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Design and synthesis of penicilloyl oxymethyl quinolone carbamates as a new class of dual-acting antibacterials

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Introduction

Penicillins, one of the safest β-lactams in clinical use, act by acylating the active site serine residue of transpeptidases, which are enzymes responsible for cross-linking peptidoglycan in the final step towards cell wall synthesis [1]. Chemically combining penicillins with 4-quinolones, which are DNA-gyrase inhibitors, can result in a synergy between their respective modes of action [2]. Albrecht *et al* [3, 4] have proved the efficacy of similar antibacterials with respect to a broad spectrum of activity and a superior pharmacokinetic profile.

In the present work, we have synthesized a new class of antibacterials in which penicillins are linked to the piperazinyl nitrogen of norfloxacin through a methylene carbamate linkage. We have evaluated these compounds for their *in vitro* antibacterial activity.

Chemistry

The bifunctional molecules were synthesized as shown in scheme 1. Chloromethyl carbamate of norfloxacin was synthesized by previously reported procedures [5]. Conversion to the corresponding iodomethyl derivative by the Finkelstein reaction proved unsuccessful.

Compound **1** was subsequently condensed with the carboxylate ion of penicillin using a modified Koening–Knorr procedure. The reaction between **1** and sodium salt of cloxacillin was initially carried out in the presence of equimolar silver oxide in dimethylformamide. However, the poor solubility of silver oxide in the reaction solvent resulted in very poor yields of the condensed product. Changing the catalyst to silver nitrate gave the dual-action penicillin **2** in 40% yield.

In the case of ampicillin, the free amino group was reacted with methyl acetoacetate to give the enamine-protected salt **3**. Subsequent deprotection, after the nucleophilic displacement, was effected in 10% HCl (pH 2.5) and the condensed product was isolated as a hydrochloride salt.

In the present study, ampicillin and cloxacillin have been used as model penicillins and norfloxacin as a representative quinolone. However, the procedure can be extended to other penicillins and quinolones with a secondary piperazinyl nitrogen at the 7 position.

Pharmacology

The two penicilloyl oxymethyl quinolone carbamates (**2** and **4**) were screened for their *in vitro* antibacterial activity. This study was carried out to estimate the inherent antibacterial activity of the compounds against Gram-positive and Gram-negative microorganisms. The traditional streak–plate method was employed, wherein the following parameters were held constant: i) media composition; ii) incubation time (24 h); and iii) incubation temperature (37°C). The results were then read as the presence or absence of growth (table I).

Results and dicussion

Table I shows a comparison of the antibacterial activities of the bifunctional penicillins (**2** and **4**)

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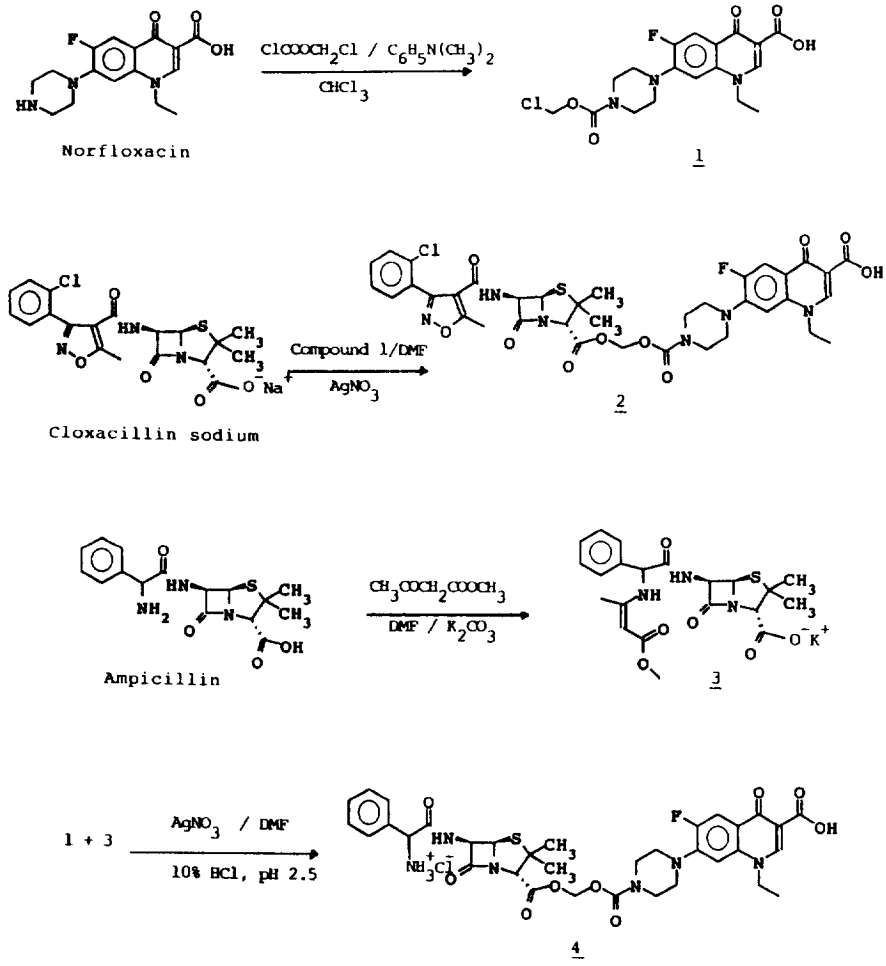

Scheme 1.

and the standard antibacterials like ampicillin and norfloxacin. In general the penicillin-quinolone carbamates showed excellent broad spectrum activity. Compound **4** was inactive against chloramphenicol-resistant *Salmonella* species, but had an MIC ≤ 2.5 μg/ml against the remaining strains. Compound **2** showed excellent activity (MIC ≤ 1.25 μg/ml) against all the strains employed in the study. The activity of **2** against chloramphenicol-resistant *Salmonella* species was noteworthy, since the reference compounds are not active against this strain.

Conclusion

The inherent antibacterial activity of the synthesized compounds, particularly against microorganisms resistant to the reference compounds, indicate that they exhibit a dual mode of action. The penicillins, like other β-lactam antibacterials, act by acylating active site residue of peptidoglycan transpeptidase and the quinolones act by inhibiting bacterial DNA gyrase.

The behaviour of these dual-action penicillins *in vivo* remains to be studied. It may be possible that the bifunctional penicillins are cleaved through the action of esterases, releasing the bioactive molecules. Alternatively, it may be capable of directly attacking either of the two targets as a single entity.

Experimental protocols

Chemistry

Melting points were determined by the open capillary method and are uncorrected. Proton magnetic resonance spectra

Table I. Antibacterial sensitivity testing.

Antibiotic	*Conc (μg/ml)*	*Staphylococcus aureus*	*Staphylococcus lutea*	*Escherichia coli*	*Bacillus subtilis*	*Salmonella*		
						Std NCTC	*Sen/CLP*[a]	*Res/CLP*[a]
Ampicillin	10.0	A	A	NA	A	A	NA	NA
	5.0	A	A	NA	A	A	NA	NA
	2.5	A	A	NA	A	A	NA	NA
Norfloxacin	10.0	A	NA	A	A	A	A	A
	5.0	A	NA	A	A	A	A	A
	2.5	A	NA	A	A	A	A	NA
2	10.0	A	A	A	A	A	A	A
	5.0	A	A	A	A	A	A	A
	2.5	A	A	A	A	A	A	A
	1.25	A	A	A	A	A	A	A
4	10.0	A	A	A	A	A	A	NA
	5.0	A	A	A	A	A	A	NA
	2.5	A	A	A	A	A	NA	NA

Sen/CLP = sensitive to chloramphenicol; Res/CLP = resistant to chloramphenicol; A = active; NA = not active.

(^{1}H-NMR) were recorded on a 60 MHz Varian EM-360L spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane. Infrared (IR) spectra were obtained on a Buck-Scientific M-500 spectrometer.

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4'-[[[6-[3-(2-chlorophenyl)-5-methylisoxazol-4-yl-carboxamido]penicillanate]-methoxy]carbonyl]-1'-piperazinyl]quinoline-3-carboxylic acid 2

Chloromethyl carbamate of norfloxacin **1** (0.82 g, 0.2 mM) [5], was dissolved in 15 ml dry dimethylformamide and 0.32 g (2.0 mM) silver nitrate was added and the mixture stirred in the dark for 30 min. Cloxacillin sodium (1.2 g, 2.6 mM) was added and stirring continued in the dark for another 8 h. The reaction was quenched with 100 ml ethyl acetate and the solution filtered through a celite bed (filter aid) to remove suspended solid matter. The filtrate washed with water (2 x 40 ml) and brine (2 x 30 ml). The organic layer dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was recrystallized twice from methylene chloride/ethanol and methylene chloride/diethyl ether, to afford 0.64 g of white solid. Yield: 40%, mp: 90–92°C. IR (KBr) cm^{-1}: 3010, 1780, 1710, 1610, 1200. ^{1}H-NMR ($CDCl_3$): 1.2 (3H, S, $-CH_3$), 1.25 (3H, t, merged, CH_2CH_3), 2.54 (3H, s, isoxazolyl $-CH_3$), 2.9–3.7 (8H, 2s, piperazine), 4.00 (2H, q, $-CH_2CH_3$), 4.13 (1H, C(3)-H), 5.1–5.9 (4H, m, C(5)-H,C(6)-H,$-OCH_2$), 6.63 (1H, d, J = 6 Hz, C(5')-H), 7.20 (4H, s, C_6H_5), 7.66 (1H, d, J = 12 Hz, C(8')-H), 8.35 (1H, s, C(2')-H). Anal $C_{37}H_{36}ClFN_6O_{10}S$ (C, H, N); calc C: 54.78, H: 4.47, N: 10.36; found: C: 55.24, H: 4.20, N: 9.88.

Potassium 6β-[N-(methoxycarbonyl propen-2-yl)-D-α-amino-α-phenyl-acetamido]penicillanate 3

To a suspension of potassium carbonate (0.76 g, 5.0 mM) in 10 ml dimethylformamide, 1.1 ml (10.0 mM) methyl acetoacetate and 2.01 g (5.0 mM) ampicillin trihydrate were added. The reaction mixture was stirred for 2 h at room temperature followed by another 4 h at 0°C. The solvent was decanted and the residue washed with 25 ml diethyl ether. The residue was dissolved in 15 ml acetone and the insoluble material filtered through celite. The filtrate was diluted with 15 ml isopropanol and evaporated *in vacuo* to remove acetone. Recrystallization was induced by scratching and the resulting suspension kept at 0°C for 12 h. The precipitated solid was washed with cold isopropanol and diethyl ether to give a fluffy white solid. Yield: 90%, IR (KBr) cm^{-1}: 1760, 1740, 1624, 1576, 1320. ^{1}H-NMR (DMSO-d_6): 1.23 (3H, s, $-CH_3$), 1.35 (3H, s, $-CH_3$), 1.55 (3H, s, vinylic $-CH_3$), 3.30 (3H, s, ester -CH), 3.60 (1H, s, benzylic -CH), 4.23 (1H, s, -CH), 4.90–5.45 (2H, m, C(5)-H, C(6)-H), 7.05 (5H, s, arom), 8.70 (1H, d, -NH, D_2O exch), 9.10 (1H, d, amide, D_2O exch).

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4'-[[[6β-(D-α-amino-α-phenylacetamido)penicillanate]methoxy]carbonyl]-1'-piperazinyl]quinoline-3-carboxylic acid 4

Chloromethyl carbamate of norfloxacin **1** (0.82 g, 2.0 mM) was dissolved in 15 ml of dry dimethylformamide and 0.32 g (2.0 mM) silver nitrate was added and the reaction mixture stirred in the dark for 30 min. The enamine-protected potassium salt of ampicillin **3** (1.46 g, 3.0 mM) was added and stirring continued in the dark for another 8 h. Ethyl acetate (75 ml) was added and the solution filtered through celite (filter aid). The filtrate washed with a saturated solution of calcium chloride (2 x 50 ml). The organic layer was concentrated to 15 ml *in vacuo* and to this was added 2 ml 2-butanol and 1 ml water. To the above well-stirred and cooled mixture (5–10°C), 10% HCl was added to adjust the pH to 2.5. Water (20 ml) and diethyl ether (50 ml) were added to the solution and the aqueous layer separated. The organic layer was again extracted with two portions 15 ml water. The combined extract was saturated with sodium chloride and extracted with methylene chloride. Evaporation of the solvent after drying over Na_2SO_4 gave the crude product, which was recrystallized from isopropanol/diethyl ether. Yield: 42.5%, mp: 178–182°C, IR (KBr) cm^{-1}: 3050, 1788, 1712, 1548, 1468, 1204. ^{1}H-NMR (DMSO-d_6): 0.85–1.41 (9H, m, 2-CH_3, $-NCH_2CH_3$), 2.82–3.50 (8H, 2s, piperazine), 4.15 (2H, q, $-NCH_2CH_3$), 4.50 (1H, s,

C(3)-H), 5.02–5.70 (4H, m, C(5-H, C(6)-H, -OCH_2O-), 7.00 (6H, m, phenyl, C(8')-H), 7.50 (1H, d, J = 12 Hz, C(5')-H), 8.50 (1H, s, C(2')-H). Anal $C_{34}H_{38}ClFN_6O_9S$ (C, H, N); calc C: 53.65, H: 5.03, N: 11.04; found: C: 53.22, H: 5.10, N: 10.56.

Pharmacology

Antibacterial sensitivity testing

Serial two-fold dilutions of the test compounds (**2** and **4**) and standard antibacterials (norfloxacin and ampicillin) were prepared in dimethylformamide, such that when diluted 10-fold in Mueller–Hinton agar gave concentrations ranging from 10 to 2.5 μg/ml. In case of **2**, further dilution was carried to 1.25 μg/ml. A blank was maintained (no compound) to study the effect of dimethylformamide. Petridishes (10 cm diameter), containing 20 ml final volume (compound + agar), were divided into eight zones. Each zone was streaked with overnight fresh subculture of either standard or test strain of microorganisms. After incubation for 24 h at 37°C, the results were read as presence or absence of growth.

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