SYNTHESIS AND BIOLOGICAL ACTIVITY OF N-ACYL-5-BROMANTHRANILIC ACIDS

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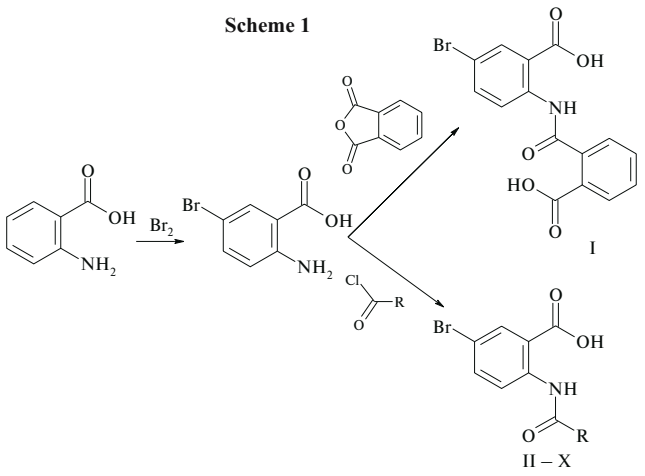
Ten N-acyl-5-bromanthranilic acids have been obtained via acylation of 5-bromanthranilic acid with appropriate anhydrides. The antiinflammatory and antibacterial properties of the synthesized compounds have been evaluated. It is established that N-(*o*-anisoyl)-5-bromanthranilic acid possesses most pronounced antiinflammatory activity (49% inhibition of carrageenan induced paw edema in rat) and antimicrobial properties (MIC = 2.0 μg/ml with respect to *St. aureus* and *E. coli*).

Halogen-substituted N-acylanthranilic acid amides exhibit a broad spectrum of biological activity, including antiviral [1, 2], antibacterial [3, 4], antifungal [5], anti-inflammatory and analgesic [3, 4, 6, 7] properties. In continuation of the search for biologically active compounds in the class of 5-bromine-substituted anthranilic acids [8 – 10], we synthesized a series of N-acyl-5-bromanthranilic acids (I – X) and studied their antiinflammatory and antimicrobial properties.

The initial 5-bromanthranilic acid was obtained by brominating anthranilic acid according to the well-known classical method [11]. The subsequent acylation of this brominated acid with phthalic anhydride in ethyl acetate at room temperature (Scheme 1) using a method described previously [12] yielded N-phthalyl-5-bromanthranilic acid (I). A series of N-acyl derivatives (II – X) were obtained via the interaction of 5-bromanthranilic acid with the corresponding acid chloroanhydrides in benzene. The reactions were carried out with heating on a water bath for 30 – 40 min [8].

The synthesized compounds appeared as white, sometimes with a yellowish tint, crystalline substances insoluble in water and soluble in organic solvents (DMSO, DMF, ethanol, acetone, dioxane). Yields, physicochemical characteristics, and parameters of the IR and ^{1}H NMR spectra of the newly synthesized N-acyl-5-bromanthranilic acids are presented in Table 1 and in the experimental chemical part below.

Scheme 1



R = *n*-Pr (II); Ph (III); C_6H_4OMe-2 (IV); $C_6H_4NO_2$-3 (V); C_6H_4Cl-4 (VI); C_6H_4Br-4 (VII); CH_2Ph (VIII); $CHPh_2$ (IX); 2-furyl (X)

EXPERIMENTAL CHEMICAL PART

The ^{1}H NMR spectra were measured on an RYa-2310 spectrometer (Russia) operating at a frequency of 60 Hz. The samples were dissolved in DMSO-d_6 and the chemical shifts were calculated relative to HMDS (internal standard). The IR spectra were recorded with a Specord M-80 spectrophotometer (Germany) using samples prepared as nujol mulls. The course of reactions was monitored and the purity of the reaction products was checked by TLC on Silufol UV-254 plates (Chemapol, Czech Republic), which were eluted in an ace-

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tone – benzene (1 : 6) mixture and developed by exposure to iodine vapor and under UV irradiation [13].

N-Phthalyl-5-bromanthranilic acid (I). To a solution of 1.08 g (0.005 mole) of 5-bromanthranilic acid in 10 ml of ethyl acetate was gradually added with stirring a solution of 0.74 g (0.005 mole) of phthalic anhydride in 10 ml of the same solvent. The mixture was stirred and kept at room temperature for 24 h. The precipitate was filtered, dried, and recrystallized from ethanol; yield of compound I, 0.90 g (49%); m.p., 194 – 196°C.

N-Phenylacetyl-5-bromanthranilic acid (VIII). To a suspension of 1.08 g (0.005 mole) of 5-bromanthranilic acid in 10 ml of benzene was added 0.93 g (0.006 mmole) of phenylacetic acid chloroanhydride and the mixture was boiled for 30 min on a water bath, after which excess benzene was distilled off. The reaction mass was cooled, poured into water (50 ml), and neutralized with sodium carbonate. The precipitate was separated by filtration, dried, and recrystallized from acetone to obtain compound VIII with a yield of 1.41 g (84%); m.p., 232 – 234°C; IR spectrum (ν_{max}, cm^{-1}): 3332 (CO<u>OH</u>), 3248 (<u>NH</u>CO), 1672 (<u>CO</u>OH, NH<u>CO</u>), 1518 (NH<u>CO</u>), 920 (CO<u>OH</u>).

TABLE 1. Yields, Physicochemical Characteristics, and Parameters of the ^{1}H NMR Spectra of N-Acyl-5-Bromanthranilic Acids (I – X)

Compound	Yield, %	M.p., °C	Empirical formula	^{1}H NMR spectrum (DMSO-d_6): δ, ppm
I	49	194 – 196	$C_{15}H_{10}BrNO_5$	7.42 – 8.75 (m, 7H, C_6H_3, C_6H_4), 11.38 (s, 1H, NH)
II	87	242 – 243	$C_{11}H_{12}BrNO_3$	0.95 (t, 3H, CH_3), 1.77 (m, 2H, CH_2), 2.55 (t, 2H, $COCH_2$), 7.65 – 8.95 (m, 3H, C_6H_3), 10.58 (s, 1H, NH), 11.78 (s, 1H, COOH)
III	46	255 – 256	$C_{14}H_{10}BrNO_3$	6.65 – 7.98 (m, 8H, C_6H_3, C_6H_5), 11.05 (s, 1H, NH)
IV	79	256 – 260	$C_{16}H_{13}BrNO_3$	4.07 (s, 3H, OCH_3), 6.80 – 8.83 (m, 7H, C_6H_3, C_6H_4), 12.27 (bs, 1H, NH)
V	92	236 – 238	$C_{14}H_9BrN_2O_5$	7.58 – 8, 85 (m, 7H, C_6H_3, C_6H_4), 10.15 (s, 1H, NH)
VI	90	252 – 253	$C_{15}H_{10}BrClNO_3$	6.88 – 9.05 (m, 7H, C_6H_3, C_6H_4), 12.22 (s, 1H, NH)
VII	71	252 – 254	$C_{15}H_{10}Br_2NO_3$	6.83 – 9.00 (m, 7H, C_6H_3, C_6H_4), 12.17 (bs, 1H, NH)
VIII	84	230 – 232	$C_{15}H_{12}BrNO_3$	3.72 (s, 2H, CH_2), 6.85 – 8.72 (m, 8H, C_6H_3, C_6H_5), 11.21 (s, 1H, NH)
IX	91	225 – 227	$C_{21}H_{16}BrNO_3$	5.12 (s, 1H, CH), 7.00 – 7.88 (m, 13H, C_6H_3, C_6H_5, C_6H_5), 11.62 (s, 1H, NH)
X	87	257 – 258	$C_{12}H_8BrNO_4$	6.62 – 8.78 (m, 6H, C_6H_3, C_4H_3O), 12.08 (s, 1H, NH)

Analogous procedures were used for the synthesis of compounds II – VII, IX, and X.

N-(3-Nitrobenzoyl)-5-bromanthranilic acid (X). IR spectrum (ν_{max}, cm^{-1}): 3140 (<u>NH</u>CO, CO<u>OH</u>), 1704 (<u>CO</u>OH), 1612 (NH<u>CO</u>), 1540 (<u>NH</u>CO), 1502, 1353, 824, 740 (NO_2), 932 (CO<u>OH</u>).

N-(2-Furanoyl)-5-bromanthranilic acid (X). IR spectrum (ν_{max}, cm^{-1}): 3180 (<u>NH</u>CO, CO<u>OH</u>), 1712 (<u>CO</u>OH), 1668 (NH<u>CO</u>), 1532 (<u>NH</u>CO), 1554, 1496 (C_4H_3O), 884 (CO<u>OH</u>).

EXPERIMENTAL BIOGICAL PART

The antiinflammatory activity of the synthesized compounds was studied on a group of white mongrel rats weighing 200 – 250 g, bearing a carrageenan-induced foot edema model [14]. The synthesized compounds (in a dose of 50 mg/kg) and the reference drug diclofenac sodium (25 mg/kg) were intraperitoneally injected (as aqueous suspensions stabilized with Tween-80) 1 h before carrageenan injections. Animals in the control group were treated with an equal volume of Tween-80 solution. The extent of edemation was estimated oncometrically, by measuring the inflamed foot volume immediately before and 3 and 5 h after edema induction. The effect was evaluated by a degree of inhibition

TABLE 2. Antiinflammatory Activity of N-Acyl-5-Bromanthranilic Acids (III – X)

Compound[1]	Dose, mg/kg (i.p.)	Percentage foot edema growth relative to initial volume ($M \pm m$)	
		3 h	5 h
III	50	54.2 ± 7.10**	56.0 ± 5.50**
Control	–	90.5 ± 7.80	90.4 ± 6.80
IV	50	46.1 ± 6.80**	46.0 ± 4.70**
Control	–	90.5 ± 7.80	90.4 ± 6.80
V	50	46.9 ± 6.50	48.7 ± 5.80*
Control	–	66.1 ± 6.30	69.6 ± 4.60
VI	50	69.0 ± 12.50	70.5 ± 8.90
Control	–	90.5 ± 7.80	90.4 ± 6.80
VII	50	78.2 ± 9.8	79.4 ± 7.30
Control	–	66.1 ± 6.30	69.6 ± 4.60
VIII	50	46.6 ± 5.30*	46.6 ± 5.30*
Control	–	66.1 ± 6.30	69.6 ± 4.60
IX	50	65.0 ± 9.70	64.1 ± 8.20*
Control	–	90.5 ± 7.80	90.4 ± 6.80
X	50	48.1 ± 7.70	51.9 ± 7.40
Control	–	66.1 ± 6.30	69.6 ± 4.60
Diclofenac	25	27.7 ± 5.00**	23.8 ± 6.00***
Control	–	73.0 ± 10.60	76.2 ± 6.00

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ relative to control; number of animals in experimental groups, $n = 5$.

TABLE 3. Antimicrobial Activity of N-Acyl-5-Bromanthranilic Acids (III – V, VII, VIII, X)

Compound	MIC, μg/ml	
	St. aureus	*E. Coli*
III	62	62
IV	2.0	2.0
V	250	250
VII	500	500
VIII	250	250
X	125	250
Dioxidine*	62.5 – 1000	3.9 – 62.5

* MIC variation interval [16].

of the edema growth relative to that in the untreated control group.

The antibacterial activity of the synthesized compounds was studied by the conventional method of double serial dilutions in a liquid nutrient medium [15] in comparison to the reference drug dioxidine [16]. The bacteriostatic effect 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 MIC values were determined with respect to the standard strains of *Staphylococcus aureus* (ATCC 6538-P) and *Escherichia coli* (ATCC 25922).

The experimental results were statistically processes in terms of Student's *t* criterion.

It is established that compounds III – V, VIII, and IX exhibit antiinflammatory properties, although the effect is less pronounced as compared to that of diclofenac (Table 2). Many of the synthesized compounds (III – V, VII, VIII, X) also exhibit a more or less pronounced antibacterial effect with respect to both standard strains studied. The most significant bacteriostatic effect was observed for compound IV, which was even superior to the reference compound dioxidine (Table 3).

It should be recalled that previously we demonstrated [8, 9] that N-acyl-5-bromanthranilic acid amides inhibit the carrageenan edema model development more effectively than the corresponding acids, in agreement with the established general rule [17]. The inverse trend is observed with respect to the antimicrobial action [10, 17].

Thus, the results of this investigation confirmed good prospects in the search for new antimicrobial agents among N-acyl-5-bromanthranilic acids and their derivatives.

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