Synthesis and *in vitro* cytotoxic activity of *N*-[2-(thienyl)-2-(chlorobenzyloxyimino)]ethyl enoxacin derivatives

Eskandar Alipour[1], Negar Mohammadhosseini[2], Loghman Firoozpour[3], Maliheh Safavi[4], Sussan Kabudanian Ardestani[5], Abbas Shafiee[6], Alireza Foroumadi[3,6*]

[1]*Faculty of Chemistry, Tehran-North Branch, Islamic Azad University, Tehran, Iran*
[2]*Young Researchers and Elite Club, Islamshahr Branch, Islamic Azad University, Tehran, Iran*
[3]*Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Biotechnology Department, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran*
[5]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran*
[6]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran*
_____

ABSTRACT

*In the present work, N-[2-(thienyl)-2-(chlorobenzyloxyimino)ethyl]enoxacin derivatives (**4a-f**) were synthesis by the reaction of enoxacin with 2-bromo-1-(thiophen) ethanone O-2-chlorobenzyl oxime derivatives (**3a-f**) in DMF in the presence of NaHCO₃ at room temperature. The synthesized compounds were tested in vitro on human tumor cell lines. Preliminary screening showed **4b** and **4e** demonstrated significant growth inhibitory potential against all evaluated cell lines. The results of structure-activity relationship study exhibited that quinolone derivatives containing 3-chlorobenzyloxime are superior in cytotoxic potential compared to 2 and 4-chloro derivatives.*

Key words: Thienyl, chlorobenzyloxyimino, Enoxacin, Cytotoxic activity
_____

INTRODUCTION

Cancer is a very serious public health problem in developed countries and finding new anticancer compounds is an important area of interest in the life sciences [1, 2].

DNA topoisomerase II is involved in tumor growth and is a target of antineoplastic drugs such as etoposide and doxorubicin [3-6]. During the last decade it showed that quinonole-type compounds may exhibit cytotoxicity activity against cancer cell lines [7, 8]. Recently, Tomita and coworkers reported veroloxin, a novel 1,8-naphthyridine analog as cytoxic reagent with no antibacterial activity, *see* **figure 1** [9-11]. These finding well validated that quinolones are not only potent broad-spectrum antibacterial agents but also they are potent cytotoxic compounds and we will find them anticancer drug in near future.

We have reported some ciprofloxacin derivatives containing *N*-2-(2-furyl)-2-(chlorobenzyloxyimino)ethyl at C-7 position that exhibited significant anticancer activity [12-15]. Results showed increasing lipophilic substituents at C-7 of quinolones led to the change in biological profile of quinolones from antibacterial to cytotoxic agents.

_____

In continuation of our research program to find new quinolone based cytotoxic agents, herein we report the preparation and *in vitro* cytotoxicity evaluation of *N*-2-(2-thienyl and 3-thienyl)-2-(chlorobenzyloxyimino) ethyl enoxacin (**4a-f**).

EXPERIMENTAL SECTION

Chemistry
All Chemicals and solvents used in this project were purchased from Aldrich Chemicals and Merck AG. Melting points were determined on a Kofler hot stage apparatus. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). ^{1}H NMR spectra were recorded using Bruker 400 spectrometer and chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Elemental analyses were carried out on a Costec rapid elemental analyzer Model 4010 (GmbH-Germany) for C and H, and the results are within ±0.4% of the theoretical calculated values.

General Procedure for the synthesis of (4a-e)
A mixture of corresponding 2-bromo-1-(thiophen) ethanone *O*-2-chlorobenzyloxime (**3a-f**) (0.55 mmol), enoxacin (0.5 mmol) and NaHCO$_3$ (0.5 mmol) in DMF (5mL) was stirred at room temperature for 6-9 days. After completion, water (20 mL) was added and the precipitate was filtered off, washed with water and re-crystallized from EtOH-CHCl$_3$ to afford target compounds **4a-e**.

7-(4-(2-(2-chlorobenzyloxyimino)-2-(thiophen-2-yl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (4a)
Yield: 27%, m.p. 145-146 $^°$C; IR (KBr, cm^{-1}) v_{max}: 1729 (C=O) 1630 (C=O). ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 1.36 (t, 3H, CH$_3$, *J*=7.0Hz), 2.51-2.52(m, 4H, Piperazine), 3.70 (s, 2H, CH$_2$), 3.70-3.77 (m, 4H, Piperazine), 4.48 (q, 2H, CH$_2$-Me, *J*=7.0Hz), 5.23 (s, 2H, OCH$_2$- Zisomer), 5.36 (s, 2H, OCH$_2$-*E* isomer), 7.09 (d, 1H, Thiophen-Zisomer, *J*=3.05 Hz), 7.19 (m, 1H, Thiophen-*E*isomer, *J*=3.05Hz), 7.35-7.37 (m, 2H, Benzyl), 7.46-7.48 (m, 1H, Thiophen-Z isomer), 7.53-7.54 (m, 2H, Benzyl), 7.61 (dd, 1H, Thiophen-*E*isomer, *J*= 1.0 Hz), 7.80-7.83 (m, 1H, Thiophen-Zisomer), 7.90-7.92 (m, 1H, Thiophen-*E*isomer), 8.07 (d, 1H, H$_5$, *J*=13.0Hz), 8.97 (s, 1H, H$_2$), 15.29 (s, 1H, COOH). Anal. Calcd for C$_{28}$H$_{27}$ClFN$_5$O$_4$S: C, 57.58; H, 4.66; N, 11.99, Found: C, 57.60; H, 4.65; N, 11.97.

7-(4-(2-(3-chlorobenzyloxyimino)-2-(thiophen-2-yl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (4b)
Yield: 45%, m.p. 118-120$^°$C; IR (KBr, cm^{-1}) v_{max}: 1729 (C=O) 1631 (C=O). ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 1.35-1.38 (m, 3H, CH$_3$), 2.52-2.62 (m, 4H, Piperazine), 3.51 (s, 2H, CH$_2$-*E* isomer), 3.70 (s, 2H, CH$_2$-Zisomer), 3.70-3.77 (m, 4H, Piperazine), 4.47(q, 2H, CH$_2$-Me, *J*=7.5Hz), 5.17 (s, 2H, OCH$_2$-Zisomer), 5.30 (s, 2H, OCH$_2$-*E* isomer), 7.09 (dd, 1H, Thiophen-Zisomer, *J*= 4.0 Hz), 7.20 (dd, 1H, Thiophen-*E* isomer, *J*= 4.0 Hz), 7.35-7.47 (m, 4H, Benzyl), 7.54 (dd, 1H, Thiophen- Z isomer, *J*= 4.0 Hz), 7.61 (dd, 1H, Thiophen-*E* isomer, *J*= 1.0 Hz), 7.83(d, 1H, Thiophen-Zisomer, *J*= 1.0 Hz), 7.90 (d, 1H, Thiophen-*E* isomer, *J*=1.0Hz), 8.08 (d, 1H, H$_5$, *J*=14.0Hz), 8.98 (s, 1H, H$_2$), 15.29 (s, 1H, COOH). Anal. Calcd for C$_{28}$H$_{27}$ClFN$_5$O$_4$S: C, 57.58; H, 4.66; N, 11.99, Found: C, 57.57; H, 4.65; N, 11.96;

7-(4-(2-(4-chlorobenzyloxyimino)-2-(thiophen-2-yl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (4c)
Yield: 51%, m.p. 132-135$^°$C, IR (KBr, cm^{-1}) v_{max}: 1731 (C=O) 1625 (C=O). ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 1.37 (t, 3H, CH$_3$, *J*=7.0 Hz), 2.58-2.60 (m, 2H, CH$_2$-*E* isomer), 2.83-2.85 (m, 4H, Piperazine), 3.69 (s, 2H, CH$_2$-Zisomer), 3.72-3.74 (m, 4H, Piperazine), 4.46 (q, 2H, CH$_2$-Me, *J*=7.0Hz), 5.15 (s, 2H, OCH$_2$-Z isomer), 5.28(s, 2H, OCH$_2$-*E* isomer), 7.05 (dd, 1H, Thiophen-Z isomer, *J*=1.0 Hz), 7.20(dd, 1H, Thiophen-*E* isomer), 7.42-7.46(m, 1H, benzyl), 7.53-7.56(m, 1H, Thiophen- Z isomer), 7.60-7.61(m, 1H, Thiophen-*E* isomer), 7.81(dd, 1H, Thiophen-Z isomer, *J*= 1.0 Hz), 7.89 (dd, 1H, Thiophen-*E* isomer, *J*= 1.0 Hz), 8.01-8.06 (m, 1H, H$_5$), 8.93 (s, 1H, H$_2$), 15.30 (s, 1H, COOH). Anal. Calcd for C$_{28}$H$_{27}$ClFN$_5$O$_4$S: C, 57.58; H, 4.66; N, 11.99, Found: C, 57.55; H, 4.67; N, 12.01;
.

7-(4-(2-(2-chlorobenzyloxyimino)-2-(thiophen-3-yl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**4d**)
Yield: 51%, m.p. 148-150 $^°$C; IR (KBr, cm^{-1}) v_{max}: 1731 (C=O) 1634 (C=O). ^{1}H NMR (400 MHz, DMSO-*d$_6$*): δ 1.37 (t, 3H, CH$_3$, *J*= 6.8 Hz), 2.55-2.65 (m, 4H, piperazine), 3.68 (s, 2H, CH$_2$), 3.70-3.80 (m, 4H, Piperazine), 4.47 (q, 2H, CH$_2$, *J*=6.8 Hz), 5.25 (s, 2H, OCH$_2$), 7.35-7.38 (m, 2H, benzyl), 7.42 (d, 1H, thiophen, *J*=4.8Hz), 7.47-7.52 (m,

_____

1H, Thiophen), 7.52-7.58 (m, 2H, benzyl), 8.02-8.09 (m, 2H, thiophen-H_5), 8.96 (s, 1H, H_2), 15.20 (s, 1H, COOH). Anal. Calcd for $C_{28}H_{27}ClFN_5O_4S$: C, 57.58; H, 4.66; N, 11.99, Found: C, 57.54; H, 4.71; N, 12.00;

7-(4-(2-(3-chlorobenzyloxyimino)-2-(thiophen-3-yl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (4e)

Yield: 31%, m.p. 118-120 $^\circ$C; IR (KBr, cm^{-1}) ν_{max}: 1730 (C=O) 1630 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): 1.36 (t, 3H, CH_3, *J*=6.6 Hz), 2.20-2.63 (m, 4H, Piperazine), 3.67(s, 2H, CH_2), 2.56-2.82 (m, 4H, Piperazine), 4.46 (q, 2H, CH_2-Me, *J*=6.6 Hz), 5.17 (s, 2H, OCH_2), 5.20 (2s, 2H, OCH_2- *E,Z* isomer), 7.32-7.42 (m, 4H, Benzyl), 7.45(s, 1H, Thiophene), 7.52 (t, 1H, Thiophene, *J*=1.0 Hz), 8.04(d, 1H, H_5, *J*=13.7 Hz), 8.95 (s, 1H, H_2), 15.33 (s, 1H, COOH). Anal. Calcd for $C_{28}H_{27}ClFN_5O_4S$: C, 57.58; H, 4.66; N, 11.99, Found: C, 57.58; H, 4.62; N, 11.96;

7-(4-(2-(4-chlorobenzyloxyimino)-2-(thiophen-3-yl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (4f)

Yield: 25%, m.p. 131-133 $^\circ$C, IR (KBr, cm^{-1}) ν_{max}: 1722Cm^{-1} (C=O) 1624Cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): 1.38 (t, 3H, CH_3, *J*= 6.8 Hz), 2.52-2.59 (m, 2H, piperazine), 3.28-3.38 (m, 4H, piperazine), 3.66 (s, 2H, CH_2), 3.76 (s, 4H, Piperazine), 4.49 (q, 2H, CH_2, *J*=6.8Hz), 5.16 (2s, 2H, OCH_2-*E,Z* isomer), 57.36-7.46 (m, 4H, benzyl), 7.50-7.54 (m, 1H, thiophen), 8.00-8.01 (m, 1H, Thiophen), 8.02 (d, 1H, H_5, *J*=13.3Hz), 8.38-8.42 (m, 1H, thiophen), 8.98 (s, 1H, H_2), 15.35 (s, 1H, COOH). Anal. Calcd for $C_{28}H_{27}ClFN_5O_4S$: C, 57.58; H, 4.66; N, 11.99, Found: C, 57.53; H, 4.69; N, 11.95;

Cytotoxic activity

The synthesized compounds (**4a-f**) were tested against different human breast tumor cell lines including SK-N-MC, KB and K562 using MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay. The cell lines were purchased from National Cell Bank of Iran (NCBI). Cells were seeded in 96-well plates at the density of 10,000 viable cells per well and incubated at 37° C in a humidified atmosphere with 5% CO_2 for 24 h to allow cell attachment. The cells were then incubated for another 48 h with various concentrations of tested compounds. The synthetic compounds were dissolved in DMSO and the final concentration of DMSO in each well was kept below 1%. Etoposide was used as a positive control for each cell line. The medium was replaced with 200 µL RPMI-1640 without phenol red containing 0.5 mg/mL MTT. An additional 4h of incubation at 37°C were done and then the medium was discarded. Dimethyl sulfoxide (100 µL) was added to each well and the solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each cell was measured by plate reader (Biotek Instruments, Winooski, Vt.) at a test wavelength of 492 nm. Three independent experiments in triplicate were performed for determination of sensitivity to each compound. The IC_{50} were calculated by linear regression analysis, expressed in mean ± SD.
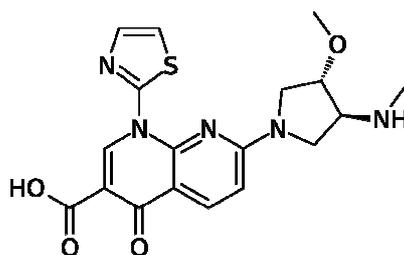
Figure 1. Voreloxin, a topoisomerase II inhibitor, under phase III clinical trial investigation for acute myelogenous leukemia (AML) and ovarian cancer

_____
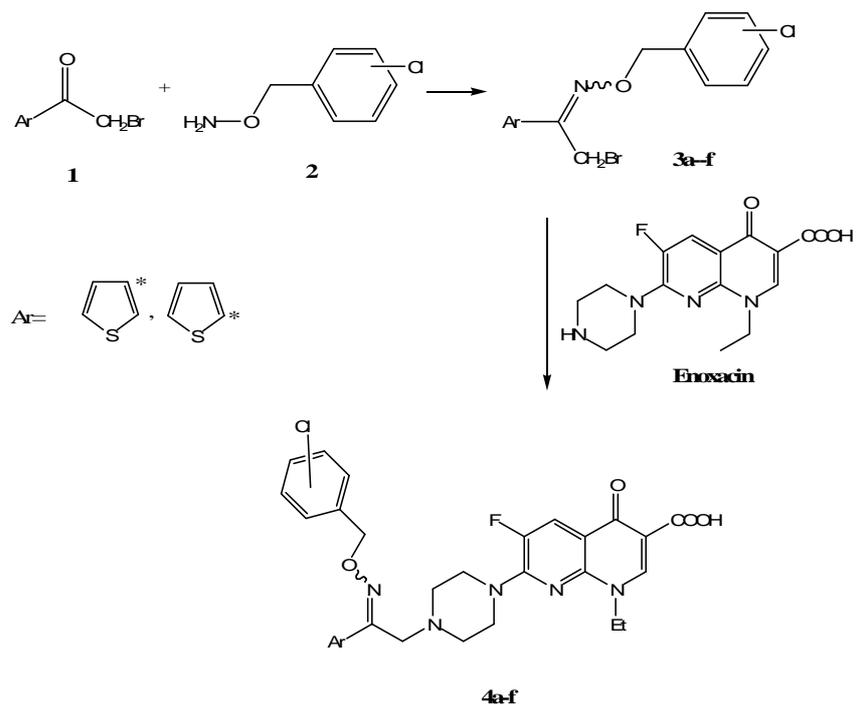
Scheme 1. Synthesis rout for preparation of 4a-f

Table 1. Structures and *in vitro* cytotoxic activity of compounds 4a-f in comparison to etoposide against selected tumor cell lines

Compound	Ar	X	Cell line IC50 (mM)		
			SK-N-MC	KB	K562
6a		2-Cl	>100	>100	>100
6b		3-Cl	4.34 ± 0.6	5.32 ± 1.95	5.43 ± 1.85
4c		4-Cl	11.47 ± 2.71	>100	15.34±8.16
4d		2-Cl	>100	>100	>100
4e		3-Cl	3.83 ± 0.74	5.06 ± 0.65	5.92 ± 2.73
4f		4-Cl	>100	>100	11.09 ± 4.66
Etoposide			21.55 ± 4.91	20.58 ± 3.84	31.5 ± 9.22

_____

RESULTS AND DISCUSSION

Reaction of 2-bromo-1-(thiophen-2-yl)ethanone with O-(chlorobenzyl)hydroxylamine hydrochloride in methanol gave 2-bromo-1 thiophen)ethanone *O*-chlorobenzyl oxime derivatives (**3a-f**). The target quinolones (**4a-f**) were synthesized from the reaction of enoxacin with **3a-f** in DMF in the presence of NaHCO$_3$.

The compounds **4a-f** were tested *in vitro* against a panel of three human tumor cell lines. The percentage of growth was evaluated using MTT colorimetric assay versus controls not treated with test agents. For each compound the 50% inhibitory concentration (IC$_{50}$) were determined and reported in Table 1. The data for Etoposide was provided for comparison. A rapid glance to the obtained results revealed that compounds (**4a, 4d, 4f**) possessed no cytotoxic activity (IC50 > 100 µM) against all cell lines. In contrast, compounds (**4b** and **4e**) showed significant activity against all tested cell lines.

The results of structure-activity relationship study demonstrated that quinolone derivatives containing 3-chlorobenzyloxime are superior in cytotoxic potential compared to 2 and 4-chloro derivatives.

REFERENCES

[1] KL Vine; JM Locke; M Ranson; SG Pyne; JB Bremner. *Bioorg. Med. Chem.,* **2007**, 15, 931.
[2] A Jemal; T Murray; E Ward; A Samuels; RC Tiwari; A Ghafoor; EJ Feuer; MJ Thun. *Cancer. J. Clin.,* **2005**, 55, 10.
[3] A McClendon; N Osheroff. *Mutat. Res.,* **2007**, 623: 83-97.
[4] J Nitiss. *Nat. Rev. Cancer*, **2009**, 9: 327-337.
[5] E Baldwin; N Osheroff. *Curr. Med. Chem. Anticancer Agents*. **2005**, 5: 363-372.
[6] J Nitiss. *Nat. Rev. Cancer*, **2009**, 9: 338-350.
[7] D Hooper. *Clin. Infect. Dis.*, **2001**, 32(1): S9-S15.
[8] Y Tsuzuki; K Tomita; K Shibamori; Y Sato; S Kashimoto; S Kashimoto; K Chiba. *J. Med. Chem*., **2004**, 47: 2097-2109.
[9] K Tomita; Y Tsuzuki; K Shibamori; M Tashima; F Kajikawa; Y Sato; S Kashimoto; K Chiba; K Hino. *J. Med. Chem*., **2002**, 45, 5564-5575.
[10] U Hoch; J Lynch; Y Sato; S Kashimoto; F Kajikawa; Y Furutani; JA. *Cancer Chemother. Pharmacol*., **2009**, 64: 53-65.
[11] C Scatena; J Kumer; J Arbitrario; A Howlett; R Hawtin; J Fox; J Silverman. *Cancer Chemother. Pharmacol.,* **2010**, 66(5):881-8.
[12] A Aliabadi; F Shamsa; SN Ostad; S Emami; A Shafiee; J Davoodi; Foroumadi A, *European Journal of Medicinal Chemistry*, **2010**, 45, 5384.
[13] Z Nazarian; S Emami; S Heydari; SK Ardestani; M Nakhjiri; F Poorrajab; A Shafiee; A Foroumadi, *European Journal of Medicinal Chemistry,***2010**, 45,1424.
[14] M Mahmoodi; A Aliabadi; S Emami; M Safavi; S Rajabalian; MA Mohagheghi MA; A Khoshzaban; A Samzadeh-Kermani; N Lamei; A Shafiee; A Foroumadi, *Archiv der Pharmazie*, **2010**, 343, 411.
[15] M Mahdavi; A Asadipour; S Rajabalian; M Vosooghi; L Firoozpour; M Nakhjiri; A Shafiee; A Foroumadi, *E-Journal of Chemistry,* **2011**, 8, 598.