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SYNTHESIS AND BIOLOGICAL EVALUATION OF *N*-(1-AZIRIDINO)-6-FLUORO-QUINOLONE-3-CARBOXYLIC ACIDS

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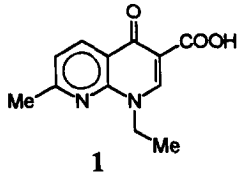
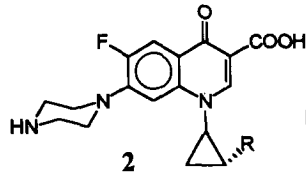
Abstract: New racemic *N*-(1-aziridino)-6-fluoro-7-(4-methylpiperazin-1-yl)-4(1*H*)-quinolone-3-carboxylic acids (**9a-i**) were synthesized and their antibacterial activities were tested against Gram-positive and Gram-negative micro-organisms. According to the MIC, all compounds studied are less active than Ciprofloxacin; two of them (**9a,b**) have similar activity as Nalidixic acid (**1**). Copyright © 1996 Elsevier Science Ltd

Introduction. The appearence of the third generation of antibacterial Fluoroquinolines (based on Nalidixic acid) in the early 1980's gave a new impulse for the intense international competition to synthesize more effective agents with broader spectrum activity[1-3]. Since then, as a result of these efforts, near to a dozen representatives of this class have been introduced into human and veterinary therapy for a broad variety of clinical indications and others are under extensive investigation[4]. During various structure-activity studies[5] the ethyl group at position 1 of Nalidixic acid (**1**) has been replaced by methylamino and cyclopropyl groups (and N-8 by CH) to give Amifloxacin[6] (**3a**) and Ciprofloxacin[7] (**2a**), one of the most clinically successful agents.

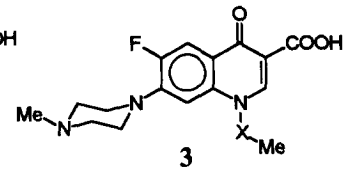
Here we report the synthesis of several fluoroquinolines containing different 1-aziridinyl moieties at position 1 and the evaluation of their *in vitro* antibacterial activities.



1
Nalidixic acid



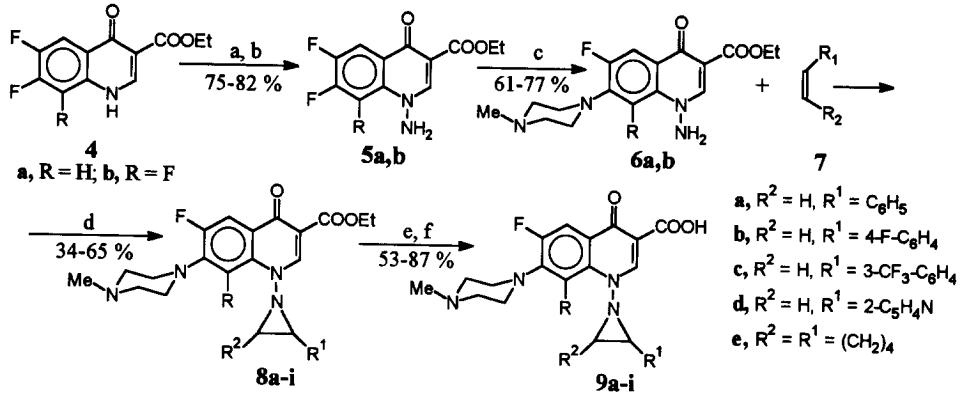
2
a, Ciprofloxacin (R = H)
b, 2-Ph-Ciprofloxacin (R = Ph)



3
a, Amifloxacin (X = NH)
b, Pefloxacin ($X = CH_2$)

Chemistry. The aza analogues of **2b**, the new racemic *N*-(1-aziridino)-6-fluoro-7-(4-methylpiperazin-1-yl)-4(1*H*)-quinolone-3-carboxylic acids (**9a-i**) were synthesized as follows: the quinolone-3-carboxylic acid esters (**4a,b**)[8] were *N*-aminated under basic conditions by the known *N*-aminating reagent *O*-(4-toluenesulfonyl)-hydroxylamine (TSH)[9]. The fluorine substituent at position C-7 of *N*-amino derivatives **5a,b** was replaced by

N-methyl-piperazinyl group to afford **6a,b**. The nitrenes generated from *N*-aminoquinolones (**6a,b**) by treatment with $Pb(OAc)_4$ underwent insertion[10] into the double C-C bond of olefins **7a-f** to give the *N*-(1-aziridino) derivatives (**8a-i**). The hydrolysis of the ester group was performed in ethanol by means of aqueous sodium hydroxide. Upon acidification with acetic acid, the *N*-(1-aziridino)-6-fluoro-7-(4-methylpiperazin-1-yl)-4(1*H*)-quinolone-3-carboxylic acids (**9a-i**) were isolated. Much effort has been made to synthesize the parent compound (**9**, R^1, R^2 = H) by this method (using ethylene as reagent) or by other possible routes. All attempts however failed to provide the desired compound.



a, K_2CO_3 (2 eqv.), DMF, RT, 2 h; **b**, TSH (1.1 eqv.), CH_2Cl_2, **c**, *N*-Methylpiperazine (excess), pyridine, refl., 5 h; **d**, **7a-e** (5 eqv.), $Pb(OAc)_4$ (1.1 eqv.), CH_2Cl_2, RT, 2 h; **e**, NaOH (aq., 2 N), EtOH, RT, 48 h; **f**, AcOH (pH = 7)

	9a	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**9i**
R	H	H	H	H	F	F	F	F	H
R^1	H	H	H	H	H	H	H	H	R^1R^2=
R^2									$(CH_2)_4$

Biological assays. The series of *N*-(1-aziridino)fluoroquinolone-carboxylic acids (**9a-i**) together with selected reference agents - Ciprofloxacin (**2a**) and Nalidixic acid (**1**) - were tested against 23 representative Gram-positive and Gram-negative organisms using a standard procedure described below.

Stock-solutions in phosphate buffer or dimethyl sulfoxide at a concentration of 1 mg/ml (or 10 μg/ml) were prepared and filtered by bacterological filter to obtain sterile solutions. These stock-solutions were then diluted by the suitable culture medium to five fold volume (200 μg/ml or 2 μg/ml). The dilution of the latter (each time to double the volume) resulted in 2 series of solutions (9 members in each).

The MIC's were determined using standard macrodilution techniques[11] (using Wassermann tubes with diameter of 16 mm, length: 90 mm) and compared in multiple experiments and recorded in Table 1. Ciprofloxacin and Nalidixic acid were used as controls and are also included in Table 1.

Table 1
Test Results of Compounds **9a-i**, Ciprofloxacin (**2a**) and Nalidixic acid (**1**)

Organism	Gram	Minimum inhibitory concentrations (MIC's, mg/l)										
		Cipro-floxacin (2a)	Nalidixic acid (1)	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**9i**
B. subtilis ATCC 6633	+	0.03	3.1	6.2	10.0	10.0	50.0	100.0	>100	50.0	>100	3.1
S. aureus SMITH	+	0.12[a]	12.5	12.5	25.0	25.0	50.0	100.0	>100	25.0	>100	50.0
S. aureus 1110 pen.rez.	+	0.25[a]	25.0	25.0	25.0	25.0	100.0	100.0	>100	50.0	>100	50.0
S. faecalis	+	1.0	>100	25.0	25.0	25.0	100.0	>100	>100	50.0	>100	>100
S. pneumoniae	+	1.0	>100	5.0	5.0	12.5	25.0	25.0	50.0	25.0	100.0	100.0
S. pyogenes A 118	+	1.0[b]	>100	10.0	25.0	25.0	50.0	>100	>100	100.0	>100	100.0
S. pyogenes A 115 ROBB	+	1.0[b]	>100	10.0	10.0	50.0	50.0	50.0	100.0	100.0	>100	100.0
M. tub. $H_{37}R_V$ (human)	+	0.2	25.0	6.2	12.5	12.5	50.0	100.0	100.0	100.0	100.0	6.2
M. tub. RAVENEL (bovin)	+	0.05	50.0	3.1	6.2	100.0	25.0	50.0	50.0	50.0	50.0	3.1
B. bronchiseptica ATCC 4617	-	0.25	3.1	6.2	12.5	50.0	100.0	100.0	>100	100.0	>100	25.0
E. coli K_{12}	-	0.008[c]	3.1	50.0	50.0	100.0	100.0	25.0	12.5	50.0	50.0	50.0
E. coli 6R	-	0.12[c]	50.0	100.0	100.0	100.0	100.0	>100	>100	100.0	>100	100.0
K. pneumoniae ATCC 10031	-	0.008[d]	0.8	3.1	6.2	12.5	50.0	12.5	12.5	12.5	12.5	0.8
P. vulgaris XL	-	0.008	>100	50.0	100.0	100.0	>100	25.0	25.0	100.0	25.0	50.0
P. pyocyanea NCTC 10490	-	0.5	>100	100.0	100.0	100.0	100.0	>100	>100	100.0	>100	100.0
S. typhy-murium 51	-	0.015	3.1	50.0	50.0	100.0	100.0	25.0	25.0	50.0	25.0	25.0
S. sonnei	-	0.015	3.1	6.2	25.0	25.0	12.5	6.2	12.5	12.5	6.2	12.5
C. perfringens 70500	+	0.4	ND	6.2	6.2	12.5	50.0	100.0	100.0	100.0	100.0	100.0
A. anitratus 150001 (not pat.)	-	0.25	ND	50.0	50.0	100.0	>100	>100	>100	100.0	>100	12.5
A. faecalis 140001	-	1.0	ND	100.0	100.0	100.0	>100	>100	>100	100.0	>100	100.0
P. inconstans NCTC 8055	-	0.03	ND	100.0	100.0	100.0	>100	50.0	25.0	100.0	50.0	100.0
S. marcescens	-	0.03	ND	100.0	100.0	100.0	100.0	50.0	25.0	100.0	25.0	50.0
B. fragilis ATCC 25285	-	6.2	ND	6.2	25.0	50.0	50.0	>100	>100	50.0	>100	25.0

ND = not detected; MIC's (μg/ml) of Ciprofloxacin (**2a**) and racemic *trans*-**2b**[13]: [a] *S. aureus* ATCC 6538P or *S. aureus* CMX 68613: 0.20 and 1.56; [b] *S. Pyogenes* 930: 0.39 and 1.56; [c] *E. coli* Juhl: 0.01 and 25; [d] *K. pneumoniae* 8045: 0.01 and 6.20

Results and Discussion. The results of measurements of MIC's summarised in Table 1 show that only two derivatives (**9a,b**) of the whole series showed similar or somewhat better activity as Nalidixic acid. These compounds - phenyl (**9a**) and 4-fluorophenyl (**9b**) substituents in the aziridine ring - are significantly more potent against Gram-positive microorganisms than Nalidixic acid and have similar potency against Gram-negative ones. Introduction of a fluorine substituent in position C-8 (derivatives **9e-h**) resulted in the loss of activity. A substituent in the aziridine ring with enhanced electronwithdrawing property (**9c,d**) or a fused ring (**9i**) also led to a decrease in the activity.

While the substitution of the methylene group of the 1-ethyl moiety of Pefloxacin (**3b**) with NH group (Amifloxacin **3a**) resulted in a slight decrease of antibacterial activity[12], the introduction of a nitrogen into position 1 of *trans*-2-Ph-Ciprofloxacin (**2b**)[13] seems to afford similar or less potent compounds (**9a-i**). Analysing the given data[13] shows that **2b** is 10 to 15 times less active against Gram positive and at least 2 orders of magnitude less potent against Gram negative microorganisms than Ciprofloxacin. A similar tendency can be seen when we compare the results of **9a** (and **9b**) and that of Ciprofloxacin[14] in Table 1 of this work.

In conclusion, the results of *in vitro* antibacterial activities of **9** against a range of Gram-positive and Gram-negative microorganisms suggest that substitution of the 1-methylene group within the N1-cyclopropyl moiety of **2b** by NH did not considerably influence the antibacterial potency.

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