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Synthesis and In-vitro Antibacterial Activity of *N*-Piperazinyl Quinolone Derivatives with a 2-Thienyl Group

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Abstract

Novel N-substituted piperazinyl quinolones have been synthesized by reaction of piperazinyl quinolones with α-bromo-2-acetylthiophene or α-bromo-2-acetylthiophene oximes and evaluated for in-vitro antibacterial activity.

Compounds with a [2-oxo-2-(2-thienyl)ethyl] group had antibacterial activity against Gram-positive and Gram-negative bacteria similar to that of reference drugs, ciprofloxacin, norfloxacin and enoxacin. The oximes were almost as potent as the corresponding ketones against staphylococci but less active against Gram-negative bacteria. Replacement of the hydrogen of the oxime with a methyl group resulted in greater antibacterial activity against both Gram-positive and Gram-negative bacteria, with minimum inhibitory concentrations (MIC) ranging from 0·0075 to 0·5 $\mu g\,mL^{-1}$; this potency was greater than that of the reference compounds. The antibacterial activity of the *O*-benzyloxime derivatives was lower. Ciprofloxacin derivatives were usually more active than norfloxacin or enoxacin derivatives.

Quinolones are structurally related to nalidixic acid. New fluorinated piperazinyl quinolones, congeners of nalidixic acid, have a much wider in-vitro antibacterial spectrum and are more potent than the parent compound (Mandell & Petri 1996).

During recent years much attention has been devoted to the synthesis of new 4-quinolone-3-carboxylates and to testing these newly prepared agents for antibacterial activity (Foroumadi et al 1997). Further advances in quinolone development are likely to provide better compounds for clinical use (Androile 1999). Quinolones exert antibactericidal activity primarily by inhibiting bacterial DNA gyrase. The inhibition of DNA gyrase and the cell permeability of the quinolones are greatly influenced by the nature of the C-7 substituent on the standard structure of the 4-quinolone-3-carboxylic acids (Domagala et al 1986); substitution at the C-7 position of the quinolone molecule by bulky functional groups is also possible (Shen et al 1989). We have previously synthesized and measured the in-vitro antibacterial activity of *N*-[2-(2-furyl)-2-oxoethyl]piperazinyl quinolones and related compounds; these compounds had significant antibacterial activity against Gram-positive and Gram-negative bacteria (Foroumadi et al 1999). We report here the synthesis and in-vitro antibacterial activity of a series of *N*-[2-oxo-2-(2-thienyl)ethyl and *N*-[2-oxyimino-2-(2-thienyl)ethyl]piperazinyl quinolones (Figure 1 and Table 1, **6a**–**c** and **8a**–**i**).

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Materials and Methods

Chemical procedures

α-Bromo-2-acetylthiophene (**2**) was prepared by bromination of 2-acetylthiophene (**1**) (Mullen et al 1988). Reaction of the **2** with hydroxylamine hydrochloride, *O*-methylhydroxylamine hydrochloride or *O*-benzylhydroxylamine hydrochloride in methanol at room temperature (Schumann et al 1964; Schaefer & Mongold 1982) gave the intermediate compounds **7a**–**c** in good yields. Reaction of quinolones (**3**, **4** or **5**) with α-bromo-2-acetylthiophene (**2**) or α-bromo-2-acetylthiophenoximes (**7a**–**c**) without protection of the 3-carboxylic acid group of the quinolones (Kondo et al 1986) in the presence of sodium bicarbonate in dimethylformamide (DMF) at room temperature afforded ketones **6a**–**c** and oximes **8a**–**i** respectively (Figure 1).

The compounds were characterized by ^{1}H NMR and IR spectroscopy and by microanalysis. The

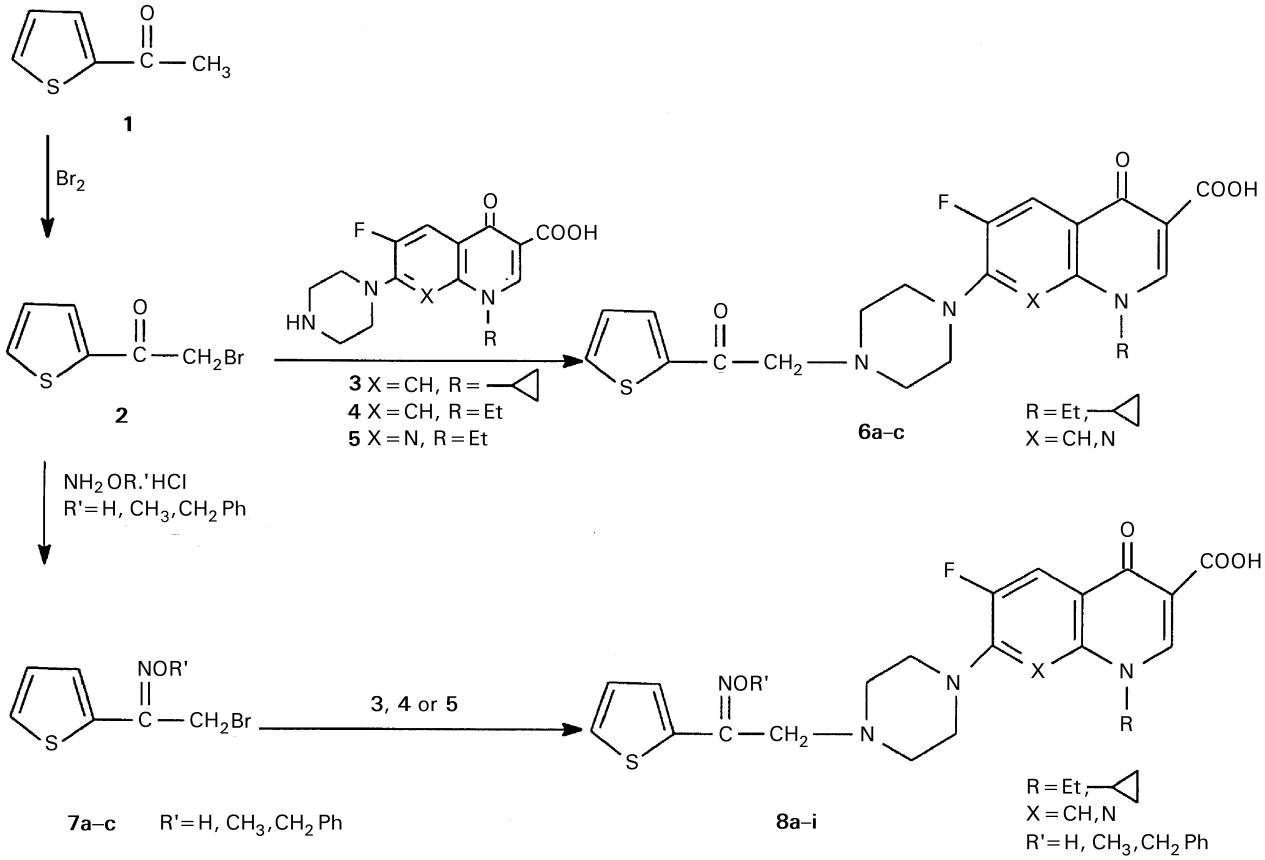


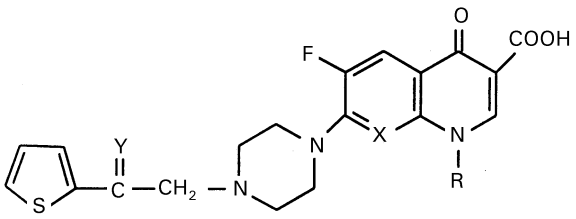
Figure 1. Synthesis of N-substituted piperazinyl quinolones.

purity of all the products was determined by thin-layer chromatography with several mobile phases of different polarity. The reaction times and physical properties of the products are listed in Table 1.

In-vitro antibacterial activity

The in-vitro antibacterial activity of the test compounds against Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-

Table 1. Physical properties of N-substituted piperazinyl quinolones.



Compound	Y	R	X	mp (°C)	Yield (%)	Crystallization solvent	Reaction time (h)
6a	O	Cyclopropyl	CH	196–198	65	EtOH	48
6b	O	Ethyl	CH	218–220	73	EtOH	48
6c	O	Ethyl	N	208–210	70	EtOH	48
8a	NOH	Cyclopropyl	CH	238–240	45	$CHCl_3$–EtOH	72
8b	NOH	Ethyl	CH	250–252	40	$CHCl_3$–EtOH	48
8c	NOH	Ethyl	N	248–250	45	$CHCl_3$–EtOH	60
8d	$NOCH_3$	Cyclopropyl	CH	250–252	40	EtOH	168
8e	$NOCH_3$	Ethyl	CH	233–235	60	EtOH	48
8f	$NOCH_3$	Ethyl	N	196–198	67	EtOH	168
8g	$NOCH_2C_6H_5$	Cyclopropyl	CH	138–140	45	EtOH	96
8h	$NOCH_2C_6H_5$	Ethyl	CH	176–178	54	EtOH	96
8i	$NOCH_2C_6H_5$	Ethyl	N	150–152	50	EtOH	168

negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*) bacteria was tested by use of conventional agar-dilution procedures (Baron & Finegold 1990) and compared with that of ciprofloxacin, norfloxacin and enoxacin.

Twofold serial dilutions of the compounds and reference drugs were prepared in Muller-Hinton agar. Drugs (6·4 mg) were dissolved in dimethylsulphoxide (DMSO; 1 mL) and the solution was diluted with water (9 mL). Further progressive double-dilutions with melted Muller-Hinton agar were performed to obtain the required concentrations of 64, 32, 16, 8, 4, 2, 1, 0·5, 0·25, 0·13, 0·06, 0·03, 0·015, 0·0075 and 0·00375 $\mu g\,mL^{-1}$. Petri dishes were inoculated with $1-5 \times 10^4$ colony-forming units and incubated at 37°C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

Results and Discussion

The nature of the functional group at the 7 position of the quinolone ring system is known to influence the range and extent of in-vitro antibacterial activity. We have previously reported a series of quinolones with *N*-(2-oxo-2-phenyl ethyl), *N*-[2-(2-furyl)-2-oxoethyl]piperazinyl quinolones and related compounds attached to the 7 position which had significant antibacterial activity against some Gram-positive and Gram-negative bacteria (Foroumadi et al 1997, 1999).

In this study a series of *N*-[2-oxo-(2-thienyl)ethyl, *N*-[2-hydroxyimino-2-(2-thienyl)ethyl], *N*-[2-methoxyimino-2-(2-thienyl)ethyl] and *N*-[2-phenylmethoxyimino-2-(2-thienyl)ethyl]piperazinyl quinolones (**6a–c** and **8a–i**) (Figure 1; Table 1) were synthesized and evaluated for in-vitro antibacterial activity.

The activity of compounds **6a–c** and **8a–i** was tested against Gram-positive and Gram-negative bacteria. The MIC was determined by use of conventional agar-dilution procedures (Baron & Finegold 1990). Ciprofloxacin (**3**), norfloxacin (**4**) and enoxacin (**5**) were chosen as standard controls. Results obtained from testing of antibacterial activity are summarized in Table 2.

Compounds with a 2-oxo-2-(2-thienyl)ethyl group (**6a–c**) had antibacterial activity similar to that of ciprofloxacin, norfloxacin and enoxacin against both Gram-positive and Gram-negative bacteria. The oximes **8a–c** were more potent than the reference compounds against Gram-positive bacteria although the potency against Gram-negative bacteria was significantly less. These findings are similar to those of our previous study (Foroumadi et al 1999).

In contrast with our previous study, which showed that replacing the hydrogen of the oxime by a methyl group reduced in-vitro antibacterial activity (Foroumadi et al 1999), in this study all three compounds with methoxyimino substitution (**8d–f**), were more active than ciprofloxacin, norfloxacin and enoxacin against both Gram-positive and Gram-negative bacteria. Replacement of the

Table 2. In-vitro antibacterial activity of piperazinyl quinolones expressed as the minimum inhibitory concentration (MIC).

Compound	MIC ($\mu g\,mL^{-1}$)				
	S. aureus ATCC 6538p	*S. epidermidis* ATCC 12228	*E. coli* ATCC 8739	*K. pneumoniae* ATCC 10031	*E. cloacae* PTCC 1003
6a	0·5	0·25	0·0075	0·25	0·06
6b	0·5	0·5	0·06	0·25	1
6c	0·5	0·25	0·25	0·5	0·25
8a	0·06	0·5	0·5	2	1
8b	0·5	0·25	4	8	4
8c	0·13	0·06	8	16	16
8d	0·5	0·06	0·0075	0·06	0·03
8e	0·5	0·5	0·06	0·25	0·13
8f	0·5	0·5	0·25	0·5	0·25
8g	0·5	0·5	4	64	8
8h	64	32	16	64	> 64
8i	64	32	64	> 64	> 64
Ciprofloxacin (**3**)	0·25	0·25	0·06	0·06	0·13
Norfloxacin (**4**)	1	1	0·25	0·25	0·13
Enoxacin (**5**)	1	0·5	0·13	0·5	0·13

hydrogen of the oxime by a benzyl group (**8g–i**) resulted in reduced in-vitro antibacterial activity against Gram-positive and Gram-negative bacteria. Similar results were obtained in our previous study (Foroumadi et al 1999). Ciprofloxacin derivatives were usually more active than norfloxacin and enoxacin derivatives.

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