



Synthesis and *In Vitro* Cytotoxic Activity of *N*-2-(2-Furyl)-2-(chlorobenzyloxyimino) Ethyl Ciprofloxacin Derivatives

ESKANDAR ALIPOUR, NEGAR MOHAMMADHOSSEINI,
FATEMEH PANA[#], SUSSAN K. ARDESTANI[#], MALIHEH SAFAVI[§],
ABBAS SHAFIEE[§] and ALIREZA FOROUMADI^{¶*}

Department of Chemistry, North Tehran Branch
Islamic Azad University, Tehran, Iran

[#]Institute of Biochemistry and Biophysics,
Department of Biochemistry, University of Tehran, Tehran, Iran

[§]Pharmaceutical Sciences Research Center
Tehran University of Medical Sciences, Tehran, Iran

[¶]Department of Medicinal Chemistry
Faculty of Pharmacy and Drug Design & Development Research Center
Tehran University of Medical Sciences, Tehran, Iran

aforoumadi@yahoo.com

Received 21 September 2010; Accepted 22 November 2010

Abstract: Quinolone are a class of potent broad-spectrum antibacterial drugs that target the bacterial type II DNA topoisomerases (DNA gyrase) and topoisomerase IV. In the present study, the synthesis and evaluation of cytotoxicity activity of a new series of *N*-piperazinyl quinolones containing *N*-2-(furyl-2-yl)-2-(chlorobenzyloxyimino) ethyl moiety (**6a-c**) have been studied. Preliminary screening showed that one of the new compounds, namely 7-(4-(2-(3-chlorobenzyloxyimino)-2-(furan-2-yl) ethyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6b**) showed significant cytotoxic activity against human breast tumor cell lines.

Keywords: Quinolones, Ciprofloxacin, Furyl, Oximes, Cytotoxicity.

Introduction

Quinolone antibacterial agents have been known to inhibit DNA gyrase and topoisomerase IV¹. Type II topoisomerases are the enzymes critical for maintaining and controlling the conformations

required for DNA replication and transcription². Indeed, DNA topoisomerase II enzyme catalyzes the double-strand breakage of DNA to allow strand passage and thereby control the topology and conformation of DNA³. DNA topoisomerases are found in both eukaryotic and prokaryotic cells and are targets for chemotherapeutic intervention in antibacterial and anticancer therapies^{4,5}. Quinolone antibacterials have been developed for clinical use in human medicine⁶. Moreover, it was shown that they could also inhibit mammalian topoisomerase and tubulin polymerization and thus act as potent antitumor drugs as well⁷. In view of the mechanistic similarities and sequence homologies exhibited by the prokaryotic type II topoisomerases (DNA gyrase and topoisomerase IV) and the eukaryotic type II topoisomerases, tentative efforts to selectively Shift from an antibacterial to an antitumoral activity was made by synthesizing novel classes of quinolones⁸⁻¹⁰. The inhibition of DNA gyrase and cell permeability of quinolones is greatly influenced by the nature of C-7 substitute on the standard structure of 4-quinolone-3-carboxylic acid¹¹. In addition, substitution of bulky functional groups is permitted at the C-7 position¹². During recent years, a number of quinolones with substitution on piperazine ring at C-7 position of the basic structure of quinolones were synthesized and evaluated for antibacterial activities¹³⁻¹⁴. The inhibition of DNA topoisomerases and the cell permeability of quinolones are greatly influenced by the nature of the C-7 substitution. In addition, a position on the quinolone molecule where substitution of bulky functional groups is permitted is at the C-7 position¹⁵. Most of the quinolones that showed anticancer activity contain aryl moiety at the C-7 position. Introduction of aryl ring at C-7 position of quinolone transforms its selectivity from bacterial to human topoisomerase II¹⁶. A number of 7-pyridinyl or 7-(4-hydroxyphenyl) quinolone derivatives such as CP-115, 953, WIN572946, A-621767 and A-852268, have been reported to display potent activity towards the eukaryotic type II enzyme and cytotoxicity against mammalian cells⁷⁻¹⁰. Recently, several compounds from *N*-substituted piperazinyl quinolone series (Figure 1) that exhibited significant anticancer activity have been reported by our group¹⁷. In continuation of our research program to find a new cytotoxic agent¹⁸⁻²¹, herein we would like to report the preparation and *in vitro* cytotoxicity of *N*-2-(2-furyl)-2-(chlorobenzoyloxyimino) ethyl ciprofloxacin (**6a-c**) derivatives as possible anticancer drug.

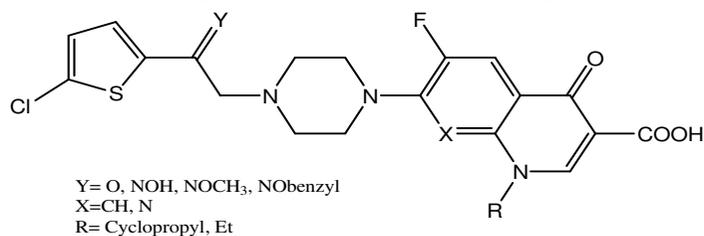


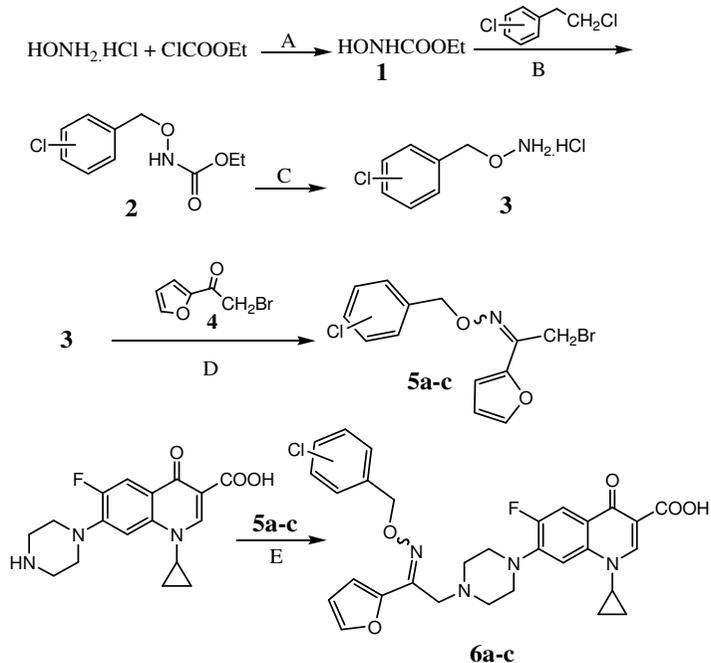
Figure 1. *N*-substituted piperazinyl quinolones with cytotoxic activities

Experimental

All starting materials, reagents and solvents were purchased from Merck and Aldrich companies. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F254 plates were used for analytical TLC. Column chromatography was performed on Merck silica gel (70-230 mesh) for purification of intermediate and final compounds. ¹H-NMR spectra were recorded using a Broker 500 spectrometer and chemical Shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). Melting points were determined on a Kofler hot stage apparatus and are uncorrected.

Chemistry

7-(4-(2-(Chlorobenzoyloxyimino)-2-(furan-2-yl)ethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acids (**6a-c**) were synthesized by reaction of ciprofloxacin, corresponding 2-bromo-1-(furan-2-yl)ethanone *O*-chlorobenzyl oxime (**5a-c**) in DMF in the presence of NaHCO₃ (Scheme 2)¹⁵. The intermediate *O*-benzylloximes **5a-c** were prepared by the reaction of substituted *O*-benzylhydroxylamine hydrochlorides (**3**) with 2-bromo-1-(2-furyl)ethanone (**2**) (Scheme 2). The structures of synthesized compounds were characterized by IR, ¹H NMR and ¹³C NMR spectral data.



Scheme 2. Synthesis of *N*-2-(2-furyl)-2-(chlorobenzoyloxyimino)ethyl ciprofloxacin derivatives (**6a-c**). Reagent and condition: A) Na₂CO₃, H₂O; B) Na, EtOH, rt; C) 1. NaOH, H₂O, heat, 2. EtOH, HCl; D) MeOH, rt; E) DMF, NaHCO₃, rt.

General Procedure for the synthesis of 7-(4-(2-(2-(chlorobenzoyloxyimino)-2-(furan-2-yl)ethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a-c)

A mixture of 2-bromo-1-(furan-2-yl)ethanone *O*-2-chlorobenzyl oxime derivatives (**5a-c**) (0.55 mmol), ciprofloxacin (0.5 mmol) and NaHCO₃ (0.5 mmol) in DMF (5 mL) was stirred at room temperature for 6-9 days. After consumption of ciprofloxacin, water (20 mL) was added and the precipitate was filtered, washed with water and crystallized from EtOH-CHCl₃ to give compounds **6a-c**.

7-(4-(2-(2-(Chlorobenzoyloxyimino)-2-(furan-2-yl)ethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6a)

Yield 47%; m.p. 200-201 °C; IR (KBr, cm⁻¹): 1628, 1733 (C=O), 3433 (COOH). ¹H NMR(DMSO-d₆)δ ppm: 1.13-1.18 (m, 2H, cyclopropyl), 1.26-1.32 (m, 2H, cyclopropyl),

2.62-2.68 (m, 4H, piperazine), 3.22-3.25 (m, 4H, piperazine), 3.63(s, 2H, CH₂), 3.77-3.82(m, 1H, cyclopropyl), 5.18 (s, 2H, OCH₂), 6.56-6.58 (m, 1H, H_{b(furyl)}), 6.98 (d, 1H, H_{a(furyl)}, *J*=3.1 Hz), 7.41-7.45 (m, 4H, H_{benzyl}), 7.54 (d, 1H, H₈, *J*=7.4 Hz), 7.62-7.64 (m, 1H, H_{c(furyl)}), 7.89 (d, 1H, H₅, *J*=13.2 Hz), 8.65 (s, 1H, H₂), 15.30 (s, 1H, COOH). ¹³C NMR (125 MHz, DMSO-d₆) δ ppm: 7.50, 35.78, 49.39, 50.59, 52.53, 73.07, 106.43, 106.70, 110.75, 110.93, 111.61, 112.33, 127.20, 129.24, 129.69, 130.36, 132.48, 134.88, 139.08, 144.19, 147.67, 147.91, 148.55, 151.98, 153.95, 165.87, 176.28.

7-(4-(2-(3-Chlorobenzoyloxyimino)-2-(furan-2-yl)ethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (6b)

Yield 30%; m.p. 279-280 °C; IR (KBr, cm⁻¹): 1627, 1730 (C=O), 3443 (COOH). ¹H NMR(DMSO-d₆)δ ppm: 1.14-1.20 (m, 2H, cyclopropyl), 1.29-1.36 (m, 2H, cyclopropyl), 2.62-2.73(m, 4H, piperazine), 3.25-3.30 (m, 4H, piperazine), 3.66 (s, 2H, CH₂), 3.75-3.84 (m, 1H, cyclopropyl), 5.22 (s, 2H, OCH₂), 6.56-6.60(m, 1H, H_{b(furyl)}), 7.00-7.03 (m, 1H, H_{a(furyl)}), 7.37-7.44(m, 3H, H_{benzyl}), 7.49(s, 1H, H_{benzyl}), 7.56(d, 1H, H₈, *J*=6.8 Hz), 7.72-7.76(m, 1H, H_{c(furyl)}), 7.90(d, 1H, H₅, *J*= 13.1 Hz), 8.66(s, 1H, H₂), 15.12 (s, 1H, COOH).. ¹³C NMR (125 MHz, DMSO-d₆) δ: 7.50, 35.77, 39.99, 50.69, 52.59, 74.69, 106.00, 106.68, 110.75, 110.93, 111.61, 112.33, 126.30, 127.44, 127.59, 130.22, 139.19, 144.12, 140.45, 147.20, 145.64, 147.86, 150.42, 153.98, 164.23, 165.92, 176.27.

7-(4-(2-(4-Chlorobenzoyloxyimino)-2-(furan-2-yl) ethyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (6c)

Yield 53%; m.p. 187-189 °C; IR (KBr, cm⁻¹): 1626, 1731 (C=O), 3441 (COOH). ¹H NMR(DMSO-d₆)δ ppm: 1.10-1.20 (m, 2H, cyclopropyl), 1.30-1.38 (m, 2H, cyclopropyl), 2.60-2.70 (m, 4H, piperazine), 3.18-3.28 (m, 4H, piperazine), 3.66 (s, 2H, CH₂), 3.75-3.84 (m, 1H, cyclopropyl), 5.27 (s, 2H, OCH₂), 6.52-6.54 (m, 1H, H_{b(furyl)}), 6.99 (d, 1H, H_{a(furyl)}, *J*=2.8 Hz), 7.40 (m, 2H, H_{benzyl}), 7.42-7.44 (m, 1H, H_{c(furyl)}), 7.50-7.58 (m, 2H, H_{benzyl}-H₈), 7.70-7.72 (m, 1H, H_{benzyl}), 7.85 (d, 1H, H₅, *J*=13.2 Hz), 8.63 (s, 1H, H₂), 15.35 (s, 1H, COOH). ¹³C NMR (125 MHz, DMSO-d₆) δ: 7.50, 35.75, 49.36, 50.58, 52.52, 74.78, 106.70, 106.74, 110.75, 110.93, 111.58, 112.21, 128.28, 129.73, 132.34, 136.74, 139.10, 144.12, 145.20, 147.26, 147.47, 147.93, 148.62, 165.86, 176.31.

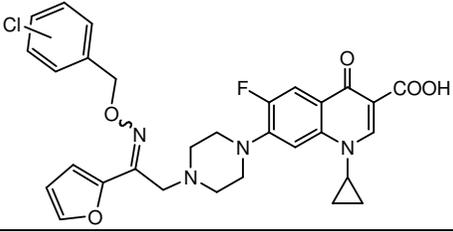
Biological activity

The synthesized compounds were tested against a human breast tumor cell lines. The cell lines were purchased from National Cell Bank of Iran (NCBI). Cells were seeded in 96-well plates at the density of 8000-10,000 viable cells per well and incubated for 48 h to allow cell attachment. The cells were then incubated for another 48-96 h (depends to cell cycle of each cell line) with various concentrations of compounds **6a-c**. Cells were then washed in PBS (Phosphate Buffer Saline) and 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution (5 mg/mL) were added to each well. An additional 4 h of incubation at 37 °C were done and then the medium was discarded. Dimethyl sulfoxide (60 μL) was added to each well and the solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each well was measured by plate reader (Anthous 2020; Austria) at a test wavelength of 550 nm against a standard reference solution at 690 nm. The amount of produced purple formazan is proportional to the number of viable cells. Two independent experiments in triplicate were performed for determination of sensitivity to each compound, the IC₅₀ were calculated by linear regression analysis, expressed in mean ± SD²².

Results and Discussion

The compounds **6a-c** were tested *in vitro* against a panel of a human cancer cell lines including Human breast tumor cell lines. The percentage of growth was evaluated using MTT colorimetric assay *versus* controls not treated with test agents. For each compound, 50% inhibitory concentration (IC_{50}) was determined and reported in Table 1. The data for tamoxifen was included for comparison. The obtained results revealed that compounds **6a**, **6c** possessed weaker activity against human cancer cell lines in comparison with **6b**, which possess comparable activity with tamoxifen. The results highlight the relationship between substitutions at 2, 3 or 4 positions of the phenyl ring and cytotoxic activity. Among these positions, substitution at 3-chlorophenyl position greatly influences their potency and spectrum of cytotoxic activity.

Table 1. Cytotoxic activity of compounds **6a-c** against human breast tumor cell lines in comparison with tamoxifen



Compound	Human breast tumor cell line ^a
6a (2-Cl)	IC_{50} = 25
6b (3-Cl)	IC_{50} =5
6c (4-Cl)	IC_{50} =10
Tamoxifen	IC_{50} =12

^a IC_{50} were presented as $\mu\text{g/mL}$

Conclusion

In conclusion, we have synthesized a novel series of 7-(4-(2-(chlorobenzoyloxyimino)-2-(furan-2-yl)ethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acids (**6a-c**), which one of them (**6b**) showed significant cytotoxic activity against human breast tumor cell lines.

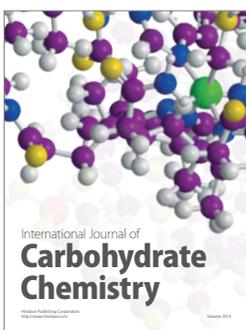
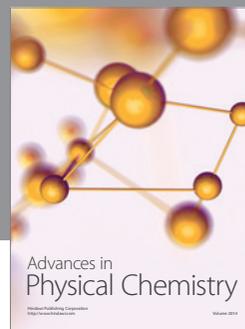
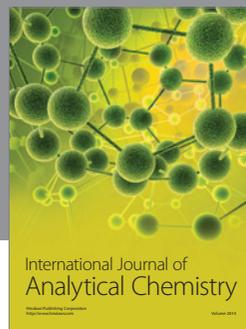
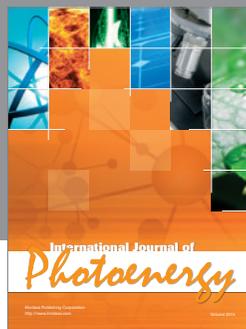
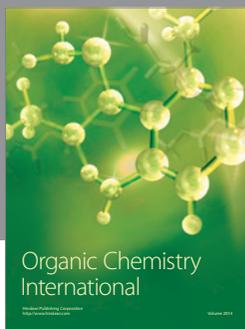
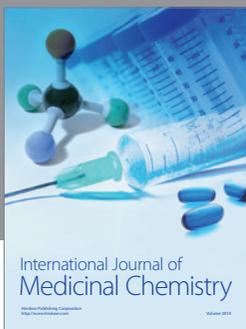
Acknowledgment

This work was supported by grants from Tehran University of Medical Sciences and Iran National Science Foundation (INSF).

References

1. Hooper D C, *Drugs*, 1999, **58**, 6-10.
2. Foroumadi A, Emami S, Rajabalian S, Badinloo M, Mohammadhosseini N and Shafiee A, *Biomed Pharmacother.*, 2009, **63**, 216-220.
3. Berger J M, Gamblin S J, Harrison S C and Wang J C, *Nature*, 1996, **379**, 225-232.
4. Gootz T D and Birghty K E, In: Andriole V T, Editor, *The quinolones* (2nd Ed.), Academic Press, San Diego, 1998, 29.
5. Spicer J, Finlay G, Baguley B, Velea L, Graves D and Denny W, *Anti-Cancer Drug Des.*, 1999, **14**, 37-45.

6. Hooper D C, *Biochim Biophys Acta*, 1998, **1400**, 45-61.
7. Chu D T W, Hallas R, Alder J and Plattner J J, *Drugs Exp Clin Res.*, 1994, **20**, 177-183.
8. Permana P A, Snapka R M, Shen L L, Chu D T W, Clement J J and Plattner J J, *Biochem.*, 1994, **33**, 11333-11339.
9. Robinson M J, Martin B A, Gootz T D and McGuirk P R, *Antimicrob Agents Chemother.*, 1992, **36(4)**, 751-756.
10. Wentland M P, Leshner G Y, Reuman M, Gruett M D, Singh B, Aldous S C, Dorff P H, Rake J B and Coughlin S A, *J Med Chem.*, 1993, **36(19)**, 2801-2809.
11. Domagala J M, Hanna L D, Heifetz C L, Hutt M P, Mich T F and Sanchez J P, Solomon M, *J Med Chem.*, 1986, **29**, 394-404.
12. Shen L L, Mitscher L A, Sharma P N, O'Donnell T J, Chu D W, Cooper C S, Rosen T and Pernet A G, *Biochem.*, 1989, **28**, 3886-3894.
13. Foroumadi A, Mansouri S, Kiani Z and Rahmani A, *Eur J Med Chem.*, 2003, **38** 851-854.
14. Foroumadi A, Emami S, Mansouri S, Javidnia A, Saeid-Adeli N, Shirazi F H and Shafiee A, *Eur J Med Chem.*, 2007, **42**, 985-992.
15. Foroumadi A, Mohammadhosseini N, Emami S, Letafat B, Faramarzi M A, Samadi N and Shafiee A, *Arch Pharm (Weinheim)*, 2007, **340(1)**, 47-52.
16. Rajabalian S, Foroumadi A, Shafiee A and Emami S, *J Pharm Pharm Sci.*, 2007, **10(2)**, 153-158.
17. Shafiee A, Haddad Zahmatkesh M, Mohammadhosseini N, Khalafy J, Emami S, Moshafi M H, Sorkhi M and Foroumadi A, *Daru J Pharm Sci.*, 2008, **16(3)**, 189-195.
18. Aliabadi A, Shamsa F, Ostad S N, Emami S, Shafiee A, Davoodi J and Foroumadi A, *Eur J Med Chem.*, 2010, **45(11)**, 5384-5389.
19. Nazarian Z, Emami S, Heydari S, Ardestani S K, Nakhjiri M, Poorrajab F, Shafiee A and Foroumadi A, *Eur J Med Chem.*, 2010, **45**, 1424-1429.
20. Mahmoodi M, Aliabadi A, Emami S, Safavi M, Rajabalian S, Mohagheghi M A, Khoshzaban A, Samzadeh-Kermani A, Lamei N, Shafiee A and Foroumadi A, *Arch Pharm (Weinheim)*, 2010, **343(7)**, 411-416.
21. Mahdavi M, Asadipour A, Rajabalian S, Vosooghi M, Firoozpour L, Nakhjiri M, Shafiee A and Foroumadi A, *E-J Chem.*, 2011, **8(2)**, 598-602.
22. Virsodia V, Manvar A, Upadhyay K, Loriya R, Karia D, Jaggi M, Singh A, Mukherjee R, Shaikh M S, Coutinho E C and Shah A, *Eur J Med Chem.*, 2009, **44(3)**, 1355-1362.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

