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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF *N*-AROYL-SUBSTITUTED MONO(DI)HALO-ANTHRANILIC ACID AMIDES AND HYDRAZIDES

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A series of new *N*-aroyl-substituted mono(di)halo-anthranilic acid amides and hydrazides (**I-XII**) were synthesized via amidation of 6-bromo-, 6-iodo-, 6,8-dichloro-, and 6,8-dibromo-2-substituted-3,1-benzoxazin-4(3*H*)-ones with the corresponding 4-methylphenyl-, benzyl-, and butylamines and hydrazine. The anti-inflammatory activity of some synthesized compounds was evaluated. Compounds containing 4-methylphenyl, butyl, and benzyl substituents in the amide were active with the percentage inhibition of the carrageenan edema inflammatory reaction ranging from 45.8 to 76.9%. This class of compounds was found to be promising in the search for biologically active substances with anti-inflammatory activity.

Keywords: anthranilic acid, amide, amidation reaction, anti-inflammatory activity.

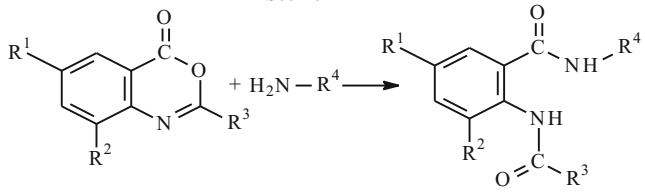
Anthranilic acid is a component part of many biologically active compounds that exhibit broad spectra of biological activity. In particular, the anthranilic acid motif is a biochemical precursor and metabolite [1] of the amino acid tryptophan and its derivatives [2]. Disrupted tryptophan metabolism may cause changes in the normal physiological condition of human and animal bodies that lead to Alzheimer's disease [3].

Anthranilic-acid derivatives form a promising class of biologically active compounds with broad spectra of biological activity such as analgesic [4], antibacterial [5, 6], and anti-inflammatory [7]. Therefore, further research on the pharmacological activity of these compounds is critical.

The present work reports the synthesis of new *N*-aroyl-substituted mono(di)halo-anthranilic-acid amides and hydrazides (**I-XII**) via amidation by the known method [8] and results from studies of the anti-inflammatory activity (AIA) of the newly obtained amides (**I-X**).

Twelve new *N*-aroyl-substituted mono(di)halo-anthranilic-acid amides and hydrazides were synthesized via reaction of 6-bromo-, 6-iodo-, 6,8-dichloro-, and 6,8-dibromo-2-substituted-3,1-benzoxazin-4(3*H*)-ones with the corresponding 4-methylphenyl-, benzyl-, and butylamines and hydrazine upon heating to 120°C in a 1:1 mixture of DMSO and HOAC (glacial) to afford *N*-aroyl-5-bromo- (5-iodo-,

Sceme 1



R^1 = Cl, R^2 = Cl, R^3 = C_6H_5, R^4 = 4-$CH_3C_6H_4$ (**I**);
R^1 = Cl, R^2 = Cl, R^3 = C_6H_5, R^4 = $CH_2C_6H_5$ (**II**);
R^1 = Br, R^2 = H, R^3 = C_6H_5, R^4 = 4-$CH_3C_6H_4$ (**III**);
R^1 = Br, R^2 = Br, R^3 = C_6H_5, R^4 = $CH_2C_6H_5$ (**IV**);
R^1 = Br, R^2 = Br, R^3 = C_6H_5, R^4 = 4-$CH_3C_6H_4$ (**V**);
R^1 = I, R^2 = H, R^3 = C_6H_5, R^4 = 4-$CH_3C_6H_4$ (**VI**);
R^1 = Cl, R^2 = Cl, R^3 = 4-$CH_3C_6H_4$, R^4 = 4-$CH_3C_6H_4$ (**VII**);
R^1 = Cl, R^2 = Cl, R^3 = 4-$CH_3C_6H_4$, R^4 = $CH_2CH_2CH_2CH_3$ (**VIII**);
R^1 = Cl, R^2 = Cl, R^3 = 4-$CH_3C_6H_4$, R^4 = $CH_2C_6H_5$ (**IX**);
R^1 = Br, R^2 = H, R^3 = 2-furyl, R^4 = 4-$CH_3C_6H_4$ (**X**);
R^1 = Br, R^2 = Br, R^3 = C_6H_5, R^4 = NH_2 (**XI**);
R^1 = Cl, R^2 = Cl, R^3 = 4-$CH_3C_6H_4$, R^4 = NH_2 (**XII**).

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3,5-dibromo-, 3,5-dichloro) anthranilic-acid amides and hydrazides (**I-XII**) [8] (Scheme 1).

EXPERIMENTAL CHEMICAL PART

IR spectra of the compounds were recorded from KBr pellets on a Specord M-80 spectrophotometer. PMR spectra were recorded in DMSO-d_6 with TMS internal standard on a Bruker 300 (300 MHz operating frequency) spectrometer. Elemental analyses were performed on an Elmer 2400 apparatus and agreed with those calculated. Mass spectra were taken in ESI^+ mode using ultra-HPLC-MS on a Waters ACQUITY UPLC I-Class system equipped with an Acquity UPLC BEH C_{18} column (1.7 μm) using a mobile phase of MeCN–H_2O at flow rate 0.6 mL/min and a Xevo TQD mass detector and an ACQUITY UPLC PDA el Detector UV detector.

***N*-Benzoyl-3,5-dichloroanthranilic acid 4-methylphenylamide (I) (general method).** A mixture of 6,8-dichloro-2-phenyl-3,1-benzoxazin-4(3*H*)-one (0.002 mol) and *p*-toluidine (0.003 mol) was treated with DMSO (4 mL) and HOAc (glacial, 4 mL). The mixture was heated to 120°C and cooled to room temperature. The resulting precipitate was filtered off, dried, and recrystallized from EtOAc–EtOH (1:1) [8]. Yield: 0.60 g (76%). White crystals, mp 244 – 246°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3232, 3104 (N-H), 1644, 1624 (C=O). PMR spectrum (δ, ppm): 2.18 (s, 3H, CH_3), 6.94 – 7.79 (m, 11H, $C_6H_2Cl_2$, C_6H_5, C_6H_4), 10.09 (s, 1H, CONH), 10.11 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 398.90 ($M + H^+$). $C_{21}H_{16}Cl_2N_2O_2$.

Compounds **II-XII** were prepared analogously to **I**.

***N*-Benzoyl-3,5-dichloroanthranilic acid benzylamide (II).** Yield: 0.68 g (86%). White crystals, mp 212 – 214°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3272, 3104 (N-H), 1752, 1704, 1656 (C=O). PMR spectrum (δ, ppm): 4.29 (d, 2H, CH_2, J 4.7 Hz), 7.10 – 7.89 (m, 12H, $C_6H_2Cl_2$, $2C_6H_5$), 8.79 (s, 1H, CONH), 10.03 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 398.85 ($M + H^+$). $C_{21}H_{16}Cl_2N_2O_2$.

***N*-Benzoyl-5-bromoanthranilic acid 4-methylphenylamide (III).** Yield: 0.71 g (87%). White crystals, mp 236 – 238°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3344, 3268 (N-H), 1660, 1632 (C=O). PMR spectrum (δ, ppm): 2.35 (s, 3H, CH_3), 7.10 – 8.40 (m, 12H, C_6H_3Br, C_6H_5, C_6H_4), 8.49 (s, 1H, CONH), 11.50 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 410.9 ($M + H^+$). $C_{21}H_{17}BrN_2O_2$.

***N*-Benzoyl-3,5-dibromoanthranilic acid benzylamide (IV).** Yield: 0.59 g (61%). White crystals, mp 218 – 220°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3344, 3264 (N-H), 1640, 1626 (C=O). PMR spectrum (δ, ppm): 4.30 (d, 2H, CH_2, J 4.7 Hz), 7.10 – 8.00 (m, 12H, $C_6H_2Br_2$, $2C_6H_5$), 8.77 (c, 1H, CONH), 10.03 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 488.75 ($M + H^+$). $C_{21}H_{16}Br_2N_2O_2$.

***N*-Benzoyl-3,5-dibromoanthranilic acid 4-methylphenylamide (V).** Yield: 0.84 g (87%). White crystals, mp 239 – 241°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3304, 3232 (N-H), 1648, 1628 (C=O). PMR spectrum (δ, ppm): 2.24 (s, 3H, CH_3), 6.90 – 7.82 (m, 11H, $C_6H_2Br_2$, C_6H_5, C_6H_4), 7.90 (c, 1H, CONH), 9.90 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 488.78 ($M + H^+$). $C_{21}H_{16}Br_2N_2O_2$.

***N*-Benzoyl-5-iodoanthranilic acid 4-methylphenylamide (VI).** Yield: 0.72 g (80%). White crystals, mp 236 – 238°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3330 (N-H), 1670, 1630, 1615 (C=O). PMR spectrum (δ, ppm): 2.35 (s, 3H, CH_3), 7.10 – 8.30 (m, 12H, C_6H_3I, C_6H_5, C_6H_4), 8.40 (s, 1H, CONH), 11.50 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 456.86 ($M + H^+$). $C_{21}H_{17}IN_2O_2$.

***N*-(4′-Methylbenzoyl)-3,5-dichloroanthranilic acid 4’-methylphenylamide (VII).** Yield: 0.66 g (81%). White crystals, mp 234 – 236°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3320, 3232 (N-H), 1648, 1628 (C=O). PMR spectrum (δ, ppm): 2.21 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 6.96 – 7.79 (m, 10H, $C_6H_2Cl_2$, $2C_6H_4$), 9.97 (c, 1H, CONH), 10.13 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 412.89 ($M + H^+$). $C_{22}H_{18}Cl_2N_2O_2$.

***N*-(4′-Methylbenzoyl)-3,5-dichloroanthranilic acid butylamide (VIII).** Yield: 0.56 g (74%). White crystals, mp 208 – 210°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3280, 3088 (N-H), 1744, 1656, 1648 (C=O). PMR spectrum (δ, ppm): 0.78 (t, 3H, CH_3), 1.26 (m, 6H, $3CH_2$), 2.35 (s, 3H, CH_3), 7.19 – 8.14 (m, 7H, $C_6H_2Cl_2$, C_6H_4 + CONH), 9.90 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 378.91 ($M + H^+$). $C_{19}H_{20}Cl_2N_2O_2$.

***N*-(4′-Methylbenzoyl)-3,5-dichloroanthranilic acid benzylamide (IX).** Yield: 0.57 g (70%). White crystals, mp 234 – 236°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3344, 3280 (N-H), 1656, 1644, 1632 (C=O). PMR spectrum (δ, ppm): 2.35 (s, 3H, CH_3), 4.30 (d, 2H, CH_2, J 5.5 Hz), 7.10 – 7.78 (m, 11H, $C_6H_2Cl_2$, C_6H_4, C_6H_5), 8.75 (c, 1H, CONH), 9.95 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 412.89 ($M + H^+$). $C_{22}H_{18}Cl_2N_2O_2$.

***N*-(2′-Furanoyl)-5-bromoanthranilic acid 4″-methylphenylamide (X).** Yield: 0.52 g (90%). White crystals, mp 203 – 205°C (EtOAc — EtOH, 1:1). IR spectrum (ν, cm^{-1}): 3288 (N-H), 1712, 1652, 1628 (C=O). PMR spectrum (δ, ppm): 2.27 (s, 3H, CH_3), 6.65 – 8.42 (m, 10H, C_6H_3Br, C_6H_4, C_4H_3O), 10.41 (c, 1H, CONH), 11.58 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 398.83 ($M + H^+$). $C_{19}H_{15}BrN_2O_3$.

***N*-Benzoyl-3,5-dibromoanthranilic acid hydrazide (XI).** Yield: 0.64 g (84%). White crystals, mp 232 – 234°C (EtOH). IR spectrum (ν, cm^{-1}): 3240 (N-H), 1700, 1640 (C=O). PMR spectrum (δ, ppm): 5.74 (d, 2H, NH_2, J 4.5 Hz), 7.40 – 7.87 (m, 7H, $C_6H_2Br_2$, C_6H_5), 8.25 (c, 1H, CONH), 8.37 (c, 1H, NHCO). Mass spectrum (ESI^+): *m*/*z* 413.67 ($M + H^+$). $C_{14}H_{11}Br_2N_3O_2$.

TABLE 1. Experimental Results from AIA Studies of Substituted *N*-Aroyl-5-bromo- (5-iodo-, 3,5-dibromo-, 3,5-dichloro)-anthranilic Acid Amides **I-X**

Compound	Increase of paw volume after 3 h, %	Inhibition of reaction, %
I	36.10 ± 5.60*	45.40
II	15.27 ± 4.65*	76.90
III	42.20 ± 9.30*	36.20
IV	36.80 ± 4.20*	45.80
V	34.80 ± 8.80*	47.30
VI	37.30 ± 8.10*	43.50
VII	37.20 ± 5.60*	43.70
VIII	17.98 ± 4.71*	72.80
IX	17.89 ± 5.03*	72.90
X	19.08 ± 7.09*	71.10
Nimesulide	33.90 ± 6.80 *	48.70
Control	66.10 ± 6.70	

* Difference statistically significant vs. the control for $p < 0.05$.

***N*-(4′-Methylbenzoyl)-3,5-dichloroanthranilic acid hydrazide (XII).** Yield: 0.45 g (74%). White crystals, mp 184 – 186°C (EtOH). IR spectrum (ν, cm^{-1}): 3355 (N-H), 1695, 1640 (C=O). PMR spectrum (δ, ppm): 2.41 (s, 3H, CH_3), 5.74 (d, 2H, NH_2, J 4.5 Hz), 7.27 – 8.14 (m, 7H, $C_6H_2Cl_2$, C_6H_4 + CONH), 8.24 (c, 1H, NHCO). Mass spectrum (ESI^+): *m/z* 337.86 ($M + H^+$). $C_{15}H_{13}Cl_2N_3O_2$.

EXPERIMENTAL BIOLOGICAL PART

Ten substituted *N*-aroyl-5-bromo- (5-iodo-, 3,5-dibromo-, 3,5-dichloro-) anthranilic acid amides (**I-X**) were studied.

All experimental manipulations with laboratory animals were conducted in compliance with generally accepted ethical standards for handling animals with observance of Good Laboratory Practice Rules for preclinical studies effective in the Russian Federation [9].

AIA was determined by evaluation the volume increase of inflamed rat paws after injection of the phlogogen carrageenan [10].

The studies used outbred mature rats of both sexes (180 – 250 g, a group included six animals) and the acute inflammatory edema model induced by subplantar injection into a rat hind paw of an aqueous carrageenan solution (0.1 mL, 1%). The volume increase of the foot indicating development of edema was estimated by oncometry before the injection and 3 h after injection of carrageenan. Tested compounds were injected intraperitoneally at a dose of 50 mg/kg 0.5 h before injection of the phlogogen.

The control group of animals received an equivalent volume of starch paste. The reference drug was nimesulide.

Statistical processing used the Student method. Inhibition of inflammation in percent vs. the control level was determined using the results. The presence of AIA was evaluated from the extent of inhibition of an inflammatory reaction. If that parameter was >30%, the result was considered positive.

RESULTS AND DISCUSSION

Compounds **I-XII** (Scheme 1) were white crystalline compounds. Derivatives **I-IX** were soluble in DMF; with heating in DMSO, and insoluble in H_2O, EtOH, Me_2CO, HOAc, toluene, and benzene. Compounds **X-XII** were soluble with heating in EtOH and Me_2CO.

IR spectra of **I-XII** exhibited absorption bands in the range 3355 – 3088 cm^{-1} for N–H stretching vibrations of acyl NH (NHCO) and amide groups (CONH). A band due to combined vibrations of carbonyls (C=O) was shifted to low-frequency at 1752 – 1615 cm^{-1}.

PMR spectra showed a group of resonances characteristic of benzene-ring protons that were recorded in the range 6.65 – 8.42 ppm. The amide resonance was observed at 7.90 – 10.41 ppm. A singlet for the acyl NH was recorded at 8.24 – 11.58 ppm.

TABLE 1 presents the results from the study of the AIA of the synthesized compounds.

The feet of the animals increased considerably by the third hour of observation after injection of the phlogogen. The growth reached 66.10%, which was taken as the control value. The reference drug nimesulide (Nimesil, Labaratorios Menarini S. A., Spain), which is successfully used in medical practice as an anti-inflammatory agent, showed statistically significant reduction of carrageenan edema by 33.90% with an analogous injection at the same dose. Preventive injection of the tested compounds inhibited the inflammatory reaction as compared to the control. The strength of the inhibition of edema during 3 h increased if radicals such as 4-methylphenyl (**I**, **III**, **V**, **VI**, **VII**, **X**) were introduced into the amide and reached from 36.20 to 71.10% as compared to the other amides of this series. Compounds containing butyl (**VIII**) and benzyl substituents (**II**, **IV**, **IX**) in the amide were also active. The AIA values fell in the range from 45.8 to 76.9%.

Four compounds with activities >70% were found as a result of the AIA study. They were *N*-benzoyl-3,5-dichloroanthranilic acid benzylamide (**II**), *N*-(4′-methylbenzoyl)-3,5-dichloroanthranilic acid butylamide (**VIII**), *N*-(4′-methylbenzoyl)-3,5-dichloroanthranilic acid benzylamide (**IX**), and *N*-(2′-furanoyl)-5-bromoanthranilic acid 4′′-methylphenylamide (**X**).

Thus, promising compounds with pronounced AIA were identified in the search for biologically active compounds in the series of anthranilic acid derivatives.

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