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Synthesis, Structure, and Antibacterial Activity of 4-Imino-1,4-dihydrocinnoline-3-carboxylic Acid and 4-Oxo-1,4-dihydrocinnoline-3-carboxylic Acid Derivatives as Isosteric Analogues of Quinolones

Chemical modification of cinnoxacin was studied with the aim of improving its antibacterial activity and spectrum. A series of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid derivatives was synthesized and their *in vitro* antibacterial activity was evaluated. These derivatives were designed as isosteric analogues of fluoroquinolones and are characterized by the presence of an imine group instead of an oxo group at the 4-position and a nitrogen atom in position 2. The crystal structure of one analogue determined by X-ray diffraction shows the dipolar form of the compound in the solid state. The *in vitro* antibacterial activity of the synthesized compounds against Gram-positive and Gram-negative bacteria was examined. The MIC of the most active compounds lies in the range of the first generation of quinolones such as nalidixic acid. The compounds with dichlorobenzyl substituent show enhanced activity against Gram-positive bacteria.

Key Words: Cinnolones; Quinolones; Antibacterial activity; DNA topoisomerase; Crystal data

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Introduction

The quinolone class of antibacterial agents has rapidly emerged as one of the most effective drugs in the treatment of bacterial diseases. Quinolinones and naphthyridinones were also found to inhibit human topoisomerase II [1–5]. The molecular target of these agents is DNA gyrase, an enzyme that catalyses the introduction of negative supercoils into circular duplex DNA. This process is necessary for DNA replication in bacteria [6–9]. Although the structure-activity relationship (SAR) of fluoroquinolone has been extensively studied, no clear conclusion has yet been reached. The most widely accepted mechanism of action of quinolones is based on their interaction with DNA bases via hydrogen bonds. Two models are usually described in the literature:

– the model based on hydrogen bonding to DNA bases and stacking with another quinolone molecule [6],
– an alternative model based on formation of Mg^{2+}-quinolone-DNA adducts and stacking with DNA bases [7].

Cinnoxacin, the chemical structure of which is characterized by a 4-oxocinnoline-3-carboxylic acid moiety, shows good antibacterial activity mainly against Gram-negative bacteria. We have been interested in the chemical modification of cinnoline-3-carboxylic acids, in the hope of developing new antibacterial agents with improved activity. As a part of a program designed to identify new DNA gyrase inhibitors, modification of the cinnolone structure was undertaken by implementation of the imine group in position 4 (Figure 1).

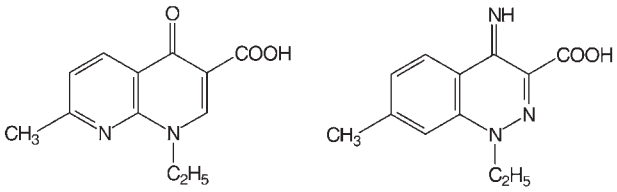

Figure 1

Also, X-ray studies of one of the resulting compounds have been undertaken. Until now only three crystal structures of 4-imine analogues of quinolones, all having cinnoline ring system, have been determined [10, 11]. However, the compounds were either dihydrochlorides (1-ethyl-7-methyl-1,4-dihydro-4-iminocinnoline-3-carboxylic acid) [10] or had a modified 3-carboxylic function, as in 3-carbamoyl-1,4-dihydro-4-imino-1-methylcinnoline [11], and the results of their structural studies could

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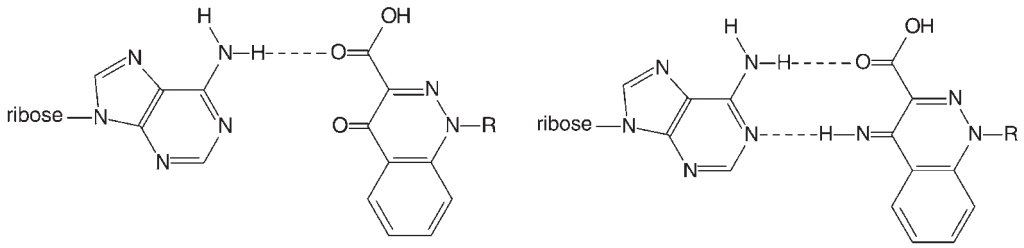


Figure 2

not be used directly for the discussion of structural similarity with neutral quinolones. The present study has been designed to investigate our hypothesis concerning the possibility of formation of hydrogen bonding 4-imine-analogues. Although the imine group is isosteric with the carbonyl group, the former group may form hydrogen bonds, both as a proton donor and as an acceptor (Figure 2).

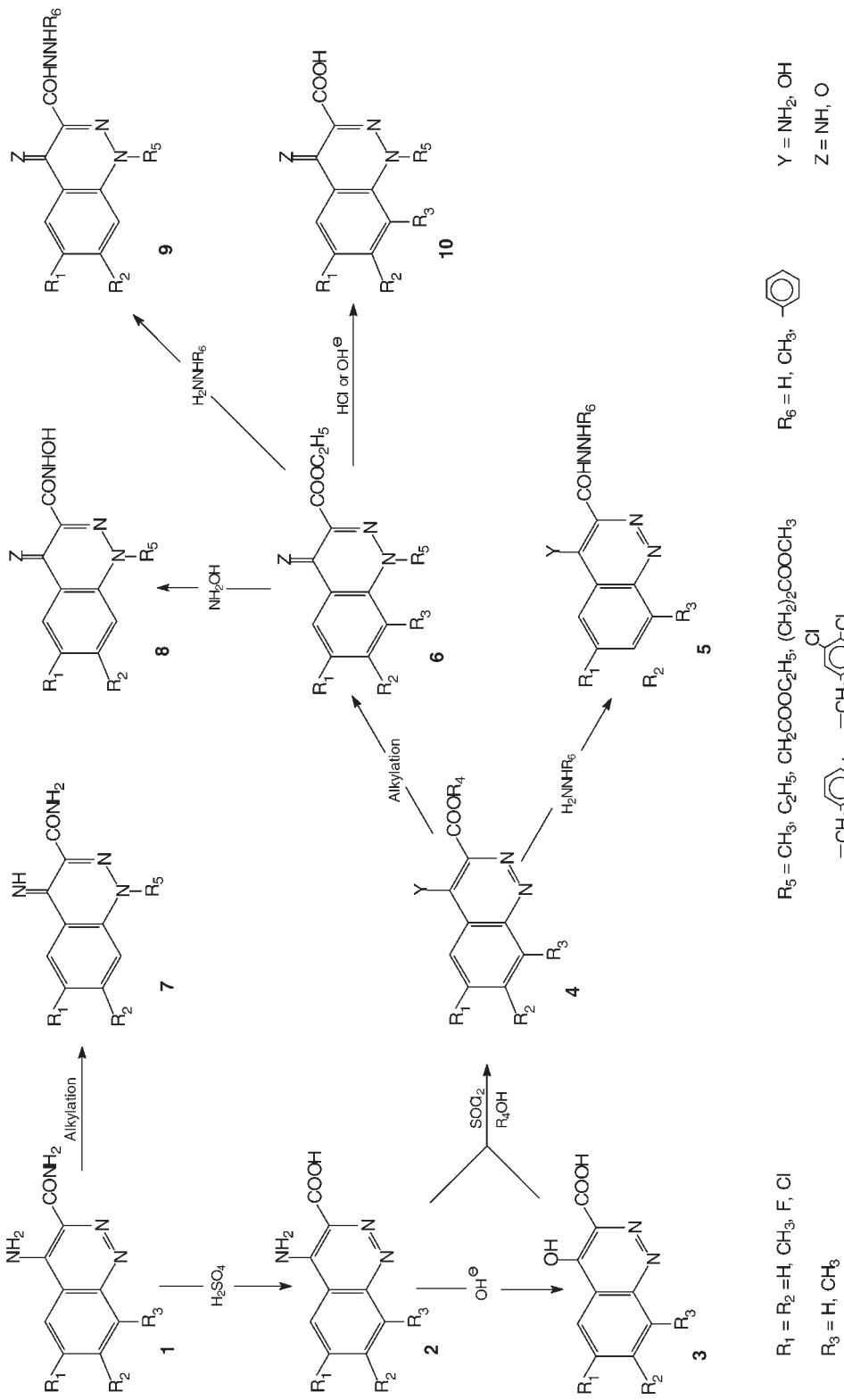
Appropriate analogues were synthesized on the basis of molecular modelling.

Investigations, results, and discussion

Chemistry

The general synthetic routes employed to prepare 4-aminocinnoline-3-carboxylic acid derivatives **2** and 4-hydroxycinnoline-3-carboxylic acids derivatives **3** are outlined in Scheme 1. The compounds **1** were prepared by the intramolecular Friedel-Crafts reaction of corresponding (arylhydrazono)(cyano)acetamides as described in the reference [12]. Hydrolysis of the amides **1** with 80 % sulphuric acid solution gave 4-aminocinnoline-3-carboxylic acids **2**. However, alkaline hydrolysis of appropriate acids **2** or amides **1** led to 4-hydroxycinnoline-3-carboxylic acids **3**.The acids **2**, **3** were converted into corresponding ethyl 4-aminocinnoline-3-carboxylates and ethyl 4-hydroxycinnoline-3-carboxylates **4** using thionyl chloride and then ethyl alcohol according to the method described in the literature [13].The esters **4** were reacted with an excess of hydrazines in the presence of DBU in dimethylformamide to give corresponding 4-amino-3-cinnolinehydrazides and 4-hydroxy-3-cinnolinehydrazides **5**.

Alkylation of the 4-hydroxycinnolines derivatives was examined extensively [14–16]. It was found that alkylation in protic solvents under basic conditions gave a mixture of *N*-1 and *N*-2 alkylated products. Similar results were reported by Miyamoto and Matsumoto [17], for alkylation of ethyl 4-hydroxycinnolinecarboxylates with alkyl iodide or dialkyl sulfate in the presence of anhydrous potassium carbonate in dimethylformamide. This gave a mixture of ethyl 1-alkyl-1,4-dihydro-4-oxocinnolinecarboxylates and anhydro-bases of 2-alkyl-3-ethoxycarbonyl-4-hydroxycinnolinium hydroxides. Based on our earlier study, we have found that the ethyl 4-hydroxy- and 4-aminocinnoline-3-carboxylates **4** were alkylated smoothly with benzyl bromides in the presence of anhydrous potassium carbonate in acetonitrile [13]. Alkylation of the 4-hydroxycinnoline-3-carboxylic esters **4** was carried out with dimethyl sulphate, diethyl sulphate, benzyl bromide, allyl bromide, ethyl bromoacetate, and 2-bromoethanol. However, there were some problems with alkylation of 4-aminocinnoline-3-carboxylic acid esters **6** with "weak" alkylating agents such as alkyl and cycloalkyl bromides. We have found that the yield of alkylation depends strongly on the solubility of esters **4** in the reaction medium, alkylating agent type, and ease of formation of the imine form of 4-amino-3-cinnolinecarboxylic acid esters **4**. In many cases the alkylation of esters failed. Structures of esters **6** were confirmed by IR[1] and H NMR spectra. In the IR spectra of **6** the two bands in the range of carbonyl area at 1700 and 1645 cm^{-1} are different from signals of esters **4**. There was clear evidence of N_1-alkylation when examining the ^1H NMR. The signals of the ethyl group was noted as second quartet (3.7–4.0 ppm) or singlet (5.8–6.2 ppm) for benzyl CH_2 protons. The singlet shift of the imine group of esters **6** an exchangeable with D_2O was about 9.6–9.9 ppm. Alkylation of the amides **1** by the same method as used for esters **4** gave 1-alkyl-1,4-dihydro-4-iminocinnoline-3-carboxamides and 1-alkyl-1,4-dihydro-4-oxocinnoline-3-carboxamides **7**. The esters **6** were converted into corresponding 1-alkyl-1,4-dihydro-4-iminocinnoline-3-carboxylic acids hydrazides **9** and 1-alkyl-1,4-dihydro-4-oxocinnoline-3-carboxylic acids hydrazides **9**. The formation of hydrazides **9** was supported by the disappearance of the ^1H NMR band corresponding to the OC_2H_5 group and the appearance two signals NH of hydrazide protons at 4.0–6.1 ppm and 10.3–11.0 ppm exchangeable with D_2O. The esters **6** were reacted with hydroxylamine hydrochloride in the presence of DBU in dimethylformamide to obtain corresponding


Alkylation
H_2SO_4
$SOCl_2$
R_4OH
$OH^{\ominus}$
Alkylation
H_2NNHR_6
NH_2OH
H_2NNHR_6
HCl or $OH^{\ominus}$
$R_1 = R_2 = H, CH_3, F, Cl$
$R_3 = H, CH_3$
$R_4 = CH_3, C_2H_5$
$R_5 = CH_3, C_2H_5, CH_2COOC_2H_5, (CH_2)_2COOCH_3$
$R_6 = H, CH_3,$
Y = NH_2, OH
Z = NH, O

Scheme 1

Table 1. Physical and analytical data of compounds (**4a–n**).

Compd.	Y	R^1	R^2	R^3	R^4	Molecular formula (weight)	Mp [°C]	Yield [%]
4a	NH_2	CH_3	H	H	CH_3	$C_{11}H_{11}N_3O_2$ (217.1)	254–256	79.6
4b	NH_2	F	H	H	CH_3	$C_{10}H_8FN_3O_2$ (221.2)	266–268	81.2
4c	NH_2	H	H	H	C_2H_5	$C_{11}H_{11}N_3O_2$ (217.1)	242–244 [13]	89.0
4d	NH_2	CH_3	H	H	C_2H_5	$C_{12}H_{13}N_3O_2$ (231.2)	242–244 [13]	80.7
4e	NH_2	CH_3	CH_3	H	C_2H_5	$C_{13}H_{15}N_3O_2$ (245.3)	263–265	79.5
4f	NH_2	H	Cl	H	C_2H_5	$C_{11}H_{10}ClN_3O_2$ (251.6)	265–267 [13]	76.8
4g	NH_2	F	H	H	C_2H_5	$C_{11}H_{10}FN_3O_2$ (235.2)	276–278	81.5
4h	NH_2	H	F	H	C_2H_5	$C_{11}H_{10}FN_3O_2$ (235.2)	284–285	76.7
4i	NH_2	Cl	Cl	H	C_2H_5	$C_{11}H_9Cl_2N_3O_2$ (286.1)	>330	85.3
4j	NH_2	F	Cl	H	C_2H_5	$C_{11}H_9ClFN_3O_2$ (269.7)	>330	80.6
4k	NH_2	Cl	H	CH_3	C_2H_5	$C_{12}H_{12}ClN_3O_2$ (265.7)	234–236	78.9
4l	OH	Cl	H	H	C_2H_5	$C_{11}H_9ClN_2O_3$ (252.6)	232–234 [13]	79.2
4m	OH	F	H	H	C_2H_5	$C_{11}H_9FN_2O_3$ (236.2)	212–213	87.7
4n	OH	H	H	F	C_2H_5	$C_{11}H_9FN_2O_3$ (236.2)	225–227	81.5

Table 2. Physical and analytical data of compounds (**6a–z**).

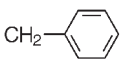
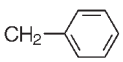
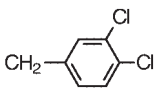
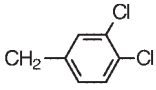
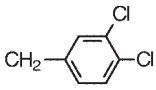
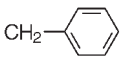
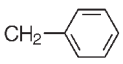
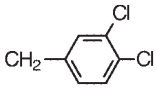
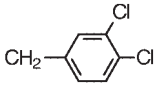
Compd.	Z	R^1	R^2	R^3	R^5	Molecular formula (weight)	Mp [°C]	Yield [%]
6a	NH	H	H	H		$C_{18}H_{17}N_3O_2$ (307.3)	215–217 [13]	74.8
6b	NH	F	H	H		$C_{18}H_{17}FN_3O_2$ (326.3)	207–208	76.9
6c	NH	CH_3	H	H		$C_{19}H_{17}Cl_2N_3O_2$ (390.3)	216–218	82.3
6d	NH	CH_3	CH_3	H		$C_{20}H_{19}Cl_2N_3O_2$ (404.3)	191–193	72.6

Table 2. (continued).

Compd.	Z	R^1	R^2	R^3	R^5	Molecular formula (weight)	Mp [°C]	Yield [%]
6e	NH	H	CH_3	H		$C_{19}H_{17}Cl_2N_3O_2$ (390.3)	224–226	80.4
6f	NH	CH_3	CH_3	H	CH_3	$C_{14}H_{17}N_3O_2$ (259.3)	>330	82.6
6g	NH	H	H	H	C_2H_5	$C_{13}H_{15}N_3O_2$ (245.3)	198–200	77.4
6h	NH	H	CH_3	H	C_2H_5	$C_{14}H_{17}N_3O_2$ (259.3)	205–207	68.4
6i	NH	H	Cl	H	C_2H_5	$C_{13}H_{14}ClN_3O_2$ (279.7)	234–236	69.9
6j	NH	Cl	Cl	H	C_2H_5	$C_{13}H_{13}Cl_2N_3O_2$ (314.2)	210–211	71.3
6k	NH	F	H	H	C_2H_5	$C_{13}H_{14}FN_3O_2$ (263.2)	227–229	77.3
6l	NH	F	Cl	H	C_2H_5	$C_{13}H_{13}ClFN_3O_2$ (297.7)	135–137	75.9
6m	NH	Cl	H	H	$(CH_2)_2COOCH_3$	$C_{17}H_{16}ClN_3O_2$ (329.8)	216–218	80.2
6n	NH	H	F	H	$CH_2COOC_2H_5$	$C_{15}H_{16}FN_3O_2$ (289.3)	254–255	79.6
6o	NH	F	Cl	H	$CH_2COOC_2H_5$	$C_{15}H_{15}ClFN_3O_2$ (323.8)	298–299	84.2
6p	O	Cl	H	H		$C_{18}H_{15}ClN_2O_3$ (342.7)	160–162 [13]	74.6
6r	O	H	CH_3	H		$C_{18}H_{18}N_2O_3$ (322.4)	205–207	77.8
6s	O	CH_3	H	H		$C_{19}H_{16}Cl_2N_2O_3$ (391.3)	215–217	69.6
6t	O	H	CH_3	H		$C_{19}H_{16}Cl_2N_2O_3$ (391.3)	224–226	68.3
6w	O	F	H	H	C_2H_5	$C_{13}H_{13}FN_2O_3$ (264.3)	187–189	80.3
6z	O	H	CH_3	H	C_2H_5	$C_{14}H_{16}N_2O_3$ (260.3)	216–217	78.9

1-alkyl-1,4-dihydro-4-iminocinnoline-3-hydroxamic acids and 1-alkyl-1,4-dihydro-4-oxo-cinnoline-3-hydroxamic acids **8**.

The ^{1}H NMR spectrum of the hydroxamic acids indicated the presence of the singlet NH proton at 8.7–9.1 ppm exchangeable with D_2O. The acidic hydrolysis of esters **6** with 20 % hydrochloric acid solution led to final 1-alkyl-1,4-dihydro-4-iminocinnoline-3-carboxylic acids **10**; however, alkaline hydrolysis of the esters **6** gave the corresponding 1-alkyl-1,4-dihydro-4-oxocinnoline-3-carboxylic acids **10j**, **10k**, similarly to unsubstituted 4-aminocinnoline-3-carboxylic acids as reported in a previous

Table 3. Physical and analytical data of compounds (**10 a–m**).

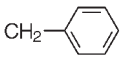
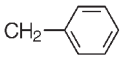
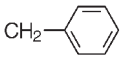
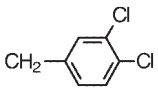
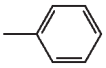
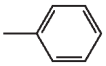
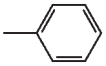
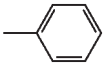
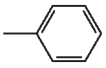
Compd.	Z	R^1	R^2	R^3	R^5	Molecular formula (weight)	Mp [°C]	Yield [%]
10 a	NH	F	H	H		$C_{16}H_{12}FN_3O_2$ (297.3)	240–242	60.4
10 b	NH	H	CH_3	H		$C_{17}H_{15}N_3O_2$ (293.3)	265–266	48.6
10 c	NH	Cl	H	H		$C_{16}H_{12}ClN_3O_2$ (313.7)	263–265	51.3
10 d	NH	H	CH_3	H		$C_{17}H_{13}Cl_2N_3O_2$ (362.2)	279–280	60.2
10 e	NH	H	H	H	C_2H_5	$C_{11}H_{11}N_3O_2 \cdot H_2O$ (235.2)	238–239 [12]	61.4
10 f	NH	CH_3	H	H	C_2H_5	$C_{12}H_{13}N_3O_2 \cdot H_2O$ (249.3)	277–278	69.4
10 g	NH	H	CH_3	H	C_2H_5	$C_{12}H_{13}N_3O_2 \cdot H_2O$ (249.3)	266–267	59.7
10 h	NH	CH_3	CH_3	H	C_2H_5	$C_{13}H_{15}N_3O_2 \cdot H_2O$ (263.3)	291–292	58.2
10 i	NH	H	Cl	H	C_2H_5	$C_{11}H_{10}ClN_3O_2 \cdot H_2O$ (269.7)	272–273	60.4
10 j	NH	F	H	H	C_2H_5	$C_{11}H_{10}FN_3O_2 \cdot H_2O$ (253.2)	261–262	55.9
10 k	NH	F	Cl	H	C_2H_5	$C_{11}H_9ClFN_3O_2 \cdot H_2O$ (287.7)	272–274	56.2
10 l	O	F	H	H	C_2H_5	$C_{11}H_9FN_2O_3$ (236.2)	228–230	76.2
10 m	O	H	CH_3	H	C_2H_5	$C_{12}H_{12}N_2O_3$ (232.2)	198–200	79.3

Table 4. Physical and analytical data of compounds (**5 a–w**).

Compd.	Y	R^1	R^2	R^3	R^6	Molecular formula (weight)	Mp [°C]	Yield [%]
5 a	NH_2	CH_3	H	H	H	$C_{10}H_{11}N_5O$ (217.2)	279–281	67.6
5 b	NH_2	CH_3	CH_3	H	H	$C_{11}H_{13}N_5O$ (231.3)	>330	73.2
5 c	NH_2	F	H	H	H	$C_9H_8FN_5O$ (221.2)	>330	71.9
5 d	NH	Cl	H	H	H	$C_9H_8ClN_5O$ (237.6)	>330	66.9
5 e	NH_2	Cl	H	CH_3	H	$C_{10}H_{10}ClN_5O$ (251.7)	>330	72.2
5 f	NH_2	F	Cl	H	H	$C_9H_7ClFN_5O$ (255.6)	>330	69.2

Table 4. (continued).

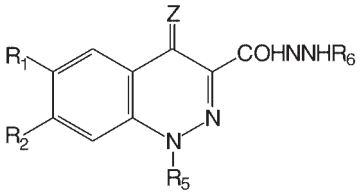
Compd.	Z	R^1	R^2	R^3	R^5	Molecular formula (weight)	Mp [°C]	Yield [%]
5g	NH_2	Cl	H	H	CH_3	$C_{10}H_{10}ClN_5O$ (251.7)	>330	61.9
5h	NH_2	H	Cl	H	CH_3	$C_{10}H_{10}ClN_5O$ (251.7)	229–232	70.6
5i	NH_2	F	H	H	CH_3	$C_{10}H_{10}FN_5O$ (235.2)	>330	62.8
5j	NH_2	F	Cl	H	CH_3	$C_{10}H_9ClFN_5O$ (269.7)	245–246	72.7
5k	NH_2	Cl	H	CH_3	CH_3	$C_{11}H_{12}ClN_5O$ (265.7)	220–222	73.1
5l	NH_2	Cl	H	H	$COCH_3$	$C_{11}H_{10}ClN_5O_2$ (267.7)	218–220	75.9
5m	NH_2	CH_3	H	H		$C_{16}H_{15}N_5O$ (293.3)	276–278	60.1
5n	NH_2	F	H	H		$C_{15}H_{12}FN_5O$ (297.3)	235–237	61.2
5o	NH_2	Cl	H	H		$C_{15}H_{12}ClN_5O$ (313.7)	248–250	57.8
5p	NH_2	Cl	H	CH_3		$C_{16}H_{14}ClN_5O$ (327.8)	245–247	60.9
5r	OH	H	H	H	H	$C_9H_8N_4O_2$ (204.2)	>330	59.9
5s	OH	Cl	H	H	H	$C_9H_7ClN_4O_2$ (238.6)	>330	61.4
5t	OH	H	H	H		$C_{15}H_{12}N_4O_2$ (280.3)	289–291	57.9
5w	OH	Cl	H	H	CH_3	$C_{10}H_9ClN_4O_2$ (352.7)	>330	62.9

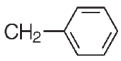
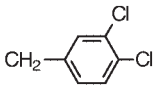
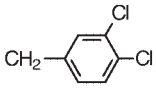
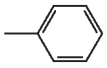
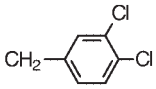
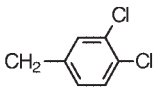
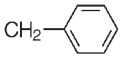
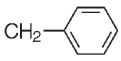
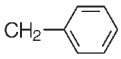
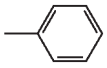
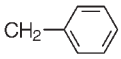
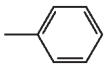
paper [14]. In a typical example, the ^{1}H NMR spectrum of the acids **10** exhibited, besides the aromatic protons, singlet or quartet for N_1 methylene group and singlet of NH protons at 10.5–11.2 ppm. We tried to substitute the chlorine atom at 7-position by piperazine and 4-methylpiperazine because this is a basic substituent in 7 position of most used flouoroquinolones. Unfortunately, attempts to perform those replacements were unsuccessful under a variety conditions. All the compounds were characterized by physical constants, elemental analysis, IR, and ^{1}H NMR spectra.

Spectral data of the obtained compounds were consistent with the assigned structure and are given for only one example because of the similarity of the spectral data in corresponding groups of compounds. (The ^{1}H NMR and IR spectra of the remaining compounds are available on request.) The physical properties of compounds **4–10** and the structures of their substituents are summarized in Tables 1–6.

We have also analysed the optimised structures (PM3, HyperChem, ChemPlus, Hypercube 1998) of compounds **6d**, **10** and compared them with that of nalidixic acid. Superimposition of the PM3 optimised conformers of the individual compounds and nalidixic acid was performed with the RMS fit procedure within ChemPlus 2.0. As is usual in studies attempting to superimpose struc-

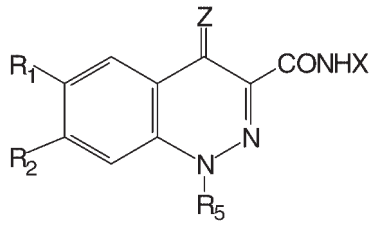
Table 5. Physical and analytical data of compounds (**9 a–l**).



Compd.	Z	R^1	R^2	R^5	R^6	Molecular formula (weight)	Mp [°C]	Yield [%]
9 a	NH	CH_3	H	C_2H_5	H	$C_{12}H_{15}N_5O$ (245.3)	265–267	68.9
9 b	NH	CH_3	H		CH_3	$C_{18}H_{19}N_5O$ (321.4)	208–210	67.2
9 c	NH	CH_3	H		H	$C_{17}H_{15}Cl_2N_5O$ (376.3)	257–259	75.7
9 d	NH	CH_3	H			$C_{23}H_{19}Cl_2N_5O$ (452.3)	278–279	65.6
9 e	NH	CH_3	CH_3		CH_3	$C_{19}H_{19}Cl_2N_5O$ (404.3)	267–269	59.8
9 f	NH	CH_3	H		CH_3	$C_{18}H_{17}Cl_2N_5O$ (390.3)	252–254	62.8
9 g	O	H	H		H	$C_{16}H_{14}N_4O_2$ (294.3)	185–187	79.8
9 h	O	Cl	H		CH_3	$C_{17}H_{15}ClN_4O_2$ (342.8)	175–177	68.9
9 i	O	Cl	H			$C_{22}H_{17}ClN_4O_2$ (404.9)	213–215	61.8
9 j	O	Cl	H		H	$C_{16}H_{13}ClN_4O_2$ (328.8)	220–222	71.7
9 k	O	H	CH_3	C_2H_5		$C_{18}H_{18}N_4O_2$ (322.4)	173–175	62.5
9 l	O	H	CH_3	C_2H_5	CH_3	$C_{13}H_{16}N_4O_2$ (260.3)	198–200	74.9

tures, we selected three pairs of atoms for the fitting procedure: N-1 atom of cinnoline ring, C-7 carbon atom of cinnoline nucleus, and C atom of the carbonyl group at 3-position of cinnoline and analogous atoms of the structure of nalidixic acid. The three points mentioned were selected as atom pairs for the fitting procedure. The superimposition of nalidixic acid with cinnoline analog **10 g** (RMS = 1.887 Å) is shown in Figure 3. However, the fit of compound **6 d**, which had the best antibacterial activity, is worse than that of compounds with poor activity (RMS = 5.139 Å). The superimposition of compound **6 d** with nalidixic acid is shown in Figure 4. It can be noted that compounds with the best activity against Gram-positive bacteria are inactive against Gram-negative species Evidently, further refinement of the model is required to explain these facts.

Table 6. Physical and analytical data of compounds (**7 a–d**, **8 a–d**).



Compd.	Z	X	R^1	R^2	R^5	Molecular formula (weight)	Mp [°C]	Yield [%]
7 a	NH	H	H	H	C_2H_5	$C_{11}H_{12}N_4O$ (216.2)	>330	60.4
7 b	NH	H	F	Cl	C_2H_5	$C_{11}H_{10}FClN_4O$ (268.7)	>330	64.1
7 c	NH	H	Cl	H	C_2H_5	$C_{11}H_{11}ClN_4O$ (250.7)	>330	61.4
7 d	NH	H	H	CH_3	CH_2–C$_6$H$_5$	$C_{17}H_{16}N_4O$ (292.3)	287–289	57.9
8 a	NH	OH	H	H	C_2H_5	$C_{11}H_{12}N_4O_2$ (232.2)	266–268	66.1
8 b	NH	OH	H	CH_3	CH_2–C$_6$H$_5$	$C_{17}H_{16}N_4O_2$ (308.3)	284–286	68.5
8 c	NH	OH	F	H	CH_2–C$_6$H$_5$	$C_{16}H_{13}FN_4O_2$ (312.3)	238–240	69.2
8 d	O	OH	Cl	H	CH_2–C$_6$H$_5$	$C_{16}H_{12}ClN_3O_3$ (313.7)	202–204	75.8

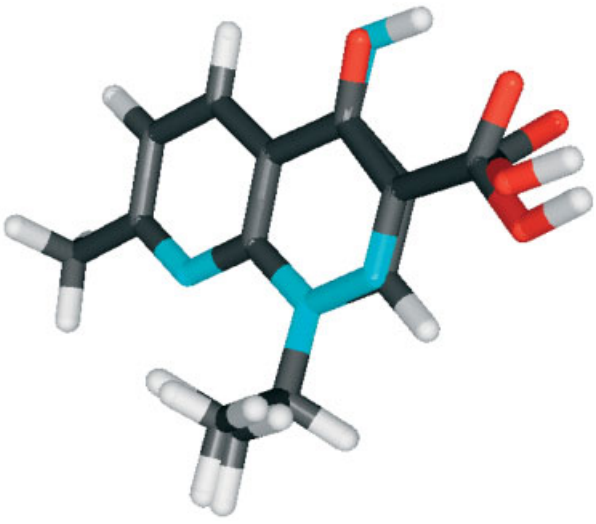


Figure 3. Superimposition of nalidixic acid with cinnoline analog (**10 g**).

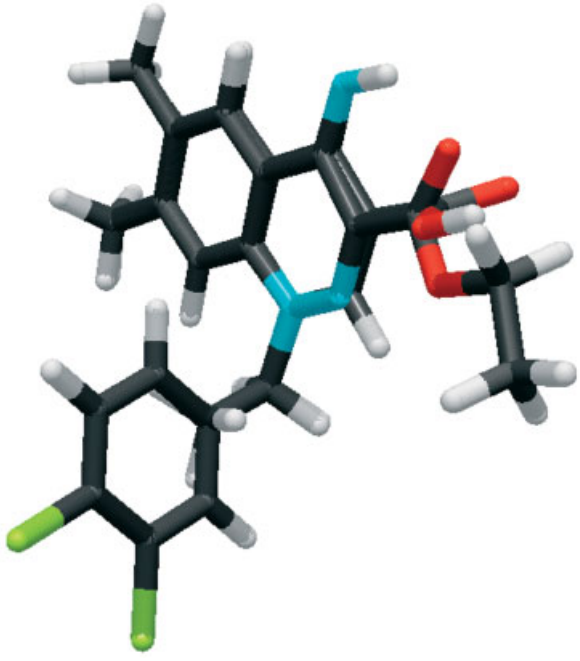


Figure 4. Superimposition of nalidixic acid with compound (**6 d**).

X-ray crystallography

The most surprising result of the X-ray study of 1-benzyl-7-methyl-1,4-dihydro-4-iminocinnoline-3-carboxylic acid **10 b** was its existence in dipolar form in the crystal state (Figure 5). It suggests that the same form may predominate in aqueous solution, especially in the case of analogues containing a more basic substituent in position 7, for example, 4-methylpiperazine. Other characteristic features of the molecular structure of **10 b** are:

– strong intramolecular hydrogen bonds between protonated 4-imine group as a hydrogen bond donor and 3-carboxylate anion as an acceptor;

– partial localization of π electrons in the benzene ring of the cinnoline system, shown by shortening of *C-5–C-6* and *C-7–C-8* bonds to 1.361(3) and 1.376(3) Å, respectively, as compared with the rest of the *C–C* bonds, being 1.399–1.408 Å;

– stacking of the flat pseudo three ring structure of **10 b** in an antiparallel fashion (Figure 3).

Similar relative orientation of quinolones and its analogues may be of great importance for their interactions with DNA [18]. We are aware that 4-imine analogues, though formally classical isosteres of quinolones, can not interact specifically with the same DNA base or in the same fashion due to reversed functionality of a 4-imine group in hydrogen bonds as compared with a 4-oxo one.

Antibacterial activity

Antibacterial activity of 4-amino-3-cinnolinecarboxylic acid derivatives was examined while indicating the minimum inhibitory concentration (MIC) for representatives of the Gram-positive (*Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, *E. faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633), and Gram-negative (*Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacterial strains. Nalidixic acid was used as a reference drug of known antibacterial activity. The MIC of the chosen compounds against Gram-positive and Gram-negative bacteria are summarized in Table 7, which includes only the compounds with MIC < 500 µg/mL. Surprisingly, the most active compounds appeared to be ester **6 d** and methyl hydrazide **9 e**, both with the same substituent at *N*-1 position. In contrast to classical quinolones, these

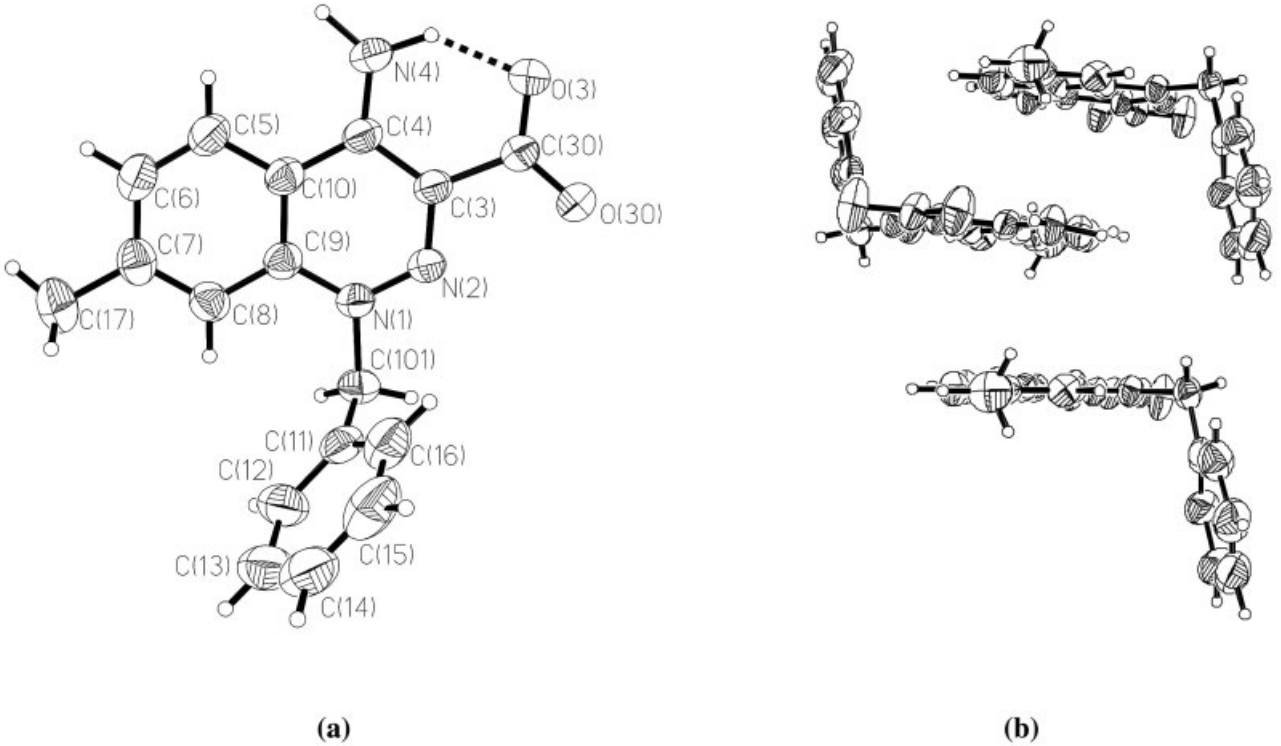


Figure 5. Crystal structure of (**10 b**). Thermal elipsoids were drawn with 50 % probability with the XP program [19]. **(a)** General view of molecule, atom numbering system, and intramolecular hydrogen bond. **(b)** Arrangement of molecules in the crystal state.

Table 7. *In vitro* antibacterial activity of the compoundds.

Compd.	Minimum inhibitory concentration (MIC) µg/mL							
	Gram-positive organisms					Gram-negative organisms		
	S. aureus ATCC 6538P	*S. aureus*[a] ATCC 29213	*S. aureus*[a] ATCC 25923	*E. faecalis*[a] ATCC 29212	*B. subtilis* ATCC 6633	*E. coli*[a] ATCC 35218	*E. coli* ATCC 25922	*Ps. aeruginosa* *ATCC* 27853
5 a	×[b]	×	×	×	×	300	300	×
5 b	62	62	62	125	125	×	×	×
5 h	250	250	250	250	250	250	250	250
5 n	62	62	62	×	62	×	×	×
6 b	500	500	500	×	500	×	×	×
6 c	125	125	125	250	125	×	×	×
6 d	25	25	25	50	100	×	×	×
7 b	375	×	×	×	187	187	93	×
8 d	250	250	250	500	250	125	125	>500
9 c	>500	>500	300	500	300	300	300	×
9 e	50	50	50	50	50	×	×	×
9 h	250	250	250	250	250	×	×	×
9 j	500	250	250	250	250	×	×	×
10 j	×	×	×	×	>400	100	200	×
10 k	>500	×	×	×	500	250	250	×
Nevigramon	>50	>50	>50	>50	6,2	6,2	<6,25	>50

[a] Multiple resistant bacteria.
[b] No activity.

two compounds showed increased antibacterial activity against Gram-positive bacterial strains and loss of activity against Gram-negative ones. Generally, among the tested compounds, there can be seen rare antibacterial activity, mainly against Gram-positive bacterial strains, which is especially surprising for final acids **10**, which are very similar to typically applied quinolones.

Acknowledgement

The authors gratefully acknowledge financial support within the project 4 P05F 024 17 from the Polish State Committee for Scientific Research.

Experimental

Apparatus

All chemicals and solvents used in this study were purchased locally from Fluka and Aldrich and were used without further purification.

Melting points were determined on an Electrothermal apparatus in open capillaries and have not been corrected. The ^{1}H NMR spectra of compounds were recorded on in DMSO-d_6 on a Varian Mercury 300 MHz spectrometer using tetramethylsilane as internal standard. The infrared spectra of the compounds were recorded on a Mattson Infinity Series FTIR spectrophotometer. ^{1}H NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Carbon, hydrogen, and nitrogen elemental analyses were performed on a Perkin Elmer series II CHNS/O Analyzer 2400 and were within 0.4 % of theoretical values.

Chemistry

Synthesis of series **1**, **2**, **3**

Compounds **1**, **2**, **3** were synthesized as described in [12].

Synthesis of ethyl 4-aminocinnoline-3-carboxylates **4** *and ethyl 4-hydroxycinnoline-3-carboxylates* **4**

Compounds **4** were synthesized as described in [13].

Physical and analytical data for **4** are shown in Table 1. Example of spectral data for **4 e**: **IR** (KBr): ν_{max} cm^{-1} = 3280–3000 (NH_2), 1690 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 1.3–1.6 (t, 3 H, CH_3), 2.6 (d, 6 H, CH_3-Ar), 3.7–4.0 (q, 2 H, CH_2), 8.0 (s, 1 H, Ar-NH), 8.2 (s, 2 H, NH_2), 8.3 (s, 1 H, Ar-H). **4 p**: **IR** (KBr): ν_{max} cm^{-1} = 3200–3000 (OH), 1700 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 1.2–1.6 (t, 3 H, CH_3), 4.1–4.6 (q, 2 H, CH_2), 7.8–8.0 (d, 2 H, Ar-H), 8.1–8.3 (d, 2 H, Ar-H + OH).

Synthesis of ethyl 1-alkyl-1,4-dihydro-4-iminocinnoline-3-carboxylates **6** *and ethyl 1-alkyl-1,4-dihydro-4-oxocinnoline-3-carboxylates* **6**

To a stirred suspension of the appropriate esters **4** (10 mmol) in CH_3CN or DMF was added 15 mmol DBU or K_2CO_3 (for benzyl derivatives) and the mixtures were refluxed for 0.5 h. After that the alkylating agents (25 mmol) were added dropwise and the mixtures were refluxed for 2 h before being evaporated down to 1/3 volume. The remaining solutions were rendered alkaline with 10 % ammonia. The crude products were filtered off and crystallized from a mixture of DMF and water.

Physical and analytical data for **6** are shown in Table 2. Example of spectral data for **6 g**: **IR** (KBr): ν_{max} cm^{-1} = 3270–3000 (NH), 1700 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 1.2–1.5 (dt, 6 H, CH_3), 3.7–3.8 (q, 2 H, N-CH_2), 4.3–4.4 (q, 2 H, –OCH_2), 7.2–7.6 (m, 2 H, Ar-H), 8.0–8.2 (d, 1 H, Ar-H), 9.6 (s, 1 H, NH); **6 r**: **IR** (KBr): ν_{max} cm^{-1} = 1700, 1680 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 1.3–1.6 (t, 3 H, CH_3), 4.2–4.5 (q, 2 H, CH_2), 5.9 (s, 2 H, CH_2-Ar), 7.5–8.0 (m, 6 H, Ar-H), 8.3 (d, 2 H, Ar-H).

Synthesis of 1-alkyl-1,4-dihydro-4-iminocinnoline-3-carboxylic acids **10**

Compounds **10 a–k** were synthesized as described in [13].

Physical and analytical data for **10 a–k** are shown in Table 3. Example of spectral data for **10 h**: **IR** (KBr): ν_{max} cm^{-1} = 3200–3050 (OH, NH), 1670 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 1.4–1.5 (t, 3 H, CH_3), 4.6–4.8 (q. 2 H, CH_2), 7.9–8.1 (m, 2 H, Ar-H), 8.3–8.4 (d, 1 H, Ar-H), 10.6 (s, 1 H, NH).

Synthesis of 1-alkyl-1,4-dihydro-4-oxocinnoline-3-carboxylic acids **10**

A stirred suspension of 5 mmol esters **6** and 2 M solution (20 cm^3) NaOH was refluxed for 2 h. After cooling the mixtures were acidified with 30 % CH_3COOH and filtered. The products were purified by crystallization from CH_3COOH with charcoal. Physical and analytical data for **10 l–m** are shown in Table 3. Example of spectral data for **10 l**: **IR** (KBr): ν_{max} cm^{-1} = 2900–2400 (OH), 1675 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 1.4–1.5 (t, 3 H, CH_3), 4.6–4.8 (q, 2 H, CH_2), 7.9–8.0 (m, 1 H, Ar-H), 8.4–8.5 (dd, 1 H, Ar-H), 8.9–9.0 (m, 1 H, Ar-H).

Synthesis of 4-aminocinnoline-3-carboxylic acid hydrazides **5** *and 4-hydroxycinnoline-3-carboxylic acid hydrazides* **5**

A solution of esters **4** or **6** (10 mmol) and DBU (15 mmol) in DMF (50 cm^3) was refluxed for 0.5 h. To the reaction mixtures the corresponding hydrazines (10 mmol) were added and the mixtures were heated under reflux for 2 h. After cooling water (50 cm^3) was added and the mixtures were left for 24 h. The solids were filtered off, washed with hot ethanol, and crystallized from mixture DMF and water.

Physical and analytical data for **5** are shown in Table 4. Example of spectral data for **5 a**: **IR** (KBr): ν_{max} cm^{-1} = 3900–3550 (NH), 1690 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 2.7 (s, 3 H, CH_3-Ar), 4.5 (bs, 2 H, NH_2), 7.8–7.9 (m, 2 H, Ar-H), 8.1–8.3 (m, 2 H, Ar-H + NH_2), 10.5 (bs, 1 H, CONH).

Synthesis of 1-alkyl-1,4-dihydro-4-iminocinnoline-3-carboxylic acids hydrazides and 1-alkyl-1,4-dihydro-4-oxocinnoline-3-carboxylic acid hydrazides **9**

Compounds **9** were synthesized as described above for compounds **5**.

Physical and analytical data for **9** are shown in Table 5. Example of spectral data for **9 b**: **IR** (KBr): ν_{max} cm^{-1} = 3950–3780 (NH), 1690 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 2.7 (s, 3 H, CH_3-Ar), 3,8 (d, 3 H, N-CH_3), 4.9 (bs, 1 H, NH-CH_3), 5.9 (s, 2 H, CH_2), 7.2–7.4 (m, 5 H, Ar-H), 7.8–7.9 (m, 2 H, Ar-H), 8.1–8.3 (m, 2 H, Ar-H + NH_2), 10.8 (bs, 1 H, CO-NH).

Synthesis of 1-alkyl-1,4-dihydro-4-iminocinnoline-3-carboxylic acid amides and 1-alkyl-1,4-dihydro-4-oxocinnoline-3-carboxylic acid amides **7**

Compounds **7** were synthesized as described above for compound **6**.

Physical and analytical data for **7** are shown in Table 6. Example of spectral data for **7 b**: **IR** (KBr): ν_{max} cm^{-1} = 3250–31250 (NH), 1680 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 1.4–1.5 (t, 3 H, CH_3), 4.7–4.9 (q, 2 H, CH_2), 8.0 (s, 1 H, Ar-H), 8.1 (s, 1 H, Ar-H), 8.2–8.4 (bs, 2 H, $CONH_2$), 10.3 (s, 1 H, NH).

Synthesis of 1-alkyl-1,4-dihydro-4-iminocinnoline-3-hydroxamic acids and 1-alkyl-1,4-dihydro-4-oxocinnoline-3-hydroxamic acids **8**

To a stirred solution of the appropriate esters **4** (10 mmol) in DMF was added DBU (15 mmol) and the mixtures were refluxed for 0.5 h. After that the hydroxylamine hydrochloride solution 25 mmol was added dropwise and mixtures were stirred for 20 h. The solvents were evaporated to 1/2 volume and acidified with 30 % CH_3COOH and filtered. The products were purified by crystallization from mixture DMF and water with charcoal.

Physical and analytical data for **8** are shown in Table 6. Example of spectral data for **8c**: **IR** (KBr): ν_{max} cm^{-1} = 3050–2860 (NH, OH), 1660 (C=O). **^{1}H NMR** (300 MHz, DMSO-d_6): δ (ppm) = 6.0 (s, 2 H, CH_2), 7.2–7.4 (m, 5 H, Ar-H), 8.4-8.5 (d, 2 H, Ar-H), 8.9–9.0 (s, 1 H, NH), 10.5 (s, 1 H, NH).

X-ray structure determination

X-ray data were collected on four-circle diffractometer KM-4 at room temperature with Cu K_α. The structure was solved by direct method and refined with SHELXL [19].

Crystal data and structure refinement details for **10 b**.

Empirical formula		$C_{17}H_{15}N_3O_2$
Crystal system and space group		monoclinic, $P2_1/c$
Unit cell dimensions (A and °)	*a*	7.579(2)
	b	14.808(3)
	c	12.679(3)
	β	90.10(3)
Volume [A] and *Z*		1456.8(6); 4
F(000)		616
Theta range in data collection [°]		3–80
No. of reflections:		
– collected		4019
– unique		2857
– observed		2254
Data/parameter ratio		10
Goodness of fit (on F^2)		1.017
Final *R* (R_w)		0.048
Largest difference peak (e/Å^3)		0.19

In vitro antibacterial activity

The minimal inhibitory concentrations (MICs) of the studied compounds were determined according to the method of serial twofold dilutions in agar, recommended by NCCLS (National Committee for Clinical Laboratory

Standards) [20]. The inoculum, containing approximately 10^4 CFU/mL, was applied to the Mueller-Hinton agar (Difco Laboratories, Detroit, MI) plates, with the use of Steer's replicator. The plates were incubated at 35 °C for 18 h and inspected immediately.

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