SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 2-IMINOCOUMARIN-3-CARBOXYLIC ACID AMIDES

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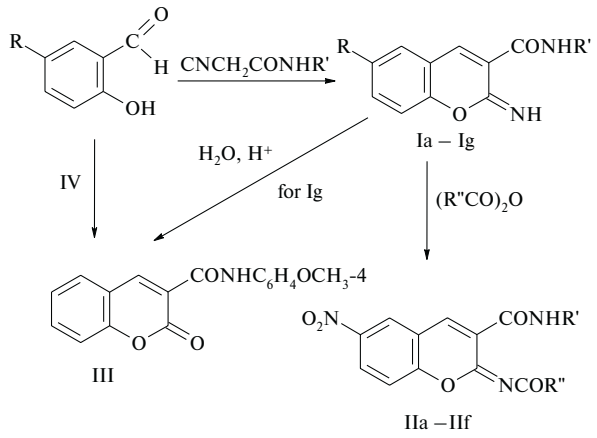
As is known, coumarin derivatives exhibit biological activity of various types [1]. Previously [2] we reported on the synthesis of 2-iminocoumarin-3-carboxylic acid amides by condensation of salicylaldehyde with cyanoacetic acid amides.

Aimed at expanding these investigations and evaluating the biological activity of compounds of this class, we studied the reactions of 5-nitrosalicylaldehyde with cyanoacetic acid arylamides and malonanilic acid ethylates.



I: R = NO_2 (a – f), H (g); R′ = C_2H_5 (a), 2-ClC_6H_4 (b), 4-$CH_3C_6H_4$ (c), $CH_2C_6H_5$ (d), 2,4-$(CH_3)_2C_6H_3$ (e), 3-$C_2H_5OC_6H_4$ (f), 4-$CH_3OC_6H_4$ (g);
II: R′ = $CH_2C_6H_5$ (a, d), 3-$C_2H_5OC_6H_4$ (b, e), 2,4-$(CH_3)_2C_6H_3$ (c, f);
R″ = CH_3 (a, b, c); C_2H_5 (d, e, f).

The reaction of 5-nitrosalicylaldehyde with substituted cyanoacetic acid amides proceeded upon boiling an alcohol solution of the initial compounds in the presence of piperidine as the catalyst. The reactions yield substituted amides of 6-nitro-2-iminocoumarin-3-carboxylic acid (Ia – If), the properties of which are listed in Table 1. Amides Ia – If appear as yellowish crystalline substances insoluble in water and soluble in dioxane. The IR spectra of these amides contain characteristic absorption bands in the region of 1610 – 1620 (C=N), 1680 – 1690 (CO), 3330 – 3340 (=NH), and 3420 cm^{-1} (CONH).

Under the action of acetic or propionic acid anhydrides, compounds Id – If were readily acylated at the imino group with the formation of the corresponding substituted amides of 2-acyliminocoumarin-3-carboxylic acid (IIa – IIf). The proposed structures of compounds IIa – IIf were confirmed by the characteristics of their IR and ^{1}H NMR spectra. The IR spectra display bands at 1660 (CO), 1700 (CO), and 3170 cm^{-1} (NH). The ^{1}H NMR spectra exhibit a singlet at 2.21 – 2.26 ppm ($COCH_3$), a pair of signals at 1.12 and 4.16 ppm (COC_2H_5), a multiplet at 7.60 – 7.86 ppm (H arom), and a singlet at 10.66 – 11.03 ppm (NH).

The treatment of 2-iminocoumarin-3-carboxylic acid 4-anisidide (Ig) [2] with concentrated hydrochloric acid leads to hydrolysis of the imino group with the formation of coumarin-3-carboxylic acid anisidide (III). The IR spectrum of compound III contain absorption bands at 1670 (CO), 1720 (CO), and 3290 cm^{-1} (NH). For comparison, compound III was also obtained by direct synthesis, using the interaction of salicylaldehyde with malonic acid monoethyl ether 4-anisidide (IV) in slightly heated alcohol solutions in the presence of a catalytic amount of piperidine. The products obtained by both methods proved to be identical.

EXPERIMENTAL CHEMICAL PART

The IR spectra of the synthesized compounds were recorded with a UR-20 spectrophotometer (Germany) using samples prepared as nujol mulls. The ^{1}H NMR spectra were measured on a RYa-2310 (60 MHz) spectrometer (Russia) using 5% solutions in DMSO-d_6 with HMDS as the internal standard. The data of elemental analyses agreed with the results of calculations using the empirical formulas.

6-Nitro-2-iminocoumarin-3-carboxylic acid amides (Ia – If). A mixture of 3 g (0.025 mole) of 5-nitrosalicylaldehyde, 0.025 mole of the corresponding cyanoacetic acid amide, and 5 drops of piperidine in 15 ml of ethanol was boiled

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TABLE 1. Characteristics of the Synthesized Compounds

Compound	Yield, %	M.p., °C	Empirical formula
Ia	49.0	130 – 132	$C_{12}H_{11}N_3O_4$
Ib	92.7	269 – 271	$C_{16}H_{10}ClN_3O_4$
Ic	92.3	235 – 237	$C_{17}H_{13}N_3O_4$
Id	42.3	187 – 188	$C_{17}H_{13}N_3O_4$
Ie	44.4	230 – 231	$C_{18}H_{15}N_3O_4$
If	92.3	206 – 207	$C_{18}H_{15}N_3O_5$
IIa	50.8	112 – 114	$C_{19}H_{15}N_3O_5$
IIb	44.6	208 – 210	$C_{20}H_{17}N_3O_5$
IIc	87.7	238 – 240	$C_{20}H_{17}N_3O_5$
IId	86.2	200 – 202	$C_{21}H_{19}N_3O_6$
IIe	66.6	196 – 198	$C_{21}H_{19}N_3O_6$
IIf	67.8	206 – 208	$C_{21}H_{19}N_3O_6$
III	95.0	210 – 211	$C_{17}H_{13}N_3O_4$

TABLE 2. Antimicrobial Activity of Compounds Ia – If and IIa – IIe

Compound	MIC, μg/ml	
	St. aureus	*E. coli*
Ia	500	125
Ib	62	62
Ic	500	500
Id	500	1000
Ie	1000	1000
If	125	62
IIa	62	31
IIb	1000	1000
IIc	500	500
IIe	1000	1000
Ethacridine	500	2000

for 1 h and cooled. The precipitate was separated by filtration, washed with ether, and crystallized from dioxane.

6-Nitro-2-acyliminocoumarin-3-carboxylic acid amides (IIa – IIf). A solution (prepared on weak heating to 40°C) of 1 g (0.003 mole) of compound Id – If in 20 ml of acetic or propionic acid anhydride was allowed to stand at 20°C for 12 h. Then the reaction mixture was diluted with water and the precipitate was separated by filtration, washed with water, and crystallized from dioxane.

Coumarin-3-carboxylic acid anisidide (III). Method A. To a cooled solution of 0.5 g (1 mmole) of anisidide Ig [2] in 15 ml of DMF was added 15 ml of concentrated hydrochloric acid and the mixture was allowed to stand for two days at room temperature. The precipitate was separated by filtration, washed with water until neutral reaction, and crystallized from dioxane.

Method B. A mixture of 0.61 g (5 mmole) of salicylaldehyde, 1.18 g (5 mmole) of malonic acid monoethyl ether 4-anisidide (IV), 10 ml of ethanol, and 5 drops of piperidine was heated for 1 h at 70°C and cooled. The precipitate was separated by filtration, washed with ether, and crystallized from dioxane.

EXPERIMENTAL BIOLOGICAL PART

The antimicrobial properties of the compounds Ia – If and IIa – IIf were studied with respect to standard strains of *Escherichia coli* and *Staphylococcus aureus*. The bacteriostatic activity was determined by the conventional method of double serial dilutions in a beef-infusion broth [3]. A daily grown culture washed by sterile physiological solution was used to prepare the initial stock solution with a bacterial load of 500×10^6 microbial cells/ml (according to bacterial standard). Then 0.1 ml of the bacterial stock solution was introduced into 2 ml of the beef-infusion broth containing a given dilution of the test compound. The final bacterial load was 250×10^3 microbial cells/ml. The results were assessed after incubation of the test and control samples for 18 – 20 h in a thermostat at 37°C. The activity was characterized by the minimum inhibiting concentration (MIC, μg/ml) corresponding to the maximum dilution leading to complete suppression of the test microbe growth. The test compounds were dissolved in DMF; the reference drug was ethacridine.

It was established that all the synthesized compounds possess antimicrobial properties (Table 2) with respect to both *St. aureus* and *E. coli*. The most active substances (Ib, If, and IIa) significantly exceed ethacridine in the bacteriostatic effect. Thus, the results of our experiments are indicative of good prospects in the search for new antimicrobial agents in the series of substituted 6-nitrocoumarin-3-carboxylic acid amides.

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