Directed synthesis and immunoactive properties of (2-hydroxyethyl)ammonium salts of 1-R-indol-3-ylsulfanyl(sulfonyl)alkanecarboxylic acids

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A general method for the synthesis of 1-alkyl(allyl)(benzyl)-substituted (indol-3-yl)-sulfanylalkanecarboxylic acids and hexane-1,6-diyl(1,4-phenylenemethylene)bisindol-3-yl-sulfanylalkanecarboxylic acids from the corresponding N-substituted indoles and bisindoles, thiourea, iodine, and halogencarboxylic acids was developed. The oxidation of substituted (indol-3-yl)sulfanylalkanecarboxylic acids for the first time afforded their analogs containing the sulfonyl group. New (2-hydroxyethyl)ammonium salts of 1-R-indol-3-ylsulfanyl(sulfonyl)-alkanecarboxylic acids, which are structural analogs of highly active immunomodulators of indacetamin and VILIM, were synthesized. Among the studied (2-hydroxyethyl)ammonium salts of 1-R-indol-3-ylsulfanylacetic and -sulfonylalkanecarboxylic acids, the compounds exhibiting high dose-dependent antiproliferative activity by the ability to affect the spontaneous and mitogen-stimulated splenocyte proliferation of mice *in vitro* were found.

Key words: indole, indolylsulfanylalkanecarboxylic acids, indolylsulfonylalkanecarboxylic acids, triethanolamine, dimethylethanolamine, methylethanolamine, immunomodulators, antiproliferative activity.

Tris(2-hydroxyethyl)ammonium salts of aroxyacetic acids, whose cation has the compact tricyclic atrane (2,8,9-trihydroprotatrane) structure favoring the penetration of a substance through cell membranes, are a new class of pharmacologically active substances.[1] They possess[2—12] anti-aggregation, membrane-stabilizing, antioxidant, antisclerotic, antiphlogistic, cardiotropic, analgesic, hypocholesterolemic, immunomodulating, and antitumor activity considerably exceeding or different from the effect of the initial biologically active acids and triethanolamine.

Earlier synthesized analogs of these compounds, *viz.*, alkanolammonium salts of (het)arylsulfanyl-(sulfonyl)acetic acids $(Het)ArS(SO_2)CH_2COO^- \cdot N^+R^1R^2(CH_2CH_2OH)_n$ (R^1, R^2 = H, Alk, CH_2CH_2OH; n = 1—3),[13—15] and their complexes with salts of biomicroelements[16,17] are lowly toxic (LD_{50} = = 1300—6000 mg kg^{-1}), also exhibit high and diverse biological activity, such as hemo- and immunotropic, cardiotropic, antiphlogistic, antithrombotic, antioxidant, adaptogenic, hypocholesterolemic, *etc.*, and are highly efficient growth-stimulating preparations for biotechnological processes.[18,19] Biological activity of alkanolammonium salts of sulfur-containing acids is not inferior and often exceeds the activity of related alkanolammonium salts of aroxyacetic acids. The activity is enhanced with an increase in the oxidation state of the sulfur atom and depends on the structure of the (2-hydroxyethyl)ammonium fragment.[14,15]

Indole derivatives,[20] in particular, (indol-3-yl)sulfanylalkanecarboxylic acids and their salts, are worthy of special attention as biologically active substances,[21] intermediates for the synthesis of medical preparations[22—24] and technically valuable products.[25]

We have earlier found by screening efficient non-toxic (LD_{50} = 1300—3000 mg kg^{-1}) immunoactive compounds, *viz.*, tris(2-hydroxyethyl)ammonium indol-3-yl- and 1-benzylindol-3-ylsulfanyl acetates (indacetamin and VILIM, respectively).

Indacetamin has a wide range of biological activity and is an efficient anti-aggregant, stabilizer of cell membranes of erythrocytes and thrombocytes, and protector during ultrasonication and γ-irradiation.[26,27]

A distinctive feature of indacetamin[28,29] and VILIM[30—32] is the pronounced antiproliferative activity in the culture

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in vitro and immunosuppressive properties *in vivo* at relatively low toxicity.

Indacetamin possesses pronounced erythro- and immunopoiesismodulating properties by the reduction of the proliferative-differentiating parameters of haemopoiesis precursors of erythropoiesis (inhibits the production of antiphlogistic cytokines IL-1 β and FNOα, decreases the number of early erythroid precursors, and stimulates the number of precursors of the granuloid-macrophagal series) in mice with different clinical variants of immunopathology, such as immunodeficiency, anaemia, and immunocomplex glomerulonephritis; eliminates anaemia; and prevents leucocyte decreasing in blood. In the experimental model of the autoimmune disease (immunocomplex glomerulonephritis), it exhibits a pronounced clinical effect (eliminates anaemia, increases the weight of an animal body, and decreases erythrocyte sedimentation rate (ESR) and proteinuria), which is due to the suppression of antiphlogistic cytokine production and a decrease in the erythropoietic hyperplasia from the early stages of erythron differentiation in sick mice. According to the data of morphological studied, the compound retards the development of mesangial proliferative glomerulonephritis.

VILIM in the culture *in vitro* suppresses the mitogen-stimulated proliferation of spleen cells in mice and lymphocytes of human blood, as well as the testosterone-stimulated proliferation of haematogenic stem cells in mice marrow.[32]

VILIM is an immunosuppressive drug with the antitumor effect. It was demonstrated by comparing this compound with the known immunosuppressive agent cyclophosphane that thymus atrophy observed in animals is not related to apoptosis but to a decrease in the proliferative activity of thymus cells ("arrest" in the phase $G_0 + G_1$).[30] VILIM was shown to possess the properties of suppressing the Th2-dependent activation of B-cells (inhibition of antibody formation, production of IgE); *i.e.*, it has antiallergic properties.

The immunosuppressive agent VILIM is characterized by a poor ability to cumulation, does not oppress haemopoiesis, and exhibits no side nephro- and hepatotoxic properties of the known immunosuppressive drugs cyclosporin A and azathioprine.[32]

It should be mentioned that indacetamin[28,29] and VILIM[30—32] can selectively affect the ratio of the function of T- or B-system of immunity inducing the deviation of the immune response in certain (Th1 or Th2) required direction.

In development of these studies with the purpose of preparing new promising immunosuppressive agents, analogs of indacetamin and VILIM, we synthesized earlier unknown 1-R-indol-3-ylsulfanyl(sulfonyl)alkanecarboxylic acids and their (2-hydroxyethyl)ammonium salts and estimated the immunomodulating activity of the latter.

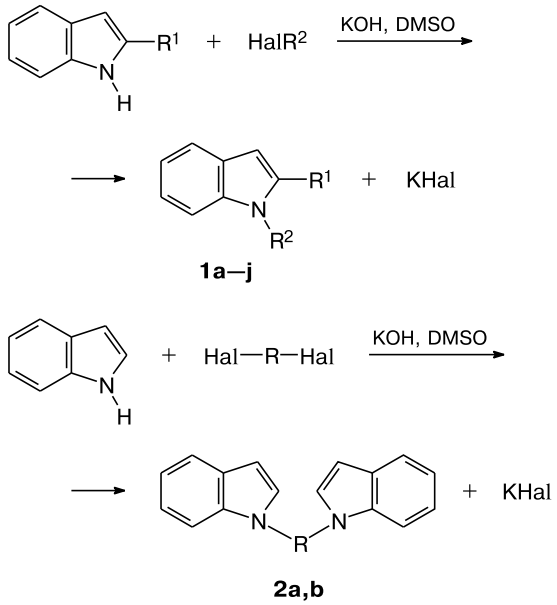
Results and Discussion

We have previously[33] developed the method for the synthesis of indol-3-ylsulfanylalkane carboxylic acids from 1*H*-, 1-methyl(benzyl)-, and 2-methylindole, thiourea, iodine, and halogencarboxylic acids; their water-soluble tris(2-hydroxyethyl)ammonium salts were also synthesized.

At the same time, the possibility of using this method for the synthesis of 1-aryl-, 1-alkyl, and 1-alkenyl-substituted indolylsulfanylalkanecarboxylic acids was not studied. The corresponding oxidized derivatives, *viz.*, indol-3-ylsulfonylalkanecarboxylic acids, and their alkanolammonium salts were unknown as well.

The starting substances for the synthesis of 1-R-indol-3-ylsulfanylalkanecarboxylic acids were N-substituted indoles **1** and **2** prepared from indole and alkyl, nitrophenyl, benzyl, or allyl halides using the known procedure,[34] which was developed for the synthesis of 1-methylindole and improved by us.[35] We succeeded to decrease the amounts of the solvent (by 2—3 times) and alkali (KOH) (by 1.5 used) by using more available NaOH as a base, and the yield of N-substituted indoles was increased by 10—15% (Scheme 1).

Scheme 1



Hal is halogen

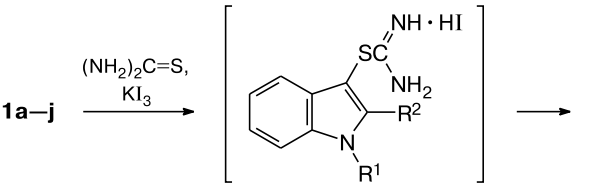
1	R^1	R^2	**1**	R^1	R^2
a	H	Me	**f**	H	4-$NO_2C_6H_4$
b	H	Et	**g**	Me	Me
c	H	Bu	**h**	Me	Et
d	H	All	**i**	Me	All
e	H	Bn	**j**	Me	Bn

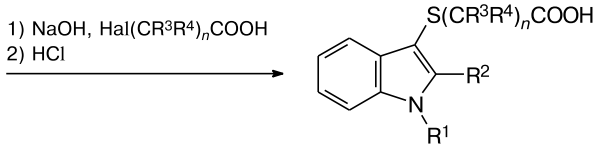
2: R = $CH_2(CH_2)_4CH_2$ (**a**), 4-$CH_2C_6H_4CH_2$ (**b**)

The constants of obtained substituted indoles **1a—h** and **2a,b** correspond to the literature data.[35] The structure of 1-allyl- and 1-benzyl-2-methylindoles **1i,j** was proved by spectral methods and confirmed by elemental analysis data.

It was shown that the reactions of indoles **1a—j** and bisindoles **2a,b** with thiourea and potassium triiodide in the ratios 1 : 2 : 1 and 1 : 4 : 2, respectively, in an inert medium afforded indolylisothiuronium iodide, whose subsequent treatment (without their preliminary isolation) with monohalogencarboxylic acids in the presence of a base resulted in indolylsulfanylalkanecarboxylic acids **3a—q** and **4a,b**, respectively, in high and stable yields (except for acids **3h,l**) (Schemes 2 and 3).

Scheme 2





3a—q

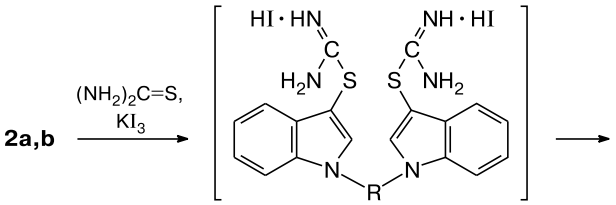
3	R^1	R^2	R^3	R^4
a	H	H	H	H
b	H	H	H	Me
c	Me	H	H	H
d	Et	H	H	H
e	Bu	H	H	H
f	All	H	H	H
g	Bn	H	H	H
h	4-$NO_2C_6H_4$	H	H	H
i	Bn	H	H	H
j	Bn	H	H	Me
k	Bn	H	H	Et
l	Bn	H	Me	Me
m	Me	Me	H	H
n	Et	Me	H	H
o	All	Me	H	H
p	All	Me	H	Et
q	Bn	Me	H	H

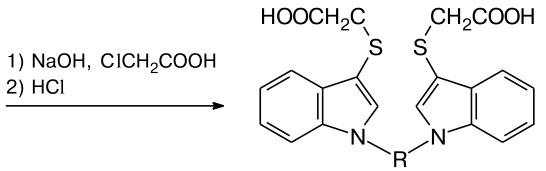
Hal = Cl, Br; n = 1 (**3a—h,j—q**), 2 (**3i**)

An advantage of the proposed method for the synthesis of N-substituted (indol-3-yl)sulfanylalkanecarboxylic acids is that isothiuronium salts of indoles formed *in situ* from available raw materials are used instead of unstable or presently non-obtained N-substituted indolethiols.

We found that the introduction of hydrazine hydrate in an amount equimolar to that of indole into the reaction

Scheme 3





4a,b

4: R = $CH_2(CH_2)_4CH_2$ (**a**), $CH_2C_6H_4CH_2$ (**b**)

process at the stage of interaction of isothiuronium salts with halogencarboxylic acids made it possible to increase the yield and purity of the final products, most likely, due to the exclusion of side processes of oxidation of indolethiol and isothiuronium salts.

The reaction can be carried out in methanol, ethanol, or propan-2-ol. The purity of the acids that were obtained by the developed method and were not subjected to additional purification was determined by the potentiometric titration of a methanol solution of the acid with a solution of sodium methylate as 94—99%.

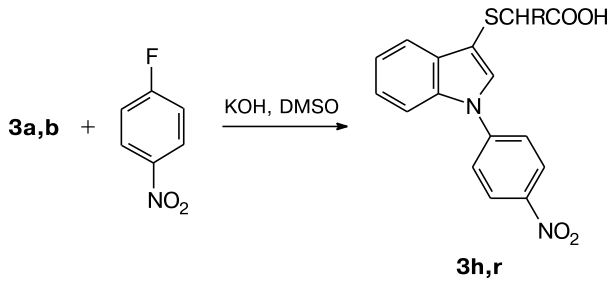
The yield of 1-(4-nitrophenyl)indol-3-ylsulfanylacetic acid (**3h**) prepared by this method did not exceed 8% even after the temperature of the process was increased to 50—80 °C and the duration was elongated to 18 h at the stage of isothiuronium salt formation. No formation of by-products was observed, and only unreacted 1-(4-nitrophenyl)indole (**1f**) was quantitatively isolated from the reaction mixture. Its low reactivity in this reaction can be explained by the decreased nucleophilicity and also by the poor solubility in alcohols even on heating and at large dilution.

N-(4-Nitrophenyl)-substituted acid **3h** and 1-(4-nitrophenylindol-3-yl-1-methylacetic acid **3r** were obtained in a yield higher than 70% by the arylation of indole-3-sulfanylalkanecarboxylic acids **3a,b** with 4-nitrofluorobenzene (Scheme 4).

In spite of the possible occurrence of side processes of esterification and decarboxylation of indolylsulfanylalkanecarboxylic acids, the approach proposed for the synthesis of acids **3h,r** (see Scheme 4) is very promising for the preparation of various 1-substituted (indol-3-yl)sulfanylalkanecarboxylic acids if the corresponding 1-unsubstituted indole derivatives are available.

The structures of earlier undescribed compounds **3c—f,h—r** and **4a,b** were determined by IR spectroscopy,

Scheme 4



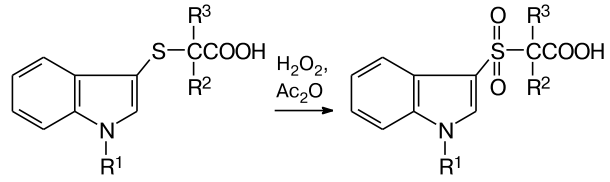
3: R = H (**h**), Me (**r**)

^{1}H NMR spectroscopy, and potentiometric titration, and the compositions were confirmed by elemental analysis data (Tables 1 and 2). The IR spectra of the synthesized acids contain absorption spectra of the heterocycle and intense absorption bands of the carboxyl groups (see Table 2).

The ^{1}H NMR spectra of acids **3** and **4** contain signals of protons of the indole fragment and substituents in positions 1—3 of indole with the characteristic multiplicity (see Table 2). It should be mentioned that the ^{1}H NMR spectra of compounds **3a**—**l**,**r** and **4a**,**b** exhibit the singlet signal of the proton in position 2 of the indole cycle, which is not observed in the spectra of acids **3m**—**q**.

The oxidation of N-substituted indolylsulfanylalkanecarboxylic acids **3g**,**h**,**j**—**l**,**r** gave earlier unknown indolylsulfonylalkanecarboxylic acids **5a**—**f** in 88—92% yields (Scheme 5). The reactions were carried out by the treatment with 50% hydrogen peroxide in acetic anhydride. The oxidation with commercial 30% H_2O_2 in acetic acid at 20 °C also affords indolylsulfonylalkanecarboxylic acids but with a lower yield (70%).

Scheme 5



3g,h,j—l,r **5a—f**

5	R^1	R^2	R^3	**5**	R^1	R^2	R^3
a	Bn	H	H	**d**	Bn	Me	Me
b	Bn	H	Me	**e**	4-$NO_2C_6H_4$	H	H
c	Bn	H	Et	**f**	4-$NO_2C_6H_4$	H	Me

The yields, physicochemical and spectral characteristics, and elemental analysis data for compounds **5a—f** are presented in Tables 3 and 4.

An attempt to perform the chemiselective oxidation of NH- and *N*-alkylindolylsulfanylalkanecarboxylic acids under the studied conditions was unsuccessful.

Table 1. Yields, physicochemical characteristics, and elemental analysis data for compounds **3c—f,h—r** and **4a,b**

Compound	Yield (%)	M.p. /°C	Found / Calculated (%) C	H	N	S	Empirical formula
3c	89	98—100	59.78 / 59.71	4.99 / 5.01	6.37 / 6.33	14.36 / 14.49	$C_{11}H_{11}NO_2S$
3d	85	102—105	61.30 / 61.25	5.52 / 5.57	5.99 / 5.95	13.66 / 13.63	$C_{12}H_{13}NO_2S$
3e	87	59—62	63.81 / 63.85	6.55 / 6.51	5.28 / 5.32	12.20 / 12.15	$C_{14}H_{17}NO_2S$
3f	83	Oil	63.20 / 63.13	5.31 / 5.30	5.69 / 5.66	12.94 / 12.97	$C_{13}H_{13}NO_2S$
3h	8[a] (74[b])	128—131	58.62 / 58.53	3.70 / 3.68	8.57 / 8.53	9.70 / 9.77	$C_{16}H_{12}N_2O_4S$
3i	53	84—86	69.49 / 69.43	5.54 / 5.50	4.44 / 4.50	10.08 / 10.30	$C_{18}H_{17}NO_2S$
3j	86	95—97	69.45 / 69.43	5.53 / 5.50	4.52 / 4.50	10.34 / 10.30	$C_{18}H_{17}NO_2S$
3k	85	119—121	70.11 / 70.12	5.85 / 5.88	4.28 / 4.30	9.83 / 9.85	$C_{19}H_{19}NO_2S$
3l	46	100—105	70.15 / 70.12	5.91 / 5.88	4.29 / 4.30	9.87 / 9.85	$C_{19}H_{19}NO_2S$
3m	90	109—112	61.27 / 61.25	5.60 / 5.57	5.61 / 5.95	13.65 / 13.63	$C_{12}H_{13}NO_2S$
3n	88	98—101	62.60 / 62.62	6.04 / 6.06	5.56 / 5.62	12.73 / 12.86	$C_{13}H_{15}NO_2S$
3o	70	Масло	64.30 / 64.34	5.82 / 5.79	5.39 / 5.36	12.30 / 12.27	$C_{14}H_{15}NO_2S$
3p	68	Масло	66.34 / 66.41	6.66 / 6.62	4.88 / 4.84	11.16 / 11.08	$C_{16}H_{19}NO_2S$
3q	90	105—108	69.55 / 69.43	5.54 / 5.50	4.55 / 4.50	10.32 / 10.30	$C_{18}H_{17}NO_2S$
3r	70[c]	109—111	59.50 / 59.64	4.11 / 4.12	8.21 / 8.18	9.30 / 9.37	$C_{17}H_{14}N_2O_4S$
4a	89	171—174	62.73 / 62.88	5.65 / 5.68	5.69 / 5.64	12.77 / 12.91	$C_{26}H_{28}N_2O_4S_2$
4b	91	120—123	65.16 / 65.09	4.70 / 4.68	5.48 / 5.42	12.36 / 12.41	$C_{28}H_{24}N_2O_4S_2$

[a] From 1-(4-nitrophenyl)indole.
[b] Synthesis by the N-arylation of indol-3-ylsulfanylacetic acid.
[c] Synthesis by the N-arylation of indol-3-ylsulfanylmethylacetic acid.

Alkanolammonium salts of indolylsulfanyl(sulfonyl)-alkanecarboxylic acids **6a—h** were obtained in yields higher than 80% on heating for 5—15 min of equimolar amounts of acids **3a,g** and **5a,b** with *N*,*N*-dimethylethanolamine, *N*-methyldiethanolamine, and triethanolamine in alcohol media (Scheme 6).

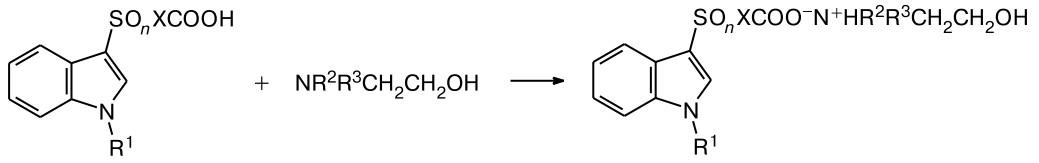
Synthesized compounds **6a—h** are hygroscopic solid or viscous odorless substances, whose color ranges from white to pink; they are highly soluble in water, alcohol, and DMSO, restrictedly soluble in acetone, and insoluble in diethyl ether.

The structures of the synthesized compounds were proved by IR spectroscopy and ^{1}H NMR spectroscopy, and the compositions were confirmed by the elemental

Table 2. ^{1}H NMR and IR spectra of compounds **3c—f,h—r** and **4a,b**

Compound	Solvent	^{1}H NMR, δ (*J*/Hz)	IR, ν/cm^{-1}
3c	$(CD_3)_2CO$	3.39 (s, 2 H, CH_2S); 3.72 (s, 3 H, NMe); 7.15—7.27 (m, 2 H, H(5), H(6)); 7.42—7.44 (m, 1 H, H(7)); 7.45 (s, 1 H, H(2)); 7.75 (m, 1 H, H(4))	1700, 1510, 1460, 1420
3d	$CDCl_3$	1.41 (t, 3 H, Me, *J* = 7.2); 3.39 (s, 2 H, CH_2S); 4.10 (q, 2 H, $NC\underline{H}_2Me$, *J* = 7.2); 7.17—7.23 (m, 2 H, H(5), H(6)); 7.30 (s, 1 H, H(2)); 7.32 (m, 1 H, H(7)); 7.74 (m, 1 H, H(4))	1690, 1500, 1460
3e	$(CD_3)_2CO$	0.87 (t, 3 H, Me, *J* = 7.4); 1.28 (d, 2 H, CH_2, *J* = 7.4); 1.75 (dd, 2 H, CH_2, *J* = 7.4, *J* = 7.1); 3.39 (s, 2 H, CH_2S); 4.15 (t, 2 H, CH_2N, *J* = 7.1); 7.10—7.43 (m, 3 H, H(5), H(6), H(7)); 7.44 (s, 1 H, H(2)); 7.70 (m, 1 H, H(4))	1700, 1500, 1460
3f	$CDCl_3$	3.31 (s, 2 H, CH_2S); 4.48 (d, 2 H, CH_2N, *J* = 9.2); 4.93 (d, 1 H, $=CH_2$, *J* = 17.2); 5.05 (d, 1 H, $=CH_2$, *J* = 9.9); 5.79 (ddd, 1 H, =CH, *J* = 9.2, *J* = 9.9, *J* = 17.2); 7.09—7.21 (m, 4 H, H(6), H(5), H(7), H(2)); 7.68 (m, 1 H, H(4))	1700, 1500, 1440
3h	$(CD_3)_2SO$	3.59 (s, 2 H, CH_2S); 7.24—7.32 (m, 2 H, H(6), H(5)); 7.69—7.72 (m, 2 H, H(7), H(4)); 7.87 (m, 2 H, $H(2)_{Ar}$, $H(6)_{Ar}$); 7.96 (s, 1 H, H(2)); 8.36 (m, 2 H, $H(3)_{Ar}$, $H(5)_{Ar}$)	1725, 1591, 1522, 1345, 1331
3i	$CDCl_3$	2.55 (t, 2 H, CH_2, *J* = 7.0); 2.89 (t, 2 H, CH_2S, *J* = 7.0); 5.26 (s, 2 H, $C\underline{H}_2Ar$); 7.08—7.34 (m, 8 H, H_{Ar}, H_{Het}); 7.27 (s, 1 H, $H(2)_{Het}$); 7.75 (m, 1 H, $H(4)_{Het}$)	1697, 1510, 1453
3j	$CDCl_3$	1.36 (d, 3 H, Me, *J* = 7.2); 3.50 (q, 1 H, CHS, *J* = 7.2); 5.19 (s, 2 H, $C\underline{H}_2Ar$); 7.01—7.33 (m, 8 H, H_{Ar}, H_{Het}); 7.24 (s, 1 H, $H(2)_{Het}$); 7.75 (m, 1 H, $H(4)_{Het}$)	1704, 1517, 1454
3k	$CDCl_3$	1.02 (t, 3 H, Me, *J* = 7.2); 1.78 (dd, 2 H, CH_2, *J* = 7.2, *J* = 8.8); 3.28 (t, 1 H, CH, *J* = 7.8); 5.22 (s, 2 H, $C\underline{H}_2Ar$); 7.03—7.34 (m, 8 H, H_{Ar}, H_{Het}); 7.25 (s, 1 H, $H(2)_{Het}$); 7.76 (m, 1 H, $H(4)_{Het}$)	1706, 1700, 1504, 1459
3l	$CDCl_3$	1.44 (s, 6 H, 2 Me); 5.21 (s, 2 H, $C\underline{H}_2Ar$); 7.01—7.39 (m, 8 H, H_{Ar}, H_{Het}); 7.24 (s, 1 H, $H(2)_{Het}$); 7.74 (m, 1 H, $H(4)_{Het}$)	1690, 1685, 1510, 1460
3m	$CDCl_3$	2.51 (s, 3 H, Me); 3.30 (s, 2 H, CH_2S); 3.67 (s, 3 H, NMe); 7.12—7.27 (m, 3 H, H(5), H(6), H(7)); 7.67 (m, 1 H, H(4))	1700, 1500, 1440
3n	$CDCl_3$	1.30 (t, 3 H, Me, *J* = 7.2); 2.49 (s, 3 H, Me); 3.29 (s, 2 H, CH_2S); 4.10 (q, 2 H, $C\underline{H}_2N$, *J* = 7.2); 7.11—7.28 (m, 3 H, H(5), H(6), H(7)); 7.67 (m, 1 H, H(4))	1700, 1680, 1510, 1450
3o	$CDCl_3$	2.46 (s, 3 H, Me); 3.30 (s, 2 H, CH_2S); 4.67 (d, 2 H, CH_2N, *J* = 2.4); 4.74 (d, 1 H, $=CH_2$, *J* = 17.1); 5.08 (d, 1 H, $=CH_2$, *J* = 10.4); 5.87 (ddd, 1 H, =CH, *J* = 2.4, *J* = 10.4, *J* = 17.1); 7.13—7.22 (m, 3 H, H(6), H(5), H(7)); 7.68 (m, 1 H, H(4))	1700, 1680, 1510, 1450
3p	$CDCl_3$	1.03 (t, 3 H, Me, *J* = 7.2); 1.78 (dd, 2 H, CH_2, *J* = 7.2, *J* = 8.8); 2.45 (s, 3 H, Me); 3.22 (d, 1 H, CHS, *J* = 7.1); 4.66 (d, 2 H, CH_2N, *J* = 2.2); 4.72 (d, 1 H, $=CH_2$, *J* = 13.2); 5.07 (d, 1 H, $=CH_2$, *J* = 10.4); 5.88 (ddd, 1 H, =CH, *J* = 2.2, *J* = 10.4, *J* = 13.2); 7.12—7.22 (m, 3 H, H(6), H(5), H(7)); 7.68 (m, 1 H, H(4))	1700, 1680, 1510, 1450
3q	$CDCl_3$	2.46 (s, 3 H, Me); 3.33 (s, 2 H, CH_2S); 5.30 (s, 2 H, CH_2N); 6.93—7.24 (m, 8 H, H_{Ar}, H_{Het}); 7.74 (m, 1 H, $H(4)_{Het}$)	1690, 1520, 1450
3r	$(CD_3)_2SO$	1.34 (d, 3 H, Me, *J* = 7.2); 3.64 (q, 1 H, CHS, *J* = 7.2); 7.27—7.35 (m, 2 H, H(5), H(6)); 7.74 (m, 2 H, H(4), H(7)); 7.95 (m, 2 H, $H(2)_{Ar}$, $H(6)_{Ar}$); 8.01 (s, 1 H, H(2)); 8.42 (m, 2 H, $H(3)_{Ar}$, $H(5)_{Ar}$)	1696, 1594, 1518, 1348, 1333
4a	$(CD_3)_2SO$	1.29 (br.s, 4 H, 2 CH_2); 1.76 (br.m, 4 H, 2 CH_2); 3.36 (s, 4 H, 2 CH_2S); 4.14 (t, 4 H, 2 CH_2N, *J* = 6.8); 7.10—7.18, 7.45—7.47 (both m, 3 H each, 2 H(6), 2 H(5), 2 H(7)); 7.49 (s, 2 H, 2 H(2)); 7.68 (m, 2 H, 2 H(4))	1700, 1500
4b	$(CD_3)_2SO$	3.38 (s, 4 H, 2 CH_2S); 5.36 (s, 4 H, 2 CH_2N); 7.11—7.13 (m, 8 H, 4 H_{Ar}, 2 H(6), 2 H(7)); 7.41 (m, 2 H, 2 H(5)); 7.62 (m, 2 H, 2 H(4)); 7.69 (s, 2 H, 2 H(2))	1695, 1530, 1450

Scheme 6



3a,g, 5a,b **6a—h**

6	R^1	R^2	R^3	X	*n*
a	H	Me	Me	CH_2	0
b	H	Me	CH_2CH_2OH	CH_2	0
c	Bn	CH_2CH_2OH	CH_2CH_2OH	CH_2	0
d	Bn	Me	CH_2CH_2OH	CH_2	0

6	R^1	R^2	R^3	X	*n*
e	Bn	Me	CH_2CH_2OH	CH_2	2
f	Bn	Me	Me	CH_2	2
g	Bn	CH_2CH_2OH	CH_2CH_2OH	CH_2	2
h	Bn	CH_2CH_2OH	CH_2CH_2OH	CHMe	2

Table 3. Yields, physicochemical characteristics, and elemental analysis data for compounds **5a—f**

Compound	Yield (%)	M.p. /°C	Found / Calculated (%) C	H	N	S	Empirical formula
5a	92	165—168	61.94 / 61.99	4.57 / 4.59	4.23 / 4.25	9.79 / 9.74	$C_{17}H_{15}NO_4S$
5b	90	184—186	62.99 / 62.96	4.98 / 4.99	4.11 / 4.08	9.37 / 9.34	$C_{18}H_{17}NO_4S$
5c	88	190—192	63.77 / 63.85	5.37 / 5.36	3.90 / 3.92	9.01 / 8.97	$C_{19}H_{19}NO_4S$
5d	88	161—163	63.81 / 63.85	5.37 / 5.36	3.95 / 3.92	8.95 / 8.97	$C_{19}H_{19}NO_4S$
5e	92	166—168	53.38 / 53.33	3.38 / 3.36	7.74 / 7.77	8.93 / 8.90	$C_{16}H_{12}N_2O_6S$
5f	91	80*	54.59 / 54.54	3.76 / 3.77	7.48 / 7.48	8.58 / 8.57	$C_{17}H_{14}N_2O_6S$

* Becomes diffuse.

analysis data (Table 5). The IR spectra of salts **6a—h** exhibits the COO^- group at 1600—1590 cm^{-1} and signals of the alkanolammonium fragments (see Table 5). In the ^1H NMR spectra of compounds **6a—h**, the signals of the indole cycles correspond to those for the initial acids.

Biological assays. Selective immunomodulators represent a new and urgent section of immunopharmacology. The effect of the existing drugs (both immunostimulators and immunosuppressive agents) is non-specific, and immunosuppressive drugs are characterized by pronounced toxicity, high risk of development side reactions, and a series of contra-indications.

Modern knowledge on pathogenesis of diseases associated with the distortion of immune processes during mismatching of the Th1/Th2 regulatory effects and the balance of the corresponding cytokines dictates the necessity to develop new immunoactive drugs for the treatment of autoimmune, immunodeficiency, lymphoproliferative, oncologic, and allergic diseases, as well as complicated diseases after transplantations of organs, marrow, *etc.*, which are related to the activation of the immunity B-system and production of various antibodies.

At present there are no drugs that are allowed for medical application and capable of selective changing the Th1/Th2 balance in a required direction.[36]

We performed screening of the immunoactive properties of (2-hydroxyethyl)ammonium salts **6a—h**, which are structural analogs of indacetamin and VILIM, but differ from them by the structure of the alkanolammonium fragment (**6c**,**g**,**h** are the triethanolamine derivatives, **6a**,**f** are the dimethylethanolamine derivatives, and **6b**,**d**,**e** are the methyldiethanolamine derivatives), which contain the sulfur atom in the di- (**6a—d**) or hexavalent (**6e—h**) states. The ability of these compounds to affect both spontaneous and mitogen-stimulated by concanavalin (Con A) in a concentration of 2 μg mL^{-1} splenocyte proliferation in mice *in vitro* (antiproliferative properties) was estimated (Table 6).

As follows from the data presented (see Table 6), the compounds manifesting the high dose-dependent activity to the spontaneous and stimulated proliferation of splenocytes were found in the series of studied (2-hydroxyethyl)ammonium salts of both 1-R-indol-3-ylsulfanylacetic and -sulfonylalkanecarboxylic acids. Compounds **6c**,**d**,**f**,**g** are most efficient (see Table 6).

Such established immunopharmacologic properties of the biologically active compounds of the new type, *viz.*, (2-hydroxyethyl)ammonium salts of indol-3-ylsulfanyl-(sulfonyl)alkanecarboxylic acids, as relative low toxicity, pronounced antiproliferative properties due to cell "arrest" in the phase of the cycle $G_0 + G_1$, the presence of the

Table 4. ^1H NMR and IR spectra of compounds **5a—f**

Compound	Solvent	^1H NMR, δ (*J*/Hz)	IR, ν/cm^{-1}
5a	$(CD_3)_2SO$	4.27 (s, 2 H, CH_2SO_2); 5.50 (s, 2 H, CH_2N); 7.19—7.28, 7.50—7.52, 7.74—7.76 (all m, 9 H, 5 H_{Ar}, H(4), H(5), H(6), H(7)); 8.27 (s, 1 H, H(2))	1705, 1510, 1460
5b	$(CD_3)_2SO$	1.42 (d, 3 H, Me, J = 6.8); 4.17 (q, 1 H, $CHSO_2$, J = 6.8); 5.57 (s, 2 H, CH_2N); 7.25—7.35 (m, 7 H, 5 H_{Ar}, H(5), H(6)); 7.56 (m, 1 H, H(4)); 7.79 (m, 1 H, H(7)); 8.31 (s, 1 H, H(2))	1704, 1517, 1454, 1310, 1136
5c	$(CD_3)_2SO$	0.89 (t, 3 H, Me, J = 7.2); 1.82—1.98 (dd, 2 H, CH_2, J = 7.4, J = 10.8); 3.98 (q, 1 H, $CHSO_2$, J = 10.8); 5.57 (s, 2 H, CH_2N); 7.26—7.57 (m, 7 H, 5 H_{Ar}, H(5), H(6)); 7.56 (m, 1 H, H(4)); 7.78 (m, 1 H, H(7)); 8.31 (s, 1 H, H(2))	1707, 1516, 1454, 1310, 1136
5d	$CDCl_3$	1.65 (s, 6 H, 2 Me); 5.37 (s, 2 H, CH_2N); 7.15—7.31 (m, 8 H, 5 H_{Ar}, H(6), H(5), H(7)); 7.74 (br.s, 1 H, H(4)); 8.01 (br.s, 1 H, H(2))	1714, 1514, 1461, 1307, 1154
5e	$(CD_3)_2SO$	4.45 (s, 2 H, CH_2SO_2); 7.42 (br.s, 2 H, H(5), H(6)); 7.73 (br.s, 1 H, H(4)); 7.99 (m, 3 H, $H(2)_{Ar}$, $H(6)_{Ar}$, H(7)); 8.45 (m, 2 H, $H(3)_{Ar}$, $H(5)_{Ar}$); 8.49 (s, 1 H, H(2))	1767, 1596, 1522, 1353, 1147
5f	$CDCl_3$	1.63 (d, 3 H, Me, J = 7.0); 4.19 (q, 1 H, $CHSO_2$, J = 7.0); 7.36—7.48 (m, 2 H, H(6), H(5)); 7.72 (m, 2 H, $H(2)_{Ar}$, $H(6)_{Ar}$); 7.98—8.02 (m, 2 H, H(7), H(4)); 8.39 (s, 1 H, H(2)); 8.41 (m, 2 H, $H(3)_{Ar}$, $H(5)_{Ar}$)	1741, 1595, 1520, 1350, 1315, 1135

Table 5. Yields, physicochemical characteristics, and elemental analysis data for compounds **6a—h**

Compound	Yield (%)	M.p. /°C	Found / Calculated (%) C	H	N	S	Empirical formula	^{1}H NMR ((CD$_3$)$_2$SO)*, δ (*J*/Hz)	IR, ν/cm^{-1}
6a	99	Oil	57.95 56.73	6.88 6.80	9.40 9.45	10.86 10.82	$C_{14}H_{20}N_2O_3S$	2.60 (s, 6 H, 2 Me); 3.03 (t, 2 H, CH_2N, *J* = 5.1); 3.70 (t, 2 H, $C\underline{H}_2OH$, *J* = 5.1)	1560, 1610
6b	92	Oil	55.39 55.19	6.72 6.79	8.55 8.58	9.87 9.82	$C_{15}H_{22}N_2O_4S$	2.63 (s, 3 H, Me); 3.02 (t, 4 H, 2 CH_2N, *J* = 5.3); 3.72 (t, 4 H, 2 $C\underline{H}_2OH$, *J* = 5.3)	1560, 1620
6c	97	92—93	61.01 61.86	6.72 6.77	6.25 6.27	7.20 7.18	$C_{23}H_{30}N_2O_5S$	2.99 (t, 6 H, 3 CH_2N, *J* = 5.3); 3.61 (t, 6 H, 3 $C\underline{H}_2OH$, *J* = 5.3)	1560, 1600, 3280
6d	72	Oil	63.30 63.44	6.72 6.78	6.59 6.73	7.76 7.70	$C_{22}H_{28}N_2O_4S$	2.64 (s, 3 H, Me); 3.03 (t, 4 H, 2 CH_2N, *J* = 5.1); 3.73 (t, 4 H, 2 $C\underline{H}_2OH$, *J* = 5.1)	1560, 1620
6e	89	138	58.35 58.91	6.22 6.29	6.20 6.25	7.10 7.15	$C_{22}H_{28}N_2O_6S$	2.65 (s, 3 H, Me); 3.05 (t, 4 H, 2 CH_2N, *J* = 5.2); 3.74 (t, 4 H, 2 $C\underline{H}_2OH$, *J* = 5.2)	1560, 1620
6f	86	Oil	60.20 60.27	6.27 6.26	6.65 6.69	7.63 7.66	$C_{21}H_{26}N_2O_5S$	2.62 (s, 6 H, 2 Me); 3.05 (t, 2 H, CH_2N, *J* = 5.1); 3.72 (t, 2 H, $C\underline{H}_2OH$, *J* = 5.1)	1560, 1610
6g	96	108—110	57.79 57.72	6.34 6.32	5.95 5.85	6.77 6.70	$C_{23}H_{30}N_2O_7S$	3.05 (t, 6 H, 3 CH_2N, *J* = 5.3); 3.63 (t, 6 H, 3 $C\underline{H}_2OH$, *J* = 5.3)	1560, 1600, 3290
6h	88	126—128	58.59 58.52	6.57 6.55	5.63 5.69	6.58 6.51	$C_{24}H_{32}N_2O_7S$	3.04 (t, 6 H, 3 CH_2N, *J* = 5.3); 3.62 (t, 6 H, 3 $C\underline{H}_2OH$, *J* = 5.3)	1560, 1600, 3285

* Only the signals of the alkanolammonium fragments are presented.

indole fragment in the structure of these compounds, and inducible activity toward a gen of cytochrome P4501A1 suggest that the immunoactive action of these compounds at the cell level follows an original mechanism involving a new molecular target: arylhydrocarboxylic receptor (AhR) for which they act as promising AhR ligands selectively modulating the immune functions.

Thus, the tests of antiproliferative activity of the new compounds indicate that the search in the series of sulfur-containing 1-R-indolylalkanecarboxylic acids for selective immunomodulators for the development of modern medical preparations is promising.

Experimental

^{1}H NMR spectra were recorded on a Bruker DPX-400 instrument (400.6 MHz). IR spectra were obtained on a Bruker IFS-25 spectrometer in KBr pellets and in microlayer. Potentio-

Table 6. Influence of compounds **6a—h** on the spontaneous and mitogen-stimulated proliferation of spleen cells of intact mice *in vitro*

Compound	Dose /μg mL^{-1}	Proliferation/pulse min^{-1} spontaneous	Con A
Reference	—	2906	44304
6a	3	3156 (+8.6%)	51247 (+15.7%)
	30	2063 (−29%)	38067 (−26%)
	300	478 (−83%)	27633 (−46%)
Reference	—	2906	44304
6b	3	5033 (+73%)	42915 (−3%)
	30	3656 (+26%)	46562 (+5%)
	300	1512 (−48%)	33936 (−23.4%)
Reference	—	179	4150
6c	3	115 (−35%)	3399 (−18.1%)
	30	48 (−73.2%)	2954 (−28.8%)
	300	19 (−89.4%)	30 (−99.3%)
Reference	—	6992	47972
6d	3	6183 (−11.6%)	48330
	30	2671 (−61.8%)	66644 (+38.9%)
	300	320 (−95.4%)	306 (−99.4%)
Reference	—	6992	47972
6e	3	7431 (+6.3%)	57307 (+19.5%)
	30	6141 (−12.2%)	56914 (+18.6%)
	300	732 (−89.5%)	38024 (−20.7%)
Reference	—	1015	20313
6f	3	472 (−53%)	19384 (−5%)
	30	349 (−66%)	13240 (−34.8%)
	300	625 (−38%)	5280 (−74%)
Reference	—	122	1652
6g	3	123	1378 (−16.6%)
	30	50 (−59%)	1160 (−29.8%)
	300	31 (−74.6%)	161 (−90.2%)
Reference	—	1015	20313
6h	3	475 (−53.2%)	21142 (+4%)
	30	368 (−63.7%)	20782 (+2%)
	300	69 (−93.2%)	10311 (−49.2%)

metric titration was carried out on an EA-74 ionometer. Melting points were determined on a Boetius heating stage (Germany). Purity of substances was monitored on a Khrom 5 chromatograph, column 2 m, Cromaton-N-AW-DMCS, 0.200—0.250 mm, impregnated with 5% Silicone, helium as carrier gas, 30 mL min^{-1}.

1-Benzyl-2-methylindole (1j). 2-Methylindole (13.18 g, 0.1 mol) was added to a solution of NaOH (12 g, 0.3 mol) in DMSO (50 mL). The mixture was stirred for 15—20 min. Then benzyl chloride (12.66 g, 0.1 mol) was slowly added dropwise on cooling to 10—15 °C. The mixture was stirred for 3 h without heating and then poured into ice-cold water (200 mL). The precipitate formed was washed with water, dried, and distilled under reduced pressure. The yield was 16.83 g (76%), b.p. 200—204 °C (15 Torr). ^{1}H NMR ($CDCl_3$), δ: 2.28 (s, 3 H, Me); 5.18 (s, 2 H, CH_2N); 6.28 (s, 1 H, H(3)); 6.86—7.18 (m, 8 H, 5 H_{Ar}, H(5), H(6), H(7)); 7.52 (m, 1 H, H(4)). Found (%): C, 86.90; H, 6.81; N, 6.35. $C_{16}H_{15}N$. Calculated (%): C, 86.84; H, 6.83; N, 6.33.

1-Allyl-2-methylindole (1i) was synthesized similarly from 2-methylindole (13.18 g, 0.1 mol) and allyl bromide (11.10 g, 0.1 mol). The yield was 12.27 g (71%), b.p. 132—135 °C (15 Torr). ^{1}H NMR ($CDCl_3$), δ: 2.31 (s, 3 H, Me); 4.56 (m, 2 H, CH_2N); 4.72—5.04 (m, 3 H, $CH{=}CH_2$); 6.25 (s, 1 H, H(3)); 7.00—7.27 (m, 8 H, 5 H_{Ar}, H(5), H(6), H(7)); 7.48 (m, 1 H, H(4)). Found (%): C, 84.23; H, 7.66; N, 8.21. $C_{12}H_{13}N$. Calculated (%): C, 84.17; H, 7.65; N, 8.18.

1-Substituted indoles **1a—h** and **2a,b** were synthesized according to the described procedure, and their constants corresponded to the literature data.[35]

Synthesis of (indol-3-yl)sulfanylalkanecarboxylic acids 3a—q and 4a,b (general procedure). A solution of iodine (25.38 g, 0.1 mol) and potassium iodide (16.60 g, 0.1 mol) in 50% ethanol (50 mL) was added to a solution of indole (0.1 mol) and thiourea (15.20 g, 0.2 mol) in ethanol (50 mL) by portions in an argon flow preventing an increase in the temperature of the reaction mixture higher than 40 °C. The reaction mixture was stored for 3 h at 30—40 °C, then hydrazine hydrate (5.00 g, 0.1 mol) was added dropwise, and a solution of NaOH (20.00 g, 0.5 mol) in water (30 mL) and monochloro(bromo)alkanecarboxylic acid (0.12 mol) in water (5 mL) were slowly added. The mixture was stored for 2 h in a boiling water bath. After the end of the reaction, the alcohol was evaporated, and the precipitate formed was dissolved on heating in water with the addition of active carbon, stored for 0.5—1 h, filtered, acidified with 10% HCl until the precipitate stopped to form (pH 1), and stored for at least 12 h at 5 °C to the complete precipitation and crystallization of the product. The precipitated acid was filtered off and dried in air.

Acids **3a,b,g** were synthesized according to the described procedure, and their constants corresponded to the literature data.[33]

[(1-Methylindol-3-yl)sulfanyl]acetic acid (3c) was synthesized from 1-methylindole (13.11 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 19.69 g.

[(1-Ethylindol-3-yl)sulfanyl]acetic acid (3d) was synthesized from 1-ethylindole (14.52 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 19.97 g.

[(1-Butylindol-3-yl)sulfanyl]acetic acid (3e) was synthesized from 1-butylindole (17.32 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 22.87 g.

{[1-(Prop-2-en-1-yl)indol-3-yl]sulfanyl}acetic acid (3f) was synthesized from 1-allylindole (15.72 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 20.50 g.

{[1-(4-Nitrophenyl)indol-3-yl]sulfanyl}acetic acid (3h). ***A.*** Acid **3h** was synthesized from 1-(4-nitrophenyl)indole (23.82 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The reaction was carried out in ethanol. A solution of KI_3 was added dropwise and the stage of isothiuronium salt formation was performed on solvent boiling. The product was isolated in a yield of 2.60 g.

B. (Indol-3-yl)sulfanylacetic acid (**3a**) (10.36 g, 0.05 mol) was added to a solution of NaOH (12 g, 0.15 mol) in DMSO (50 mL). The mixture was stirred for 15—20 min. Then 4-fluoronitrobenzene (7.06 g, 0.05 mol) was slowing added dropwise on cooling to 10—15 °C. The reaction mixture was stirred for 3 h at room temperature and then poured into ice-cold water (200 mL). The precipitate formed was filtered off, washed with water, and dried. The product was isolated in a yield of 12.15 g.

[(1-Benzylindol-3-yl)sulfanyl]propionic acid (3i) was synthesized from 1-benzylindole (20.72 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and 3-bromopropionic acid (18.36 g, 0.12 mol). The product was isolated in a yield of 16.51 g.

2-[(1-Benzylindol-3-yl)sulfanyl]propionic acid (3j) was synthesized from 1-benzylindole (20.72 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and 2-chloropropionic acid (13.02 g, 0.12 mol). The product was isolated in a yield of 26.80 g.

2-[(1-Benzylindol-3-yl)sulfanyl]butanoic acid (3k) was synthesized from 1-benzylindole (20.72 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and 2-bromobutanoic acid (20.04 g, 0.12 mol). The product was isolated in a yield of 27.66 g.

2-[(Benzylindol-3-yl)sulfanyl]-2-methylpropionic acid (3l) was synthesized from 1-benzylindole (20.72 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and 2-bromo-2-methylpropionic acid (20.04 g, 0.12 mol). The product was isolated in a yield of 14.95 g.

[(1,2-Dimethylindol-3-yl)sulfanyl]acetic acid (3m) was synthesized from 1,2-dimethylindole (14.52 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 21.16 g.

[(2-Methyl-1-ethylindol-3-yl)sulfanyl]acetic acid (3n) was synthesized from 1-ethyl-2-methylindole (15.92 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 21.88 g.

{[2-Methyl-1-(prop-2-en-1-yl]indol-3-yl)sulfanyl}acetic acid (3o) was synthesized from 1-allyl-2-methylindole (17.12 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (1.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 18.21 g.

2-{[2-Methyl-1-(prop-2-en-1-yl)indol-3-yl]sulfanyl}butanoic acid (3p) was synthesized from 1-allyl-2-methylindole (17.12 g, 0.1 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (1.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and 2-bromobutanoic acid (20.04 g, 0.12 mol). The product was isolated in a yield of 19.73 g.

[(1-Benzyl-2-methylindol-3-yl)sulfanyl]acetic acid (3q) was synthesized from 1-benzyl-2-methylindole (22.13 g, 0.1 mol). thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (1.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 28.06 g.

2-{[1-(4-Nitrophenyl)indol-3-yl]sulfanyl}propionic acid (3r) was synthesized similarly to **3h** (method ***B***) from NaOH (12 g, 0.15 mol), 2-(indol-3-yl)sulfanylpropionic acid (11.07 g, 0.05 mol), and 4-fluoronitrobenzene (7.06 g, 0.05 mol). The product was isolated in a yield of 12.01 g.

Hexane-1,6-diylbis(3-carboxymethylsulfanylindole) (4a) was synthesized from 1,1′-hexane-1,6-diylbisindole (15.82 g, 0.05 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 22.14 g.

1,4-Phenylenemethylenebis(3-carboxymethylsulfanylindole) (4b) was synthesized from 1,1′-(benzene-1,4-diyldimethane-diyl)bisindole (16.82 g, 0.05 mol), thiourea (15.22 g, 0.2 mol), iodine (25.38 g, 0.1 mol), potassium iodide (16.60 g, 0.1 mol), hydrazine hydrate (5.00 g, 0.1 mol), NaOH (20.00 g, 0.5 mol), and monochloroacetic acid (11.40 g, 0.12 mol). The product was isolated in a yield of 23.48 g.

Synthesis of 1-R-(indol-3-yl)sulfonylalkanecarboxylic acids 5a—f (general procedure). 1-R-(Indol-3-yl)sulfanylalkanecarboxylic acid (0.01 mol) was dissolved in acetic anhydride (5 mL). The reaction mixture was cooled to 0 °C, and a 50% solution of hydrogen peroxide (0.05 mol) was added dropwise. The temperature of the reaction mixture was slowly increased to 20 °C, and the mixture was stirred for 24 h. Then cold water (100 mL) was added to the solution. The precipitated target product was filtered off, washed with water, and dried in air.

[(1-Benzylindol-3-yl)sulfonyl]acetic acid (5a) was synthesized from acid **4g** (2.97 g, 0.01 mol). The product was isolated in a yield of 3.03 g.

2-[(1-Benzylindol-3-yl)sulfonyl]propionic acid (5b) was synthesized from acid **3j** (3.11 g, 0.01 mol). The product was isolated in a yield of 3.08 g.

2-[(1-Benzylindol-3-yl)sulfonyl]butanoic acid (5c) was synthesized from acid **3k** (3.25 g, 0.01 mol). The product was isolated in a yield of 4.01 g.

2-[(1-Benzylindol-3-yl)sulfonyl]-2-methylpropionic acid (5d) was synthesized from acid **3l** (3.25 g, 0.01 mol). The product was isolated in a yield of 4.05 g.

{[1-(4-Nitrophenyl)indol-3-yl]sulfonyl}acetic acid (5e) was synthesized from acid **3h** (3.28 g, 0.01 mol). The product was isolated in a yield of 3.30 g.

2-{[1-(4-Nitrophenyl)indol-3-yl]sulfonyl}propionic acid (5f) was synthesized from acid **3r** (3.42 g, 0.01 mol). The product was isolated in a yield of 3.42 g.

Synthesis of alkanolammonium salts of *N*-substituted indolylsulfanyl(sulfonyl)alkanecarboxylic acids 6a—h (general procedure). A solution of alkanolamine (0.01 mol) in ethanol (10 mL) was poured to a solution of (indol-3-yl)sulfanyl(sulfonyl)-alkanecarboxylic acid (0.01 mol) in ethanol (10 mL). The reaction mixture was heated for 10—15 min to 45—50 °C and then stored for 30 min at 20—22 °C. At the end of the reaction, ethanol was partially evaporated in air, then diethyl ether was added with vigorous stirring to the reaction mixture until the undisappeared precipitate was formed, and the mixture was stored for 24 h at 5—10 °C. The precipitate formed was filtered off, washed with diethyl ether, and dried *in vacuo* over P_2O_5.

2-Hydroxy-*N*,*N*-dimethylethaneammonium(indol-3-ylsulfanyl)acetate (6a) was synthesized from acid **3a** (2.07 g, 0.01 mol) and *N*,*N*-dimethylethanolamine (0.89 g, 0.01 mol). The product was isolated in a yield of 2.95 g.

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*-methylethaneammonium-(indol-3-ylsulfanyl)acetate (6b) was synthesized from acid **3a** (2.07 g, 0.01 mol) and *N*-methylbis(2-hydroxyethyl)amine (1.19 g, 0.01 mol). The product was isolated in a yield of 3.0 g.

2-Hydroxy-*N*,*N*-bis(2-hydroxyethyl)ethaneammonium[(1-benzylindol-3-yl)sulfanyl]acetate (6c) was synthesized from acid **3g** (2.97 g, 0.01 mol) and triethanolamine (1.49 g, 0.01 mol). The product was isolated in a yield of 4.32 g. The physicochemical properties of alkanolammonium salt **6c** correspond to the literature data.[30—32]

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*-methylethaneammonium-[(1-benzylindol-3-yl)sulfanyl]acetate (6d) was synthesized from acid **3g** (2.97 g, 0.01 mol) and *N*-methylbis(2-hydroxyethyl)-amine (1.19 g, 0.01 mol). The product was isolated in a yield of 3.01 g.

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*-methylethaneammonium-[(1-benzylindol-3-yl)sulfonyl]acetate (6e) was synthesized from acid **5a** (3.29 g, 0.01 mol) and *N*-methylbis(2-hydroxyethyl)-amine (1.19 g, 0.01 mol). The product was isolated in a yield of 3.98 g.

2-Hydroxy-*N*,*N*-dimethylethaneammonium[(1-benzylindol-3-yl)sulfonyl]acetate (6f) was synthesized from acid **5a** (3.29 g, 0.01 mol) and *N*,*N*-dimethylethanolamine (0.89 g, 0.01 mol). The product was isolated in a yield of 3.70 g.

2-Hydroxy-*N*,*N*-bis(2-hydroxyethyl)ethaneammonium[(1-benzylindol-3-yl)sulfonyl]acetate (6g) was synthesized from acid **5a** (3.29 g, 0.01 mol) and triethanolamine (1.49 g, 0.01 mol). The product was isolated in a yield of 4.59 g.

2-Hydroxy-*N*,*N*-bis(2-hydroxyethyl)ethaneammonium-2-[(1-benzylindol-3-yl)sulfonyl]propanoate (6h) was synthesized from acid **5b** (3.43 g, 0.01 mol) and triethanolamine (1.49 g, 0.01 mol). The product was isolated in a yield of 4.34 g.

Biological assays. Screening of the immunoactive properties of compounds **6a—h** was carried out in the culture *in vitro*. The ability of these compounds to affect the spontaneous and mitogen-stimulated proliferation of splenocytes of mice was estimat-

ed. Healthy pubescent mice, *viz.*, hybrids (CBAxC57BL/6)F1 (CBF1), both male and female, 8—10 weeks age, with the body weight 18—20 g, were used. The scatter in groups by the initial weight of the body did not exceed ±10%. Reference and tested animals of the same age were obtained simultaneously from one breeding nursery. Before and during experiments, the reference and tested animals were contained in a vivarium on a standard food allowance. All tests were carried out at the same time (in the morning). Tests were carried out according to the rules accepted by the European Convention for the Protection of Animals (Strasbourg, 1986) and approved by the Committee on Biomedical Ethics of the Research Institute of Clinical Immunology of the Russian Academy of Medical Sciences.

Studies of the spontaneous and Con A-induced proliferation of spleen cells. Spleen cells in mice were cultured in round-bottom trays for immunologic reaction (Linbro) at 37 °C under CO_2 (5%) and air (95%) atmosphere. The absolute number of cells introduced into a well was 200 000. The cells were stimulated by mitogens, namely, concanavalin A (Con A, Sigma). The mitogen concentration was selected by preliminary titration and used in the optimal dose, being for Con A 2 μg mL^{-1}. The compounds in three doses (3, 30, and 300 mg kg^{-1}) were introduced into wells simultaneously with mitogens. The proliferative activity of the cells was estimated by the inclusion of H3-thymidine into DNA of dividing cells. A label was introduced 16 h before the end of cultivation an amount of 1 μCi into each well of the tray. For this purpose, the basic solution of H3-thymidine was first dissolved in RPMI-1640 medium to a concentration of 100 μCi mL^{-1}, and then 10 μL of the solution was added to each well of the tray. After the end of incubation, the cells were collected on glass-fiber filters (Flow Lab) using a Harvester apparatus (Titertek). The filters were dried and placed into vials for scintillation counting; radioactivity was counted in a toluene scintillator (4 g of diphenyloxazole, 0.1 g of diphenyloxazolylbenzene per liter of toluene) in a Delta liquid scintillation counter (USA). The results were expressed in pulse min^{-1} of included thymidine per $2 \cdot 10^5$ cells. The data averaged over triplet are presented. The data were processed by the non-parametric Wilcoxon—Mann—Whitney U test.

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