulla and C. N. Venkaiah, *PCT Int. Appl*., 2001, **WO 085 692** *(Chem. Abstr.*, 2001, **135**, 371649).
13. J. P. Sanchez, J. M. Domagala, S. E. Hagen, C. L. Heifetz, M. P. Hutt, J. B. Nichols, and A. K. Trehan**,** *J. Med. Chem.*, 1988, **31**, 983.
14. (a) R. R. Frazer, M. A. Petit, and J. K. Sanders, *J. Chem. Soc., (D): Chem. Connun.*, 1971, 1450. (b) A. F. Cockerill, G. L. O. Davis, R. C. Harden, and D. M. Racham, *Chem. Rev.*, 1973, **73**, 553. (c) R. R. Frazer in, Asymmetric Synthesis, Vol. **1**, ed. by J. D. Morrison, Academic Press, New York, 1983, p. 173. (d) T. J. Wenzel and J. Zaia, *Anal. Chem.*, 1987, **59**, 562.
15. G. Bocci, K. C. Nicolaou, and R. S. Kerbel, *Cancer Res.*, 2002, **62**, 6938.
16. J. Nakamura, E. R. Purvis, and J. A. Swenberg, *Nucleic Acids Res.*, 2003, **31**, 61790.
17. (a) A. Souid, H. S. Penefsky, P. D. Sadowitz, and B. Toms, *Mol. Pharm.*, 2006, **3**, 307. (b) H.- I. Hsiao, W.- S. Wang, P.- M. Chen1, and Y. Su, *Carcinogenesis*, 2006, **27**, 936.
18. (a) J. S. Damiano, A. E. Cress, L. A. Hazlehurst, A. A. Shtil, and W. S. Dalton, *Blood*, 1999, **93**, 1658. (b) T. Sethi, R. C. Rintoul, S. M. Moore, A. C. MacKinnon, D. Salter, C. Choo, E. R. Chilvers, I. Dransfield, S. C. Donnelly, R. Strieter, and C. Haslett, *Nat. Med.*, 1999, **5**, 662.
19. (a) J. E. Wolff, K. P. Pascha, H. Poppenborg, R. Anderson, C. C. Thulig, and B. Krebs, *Anti- cancer Drugs***,** 1998, **9**, 803. (b) G. Tallen, C. Mock, S. B. Gangopadhyay, B. Kangarloo, B. Krebs, and J. E. Wolff, *Anticancer Res.*, 2000, **20**, 445. (c) K. Gharehbaghi, T. Szekeres, J. A. Yalowitz, M. Fritzer-Szekeres, Y. G. Pommier, and H. N. Jayaram, *Life Sci.*, 2000, **68**, 1. (d) F. de Longueville, M. Lacroix, A. M. Barbuto, V. Bertholet, D. Gallo, D. Larsimont, L. Marcq, N. Zammatteo, S. Boffe, G. Leclercq, and J. Remacle, *Int. J. Oncol.*, 2005, **27**, 881.