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ANALYSIS

DETERMINATION OF LIPOPHILICITY AND pK_a MEASUREMENT OF SOME 4-IMINO-1,4-DIHYDROCINNOLINE-3-CARBOXYLIC ACID AND 4-OXO-1,4-DIHYDROCINNOLINE-3-CARBOXYLIC ACID DERIVATIVES – ISOSTERIC ANALOGUES OF QUINOLONES

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Abstract: As a continuation of our study, physicochemical properties of some antibacterial active 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives were investigated. Partition coefficient (log P), and dissociation constant (pK_a) were experimentally determined and also calculated by the use of ACDLabs system software. The obtained values were correlated with experimental data.

Keywords: 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives, isosteric analogues of quinolones, partition coefficient (log P), RP-TLC, pK_a measurements.

The quinolone group of antibacterial agents is one of the most effective classes of drugs in the treatment of bacterial diseases. Cinoxacin, the chemical structure of which is characterized by a 4-oxocinnoline-3-carboxylic acid moiety shows also antibacterial activity, mainly against Gram-negative bacteria (1, 2). In our previous study, chemical modification of cinoxacin was studied with the aim of improving its antibacterial activity and spectrum (3). Therefore, the series of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid derivatives were synthesized and their *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria were tested. The minimum inhibitory concentration (MIC) of the most active compounds was in the range of the first generation quinolones such as nalidixic acid. These derivatives were designed as isosteric analogues of fluoroquinolones and were characterized by the presence of an imine- group instead of an oxo-group at the 4-position and nitrogen atom in position 2. The chemical structure and minimum inhibitory concentration (MIC) of some of them are described in Table 1. Though it is known that quinolones are active against topoisomerases of type II and IV, the molecular details of their mode of action remain unclear (4). The variety of models propose binding of quinolones with DNA or non-covalent DNA-quinolone complexes (5). The chemical structure of synthesized compounds may suggest their antibacterial activity as potential intercalators. They have a planar polyaromatic system and ability to form hydrogen bond with DNA base. The tested compounds exhibit good polarizability as well and can be good electron acceptors to DNA base pairs.

Intercalators are the most important group of compounds that interact reversibly with the DNA double helix. The flat molecules of these ligands intercalate between pairs of DNA helix, lengthening and unwinding this structure at the intercalation sites. Intercalating agents share common structural features such as the presence of planar polyaromatic system, which binds by insertion between DNA base pairs, with a marked preference for 5'- pyrimidine – purine – 3'steps (6).

Six derivatives from the synthesized series were selected in our study to explain structure-activity relationships. The compounds were chosen considerably differing in the antibacterial activity to measure or calculate some physicochemical properties.

Biological activity of chemical compounds is associated with their physicochemical properties. The physicochemical constant (the dissociation constant K and partition coefficient log P) and some structural parameters (topological, steric and electronic) are useful for understanding the behavior of drug molecules. Therefore, the physicochemical parameters (log P and pK_a) of synthesized 4-imino-

1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives, influencing the biological availability were determined experimentally and calculated by the use of ACDLabs system software (7).

To evaluate the validity of pK_a constants predicted by the ACDLabs method, UV-visible spectroscopic measurements were made at different pH values (8). Spectra of **I-VI** were recorded in the range of 200-400 nm, as a function of $A = f(pH)$ and the pK_a value was calculated according to the Henderson-Hasselbalch equation. The obtained results are presented in Table 2.

The experimental pK_a values were correlated with those calculated (ACDLabs system software). The comparison of experimental and calculated data gave the equation:

$$\text{clog } pK_a = a\ pK_a + b \qquad (1)$$

where:

a = 1.0281, b = -0.0757, r = 0.9977, s = 0.2387, F = 1279.013

Comparison of these results permits to define usefulness of computer software for calculation of physicochemical parameters of the compounds.

The partition coefficient (log P) is also a very functional parameter, which may be used in combination with pK_a to predict the distribution of tested compounds in biological systems as a measure of their lipophilicity. The relative lipophilicity of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives **I-VI** were determined by reversed-phase thin-layer chromatography (RP-TLC). The chromatographic parameter of hydrophobicity in this method is the R_M value, defined as:

$$R_M = \log\left(\frac{1}{R_f} - 1\right) \qquad (2)$$

Its value decreases linearly with the increase of concentration of organic modifier (DMF) in the mobile phase. Correlation of both sets of values and extrapolation to zero values of the organic modifier (DMF) permits to obtain normalized chromatographic parameter R_{MW}.

The experimental lipophilicity values (log P_{TLC}) at pH 7.4 of all compounds **I-VI** were determined by the use of the calibration curve, which was obtained under the same measurement conditions for the set of six standards of known experimental lipophilicity (9). As the standards were used: acetanilide (**1**), 4-ethoxyacetanilide (**2**), 3,4-dichloraniline (**3**), benzophenone (**4**), 1-bromonaphthalene (**5**), and 1,2,3,4-tetrabromobenzene (**6**). Correlation of both sets of values gave the calibration equation:

$$\log P_{TLC} = 1.0428\ R_{MW} - 0.1005 \qquad (3)$$

r = 0.9997, F = 9662.0671, s = 0.03, n = 6

The literature values of log P and R_{MW} of the standards are presented in Table 3.

Terms of the linear equations correlating the R_{MW} values of derivatives **I-VI** with the concentration of DMF [c (%, v/v)] in the mobile phase and calculated results of log P_{TLC} (pH = 7.4) are presented in Table 4.

Because the distribution coefficient at the given pH is denoted log D, the log P_{TLC} value determined in this study, at pH 7.4, was denoted log $D_{7.4}$. The experimental values of partition coefficient (log P_{TLC}) of compounds **I-VI** were correlated with the calculated values (log $P_{ACDLabs}$), which predicts the logarithm of the partition coefficient of compounds in *n*-octanol – water system, to define usefulness of computer software for calculation of the log P parameters. The obtained values, compared with the experimental values of log P_{TLC} (log $D_{7.4}$) are presented in Table 5. The comparison of experimental and calculated data gave the equation:

$$\text{clog } P = a \log P_{TLC} + b \qquad (4)$$

where for ACDLabs system software:

a = 1.3277, b = -1.0986, r = 0.9600, s = 0.3737, F = 95.9456

EXPERIMENTAL

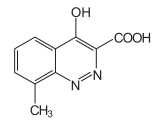
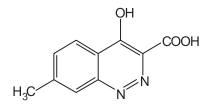
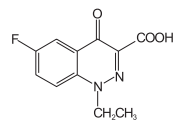
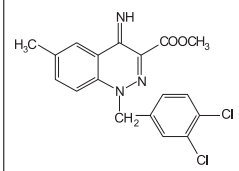
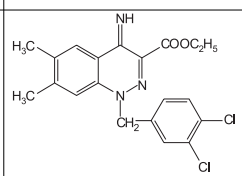
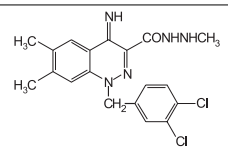
Chemistry and biological activity of tested compounds were described earlier (3).

UV spectrophotometric method of determination of pK_a values

The spectrophotometric measurements were recorded with double-beam spectrophotometer UV/VIS PAY-4 (Unicam, Denmark) with a fixed slit of 2 nm. A numerical pH-meter PHM-8 (Radiometer, Denmark) and a special glass electrode (type GK 2725 Radiometer, Denmark) for pH measurements in the $1 < pH < 10$ range were used. The glass electrode was calibrated according to standard procedures with a phosphate buffer pH = 6.88, borate buffer pH = 9.22 and citrate buffer pH = 3.0 (Merck). The temperature was kept constant at 20°C. The UV spectra of all compounds were recorded in 1 cm quartz cells over the range 200 – 400 nm. The stability of the compounds was studied spectrophotometrically at concentration $c \approx 1 \times 10^{-4}$ M in 1%, v/v aqueous solutions of DMF, $\mu = 0.1$.

Spectrophotometric method was selected due to its applicability in highly diluted solutions of compounds poorly soluble in water. Standard solutions were prepared by dissolving weighted amounts of

Table 1. Structure and MIC some of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives.

No.	Compound	*In vitro* antibacterial activity of the compounds [Minimum inhibitory concentration (MIC) µg/mL]				
		S. aureus ATCC 6538 P	*S. aureus* ATCC 29213*	*S. aureus* ATCC 25923*	*E. faecalis* ATCC 29212*	*B. subtilis ATCC 6633*
I		25	-	-	25	-
II		-	25	25	25	-
III		-	-	-	-	>400
IV		125	125	125	250	125
V		25	25	25	50	25
VI		50	50	50	50	50

* multiple resistant bacteria

particular compounds (3 – 8 mg) in 1 mL of DMF, and then filling with water to 100 mL in calibrated flasks. The analyzed solutions were made from standard solutions by dilution. Then, spectra of A = f (pH) within the range 200-400 nm were recorded. In order to choose the optimal wavelength at which the difference in absorbance between the two forms is the biggest, absorption curves for dissociated and undissociated forms were drawn. The change in pH of the solutions was obtained using various concentrations of $HClO_4$ (c = 1.0; 0.1; 0.01; 0.001 M) and solutions of anhydrous NaOH (c = 1.0; 0.1; 0.01; 0.001 M).

Chromatographic system and conditions

Chromatography was carried out on TLC glass plates (5 cm × 10 cm) coated with RP-18 F_{254s} (Merck, Darmstadt, Germany). The mobile phase was DMF – aqueous buffer TRIS, of pH = 7.4 and ionic strength 0.2 M. The organic modifier (DMF) content varied from 40 to 85% (v/v) in 5% increments. Migration of 8.5 cm was obtained on all the plates by cutting the layer at 9.5 cm and spotting the compounds on a 1 cm line of the plate. Compounds were dissolved in methanol (1.5 mg/mL) and 2 µL of the solution was spotted on the plates. After developing and drying, the spots were observed under UV light and the R_F values are mean from three determinations.

The experimental lipophilicity (log P_{TLC}) of all compounds was determined by the use of the calibration curve (eq. 3), which was obtained under the same measurement conditions for the set of six standards of known experimental lipophilicity.

Table 2. Spectrophotometric determination of pK_a values of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives.

Compd. No.	Concentration [M]	λ [nm]	*pH*	A	pK_a	pK_a (average values)
1	2	3	4	5	6	7
I	5.39×10^{-6}	290	4.58	0.37	3.95	4.08 ± 0.35
			4.00	0.41	3.72	
			3.25	0.55	4.13	
			3.09	0.57	4.49	
			2.87	0.70	4.15	
	1.13×10^{-4}	343	8.64	0.33	9.53	10.10 ± 0.60
			9.42	0.39	10.02	
			10.14	0.49	10.42	
			10.60	0.67	10.10	
			11.00	0.80	10.45	
II	8.33×10^{-4}	270	3.16	0.70	3.33	3.50 ± 0.20
			3.69	0.75	3.42	
			4.37	0.77	3.41	
			4.60	0.80	3.70	
			4.90	0.81	3.65	
	1.66×10^{-4}	360	5.79	0.70	10.35	10.38 ± 0.40
			6.44	0.68	10.05	
			6.93	0.60	10.50	
			7.57	0.50	10.29	
			8.06	0.45	10.09	
			8.55	0.42	10.98	
III	5.42×10^{-4}	275	3.24	1.74	3.89	3.84 ± 0.20
			3.5.4	1.56	3.71	
			3.71	1.40	4.06	
			3.90	1.24	3.85	
			4.30	0.95	3.70	
IV	5.20×10^{-4}	365	4.23	0.38		3.50 ± 0.30
			3.84	0.40	3.60	
			3.13	0.47	3.38	
			2.48	0.54	3.34	
			1.70	0.55	3.42	
V	5.20×10^{-5}	362	4.10	0.80	4.24	4.24 ± 0.08
			4.30	0.73	4.25	
			4.57	0.65	4.32	
			4.70	0.58	4.24	
			4.96	0.50	4.18	
VI	5.90×10^{-5}	359	4.70	0.58	4.09	4.01 ± 0.10
			4.41	0.55	4.00	
			4.12	0.51	3.92	
			3.90	0.45	4.02	
			3.52	0.36	4.05	
			3.10	0.34	3.99	
	2.45×10^{-5}	368	9.95	0.58	9.18	9.01 ± 0.20
			9.52	0.65	8.95	
			9.25	0.72	9.07	
			8.85	0.80	8.77	
			8.40	0.95	9.05	

Table 3. Comparison of literature (log P) and experimental values of lipophilicity (R_{MW}) for the standards used*.

	1	**2**	**3**	**4**	**5**	**6**
log P	1.16	1.58	2.69	3.18	4.06	5.13
R_{MW}	1.19	1.62	2.71	3.10	3.98	5.02
-b	0.0214	0.0257	0.0354	0.0415	0.0512	0.057
r	0.9991	0.9977	0.9962	0.9978	0.9965	0.9949
log P_{TLC}	1.14	1.58	2.72	3.13	4.05	5.13

*acetanilide (**1**), 4-ethoxyacetanilide (**2**), 3,4-dichloraniline (**3**), benzophenone (**4**), 1-bromonaphthalene (**5**), and 1,2,3,4-tetrabromobenzene (**6**).

Table 4. Parameters of linear correlations between the R_{MW} values of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives and the concentration of DMF [c (%, v/v)] in the mobile phase.

Compd. No.	R_{MW}	-b	n	r	log P_{TLC} (pH = 7.4)
I	1.821	0.0235	9	0.9962	1.798
II	1.931	0.0321	8	0.9978	1.913
III	1.442	0.0231	8	0.9979	1.403
IV	3.460	0.0463	10	0.9957	3.507
V	4.502	0.0618	9	0.9964	4.594
VI	3.169	0.0427	9	0.9963	3.204

Table 5. Comparison of the experimental and predicted values of physicochemical constants of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives.

Compd. No.	pK_a (experimental)	pK_a (ACDLabs)	log P_{TLC} (log $D_{7.4}$)	Log P (ACDLabs)
I	4.08 ± 0.35 10.10± 0.60	4.02 ± 0.50 10.40 ± 0.50	1.798	1.23 ± 1.02
II	3.50 ± 0.20 10.38 ± 0.40	3.93 ± 0.50 10.49 ± 0.50	1.913	1.23 ± 1.02
III	3.84 ± 0.20	3.86 ± 0.20	1.403	0.75 ± 0.69
IV	3.50 ± 0.30	3.53 ± 0.40	3.507	4.14 ± 0.75
V	4.24 ± 0.08	3.91 ± 0.40	4.594	4.60 ± 0.75
VI	4.01 ± 0.10 9.01 ± 0.20	3.99 ± 0.40 9.27 ± 0.20	3.204	3.26 ± 0.75

RESULTS AND DISCUSSION

Chemistry

SAR analysis

For 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives **I-VI,** a comparative computer analysis was done. All the structures of the studied compounds were geometrically optimized by the use of semiempirical method INDO [algorithm Polak-Ribiere, RMS grad = 0.01 kcal/(Å mol), *in vacuo*, HyperChem 5.1] (10). The systematic conformational analysis was not used. The physicochemical parameters of the compounds, received from ChemPlus modules of HyperChem are summarized in Table 6.

Geometric optimization of the studied compounds revealed a flat character of the basic core, what is the primary condition of intercalators structure. It seems that their biological activity is connected with:

1. A size of molecules, because many antibacterial compounds possess Van der Waals surface of molecule (GRID) above 300 Å^2 and similar volume, above 300 Å^3. Thus their capacities are like other small organic molecules, applied as intercalating agents, such as naphthalimides.

Table 6. Some physicochemical parameters of of 4-imino-1,4-dihydrocinnoline-3-carboxylic acid and 4-oxo-1,4-dihydrocinnoline-3-carboxylic acid derivatives.

Compd. No.	Van der Waals area of molecule (GRID) [Å^2]	Van der Waals volume [Å^3]	Refractivity	Polarizability [Å^3]	Hydrogen bond acceptors*	Hydrogen bond donors*	Log P (ChemPlus)	Dipole moment [D]
I	199.40	169.18	52.96	21.32	5	2	1.96	2.61
II	200.39	169.08	52.96	21.32	5	2	1.96	3.10
III	227.22	190.20	57.44	21.84	5	1	2.16	5.62
IV	376.78	328.91	103.31	39.96	6	1	5.50	3.55
V	365.91	332.94	108.35	41.80	6	1	5.97	6.79
VI	377.34	337.55	108.85	42.03	6	3	4.93	6.76

*Interactive Analysis Predictor (http://www.logp.com/)

2. Higher values of refractivity and polarizability, which can determine the flexibility of these derivatives during interaction with biological target. This is connected with growing values of dipole moments as well.

3. Ability to create hydrogen bonds; more active compounds are good hydrogen bond acceptors.

4. Higher values of lipophilicity. Hydrophobic/hydrophilic properties of the compounds guarantee their effective transport and consequently the biological activity. The lipophilicity parameters of the synthesized compounds, determined by RP-TLC and expressed as log P_{TLC} (log $D_{7,4}$) values are within the range 1.798-4.594 and effect the bioactivity of the derivatives. And though organic solvents with a log P between 1.5 and 4.0 are extremely toxic for microorganisms and other living cells, the most active compound **V** possesses higher log P_{TLC} (log $D_{7,4}$) value.

A size of substituent at position 1. A nontypical dichlorobenzyl substituent with aromatic ring can be stacking with DNA bases, deciding about size and increasing lipophilicity of the molecule.

pK_a and log P values of compounds **I-VI** were calculated as well by the use of the ACDLabs system software. The obtained results show a very good linear correlation ($r > 0.95$). It can suggest that methodologies are suitable for dissociation constant and lipophilicity calculation.

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