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Synthesis and antibacterial activity of new fluoroquinolones containing a substituted *N*-(phenethyl)piperazine moiety

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Abstract—*N*-(Phenethyl)piperazinyl quinolone derivatives that bear a methoxyimino-substituent have been synthesized and evaluated for antimicrobial activity against Gram-positive and Gram-negative microorganisms. In addition, to define structure–activity relationships, ciprofloxacin derivatives containing 2-oxo-2-phenylethyl or 2-hydroxyimino-2-phenylethyl moieties at N-4 position of piperazine ring were prepared and tested. Ciprofloxacin derivatives, containing a *N*-(chloro-substituted phenethyl) residue, showed in vitro Gram-positive and Gram-negative activity generally comparable or superior to that of reference quinolones.

Quinolones are synthetic antibacterial compounds based on a 4-quinolone skeleton. Fluoroquinolones have been clinically successful and are used to treat bacterial infections in both community and hospital settings. Quinolones target bacterial type II topoisomerases, generally DNA gyrase in Gram-negative bacteria and DNA topoisomerase IV in Gram-positive bacteria.[1–3] Most of the quinolones currently on the market or under development have only moderate activity against many Gram-positive cocci, including Staphylococci and Streptococci.[4,5] This insufficient activity has not only limited their use in infections caused by these organisms, such as respiratory tract infections, but has also been believed to be one of the reasons for the rapidly developing quinolone resistance. Therefore, recent efforts have been directed toward the synthesis of new quinolone antibacterials that can provide improved Gram-positive antibacterial activity, while retaining good Gram-negative activity.[4,5]

The synthesis and evaluation of over 10,000 quinolone derivatives resulted in thorough knowledge of the structure–activity relationship for many quinolone substituents.[6] The most intensive structural variation has been carried out on amines at the 7-position, partially due to the ease of their introduction through a nucleophilic aromatic-substitution reaction on the corresponding halide.[7,8] Piperazine, aminopyrrolidine, and their substituted derivatives have been the most successfully employed side chains, as evidenced by the compounds currently on the market.[7,8] Originally, the newer fluoroquinolones arose with the development of 7-piperazinyl quinolones, such as norfloxacin **1**, ciprofloxacin **2**, and enoxacin **3** (Fig. 1), which combined structural features of flumequine (C-6 fluorine atom) and pipemidic acid (C-7 piperazine side chain).[8–12]

According to the inhibition mechanisms of the quinolones, proposed by Shen et al.,[13–15] the site near the C-7 substituent is regarded as drug–enzyme interaction domain. In addition, Klopman et al. also concluded that the cell permeability is dominantly controlled by C-7 substituent.[16] The piperazine moiety of 7-piperazinyl quinolones possesses enough structural flexibility to allow product optimization. In addition, the C-7 substituent affects the interaction with the target, and both

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Keywords: Piperazinyl quinolones; Ciprofloxacin derivatives; Antimicrobial activity; Methoxyimino-substituent.

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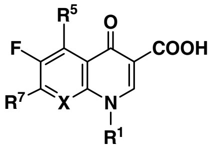


1, Norfloxacin; R^1= ethyl, R^5= H, R^7= piperazin-1-yl, X= CH

2, Ciprofloxacin; R^1= cyclopropyl, R^5= H, R^7= piperazin-1-yl, X= CH

3, Enoxacin; R^1= ethyl, R^5= H, R^7= piperazin-1-yl, X= N

Figure 1. Common pharmacophore of fluoroquinolones and structure of three piperazinyl quinolones.

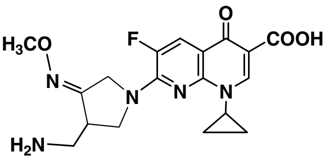
the activity spectrum and kinetic profile can be controlled at C-7.[17,18]

Recently, as part of an ongoing program to find potent and broad-spectrum antibacterial agents that display strong Gram-positive activity, we have focused our attention on modification of the C-7 basic group of the quinolone.[19–21] Therefore, our strategy to achieve a better antimicrobial profile has focused on introducing new functionality on the piperazine ring. From our research in C-7 piperazine modifications of the quinolones,[19–21] we were able to identify a series of *N*-substituted piperazinyl quinolones in which the N-4 hydrogen of piperazinyl group of norfloxacin **1**, ciprofloxacin **2**, and enoxacin **3** is replaced with various 2-oxoethyl or 2-oxyiminoethyl moieties and displays in vitro microbiological activity against Gram-positive organisms comparable or higher than respective parent quinolones. However, the improved activity against Gram-positive bacteria was generally at the expense of activity against Gram-negative bacteria (specially against *Pseudomonas aeruginosa*). Moreover, when we exchanged oxime with a bulky *O*-benzyloxime group, the synthesized compounds did not maintain activity against both Gram-positive and negative bacteria.[19–21] Since our goal was the discovery of new quinolone antibacterial agents with both strong Gram-positive and Gram-negative bacteria, we designed novel *N*-substituted piperazinyl quinolones **5a–l** (Fig. 2) and related compounds **5m–r** that possessed a methoxyiminoethyl substituent in the piperazine ring. These modifications were made on the basis of gemifloxacine **4**, a well-known quinolone antibacterial agent possessing methoxyimino group attached to the pyrrolidine ring at C-7 position (Fig. 2).[22]

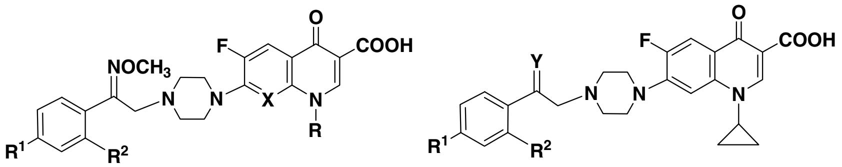
The *N*-[2-aryl-2-methoxyiminoethyl]piperazinyl quinolone analogues **5a–n** were prepared by the synthetic route diagrammed in Scheme 1. We have previously reported our synthesis of the versatile α-halooxime intermediates, which allowed us to vary the aromatic ring and the center oxime for our structure–activity relationship studies.[19–21] In this case, α-chloroketone **6** was converted to α-chloro-*O*-methyloxime intermediate **7** by stirring with excess of methoxyamine hydrochloride in methanol at room temperature. Reaction of piperazinyl quinolones (**1**, **2** or **3**) with α-chloro-*O*-methyloxime **7** in DMF, in the presence of $NaHCO_3$ at room temperature, afforded *N*-[2-aryl-2-methoxyiminoethyl]piperazinyl quinolone analogues **5a–n** as a mixture of (*E*)- and (*Z*)-isomers.

Preliminarily, compounds **5a–l** (Table 1) were evaluated for their antibacterial activity against Gram-positive (*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, and *Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, and *P. aeruginosa* ATCC 9027) bacteria using a conventional agar-dilution method.[23] The MIC (minimum inhibitory concentration) values were determined and compared to norfloxacin **1**, ciprofloxacin **2**, and enoxacin **3** as reference drugs.

Table 1 reveals that compound **5j** followed by **5d–g** are superior in inhibiting the growth of Staphylococci (MIC = 0.39–6.25 μg/mL), while the remaining compounds are statistically equivalent in antibacterial activity against these microorganisms with moderate activity (MIC = 12.5–25 μg/mL). Most tested compounds had respectable in vitro activity against *B. subtilis*, but were less active than the reference drugs. Generally, most compounds showed moderate to good activity against Gram-negative bacteria, with the exception for *P. aeruginosa*. Compound **5j** was the



4, Gemifloxacin



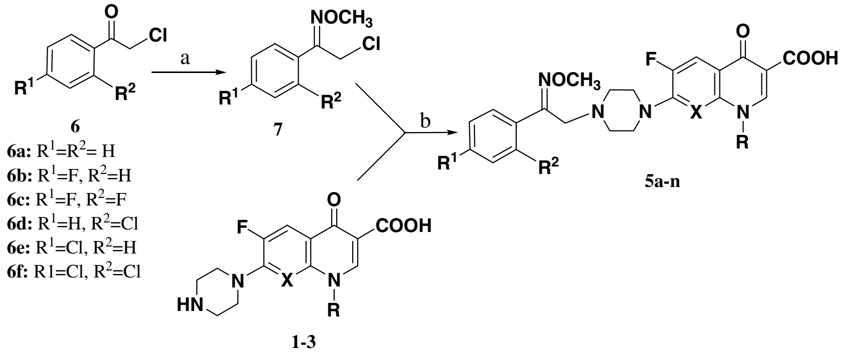
X= CH or N
R= cyclopropyl or ethyl
R^1& R^2= H, Cl, F

5a-l

R^1& R^2= H, Cl, F
Y= O, NOH, NOMe

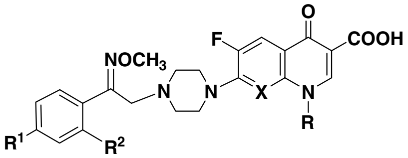
5m-r

Figure 2.



Scheme 1. Synthesis of the compounds **5a–n**. Reagents and conditions: (a) *O*-methylhydroxylamine hydrochloride, MeOH, rt; (b) DMF, $NaHCO_3$, rt.

Table 1. Structures and in vitro antibacterial activities of compounds **5a–l** against selected strains (MICs in µg/mL)



Compound	X	R	R^1	R^2	*S. aureus*	*S. epidermidis*	*B. subtilis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
5a	CH	Cyclopropyl	H	H	12.5	12.5	6.25	6.25	3.13	100
5b	CH	Ethyl	H	H	25	25	6.25	12.5	12.5	>100
5c	N	Ethyl	H	H	25	25	12.5	12.5	12.5	>100
5d	CH	Cyclopropyl	F	H	6.25	6.25	6.25	3.13	3.13	50
5e	CH	Ethyl	F	H	6.25	1.56	3.13	3.13	0.78	100
5f	N	Ethyl	F	H	12.5	6.25	6.25	6.25	1.56	100
5g	CH	Cyclopropyl	F	F	6.25	6.25	0.78	1.56	0.78	12.5
5h	CH	Ethyl	F	F	12.5	12.5	1.56	25	12.5	>100
5i	N	Ethyl	F	F	25	25	1.56	50	25	>100
5j	CH	Cyclopropyl	Cl	Cl	0.39	0.39	0.025	0.025	0.012	1.56
5k	CH	Ethyl	Cl	Cl	12.5	12.5	1.56	12.5	6.25	>100
5l	N	Ethyl	Cl	Cl	25	25	3.13	12.5	6.25	50
1	Norfloxacin				0.78	0.78	0.049	0.049	0.049	3.13
2	Ciprofloxacin				0.39	0.39	0.025	0.025	0.025	0.78
3	Enoxacin				1.56	0.78	0.098	0.098	0.098	6.25

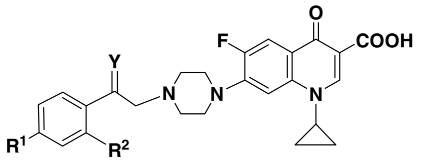
most potent against *P. aeruginosa*, with MIC value of 1.56 µg/mL. Its activity was found to be comparable to reference drugs.

Indeed, all compounds (Table 1) are less active than reference drugs with the exception of **5j**. Compound **5j** with a cyclopropyl substituent at N-1, a CH at the position-8 and a 2,4-dichlorophenyl group ($R_1 = R_2 = Cl$) exhibited the most potent inhibitory activity against both Gram-positive and Gram-negative bacteria. The MIC values of this compound were relatively equivalent to that of reference drug ciprofloxacin and were lower than other standard quinolones (norfloxacin and enoxacin).

We have briefly investigated the SAR of the methoxyimino-functionalized piperazinyl quinolones and the basic molecule has been modified at the N-1 position, with different groups added to the side-chain phenyl ring, and position 8. These modifications result in changes in the potency and antibacterial activity profile of the quinolones and indicate that only introduction of 2-(2,4-dichlorophenyl)-2-methoxyiminoethyl residue at the N-4 position of piperazine ring in ciprofloxacin molecule is well tolerated in terms of activity against both Gram-positive and Gram-negative bacteria.

As can be deduced from these data, the impact of the 2,4-dichloro- on the phenethyl side chain is highly dependent on the type of substituents at N-1 and position-8. In view of the results obtained with 2-(2,4-dichlorophenyl)-2-methoxyiminoethyl residue at the N-4 position of piperazine ring in ciprofloxacin molecule (compound **5j**), we proceeded to survey the impact of methoxyimino-moiety and chloro-substituent at 2- and 4-positions of phenethyl moiety. For this purpose we prepared several new *N*-phenethyl ciprofloxacin derivatives **5m–r** according to our previously described method,[19–21] and evaluated their antibacterial activity (Table 2). In addition, this series of compounds were tested in vitro against two clinically isolated strains of

Table 2. Structures and in vitro antibacterial activities of compounds **5j** and **5m–r** against selected strains (MICs in μg/mL)



Compound	Y	R^1	R^2	*S. aureus*	MRSA[a] I	MRSA II	*S. epidermidis*	*B. subtilis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
5j	$NOCH_3$	Cl	Cl	0.39	0.39	0.39	0.39	0.025	0.025	0.013	1.56
5m	$NOCH_3$	H	Cl	0.39	0.39	0.39	0.195	0.049	0.025	0.013	6.25
5n	$NOCH_3$	Cl	H	0.78	0.39	0.39	0.39	1.56	1.56	0.78	>100
5o	O	H	Cl	0.39	0.39	0.39	0.195	0.049	0.098	0.025	6.25
5p	O	Cl	H	0.39	0.39	0.39	0.195	0.049	0.025	0.013	1.56
5q	NOH	H	Cl	0.39	0.39	0.39	0.195	0.78	0.78	0.025	25
5r	NOH	Cl	H	0.39	0.195	0.098	0.195	0.78	0.78	0.049	100
1				0.78	0.78	0.78	0.78	0.049	0.049	0.049	3.13
2				0.39	0.39	0.39	0.39	0.025	0.025	0.025	0.78

[a] MRSA, methicillin-resistant *S. aureus*.

methicillin-resistant *S. aureus* (MRSAs I and II). To determine the effect of adding 2,4-dichloro-substitutions, we prepared both the *ortho*- and *para*-chloro derivatives **5m** and **5n**. When compared to **5j**, both compounds had similar in vitro activities against Staphylococci, but the susceptibility against Gram-negative bacteria was reduced in 4-chloro-derivative **5n**. Moreover, the susceptibility against *P. aeruginosa* was lost in **5n** and maintained in **5m**. Finally, for investigating the importance of methoxyimino-moiety in ciprofloxacin series, we compared compounds **5m** and **5n** with oxime containing compounds **5q** and **5r**, and also with ketones **5o** and **5p**. All these compounds had potent activities against Staphylococci. 4-Chloro-oxime analogue **5r** was the most potent compound against MRSAs, while its activity against *P. aeruginosa* was lost similar to 4-chloro-*O*-methyloxime derivative **5n**. Most of the new *N*-phenethyl ciprofloxacin derivatives (**5j** and **5m–r**) containing a chloro-substituent on phenyl ring showed potent antibacterial activity and modification of the ketone, oxime, and *O*-methyloxime on chlorophenethyl side chain produced only relatively minor changes in activity. Thus, in the *N*-phenethyl ciprofloxacin series, antibacterial activity can be positively modulated through the introduction of chlorine atom and the functionality on the ethyl spacer in combination with the chloro-substitution pattern is important for activity against *P. aeruginosa*.

The physicochemical properties of quinolones (e.g., relative hydrophobicity, charge or molecular mass) are important for penetration into bacterial cell and have a different role in Gram-negative and Gram-positive bacteria. Increasing molecular mass and bulkiness of substituent at C-7 position hinder penetration of quinolones into Gram-negative organisms through the porin channels.[24–26] Therefore, the size and lipophilicity of the substitution on the phenethyl moiety were considered to be key factors in determining antibacterial activity. Halogens, like chlorine or fluorine, are very useful to modulate the electronic effects on phenyl rings of drugs. Moreover, these atoms may also influence the steric characteristics and the hydrophilic–hydrophobic balance of the molecules. On the other hand, carbonyl related functional groups (ketone, oxime, and *O*-methyloxime) have different steric, electronic, and lipophilic characteristics. Thus, these structural modifications of the *N*-phenethylpiperazinyl quinolones were expected to allow modulation of the physical properties of the corresponding quinolones, while retaining the strong biological activity of the piperazinyl quinolones (especially ciprofloxacin).

In conclusion, we have designed and synthesized novel quinolone agents bearing a methoxyiminoethyl substituent or related residues in the 4-position of the piperazine ring. It was found that chloro-substituted phenethyl group attached to piperazine ring served as promising C-7 substituents for piperazinyl quinolone antibacterials. Among these compounds, ciprofloxacin derivatives **5j** and **5p** provided in vitro Gram-positive and Gram-negative activity generally comparable or superior to that of reference quinolones.

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