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SYNTHESIS, STRUCTURE AND RESEARCH OF THE PHARMACOLOGICAL ACTIVITY OF METHYL ESTERS OF 6-NITRO-N-PHENILANTHRANILIC ACIDS

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Key words: synthesis; methyl esters of N-phenylanthranilic acids; pharmacological activity

Analysis of scientific and patent literature shows the promising results of searching biologically active compounds among derivatives of aromatic aminoacids. For many years at the Medical Chemistry department of the National University of Pharmacy the research has been conducted in the field of development of synthetic methods and study of physico-chemical and pharmacological properties of aromatic acids, in particular, N-phenylanthranilic acids and products of their transformation in order to search active and harmless medicines. The synthesis of methyl esters of 6-nitro-N-phenylanthranilic acids has been carried out by Fisher esterification in the absolute methanol medium in the presence of concentrated sulfuric acid. Substituted 6-nitro-N-phenylanthranilic acids have been obtained by Ullmann reaction by the interaction of 6-nitro-2-chlorobenzoic acids with arylamines and by arylation of 6-nitro-N-phenylanthranilic acids by halogenobenzenes derivatives in the medium of n-amylalcohol, in the medium of dimethylformamide, without a solvent in the presence of copper or CuO. The structure of the compounds has been confirmed by elemental analysis, IR- and NMR-spectroscopy. The purity has been controlled by the method of thin-layer chromatography in methanol-hexane (1:1.5) and ethylacetate-methanol-ammonia (8.5:1:0.5). The computer prognosis of possible types of the biological activity of 9 methyl esters of 6-nitro-N-phenylanthranilic acids synthesized for the first time has been conducted with the help of PASS programme. It has been found experimentally that the substances synthesized possess the anti-inflammatory, analgesic, diuretic, bacteriostatic, fungistatic and antidiuretic activities. According to the classification by K.K.Sydorov the compounds synthesized when introduced intragastrically belong to low toxic compounds (DL_{50}=1200-2500 mg/kg). Some regularities of the "structure – biological activity – toxicity" relationship have been determined.

Derivatives of N-phenylanthranilic acids (N-PAA) are used in medicine as nonsteroidal anti-inflammatory drugs. Some of them have proven to be the most effective by the nonspecific action, and do not cause degradation of glycosaminoglycans and collagen in joints [7, 14, 15]. Long-term studies in the field of the series of N-arylanthranilic acids and their derivatives by researchers have led to the creation of effective medicines (mefenamic acid and its alkali salt, antral, diphtorant), which are widely applied in medical practice as anti-inflammatory, hepatoprotective and antipsoriatic medicines [7, 8]. Data of the research of domestic and foreign scientists indicate that derivatives of N-phenylanthranilic acids have a wide synthetic and pharmacological potental [2-4, 6, 8, 10-15]. These circumstances determined to synthesize methyl esters 6-nitro-N-phenylanthranilic acids and study their biological activity.

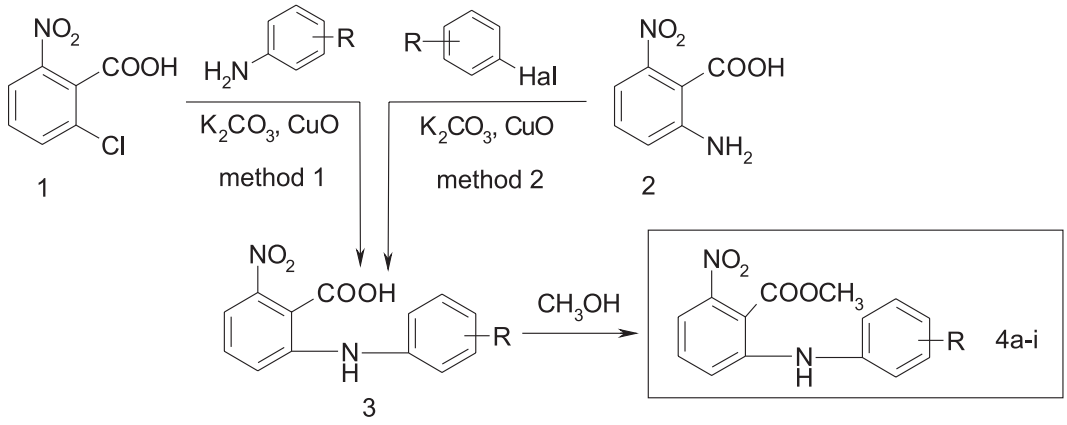
Substituted 6-nitro-N-PAA (3) have been obtained by Ullmann reaction by the interaction of 6-nitro-2-chlorobenzoic acid (1) with arylamines (Method 1) and by arylation of 6-nitro-N-phenylanthranilic acids (2) by halogenobenzenes derivatives (Method 2) in the medium of n-amylalcohol, in the medium of dimethylformamide, without a solvent in the presence of copper or copper (II) oxide [6, 8] (Scheme).

The synthesis of methyl esters of 6-nitro-N-PAA (4a-i) has been carried out by Fisher esterification in the medium of the absolute methanol in the presence of the concentrated sulfuric acid (Scheme).

The structure and identity of methyl esters of 6-nitro-N-PAA (4a-i) have been confirmed by elemental, IR- and NMR-spectroscopy, chromatographic analysis and qualitative reactions (Tab. 1, 2).

In the NMR-spectra of esters the signals of aromatic protons in the range of 6.59-7.55 ppm have been identified. The proton signal of the secondary amino-group appears as a broad singlet in the region of 7.44-7.64 ppm. The characteristic signal of methyl esters is the signal of $COOCH_3$-group, which is registered in the range of 3.95-4.15 ppm (Tab. 2).

IR-spectra of methyl esters of 6-nitro-N-PAA are characterized by a number of intense bands, which correspond to the main structural fragments of molecules of the substances synthesized. In the region of 1728-1698 cm^{-1} an intense band corresponding to stretching vibrations of the ester group carbonyl has been interpreted ($\nu_{C=O}$). In the regions of 1282-1270 cm^{-1} and 1155-1145 cm^{-1} there are stretching vibration bands of C-O-C- (ν^{acid}_{C-O}, $\nu^{alcohol}_{C-O}$), respectively. The first band refers to the stretching vibration of C-O-C – group, to which the main contribution is made by fluctuations of the acidic fragment of the molecule, the main contribution to the second band vibrations is made by the alcoholic fragment of the molecule. Symmetric and asymmetric vibrations of the nit-



Scheme

Table 1

Physical and chemical properties of methyl esters of 6-nitro-N-phenylanthranilic acids

Compound	R	Yield, %	M.p.°C[1]	Found, %			Empirical formula	Calculated, %			Rf[2]	
				C	N	H		C	N	H	1	2
4a	H	81	158-161	61.74	10.32	4.38	$C_{14}H_{12}N_2O_4$	61.76	10.29	4.44	0.62	0.68
4b	2′-CH_3	88	152-155	62.91	9.83	5.01	$C_{15}H_{14}N_2O_4$	62.93	9.79	4.93	0.51	0.60
4c	4′-CH_3	89	167-169	62.87	9.75	4.88	$C_{15}H_{14}N_2O_4$	62.93	9.79	4.93	0.48	0.59
4d	3′,4′-$(CH_3)_2$	85	182-184	63.92	9.37	5.33	$C_{16}H_{16}N_2O_4$	62.99	9.33	5.37	0.44	0.50
4e	4′-OCH_3	84	105-107	59.63	9.34	4.61	$C_{15}H_{14}N_2O_5$	59.60	9.27	4.67	0.45	0.49
4f	4′-OC_2H_5	85	152-154	60.76	8.91	5.05	$C_{16}H_{16}N_2O_5$	60.75	8.86	5.10	0.42	-
4g	4′-OC_3H_7	77	157-159	61.78	8.54	5.47	$C_{17}H_{18}N_2O_5$	61.81	8.48	5.49	0.40	-
4h	4′-Cl	79	162-164	54.88	9.15	3.61	$C_{14}H_{11}ClN_2O_4$	54.83	9.13	3.65	0.38	0.43
4i	4′-Br	80	170-173	47.85	8.02	3.12	$C_{14}H_{11}BrN_2O_4$	47.28	7.98	3.16	0.33	0.39

Notes: [1] Compounds 4a-i were recrystallized from methanol. [2] Rf values are given in the solvent systems: 1 – methanol-hexane (1:1,5); 2 – ethylacetate-methanol-ammonia (8,5:1:0,5).

Table 2

Data of IR- and NMR-spectra of methyl esters of 6-nitro-N-phenylanthranilic acids

Compound	IR-spectra, absorption maxima, ν (cm^{-1})								NMR-spectra, chemical shifts, δ (ppm)				
	ν_{NH}	$\nu^{acid}_{C=O}$	$\nu_{C=O}$	ν^{acid}_{C-O}	$\nu^{alcohol}_{C-O}$	$\nu^{as}_{NO_2}$ / $\nu^{s}_{NO_2}$	ν_{C-Ph}	δ_{NH}	NH (1H, s)	CH_3 (3H, s)	CH_2 (2H, k)	$COOCH_3$ (3H, s)	Ar H
4a	3340	1685	1705	1280	1152	1536 / 1350	1598	1572	7.62	–	–	4.01	6.88-7.46 (8H, m)
4b	3328	1670	1698	1275	1145	1533 / 1340	1600	1576	7.44	2.08	–	4.05	6.86-7.38 (7H, m)
4c	3338	1675	1720	1272	1148	1537 / 1352	1602	1578	7.55	2.30	–	3.98	6.92-7.45 (7H, m)
4d	3334	1668	1712	1270	1145	1535 / 1348	1596	1570	7.64	2.15 2.25	–	4.15	6.59-7.55 (6H, m)
4e	3344	1682	1722	1278	1156	1540 / 1357	1602	1578	7.56	3.65	–	4.03	6.80-7.40 (7H, m)
4f	3346	1684	1725	1282	1164	1528 / 1352	1604	1582	7.57	1.38	3.44	4.02	6.88-7.44 (7H, m)
4g	3345	1686	1728	1280	1166	1530 / 1354	1605	1584	–	–	–	–	–
4h	3322	1678	1714	1278	1158	1538 / 1332	1600	1575	–	–	–	–	–
4i	3326	1676	1716	1274	1160	1534 / 1328	1598	1576	7.62	–	–	3.95	6.75-7.41 (7H, m)

Table 3

Pharmacological activities of methyl esters of 6-nitro-N-phenylanthranilic acids

Compound	Anti-inflammatory, % in the dose of 20 mg/kg	Analgesic, % in the dose of 20 mg/kg	Diuretic, % in the dose of 50 mg/kg (control group 100%)	Bacteriostatic, MIC, μg/ml				Fungistatic, MIC, μg/ml		DL_{50}, mg/kg intragastrically (in mice)
				Staphyloco-ccus aureus ATCC 25923	*Bacillus subtilis*, ATCC 6639	*Escherichia coli*, ATCC 25922	*Pseudomonas aeuruginosa*, ATCC 97853	*Candida albicans*, ATCC 653/885	*Candida tropicalis* Y 2473	
4a	9.8	15.3	108	125	125	125	250	125	500	–
4b	9.2	9.3	82	125	250	125	250	250	125	1200
4c	0	29.4	112	125	250	250	125	125	125	1500
4d	19.5	29.2	140	62.5	125	62.5	250	250	125	1800
4e	14.3	0	72	125	250	125	125	125	125	–
4f	0	15.2	152	125	500	125	125	125	500	–
4g	0	0	201	125	250	125	250	250	250	2000
4h	31.4	33.8	184	31.2	250	31.2	125	125	125	2500
4i	32.3	34.3	162	31.2	250	31.2	125	125	125	2500
Diclofenac (DE_{50}=8 mg/kg)	37.5	–	–	–	–	–	–	–	–	360
Mefenamic acid in the dose of 100 mg/kg	30	–	–	–	–	–	–	–	–	628
Metamizole sodium (DE_{50}=55 mg/kg)	–	52.0	–	–	–	–	–	–	–	1197
Hydrochlorothiazide in the dose of 50 mg/kg	–	–	212	–	–	–	–	–	–	320
Ethacridine	–	–	–	31.2	15.6	31.2	62.5	–	–	–
Phthalylsulfathiazole	–	–	–	7.8	7.8	250	–	–	–	–
Nitrofural	–	–	–	–	–	–	–	64	64	–

rogroup in the spectrograms appear in the regions of 1540-1528 cm^{-1} ($\nu^{as}_{NO_2}$) and 1357-1328 cm^{-1} ($\nu^{s}_{NO_2}$). Deformation vibrations of NH-group (δ_{NH}) are presented in the spectrograms by peak in the region of 1584-1570 cm^{-1} (Tab. 2).

Using the PASS programme the analysis of the computer prognosis results shows that 6-nitro-N-PAA (4a-i) methyl esters synthesized for the first time are most likely to reveal the anti-inflammatory, antitumor, diuretic, bacteriostatic, fungistatic activity.

According to Sydorov K.K. classification, methyl esters belong to the class of low toxic substances, their DL_{50} with the internal administration in mice is within the range of 1200-2500 mg/kg (Tab. 3). As expected, methyl esters are more toxic than initial acids [6].

Among the esters of 6-nitro-N-PAA (Tab. 3) the strongest diuretic activity is revealed by compound (4g), but it is inferior to hypothiazide. It has been found that the carboxyl group esterification in 6-nitro-N-PAA (4a-i) leads to decrease of the diuretic, analgesic, anti-inflammatory effect and increase of acute toxicity. Compounds (4b, 4e) possess the antidiuretic activity, but they are inferior to adiurecrine (65%). The pharmacological screening on the anti-inflammatory and analgesic activity (Tab. 3) in the dose of 20 mg/kg has revealed the substances (4h, 4i) with the anti-exudative effect at the level of mefenamic acid (30%). It should be noted that methyl esters of 6-nitro-N-PAA (4a-i) are less active than initial acids (3). The anti-inflammatory activity of N-PAA esters and their derivatives (4a-i) is shown to be in close connection with their structure; by the anti-exudative action they can be ranged in the following way: D-glucosylammonium salts o0f N-PAA > acids > methyl esters. The microbiological research shows that the substances synthesized inhibit the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeuruginosa* in the concentrations of 31.2-500 μg/ml (Tab. 3). Esters (4h, 4i), which contain covalently bound chlorine and bromine in their structure in the neoanthranilic fragment of the molecule, reveal the most expressed bacteriostatic activity. These substances inhibit *Staphylococcus aureus* and *Escherichia coli* more selectively (MIC=31.2 μg/ml). The fungistatic activity of methyl esters of 6-nitro-N-PAA (4a-i) is 31.2-500 μg/ml in relation to *Candida albicans* and *Candida tropicalis*. Compounds (4h, 4i) exceed twice the effect of nitrofural by their fungistatic activity (Tab. 3) and are less toxic.

Experimental Part

IR-spectra of the substances synthesized were measured on a "Specord M-80" spectrophotometer in KBr tab-

lets (with the concentration of 1%). The chromatographic analysis was performed by the method of thin-layer chromatography on "Silufol UV-254" plates produced by "Avalier" firm (Czech Republic); chromatograms were developed by iodine vapours or using UV-radiation. The NMR-spectra were registered on a "Varian M-200" spectrophotometer with the operation frequency of 200 MHz, DMSO-d_6 as a solvent and TMS as an internal standard were used.

Methyl ester of 6-nitro-N-phenylanthranilic acid (4a). Heat the mixture of 2.58 g (0.01 mol) of 6-nitro-N-phenylanthranilic acid, 0.75 ml of the concentrated sulfuric acid in 30 ml of absolute methanol with a reflux condencer for 5 hours. After cooling pour the reaction mixture into water and neutralize by sodium hydrocarbonate. Filter the precipitate and dry. The product's yield was 2.20 g (81%). Compounds 4b-i were obtained similarly.

To reveal the anti-inflammatory activity of new compounds their ability to inhibit edema development in acute inflammation caused by carrageenin subplantary injection in the mouse paw was researched [5]. The compounds examined were taken orally as a suspension stabilized by emulsifier Tween-80 in the dose of 20 mg/kg of the animal's body mass. The analgesic action of substances (4a-i) was studied in white rats (weighing 160-200 g) using the acetic acid-induced writhing test in the dose of 20 mg/kg [5]. The diuretic effect of N-PAA esters (4a-i) was studied by Berkhin Ye.B. method [1]. The substances under research and the reference medicine (hydrochlorothiazide) were injected intraperitoneally 30 min before the water load in the dose of 50 mg/kg (Tab. 3). The study of the bacteriostatic and fungistatic activity of substances (4a-i) *in vitro* was performed by the two-fold serial dilution method in the liquid nutrient medium.

Acute toxicity of the substances synthesized was studied in white mice when introduced intragastrically [5].

The PASS programme was used for computer prognosis of the biochemical activity of 6-nitro-N-PAA methyl esters [10].

CONCLUSIONS

1. To search biologically active substances the synthesis of 6-nitro-N-phenylanthranilic acids methyl esters has been done, and their structure and purity has been studied.

2. Using the PASS programme computer prognosis of possible types of the biological activity has been conducted. It has been found experimentally that the substances synthesized possess a moderate anti-inflammatory, analgesic, diuretic, antidiuretic, bacteriostatic and fungistatic antidiuretic activities. Some regularities of the "structure – biological activity – toxicity" relationship among the N-phenylanthranilic acid derivatives have been determined.

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СИНТЕЗ, БУДОВА ТА ДОСЛІДЖЕННЯ ФАРМАКОЛОГІЧНОЇ АКТИВНОСТІ МЕТИЛОВИХ ЕСТЕРІВ 6-НІТРО-N-ФЕНІЛАНТРАНІЛОВИХ КИСЛОТ

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Ключові слова: *синтез; метилові естери 6-нітро-N-фенілантранілових кислот; фармакологічна активність*

Похідні N-фенілантранілових кислот широко використовуються в медичній практиці. Продовжуючи пошук нових біологічно активних сполук серед похідних антранілової кислоти, ми провели роботу щодо розробки методів синтезу та експериментальних досліджень метилових естерів 6-нітро-N-фенілантранілових кислот для вивчення їх біологічної активності. Синтез метилових естерів 6-нітро-N-фенілантранілових кислот був здійснений на кафедрі медичної хімії НФаУ. Будову 9 синтезованих сполук підтверджено даними елементного аналізу, ІЧ-, ПМР-спектрів. Чистоту контролювали методом тонкошарової хроматографії. Біологічний скринінг нових сполук проведений на кафедрі мікробіології, вірусології та імунології НФаУ. Встановлено, що синтезовані речовини проявляють протизапальну, аналгетичну, діуретичну, антидіуретичну, бактеріостатичну, фунгістатичну активність. За класифікацією К.К.Сидорова синтезовані естери при внутрішньошлунковому введенні відносяться до класу малотоксичних сполук (DL_{50}=1200-2500 мг/кг). Встановлені деякі закономірності зв'язку «структура – біологічна активність – токсичність».

СИНТЕЗ, СТРОЕНИЕ И ИССЛЕДОВАНИЕ ФАРМАКОЛОГИЧЕСКОЙ АКТИВНОСТИ МЕТИЛОВЫХ ЭСТЕРОВ 6-НИТРО-N-ФЕНИЛАНТРАНИЛОВЫХ КИСЛОТ

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Ключевые слова: *синтез; метиловые эстеры 6-нитро-N-фенилантраниловых кислот; фармакологическая активность*

Производные N-фенилантраниловых кислот широко используются в медицинской практике. Продолжая поиск новых биологически активных веществ среди производных антраниловой кислоты, мы провели работу по разработке методов синтеза и экспериментального исследования метиловых эфиров 6-нитро-N-фенилантраниловых кислот. Синтез метиловых эфиров 6-нитро-N-фенилантраниловых кислот был осуществлен на кафедре медицинской химии НФаУ. Строение 9 синтезированных веществ подтверждено данными элементного анализа, ИК-, ПМР-спектров. Чистоту контролировали методом тонкослойной хроматографии. Биологический скрининг новых соединений проведен на кафедре микробиологии, вирусологии и иммунологии НФаУ. Установлено, что синтезированные вещества проявляют противовоспалительную, анальгетическую, диуретическую, антидиуретическую, бактериостатическую и фунгистатическую активность. По классификации К.К.Сидорова синтезированные эстеры при внутрижелудочном введении относятся к классу малотоксичных соединений (DL_{50}=1200-2500 мг/кг). Выявлены некоторые закономерности связи «структура – биологическая активность – токсичность».