Functionalized N-(2-oxyiminoethyl) piperazinyl quinolones as new cytotoxic agents

Saeed Rajabalian [1], Alireza Foroumadi [1,2], Abbas Shafiee [2], Saeed Emami [3]

[1]Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran, [2]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran, [3]Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

Received October 18, 2006; Revision received November 01, 2006; Accepted November 02, 2006, Published June 14th 2007

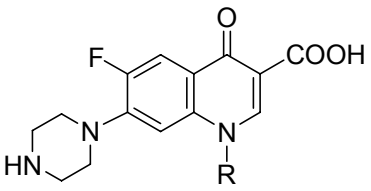
ABSTRACT – Purpose: The prokaryotic type II topoisomerases (DNA gyrase and topoisomerase IV) and the eukaryotic type II topoisomerases represent the cellular targets for quinolone antibacterial agents and a wide variety of anticancer drugs, respectively. In view of the mechanistic similarities and sequence homologies exhibited by the two enzymes, tentative efforts to selectively shift from an antibacterial to an antitumoral activity was made by synthesizing a series of functionalized *N*-(2-oxyiminoethyl)piperazinyl quinolones, in which the C-7 piperazine ring of antibacterial quinolones, ciprofloxacin and norfloxacin, is attached by a certain *N*-[2-(furan-2-yl)-2-oxyiminoethyl] and *N*-[2-(thiophen-2-yl)-2-oxyiminoethyl] moieties. Thus, as part of a continuing search for potential anticancer drug candidates in the *N*-substituted piperazinyl quinolones series, the cytotoxicity evaluation of functionalized *N*-(2-oxyiminoethyl) piperazinyl quinolones was our interest. **Methods:** The growth inhibitory activities of synthesized *N*-[2-(furan-2-yl)-2-oxyiminoethyl] and *N*-[2-(thiophen-2-yl)-2-oxyiminoethyl] piperazinyl quinolones were determined against seven cancer cell lines using an *in vitro* cell culture system (MTT assay). **Results:** Preliminary screening showed that some of *N*-(2-oxyiminoethyl) piperazinyl quinolone analogs containing *O*-benzyl group displayed *in vitro* cytotoxic activity comparable or higher than reference drug etoposide. **Conclusions:** These studies demonstrate that 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. As can be deduced from these data, *O*-benzyl functionalized *N*-(2-oxyiminoethyl) piperazinyl quinolones have excellent potential as a new class of cytotoxic agents.

INTRODUCTION

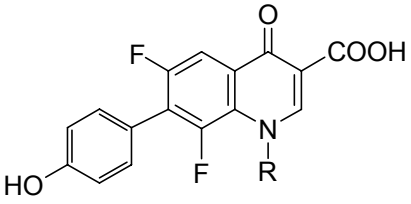
Quinolones (e.g. ciprofloxacin **1** and norfloxacin **2**) are a very important family of antibacterial agents that are widely prescribed for the treatment of infections in humans (1). They corrupt the activities of prokaryotic type II topoisomerases, DNA gyrase and topoisomerase IV, and induce them to kill cells by generating high levels of double-stranded DNA breaks. Type II topoisomerases modulate the topological state of the genetic material by passing an intact DNA helix through a transient double-stranded break that they generate in a separate DNA segment (2-4). Like bacterial cells, eukaryotic species require a type II topoisomerase, known as topoisomerase II, for viability (5,6). Thus, in addition to the antibacterial quinolones, specific members of this drug family display high activity against eukaryotic type II topoisomerases, as well as cultured mammalian cells and *in vivo* tumor models (7,8). These antineoplastic quinolones represent a potentially important source of new anticancer agents.

Several novel quinolones have been synthesized that display significant activity against eukaryotic type II topoisomerases (9,10). Structures of selected cytotoxic quinolones **3-5,** which built on the ciprofloxacin **1** or norfloxacin **2** nucleus are shown in Figure 1. Although these compounds commonly display high activity against DNA gyrase or topoisomerase IV, they are distinguished from the antibacterial quinolones by the presence of an aromatic substituent at the C-7 position (7,11-13). Recently, we have synthesized novel *N*-substituted piperazinyl quinolones **6** differing from ciprofloxacin **1** or norfloxacin **2** solely by the linkage of various 2- (furan-2-yl)-2-oxyiminoethyl and 2-(thiophen-2-yl)-2-oxyiminoethyl groups to

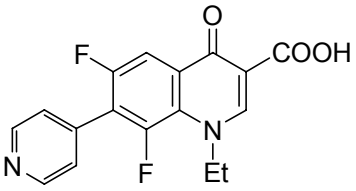
Corresponding author: Dr. Saeed Emami, Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, P.O. Box 48175-861, Khazar Abad Road, Sari, IRAN E-mail: sd_emami@yahoo.com



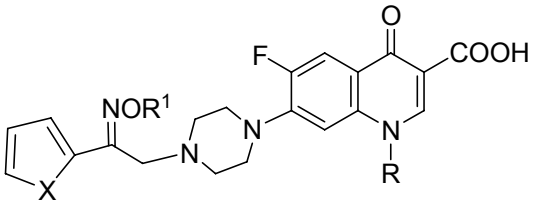
1, Ciprofloxacin; R= cyclopropyl
2, Norfloxacin; R= ethyl



3, CP-115,953; R= cyclopropyl
4, CP-67,804; R= ethyl



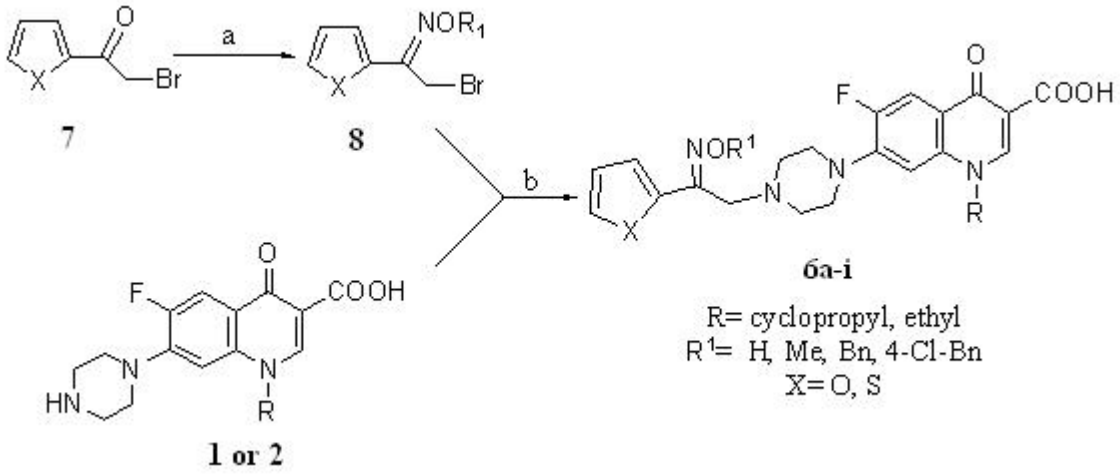
5, CP-67,015



6

R= cyclopropyl, ethyl
R^1= H, Me, Bn
X= O, S

Figure 1: Structures of compounds **1-6**.



6a-i

R= cyclopropyl, ethyl
R^1= H, Me, Bn, 4-Cl-Bn
X= O, S

Figure 2: Synthesis of compounds **6a-i**. Reagents and conditions: (a) hydroxylamine hydrochloride or methoxyamine hydrochloride or *O*-benzyl hydroxylamine hydrochloride or *O*-(4-chlorobenzyl hydroxylamine hydrochloride, MeOH, rt; (b) $NaHCO_3$, DMF, rt.

the piperazinyl residue at C-7 of the parent drug and explored their antibacterial activities (14,15). Due to ability of quinolones to preferentially target the different prokaryotic and eukaryotic type II topoisomerases, herein we report the cytotoxic activity of some *N*-[2-(furan-2-yl)-2-oxyiminoethyl] and *N*-[2-(thiophen-2-yl)-2-oxyiminoethyl] piperazinyl quinolones **6a-i**. Our synthetic route to target compounds **6a-i** is diagrammed in Figure 2. Reaction of 7-piperazinyl quinolones (**1** or **2**) with α-bromooxime derivative **8** in DMF in the presence of $NaHCO_3$, at room temperature afforded compounds **6a-i**. The intermediate α-bromooxime **8** was prepared according to the known method by the reaction of related α-bromoketone **7** with requisite hydroxylamine hydrochloride (14,15).

MATERIAL AND METHODS

Reagents and Materials

N-[2-(furan-2-yl)-2-oxyiminoethyl] and *N*-[2-(thiophen-2-yl)-2-oxyiminoethyl] piperazinyl quinolones **6a-i** were prepared according to the general synthetic procedures previously described by us, as diagrammed in Figure 2 (14,15). Etoposide, 3-(4',5'-dimethylthiazol-2'-yl)-2,5-diphenyl-tetrazolium bromide (MTT), penicillin and streptomycin were purchased from Sigma-Aldrish Co., USA. The test compounds were dissolved in dimethyl sulfoxide (DMSO), diluted with media and stored as the stock solutions with a concentration of 1.0 mg/mL at –20 ºC (The concentration of DMSO was different in the final serial diluted media but was less than 1%). All other solvents and chemicals were of analytical grade and were obtained from Merck, Germany.

Seven tumoral cell lines [ACHN renal cancer, MCF-7 breast cancer, A172 glioma, SKMEL-3 melanoma, A549 lung cancer, A2780-CP ovarian cancer (cisplatin resistance) and KB oral cancer cell lines] were purchased from National Cell Bank of Iran (NCBI). The cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (from Gibco-BRL, UK) and 100 μg/mL streptomycin and 100u/mL penicillin.

Cytotoxic Assay

The *in vitro* cytotoxic activity of the test compounds **6a-i** was investigated in comparison with etoposide using MTT colorimetric assay (16). The assay itself is based on the reduction of 3-(4', 5'-dimethylthiazol-2'-yl)-2,5-diphenyltetrazolium bromide (MTT, yellow colour) by mitochondrial dehydrogenases of metabolically active cells to a purple-blue formazan. Briefly, cultures in the exponential growth phase were trypsinized and diluted in complete growth medium to give a total cell count of 5×10^4 cells/mL. One hundred microliter of suspension was added to wells of sterile 96-well plates (NUNC, Denmark). After plating, 50 μL of a serial dilution of every agent was added. Each compound dilution was assessed in triplicate. Three wells containing only tumor cells suspended in 150 μL of complete medium were used as controls for cell viability. The plates were then incubated for 72 h. After incubation, 30 μL of a 5mg/mL solution of MTT was added to each well and the plate was incubated for another 1 h. After incubation, the culture medium was replaced with 100 μL of DMSO. Then, the absorbance of each well was measured by using a microplate reader at 492 nm wavelengths. For each compound, dose-response curves for each cell line were measured with different drug concentrations, and the concentration causing 50% cell growth inhibition (IC_{50}, equating to cytostatic activity) compared with the control were calculated. For each agent, the overall mean IC_{50} was determined, and that was the mean of the values for all cell lines.

RESULTS

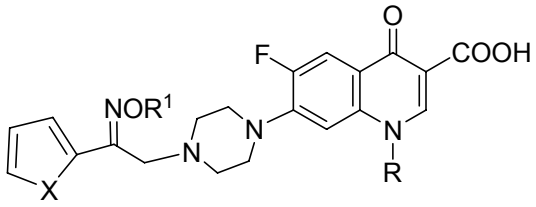
The *in vitro* cytotoxic activity of the test compounds **6a-i** was investigated in comparison with etoposide against seven tumor cell lines using MTT colorimetric assay (16). The inhibitory activities of compounds **6a-i** against the cell lines are presented in Table 1 as IC_{50} values, together with the related mean values.

The IC_{50} values of compounds **6a-d** against all tumor cell lines indicate that all these compounds possessed poor activity (IC_{50} > 100 μM) with the exception of compound **6a** that showed marginal activity against SKMEL-3 cell line (IC_{50} = 74 μM). In contrast, compounds **6e-i** showed significant activity against all tested cell lines comparable to reference drug etoposide (as a known topoisomerases II inhibitor). In addition, the IC_{50} values of derivatives **6e-i** against A127 and KB cell lines indicate that all these compounds possessed a better activity with respect to etoposide.

DISCUSSION

It is possible to discern some quite prominent structure–activity relationships for the compounds. Introduction of methyl group appended on the oxime moiety, slightly increased cytotoxicity (**6c** versus **6a** and **6d** versus **6b**). The cytotoxicity was further enhanced by converting the oxime group of **6a,b** to their respective *O*-benzyl or *O*-(4-chlorobenzyl)oximes **6e-g**. For example, comparison of activity of oxime **6a** and their corresponding *O*-benzyl and *O*-(4-chlorobenzyl) analogs (**6e** and **6f**, respectively) showed that the cytotoxicity increased 28–38 times (based on Mean

Table 1: Structures and *in vitro* cytotoxic activity of compounds **6a-i** against selected tumor cell lines [average IC_{50} (μM)] [a]



Compound	X	R	R^1	ACHN	MCF-7	A172	SKMEL-3	KB	A549	A2780	Mean[b]
6a	O	Et[c]	H	137	138	177	74	113	121	156	131
6b	O	*c*-Pr[d]	H	116	117	141	125	108	108	128	120
6c	O	Et	CH_3	106	127	123	134	105	125	125	121
6d	O	*c*-Pr	CH_3	104	116	132	127	102	111	126	117
6e	O	Et	Bn[e]	2.7	2.0	5.2	3.7	4.5	6.1	7.4	4.6
6f	O	Et	4-Cl-Bn	1.7	2.5	6.4	3	2.9	1.4	5.7	3.4
6g	O	*c*-Pr	4-Cl-Bn	1	3.6	8.3	3.1	2.3	2.1	7.6	4
6h	S	Et	Bn	1.3	1.7	6.1	3.2	2.1	1.6	10	3.7
6i	S	*c*-Pr	Bn	3.8	1.8	7	3.3	1.9	1.6	8.4	4
Etoposide				0.17	1.3	19.8	0.34	4.8	1.5	6.6	4.9

[a] The IC_{50} values represent an average of three experiments.
[b] Mean values over all cell lines tested.
[c] Et: ethyl
[d] *c*-Pr: cyclopropyl
[e] Bn: benzyl

values). As we can see, most of the new *N*-substituted piperazinyl quinolones (**6e-i**) containing benzyl substituent on oxime moiety showed potent cytotoxic activity and modification of five-membered-ring, alkyl substituent at N-1 and 4-chloro- substitution at benzyl group produced a relatively minor change of activity. Thus, in *N*-(2-oxyimino) piperazinyl quinolone series, cytotoxic activity can be positively modulated through the introduction of *O*-benzyl group.

Although the previous studies demonstrated that the antibacterial properties and cytotoxic activity of compounds with 4-quinolone pharmacophore are related to their inhibitory activity against topoisomerases (6-9), but in this study, no enzyme inhibition assay was provided to demonstrate that compounds are actually inhibiting type II topoisomerases. However, we know that most drugs with the same mechanism of action will show similar fingerprints against a cancer cell lines. Therefore, a correlation analysis using Pearson's correlation coefficient was used to compare the log IC_{50} values of compounds **6e-i** with those of etoposide (as a well known topoisomerases II inhibitor). Correlation with etoposide activity pattern against the cell lines was high for **6g** (r = 0.78, $P < 0.02$), **6f** (r = 0.73, $P < 0.05$), **6e** (r = 0.68, $P < 0.05$) and moderate for **6h** (r = 0.66, $P < 0.1$) and low for **6i** (r = 0.38). These data suggest that these series of quinolone derivatives share a similar mechanism of action with etoposide.

The first information obtained in this study is that *O*-benzyl oximes **6e-i** show more potent cytotoxic activity than oximes **6a,b** and *O*-methyl oximes **6c,d** against all tumor cell lines. In contrast, according to previous antibacterial studies (14, 15,

17), among oxime, *O*-methyl oxime and *O*-benzyl oxime derivatives of (2-oxyiminoethyl) piperazinyl quinolones, lower susceptibilities (higher MICs) were observed with *O*-benzyl oxime derivatives. For example, comparison between MIC values of *O*-benzyl oxime analog **6h** (the MICs for *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* were 64, 16, 64 and >64 μg/ml, respectively), and its oxime counterpart (the MICs for *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* were 0.5, 4, 8 and 4 μg/ml, respectively) revealed that introduction of *O*-benzyl on oxime group caused a significant diminution in antibacterial activity against most bacteria species (15). Therefore, compound **6h** did not show antibacterial activity against tested strains at concentrations ≤16 μg/ml (≈ 30 μM), while exhibited cytotoxic activity against all tested cell lines at concentrations ≤10 μM (IC_{50} = 1.3-10 μM). By calculation of selectivity index (IC_{50}/MIC) basis on available antibacterial and cytotoxic data for oxime **6a** and *O*-benzyl oxime **6e** we can find similar change in biological activity profile by *O*-benzyl substitution (14). The oxime derivative **6a** shows selectivity index ≈ 17.5, while this value for *O*-benzyl counterpart **6e** is about 0.55 (calculated based on means values of IC_{50} and MIC). Thus, introduction of *O*-benzyl moiety on oxime group of *N*-(2-oxyiminoethyl) piperazinyl quinolone series changes the biological profile of piperazinyl quinolones from antibacterials to cytotoxic agents. Although antibacterial data is not available for all *O*-benzyl oxime derivatives **6e-i** but according to available data, compound **6h** is selected example that showed high cytotoxic activity with minimal antibacterial property (IC_{50}/MIC ≈ 0.04).

The alteration of biological activity profile of *O*-benzyl functionalized *N*-(2-oxyiminoethyl) piperazinyl quinolones may be the result of a permeability mechanism or due to the change of selectivity to target enzyme. It appears that the outer membrane of bacteria is the major permeability barrier for quinolones to access their target site and to develop their antibacterial activity (18, 19), while quinolones may diffuse directly across the cytoplasmic membrane of tumor cells. Therefore, it could be hypothesized that the increasing of molecular mass and bulkiness of substituent at C-7 position hinder penetration of quinolones **6e-i** into microorganisms and decrease antibacterial activity. On the other hand, although quinolones are relatively simple in structure, mechanistically they are quite complex. The fact that quinolones bind preferentially to enzyme-DNA complexes suggests that quinolones entered the enzyme-drug-DNA ternary complex through interactions with the enzyme-DNA complex, rather than through an association with free nucleic acids (9). Recently it is investigated that the mode of action of quinolones involves interaction with both prokaryotic and eukaryotic type II topoisomerases (9, 10). Although the structural features responsible for the interaction of quinolones with the binding sites on prokaryotic or eukaryotic type II topoisomerases are not yet understood fully, position 7 of quinolone structure is considered to be one that directly interacts with topoisomerase enzyme in enzyme-drug-DNA ternary complex, and determines target preference of quinolones (10, 20). Thus, it is possible that the particular interactions of quinolones **6e-i** with these two target enzymes could lead to differences in susceptibility. Clearly, further investigation is required to clarify the action mechanism of this novel compound and understanding the ability of these quinolones to preferentially target the different prokaryotic and eukaryotic type II topoisomerases.

In conclusion, from our own research in C-7 piperazine modifications of the piperazinyl quinolones, we were able to identify a series of *N*-substituted piperazinyl quinolones **6e-i** in which the N-4 position of piperazinyl group of ciprofloxacin **1** and norfloxacin **2** replaced with various 2-oxyiminoethyl derivatives moieties with *in vitro* cytotoxic activity comparable or higher than reference drug etoposide.

ACKNOWLEDGEMENT

This study was supported by grants from the Kerman University of Medical Science and Iran National Science Foundation.

REFERENCES

[1] Piddock L.J.V. Mechanisms of fluoroquinolone resistance: an update 1994-1998. Drugs, 58 (Suppl. 2): 11-18, 1999.

[2] Drlica K., Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol. Mol. Biol. Rev.,

61: 377-392, 1997.

[3] Drlica K. Mechanism of fluoroquinolone action. Curr. Opin. Microbiol., 2: 504-508, 1999.

[4] Drlica K., Zhao X. DNA topoisomerase IV as a quinolone target. Curr. Opin. Antiinfect. Investig. Drugs, 1: 435-442, 1999.

[5] Wang J.C. DNA topoisomerases. Annu. Rev. Biochem., 65: 635-692, 1996.

[6] Burden D.A., Osheroff N. Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. Biochem. Biophys. Acta, 1400: 139-154, 1998.

[7] Coughlin S.A., Danz D.W., Robinson R.G., Klingbeil K.M., Wentland M.P., Corbett T.H., Waud W.R., Zwelling L.A., Altschuler E., Bales E., Rake J.B. Mechanism of action and antitumor activity of (S)-10-(2,6-dimethyl-4-pyridinyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyridol1,2,3-de-1,4benzothiazine-6-carboxylic acid (WIN 58161). Biochem. Pharmacol., 50: 111-122, 1995.

[8] Clement J.J., Burres N., Jarvis K., Chu D.T., Swiniarski J., Alder J. Biological characterization of a novel antitumor quinolone. Cancer Res., 55: 830-835, 1995.

[9] Anderson V.E., Osheroff N. Type II topoisomerases as targets for quinolone antibacterials: turning Dr. Jekyll into Mr. Hyde. Curr. Pharm. Design, 7: 339-353, 2001.

[10] Sissi C., Palumbo M. The quinolone family: from antibacterial to anticancer agents. Curr. Med. Chem. Anti-Cancer Agents, 3: 439-450, 2003.

[11] Robinson M.J., Martin B.A., Gootz T.D., McGuirk P.R., Moynihan M., Sutcliffe J.A., Osheroff N. Effects of quinolone derivatives on eukaryotic topoisomerase II. A novel mechanism for enhancement of enzyme-mediated DNA cleavage. J. Biol. Chem., 266: 14585-14592, 1991.

[12] Robinson M.J., Martin B.A., Gootz T.D., McGuirk P.R., Osheroff N. Effects of novel fluoroquinolones on the catalytic activities of eukaryotic topoisomerase II: Influence of the C-8 fluorine group. Antimicrob. Agents Chemother., 36: 751-756, 1992.

[13] Elsea S.H., McGuirk P.R., Gootz T.D., Moynihan M., Osheroff N., Drug features that contribute to the activity of quinolones against mammalian topoisomerase II and cultured cells: correlation between enhancement of enzyme-mediated DNA cleavage in vitro and cytotoxic potential. Antimicrob. Agents Chemother., 37: 2179-2186, 1993.

[14] Foroumadi A., Emami S., Haghighat P., Moshafi M.H. Synthesis and in-vitro antibacterial activity of new N-substituted piperazinyl quinolones. Pharm. Pharmacol. Commun., 5: 591-594, 1999.

[15] Mirzaei M., Foroumadi A. Synthesis and in-vitro antibacterial activity of N-piperazinyl quinolone derivatives with a 2-thienyl group. Pharm. Pharmacol. Commun., 6: 351-354, 2000.

[16] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63, 1983.

[17] Foroumadi A., Emami S., Mehni M., Moshafi M. H., Shafiee A. Synthesis and antibacterial activity of N-2-(5-bromothiophen-2-yl)-2-oxoethyl and N-(2-5-bromothiophen-2-yl)-2-oxyiminoethyl derivatives of piperazinyl quinolones. Bioorg. Med. Chem. Lett., 15: 4536-4539, 2005.

[18] Nikaido H., Vaara M. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev., 49: 1-32, 1985.

[19] Neves P., Berkane E., Gameiro P., Winterhalter M., de Castro B. Interaction between quinolones antibiotics and bacterial outer membrane porin OmpF. Biophys. Chem., 113: 123-128, 2005.

[20] Alovero F., Pan X.-S., Morris J.E., Manzo R.H., Fisher L.M. 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: 320-325, 2000.