STRUCTURAL MODIFICATION AND NEW METHODS FOR PREPARATION OF OFLOXACIN ANALOGS

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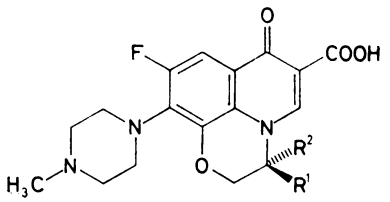
Reaction of ethyl 2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)-3-ethoxyacrylate *IIb* with 2-amino-1--propanol provided corresponding compound *IIIb* which under alkaline conditions underwent an aromatic denitrocyclization reaction which after alkaline saponification provided 10-chloro--9-fluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid *Vd*. Treatment of 8-hydroxyquinolone *VIII* with 3-bromopropyne in the presence of sodium hydrogen carbonate provided methylene derivative *VIIb* which was saponified into appropriate acid *VIIc*. Compound *VIIb* treated with N-methylpiperazine and then saponified yielded *VIIa*. Hydrogenation of 3-methylene derivative *VIIb* provided 3-methyl derivative *Va*.

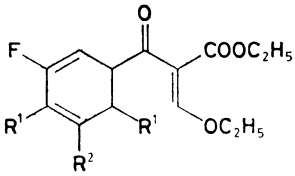
Ofloxacin (*Ia*) has become one of the leading antibacterial fluoroquinolones currently available on the European market[1]. Though the drug has an asymmetric center at position 3, it is marketed as a racemate. Recently its optically active enantiomers have been isolated by high-performance column chromatography of a racemate intermediate[2,3]. After revealing that *S*-(−)-ofloxacin is substantially more active than the *R*-(+) isomer[2,3] (from 8 to 128 times, depending on the bacterial strain used) further development in the field was directed into two areas.



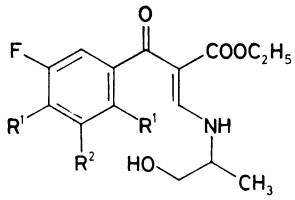
Ia, $R^1, R^2 = CH_3, H$ (ofloxacin)
Ib, $R^1 = CH_3$; $R^2 = H$ (*S*-(-) ofloxacin)
Ic, $R^1 = H$; $R^2 = CH_3$ (*R*-(+) ofloxacin)
Id, $R^1 = R^2 = CH_3$
Ie, $R^1 + R^2 = CH_2—CH_2$

Since the method originally used for preparation of ofloxacin[4] could not be used for synthesis of pure enantiomers, the first area of research on this field was centered

to the development of new methods of preparation[5–7]. In all of these methods reactive intermediate *IIa* was treated with 2-amino-1-propanol to yield *IIIa*. Key step in the synthesis of 2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6--carboxylic acid moiety consists of a double intramolecular nucleophilic cyclization

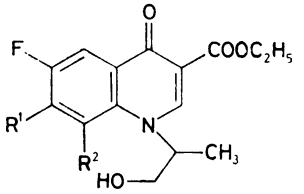


IIa, $R^1 = R^2 = F$
IIb, $R^1 = Cl$ $R^2 = NO_2$

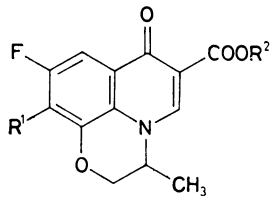


IIIa, $R^1 = R^2 = F$
IIIb, $R^1 = Cl$ $R^2 = NO_2$

reaction of *IIIa* into *Va* with or without isolation of intermediates *IVa*. Using pure enatiomers of 2-amino-1-propanol affords the respective stereoisomers which could be easily transformed into ofloxacin enantiomers.



IVa, $R^1 = R^2 = F$
IVb, $R^1 = Cl$; $R^2 = NO_2$
IVc, R^1 = 4-methyl-1-piperazinyl ; $R^2 = NO_2$



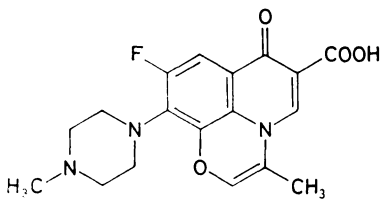
Va, $R^1 = F$; $R^2 = C_2H_5$
Vb, $R^1 = Cl$; $R^2 = C_2H_5$
Vc, $R^1 = F$; $R^2 = H$
Vd, $R^1 = Cl$; $R^2 = H$

Since we have good experience with aromatic denitrocyclization reactions[8] we decided to try this approach for the synthesis. We started from 2,4-dichloro-5-fluoro--3-nitrobenzoic acid[9] which can be easily prepared from 2,4-dichloro-5-fluoroacetophenone which is an usual intermediate in the fluoroquinolone chemistry[10]. The acid was transformed by described way[9] into *IIb*. This reactive intermediate treated with 2-amino-1-propanol yielded compound *IIIb*. Then we needed to develop reaction conditions so that only cyclization into *IVb* would take place. Compound *IVb*, due to the activation of 7-chloro atom by the 8-nitro group, could be supposed to react with N-methylpiperazine into *IVc* which was supposed to yield ofloxacin ethyl ester by denitrocyclization reaction. We tried various conditions for the cyclization of *IIIb*, but the only product was *Vb*. It is evident that the 8-nitro group of intermediate *IVb* is better leaving group than 2-chloro substituent of *IIIb*. Compound *Vb* was easily hydrolyzed into corresponding acid *Vd* which is not suitable as an inter-

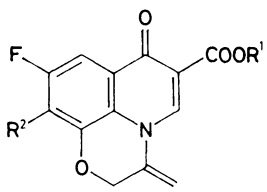
mediate for the synthesis of ofloxacin. This compound did not react with N-methylpiperazine under usual conditions and at higher temperature substitution of a fluorine atom at position 9 occured instead[4]. We tried to perform the nucleophilic displacement reaction of this compound into ofloxacin via appropriate boron esters, which were reported to facilitate this type of substitution[3], but we failed.

Second area of the research was centered to further modification of ofloxacin structure at position 3, i.e. on the asymmetric carbon atom. These modifications could help to understand structural requirements necessary for optimal antibacterial activity.

The more active *S*-(−)-ofloxacin *Ib* has the methyl group above the plane of the nearly planar core and the *R*-(+)-isomer *Ic* has the opposite arrangement at this position. The fact that similar 3,3-dimethyl derivative *Id* is practically inactive[11] suggests that there is an unfavourable steric interaction caused by the projection of a methyl group below the plane. Less sterically demanding 3-spirocyclopropyl analog *Ie* has activity smaller than ofloxacin but higher than the 3,3-dimethyl derivative[12] which supports the idea. Therefore we decided to prepare ofloxacin analogs having at position 3 a one-carbon group approximately in the plane of the skeleton. There are two principal compounds which could meet the requirements, the first is 2,3-dehydroofloxacin *VI* and the other possibility is compound *VIIa* having an exocyclic methylene group[13].



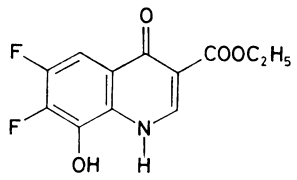
VI



VII a, R^1 = H ; R^2 = 4-methyl-1-piperazinyl
VII b, R^1 = C_2H_5 ; R^2 = F
VII c, R^1 = H ; R^2 = F
VII d, R^1 = C_2H_5 ; R^2 = 4-methyl-1-piperazinyl

For the preparation of 3-methylene analogs *VII* we chose as a starting material 8-hydroxyquinolone *VIII* the preparation of which we reported earlier[8]. This compound treated with 3-bromopropyne in N,N-dimethylformamide in the presence of sodium hydrogen carbonate provided *VIIb*. The same compound was obtained from *VIII* using dicyclohexylcarbodiimide and 2-propyne-1-ol in pyridine. Alkaline saponification of *VIIb* yielded corresponding acid *VIIc*. Reaction of *VIIb* with N-methylpiperazine provided *VIId* which was without isolation saponified into *VIIa*.

Compound *VIIb* was hydrogenated on palladium providing *Va* which is a known intermediate for the synthesis of ofloxacin[4].



VIII

During the course of this work a paper describing a tedious synthesis of 2-dehydroofloxacin *VI* appeared[14]. Since its activity was only mild we did not try to prepare this compound by a simpler way.

All the prepared compounds were tested for their antimicrobial activity in vitro against Gram positive bacteria (*Staphylococcus aureus 1/45*, *Streptococcus pyogenes 4/49*, *Streptococcus faecalis D 16/66*) and Gram negative organisms (*Escherichia coli 326/61*, *Proteus vulgaris 2/35*, *Pseudomonas aeruginosa 26/56*) at the Department of Microbiology of the Institute (Dr V. Hola, Head). The organisms are from the State Collection of Strains, Prague. Ofloxacin was used as a standard. The minimum inhibitory concentrations in mg/l are given unless they exceed 128 mg/l: *S. aureus*: *Ia* 0·5, *VIIc* 16, *VIIe* 0·25; *S. pyogenes*: *Ia* 2, *VIIc* 32, *VIIe* 8; *S. faecalis*: *Ia* 2, *VIIc* 128, *VIIe* 4; *E. coli*: *Ia* 0·25, *VIIc* 4, *VIIe* 0·125; *P. vulgaris*: *Ia* 0·25, *VIIc* 0·5, *VIIe* 0·125; *P. aeruginosa*: *Ia* 1, *VIIc* 32, *VIIe* 0·5.

EXPERIMENTAL

The melting points were determined on a Mettler FP 5 appartus and were not corrected. IR spectra were taken on a Unicam SP-2006 spectrometer in KBr pellets; wavenumbers are given in cm^{-1}. UV spectra were taken on a Unicam PU 8800 spectrophotometer in ethanol, molar absorption coeffificients (ε) are given in $m^2\ mol^{-1}$, wavelengths (λ) in nm. Mass spectra were measured on MCH 1320 and MAT 44 S spectrometers. ^1H NMR spectra (100 MHz) and ^{13}C NMR spectra (25·14 MHz) were measured on an apparatus BS-487 (Tesla Brno) 100 MHz in hexadeuterated dimethylsulfoxide (^{13}C NMR at 100°C, unless otherwise stated). The standard for ^1H NMR spectra was 3-trimethylsilylpropanoic aicd, unless otherwise stated, the ^{13}C NMR spectra were referenced to tetramethylsilane. Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. The assignments indicated by an asterisk may be interchanged.

Ethyl 2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)-3-ethoxyacrylate (*IIb*)

A mixture of ethyl 2,4-dichloro-5-fluoro-3-nitrobenzoylacetate (7·65 g, 23·6 mmol), ethyl orthoformate (5 h, 33·7 mol), and acetic anhydride (5·6 g, 54·8 mmol) was stirred at 130°C for 4 h. Volatile fractions were distilled off in vacuo providing 8·5 g (95%) of a syrup which was used for further step without purification.

Ethyl 2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)-3-[1-hydroxymethyl(ethylamino)]acrylate (*IIIb*)

2-Amino-1-propanol (1·75 g, 28·6 mmol) was added dropwise to a cold solution of *IIb* (8·5 g, 22·4 mmol) in absolute ethanol (22 ml) and the temperature was maintained under 5°C. The mixture was stirred at room temperature for 4 h and then was allowed to stand overnight at this temperature. The solvent was distilled off at reduced pressure and the residue (8·3 g, 91%) was used for further reactions without any purification.

10-Chloro-9-fluoro-3-methyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (*Vd*)

A) Sodium hydride (0·2 g, 80% oil suspension, 6·6 mmol) was added to a solution of *IIIb* (2·0 g, 4·9 mmol) in dioxane (6 ml) and the mixture was refluxed for 10 h, dioxane was distilled off in vacuo and the residue was treated with water (10 ml). The mixture was acidified with acetic acid and extracted with trichloromethane and the extract was dried with anhydrous magnesium sulfate. A solution of sodium hydroxide (0·8 g, 20 mmol) in water (25 ml) was added to the residue obtained after removing of trichloromethane and the mixture was refluxed for 3 h. The mixture was poured into water (10 ml) and acidified with acetic and insoluble portion was filtered off. Recrystallization from ethanol provided 0·94 g (65%) of *Vd*, m.p. 260—263°C. For $C_{13}H_9Cl.FNO_4$ (297·7) calculated: 52·46% C, 3·05% H, 11·91% Cl, 6·38% F, 4·70% N; found: 52·26% C, 3·17% H, 11·93% Cl, 6·29% F, 4·52% N. ^{1}H NMR spectrum: 9·08 s, 1 H (H-5); 7·78 d, 1 H (H-8, $J_{F,H} = 9$); 5·00 m, 1 H (H-3); 4·62 m, 2 H (H-2); 1·50 d, 3 H (CH_3, $J = 6$). Mass spectrum, $m/z = 297$ (M^+).

B) A mixture of *IIIb* (2·0 g, 4·9 mmol), sodium hydrogen carbonate (0·8 g, 9·5 mmol) and N,N-dimethylformamide (20 ml) was refluxed for 12 h, then the solvent was distilled off under reduced pressure. The same work up as described for *A*) provided 0·88 g (61%) of *Vd*.

C) A mixture of *IIIb* (2 g, 4·9 mmol), potassium fluoride (0·6 g, 10 mmol), and N,N-dimethylformamide was stirred at 110°C for 10 h, the solvent was distilled off in vacuo and the residue was worked up in the same way as described for *A*) yielding 0·96 g (66%) of *Vd*.

Ethyl 9,10-Difluoro-2,3-dihydro-3-methylene-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (*VIIb*)

A) A solution of 3-bromopropyne (1·5 g, 13 mmol) was added dropwise during 4 h to a mixture of *VIII* (3·0 g, 11 mmol), sodium hydrogen carbonate (1·4 g, 17 mmol) and N,N-dimethylformamide (33 ml) at 90°C and then the mixture was stirred at this temperature for additional 4 h. The mixture was evaporated in vacuo and the residue was triturated with water (50 ml), insoluble portion was filtered off, washed with water and dried. Crystallization from 95% aqueous ethanol provided 2·3 g (67%) of *VIIb*, m.p. 258—261°C. For $C_{15}H_{11}F_2NO_4$ (307·25) calculated: 58·63% C, 3·61% H, 12·37% F, 4·56% N; found: 58·37% C, 3·72% H, 12·49% F, 4·64% N. ^{13}C NMR spectrum: 13·31 q (CH_3), 59·61 t (CH_2 of ethyl), 66·47 t (C-2), 103·83 t (CH_2=), 112·75 s (C-6), 122·60 s* (C-7a), 124·50 s* (C-11a), 134·52 s (C-3), 135·60 s (C-11), 140·00 s (C-10), 140·35 d (C-5), 148·20 s (C-9), 163·52 s (COO), 171·35 s (C=O).

B) Compound *VIII* (1·0 g, 3·7 mmol) and dicyclohexylcarbodiimide (0·9 g, 4·4 mmol) were dissolved in dry pyridine (8 ml) at 110—120°C and a solution of 2-propyne-1-ol (0·35 ml, 6 mmol) in pyridine (3 ml) was added dropwise during 2 h and the mixture was stirred for additional 1 h at the same temperature. Then additional portions of dicyclohexylcarbodiimide (0·5 g, 2·5 mmol) and 2-propyne-1-ol (0·2 ml, 3·5 mmol) were added and the mixture was stirred at 120°C for 16 h. Then the mixture was evaporated under reduced pressure, triturated with tri-

chloromethane (20 ml), the mixture was filtered through a short column of silica gel, the filtrate was evaporated and the residue (1·5 g) was purified by column chromatography on silica gel (benzene–acetone 8 : 2). Crystallization of the corresponding residue provided 0·4 g (35%), m.p. 258–261°C. IR spectrum was identical with the sample prepared by the method *A*).

9,10-Difluor-2,3-dihydro-3-methylene-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (*VIIc*)

Aqueous solution of sodium hydroxide (1·1 ml 1 mol l^{-1}) was added dropwise to a stirred suspension of *VIIb* (0·31 g, 1 mmol) in 50% aqueous ethanol (28 ml) and the mixture was stirred at 55°C for 2·5 h, the formed solution was stirred with a small amount of charcoal, the charcoal was filtered off and the filtrate was acidified with hydrochloric acid. The mixture was cooled down and left to stand overnight in a refrigerator. The precipitate was filtered off and crystallized from 95% aqueous ethanol; yields 0·15 g (54%), m.p. 276–281°C. For $C_{13}H_7N_2NO_4$ (279·2) calculated: 55·92% C, 2·53% H, 13·61% F, 5·02% N; found: 55·81% C, 2·62% H, 13·41% F, 5·07% N. IR spectrum: 3 320 (OH); 1 710 (COOH); 1 660 (CH_2=); 1 620 (C=O); 1 485, 1 515, 1 560, 1 575 (aromat. system); 1 120 (cyclic ether). UV spectrum, λ_{max} (log ε): 335 (3·08), 231 (3·31), 207 (3·25). ^{13}C NMR spectrum (135°C): 66·48 t (C-2), 103·14 (C-8), 106·37 t (CH_2=), 108·91 (C-6), 120·50 s* (C-7a), 124·60 s* (C-11a), 135·45 s (C-3), 136·80 s (C-11), 141·85 s (C-10), 142·85 s (C-5), 148·10 s (C-9), 163·88 s (COOH), 176·28 s (C=O).

9-Fluoro-2,3-dihydro-3-methylene-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (*VIIa*)

A mixture of *VIIb* (0·9 g, 3 mmol), N-methylpiperazine (1 g, 10 mmol) and dimethyl sulfoxide (30 ml) was stirred at 110–120°C for 10 h. The solution was evaporated in vacuo, the residue was suspended in ethanol(90 ml) and a solution of sodium hydroxide (0·4 g, 10 mmol) in water (10 ml) was added dropwise and the mixture was stirred at 50°C for 30 min. The formed solution was evaporated, the residue was dissolved in water (10 ml), acidified with hydrochloric acid, treated with charcoal and neutralized with 5% aqueous solution of sodium hydrogen carbonate. The mixture was extracted with trichloromethane, dried with magnesium sulfate and the residue after evaporation was crystallized from 95% aqueous ethanol; yields 0·7 g (65%), m.p. 203–205°C. For $C_{18}H_{18}FN_3O_4$ (359·3) calculated: 60·16% C, 5·03% H, 5·29% F, 11·69% N; found: 59·66% C, 5·03% H, 5·20% F, 11·40% N. IR spectrum: 3 320 (OH); 1 710 (COOH); 1 660 (CH_2=); 1 610 (C=O); 1 540, 1 520, 1 490 (aromat. system); 1 080 (cyclic eher). UV spectrum, λ_{max} (log ε): 338 (3·14), 306 (3·42), 223 (3·25). ^{1}H NMR spectrum (90°C): 2·29 s, 3 H (CH_3); 2·48 bt, 4 H (H-3′, H-5′ of piperazine); 3·32 bt, 4 H (H-2′, H-6′ of piperazine); 5·00 s, 2 H (H-2); 5·50 d, 5·92 d, 2 H (CH_2=); 7·60 d, 1 H (H-8, $J_{H,F}$ = 10); 8·90 s, 1 H (H-5). ^{13}C NMR spectrum: 13·37 q (CH_3 of ethyl), 45·34 q (N—CH_3), 49·60 t, 49·75 t (C-2′, C-6′ of piperazine), 54·83 t (C-3′, C-5′ of piperazine), 65·81 t (C-2), 103·11 d (C-8, $J_{F,C}$ = 24), 105·17 t (CH_2=), 107·79 s (C-6), 118·09 s (C-7a, $J_{F,C}$ = 5), 124·99 s (C-11a), 132·14 s (C-10, $J_{F,C}$ = 13), 135·42 s (C-3), 140·20 s (C-11, $J_{F,C}$ = 4), 141·17 d (C-5), 155·03 s (C-9, $J_{F,C}$ = 246), 164·70 s (COOH), 176·65 q (C=O).

Ethyl 9,10-Difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazin-6-carboxylate (*Va*)

A solution of *VIIb* (0·31 g, 1 mmol) in ethanol (150 ml) was hydrogenated at room temperature with vigorous shaking on 5% palladium on carbon (Fluka) until hydrogen consumpiton was observed. Then the catalyst was filtered off, the filtrate was evaporated in vacuo and the residue

was crystallized from ethanol; yields 0·2 g (65%), m.p. 258–260°C. ^{1}H NMR spectrum (100°C): 1·32 t, 3 H (CH_3 of ethyl, $J = 7$); 1·45 d, 3 H (CH_3, $J = 7$); 4·26 q, 2 H (CH_2 of ethyl, $J = 7$); 4·55 m, 2 H (H-2); 4·80 m, 1 H (H-3); 7·66 dd, 1 H (H-8, $J_{H,F} = 10, 8$); 8·73 s, 1 H (H-5). ^{13}C NMR spectrum: 13·37 q (CH_3 of ethyl), 16·28 q (CH_3), 53·18 d (C-3), 59·08 t (CH_2 of ethyl), 68·57 t (C-2), 103·23 d (C-8), 110·33 s (C-6), 12·99 s* (C-11a), 125·60 s* (C-7a), 135·12 s (C-11), 144·91 d (C-5), 141·80 s (C-10), 147·96 s (C-9), 163·58 s (COO), 170·46 s (C=O).

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