SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 1,3,4-THIA(OXA)DIAZINO[6,5,4-i,j]QUINOLINE DERIVATIVES

É. V. Nosova,[1] G. N. Lipunova,[1] and V. N. Charushin[1]

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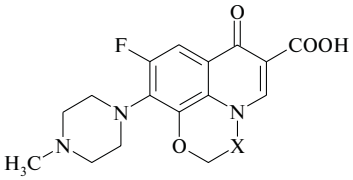
Synthetic compounds of the fluoroquinolone group, representing derivatives of 4-oxo-1,4-dihydro-3-quinolinecarboxylic acid, are widely used as antibacterial drugs (pefloxacin, norfloxacin, ciprofloxacin, etc.) [1 – 3]. Besides the therapeutic breadth and high level of activity, an important advantage of fluoroquinolones in comparison to β-lactams (penicillins, cephalosporins), aminoglycosides, and some other antibiotics is their high chemical stability. Experiments on the chimeric modification of fluoroquinolones have been conducted for more than a decade [3 – 5]. In recent years, considerable attention of researchers has been devoted to the condensed fluoroquinolones, the most widely known representatives of which are ofloxacin and marbofloxacin [6, 7].

We have developed new approaches to the synthesis of annelated fluoroquinolones [8, 9] based on the intramolecular cyclization of ethyl esters of 3-[R-carbonyl(thiocarbonyl)hydrazino]-2-tetra(penta)fluorobenzoylacrylic acid. Using this method, we obtained ethyl esters of 1,3,4-thia(oxa)diazino[6,5,4-i,j]quinoline-6-carboxylic acids and determined the conditions for hydrolysis of the ester groups and for replacement of the fluorine atoms in positions 8 and 10. This allowed us to synthesize a series of modified fluoroquinolones Ia – Ik and IIa – IIe.

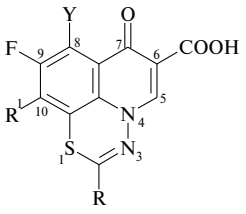
The synthesis of ethyl esters of 1,3,4-thia(oxa)- diazino-[6,5,4-i,j]quinoline-6-carboxylic acid was described in detail in [9, 10] and the synthesis of compounds Ia – Ik was reported in [11]. For this reason, below we describe only the synthesis of previously unreported 1,3,4-oxadiazino-[6,5,4-i,j]quinolines (IIa – IIe).

Since compounds Ia – Ik are representatives of a new heterocyclic system containing fluoroquinolone fragments and compounds IIa – IIe are the analogs of marbofloxacin, it was interesting to evaluate the antimicrobial activity of these substances.

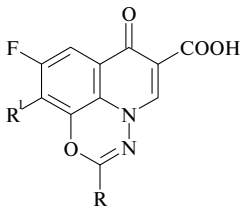


X = CH—CH_3 (ofloxacin)

X = N—CH_3 (marbofloxacin)



Ia – Ik



IIa – IIf

(for R, R^1, and Y see Table 1)

EXPERIMENTAL CHEMICAL PART

2-Phenyl-7-oxo-7-H-9,10-difluoro[1,3,4]oxadiazino[6,5,4-i,j]quinoline-6-carboxylic acid (IIa). To 2.85 g (7.7 mmole) of 2-phenyl-7-oxo-7-H-9,10-difluoro[1,3,4]-oxadiazino[6,5,4-i,j]quinoline-6-carboxylic acid ethyl ester was added 70 ml of an HCl – AcOH (1 : 4) acid mixture. The reaction mixture was boiled for 3.5 h and cooled. The precipitate of compound IIa was separated by filtration and recrystallized from DMSO; yield, 1.9 g (73%); m.p., 232 – 234°C; $C_{17}H_8F_2N_2O_4$; ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.85 (dd, 1H, ^{3}J 10.4, ^{4}J 7.6 Hz, 8-H), 7.96 (m, 3H, 3′-H, 4′-H, 5′-H), 8.06 (m, 2H, 2′-H, 6′-H), 8.80 (s, 1H, CH=), 14.3 (bs, 1H, COOH).

2-(3-Nitrophenyl)-7-oxo-7-H-9,10-difluoro[1,3,4]oxadiazino[6,5,4-i,j]quinoline-6-carboxylic acid (IIf). Compound IIe was obtained using a procedure analogous to that described above; yield, 78%; m.p., 252 – 254°C;

[1] Ural State Technical University, Yekaterinburg, Russia.

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$C_{17}H_7F_2N_3O_6$; ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.76 (m, 1H, 2′-H), 7.78 (dd, 1H, ^{3}J 10.4, ^{4}J 7.6 Hz, 8-H), 7.93 (dd, 1H, ^{3}J 7.9 Hz, 5′-H), 8.43 (m, 1H, 4′-H, 6′–H), 8.53 (m, 1H, 4′-H, 6′-H), 8.84 (s, 1H, CH=), 14.1 (bs, 1H, COOH).

2-Aryl-7-oxo-7-H-9-fluoro-10-cycloalkylimino[1,3,4]-oxadiazino[6,5,4-i,j]quinoline-6-carboxylic acids (IIb – IId). To a suspension of 0.6 g (1.9 mmole) of acid IIa 15 ml of anhydrous acetonitrile was added 0.8 g (8 mmole) of morpholine and 6 drops of diazabicycloundecene (DBU). The reaction mixture was boiled for 4 h and cooled. The precipitate of compound IIc was separated by filtration and recrystallized from DMSO; yield, 0.48 g (62%); m.p., 212 – 214°C; $C_{21}H_{16}FN_3O_5$; ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 3.38 [m, 4H, N$(CH_2)_2$], 3.79 [m, 4H, O$(CH_2)_2$], 7.54 (d, 1H, ^{3}J 12.5 Hz, 8-H), 7.67 (m, 3H, 3′-H, 4′-H, 5′-H), 8.00 (m, ^{2}H, 2′-H, 6′-H), 8.60 (s, 1H, CH=), 14.6 (bs, 1H, COOH).

Compound IIb was obtained by an analogous procedure using acid IIa and pyrrolidine; yield 57%; m.p., 261 – 263°C; $C_{21}H_{15}FN_3O_4$; ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 1.98 [m, 4H, $(CH_2)_2$], 3.78 [m, 4H, N$(CH_2)_2$], 7.39 (d, 1H, ^{3}J 14.3 Hz, 8-H), 7.58 (m, 3H, 3′-H, 4′-H, 5′-H), 8.39 (s, 1H, CH=), 8.89 (m, 2H, 2′-H, 6′-H), 14.5 (bs, 1H, COOH).

Compound IId was obtained by an analogous procedure using acid IIf: yield, 81%; m.p., 168 – 170°C; $C_{21}H_{15}FN_4O_6$; ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 2.00 [m, 4H, $(CH_2)_2$], 3.47 [m, 4H, N$(CH_2)_2$], 7.61 (d, 1H, ^{3}J 12.8 Hz, 8-H), 7.4 – 7.7 (m, 2H, 2′-H, 5′-H), 8.15 (m, 1H, 4′-H, 6′–H), 8.32 (m, 1H, 4′-H, 6′-H), 8.52 (s, 1H, CH=), 14.5 (bs, 1H, COOH).

2-(Pyridin-4-yl)-7-oxo-7-H-9-fluoro-10-(pyrrolidin-1-yl)-[1,3,4]oxadiazino[6,5,4-i,j]quinoline-6-carboxylic acid (IIe). To a solution of 0.75 g (2.0 mmole) of 2-(pyridin-4-yl)-7-oxo-7-H-9,10-difluoro[1,3,4]oxadiazino-[6,5,4-i,j]quinoline-6-carboxylic acid ethyl ester in 12 ml of acetonitrile was added 0.57 ml (8.0 mmole) of pyrrolidine and 0.2 ml (1.5 mmole) of triethylamine. The reaction mixture was boiled for 3.5 h and cooled and the precipitate was separated by filtration. To this product were added 5 ml of ethanol and 3 ml of 2 N hydrochloric acid and the mixture was allowed to stand for 15 min. The precipitate of compound IIe was separated by filtration and recrystallized from DMSO; yield 0.6 g (73%); m.p., > 300°C; $C_{20}H_{15}FN_4O_4$; ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 1.92 [m, 4H, $(CH_2)_2$], 3.67 [m, 4H, N$(CH_2)_2$], 7.28 (d, 1H, ^{3}J 12.2 Hz, 8-H), 8.03 (d, 2H, ^{3}J 6.0 Hz, 2′-H, 6′-H), 8.62 (s, 1H, CH=), 8.86 (d, 2H, ^{3}J 6.0 Hz, 3′-H, 5′-H), 14.3 (bs, 1H, COOH).

EXPERIMENTAL BIOLOGICAL PART

The antibacterial activity of compounds Ia – Ik and IIa – IIe *in vitro* was determined by the conventional method of double serial dilutions in liquid nutrient medium. The experiments were performed with respect to the following microbes: *Bacillus subtilis* ATCC 6633, *Bacillus pumilus*

TABLE 1. Antibacterial Activity *in vitro* of the Synthesized 1,3,4-Thia(oxa)diazino[6,5,4-i,j]quinoline-6-Carboxylic Acid Derivatives

Compound	Y	R	R^1	MIC, μg/ml			
				Bacillus subtilis ATCC 6633	*Bacillus pumilus* NCTC 8241	*Staphylococcus aureus* 209 P	*Escherichia coli* 157
Ia	H	Pyrrolidin-1-yl	F	2 – 4	2 – 4	8	32
Ib	F	-“-	-“-	4	8	8	64
Ic	H	-“-	Pyrrolidin-1-yl	–	2 – 4	> 128	> 128
Id	H	-“-	4-Methylpiperazin-1-yl	8	8	8	16
Ie	H	-“-	Morpholin-1-yl	1	2	4	> 128
If	H	-“-	4-Ethoxycarbonylpiperazin-1-yl	> 32	> 32	> 32	> 32
Ig	H	Cyclohexylamino	Pyrrolidin-1-yl	> 128	–	> 128	> 128
Ih	Pyrrolidin-1-yl	Pyrrolidin-1-yl	-“-	32	32	16	16
Ii	4-Ethoxycarbonylpiperazin-1-yl	-“-	4-Ethoxycarbonylpiperazin-1-yl	> 32	> 32	> 32	> 32
Ij	$NH(CH_2)_3N(CH_3)_2$	-“-	$NH(CH_2)_3N(CH_3)_2$	16	32	64	32 – 64
Ik	Pyrrolidin-1-yl	Cyclohexylamino	Pyrrolidin-1-yl	> 128	64 – 128	64	> 128
IIa	–	Phenyl	F	4	–	4	> 128
IIb	–	-“-	Pyrrolidin-1-yl	1	2	1	> 32
IIc	–	-“-	Morpholin-1-yl	4	–	4	> 128
IId	–	3-Nitrophenyl	Pyrrolidin-1-yl	> 32	> 32	> 32	> 32
IIe	–	Pyridin-4-yl	-“-	> 32	> 32	> 32	> 128
		Pefloxacin		2	–	1	0.5

NCTC 8241, *Staphylococcus aureus* 209P, and *Escherichia coli* 157. The tests with *St. aureus* 209P, *B. pumilus* NCTC 8241, and *E. coli* 157 were conducted in Hottinger broth (110 – 130 mg% amine nitrogen, pH 7.2 – 7.4), and with *B. subtilis* ATCC 6633, in a glucose-free meat-infusion broth (pH 7.0 – 7.2).

Exactly weighed amounts of the synthesized compounds were dissolved in DMSO and then diluted in Hottinger or meat-infusion broth. The antibacterial activity was evaluated in the concentration range from 0.015 to 128.0 μg/ml at a microbial load of 2×10^5 CFU/ml. The activity was expressed in terms of the minimum inhibiting concentration (MIC, μg/ml) representing the minimum concentration suppressing the growth of test microbes. The reference drug was pefloxacin.

RESULTS AND DISCUSSION

Among the series of 1,3,4-thiadiazino[6,5,4-i,j]- quinoline-6-carboxylic acid derivatives (I), the maximum antibacterial activity with respect to Gram-positive species was compound Ie characterized by MIC = 1 – 4 μg/ml. It is interesting to note that substituting of only a morpholine residue for F 10 in quinolone Ia leads to an increase in the activity with respect to Gram-positive microbes. On the contrary, introduction of pyrrolidine, 4-methylpiperazine, and 4-ethoxycarbonylpiperazine in position 10 (compounds Ic, Id, and If) reduces the activity. The presence of fluorine in position 8 decreases the antibacterial activity in comparison to that of 8-unsubstituted derivatives. For example, compound Ib is conditionally active with respect to Gram-positive microorganisms (MIC = 4 – 8 μg/ml). The substitution of pyrrolidine, 4-ethoxycarbonylpiperazine, and N,N′-dimethyl-1,3-propanediamine (derivatives Ih – Ij) for F 8 leads to a further decrease in the antibacterial activity. Compounds Ig and Ik containing cyclohexylamine in position 2 are less active than the analogs with pyrrolidine in this position.

In the series of 1,3,4-oxadiazino[6,5,4-i,j]quinoline-6-carboxylic acid derivatives (II), the most active was compound IIb containing a phenyl substituent in position II. This substance suppressed the growth of Gram-positive microbes with MIC = 1 – 2 μg/ml). Compounds IIa and IIc were also conditionally active with respect to Gram-positive species (MIC = 4 μg/ml).

It should be noted that the synthesized compounds (except for Id and Ih with MIC = 16 μg/ml) exhibited a rather low activity with respect to *E. coli* 157.

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