cis-4-[Di-(2'-chloroethyl)amino]-L-proline Ethyl Ester Dihydrochloride (Ia). A mixture of 0.474 g (1 mmole) of Va and 0.188 g (2 mmoles) of phenol is dissolved in 2 ml of acetic acid and saturated with gaseous HBr, and the solution is left to stand for 26 h at 20-25°C. Compound Ia is precipitated from the reaction mixture by ether, converted into base (as described for Va), but using 2 ml of water and 10 ml of chloroform, and then into the dihydrochloride. The dihydrochloride is reprecipitated twice from 2 ml of absolute ethanol, and dried in vacuo over P_2O_5. The product is hygroscopic. Yield, 250 mg (71%). Found, %: C 37.1; H 6.1; Cl 38.5; Cl 19.0; N 8.6. $C_{11}H_{20}Cl_2N_2 \cdot 2HCl$. Calculated, %: C 37.1; H 6.2; Cl 39.8; Cl 19.9; N 9.0.

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SYNTHESIS OF NEW SULFANILAMIDES AND THEIR ANTIMICROBIAL PROPERTIES

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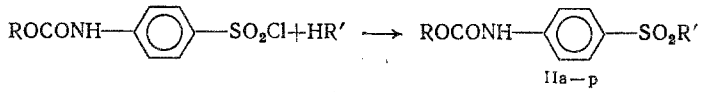
Depending on the substituents and their position in the molecule, derivatives of sulfanilic acid (I) have different biological activity: antibacterial, antiinflammatory, hypoglycemic, antitumor, antiepileptic, etc. [1-4].

Searches in the series of amino acid derivatives of I led to interesting biologically active compounds and revealed certain regularities in the dependence of their action on structure [5-9].

In continuation of studies in the field of the derivatives of I, we synthesized compounds of the general formula II-IV to determine the role of different structural components in the appearance of a biological effect, and to conduct a directed search for biologically active compounds.

Compounds II were obtained by the action of N-alkoxycarbonylsulfanilyl chlorides on the corresponding aliphatic or cyclic amines or amino esters in solvents such as ether, benzene, petroleum ether, and chloroform, in the presence of triethylamine and an excess of the amine ester, according to the method described in [10, 11].



IIa—p

a: $R = C_2H_5$, $R' = N(CH_2CH_2Cl)_2$;
b: $R = C_2H_5$, $R' = N(CH_2CH_2OH)_2$;
c: $R = CH_3$, $R' = NHCH_2CH_2OH$;
d: $R = C_2H_5$, $R' = N(CH_2)_5$;
e: $R = C_2H_5$, $R' = N(CH_2)_6$;
f: $R = C_2H_5$, $R' = N(CH_2CH_2)_2O$;
g: $R = C_2H_5$, $R' = NH(CH_2)_3N(CH_2CH_2)_2O$;
h: $R = CH_3$, $R' = NHCH(CH_3)_2$;
i: $R = C_2H_5$, $R' = NHCH(CH_3)_2$;
j: $R = iso\text{-}C_4H_9$, $R' = NHCH(CH_3)_2$;
k: $R = C_2H_5$, $R' = N[CH(CH_3)_2]_2$;
l: $R = iso\text{-}C_4H_9$, $R' = NHCH_2COOC_2H_5$;
m: $R = iso\text{-}C_4H_9$, $R' = NHCH(CH_2C_6H_5)COOC_2H_5$;
n: $R = C_2H_5$; $R' = NHCH(CH_2C_6H_5)COOC_2H_5$;
o: $R = C_2H_5$, $R' = NHCH(COOCH_3)(CH_2)_2COOCH_3$;
p: $R = C_2H_5$, $R' = NHCH(COOC_2H_5)(CH_2)_2COOC_2H_5$.

Compounds III were synthesized by the mixed anhydrides method [10]: by the action of ethyl chloroformate (V) and sulfanilamide (VI) on N-acylglycines in the presence of triethylamine in dry tetrahydrofuran (THF).

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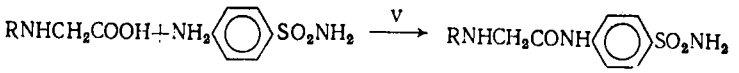
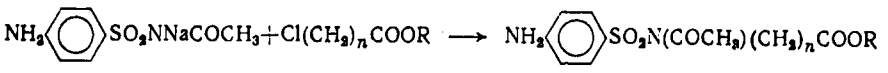


III a-e

a: R = CH_3CO;
b: R = C_6H_5CO;
c: R = $CH_3OC_6H_3CO$;
d: R = $C_2H_5OC_6H_4CO$;
e: R = $C_4H_9OC_6H_4CO$.

Compounds IV were obtained by the reaction of sulfanilacetamide sodium salt (VII) with the corresponding esters of chloroformic or haloacetic acids in an aqueous or alcoholic medium



IV a-e

a: R = CH_3, $n = 0$;
b: R = C_2H_5, $n = 0$;
c: R = H, $n = 1$;
d: R = CH_3, $n = 1$;
e: R = C_2H_5, $n = 1$.

All the compounds obtained are crystalline substances, insoluble in water and soluble in acetone.

The identity and purity of the compounds obtained were established by elemental analysis, IR spectra, and TLC. In the IR absorption spectra bands are observed in the 1170 and 1230 cm^{-1} region, corresponding to the SO_2 group; in the 3370, 1680, and 1560 cm^{-1} region, corresponding to the amide groups; in the 1540, 3300, and 3320 cm^{-1} region (NH_2 group), and at 1720 cm^{-1} (CO of the ester group).

The chemotherapeutic action of the compounds synthesized was studied. A marked activity of isopropylamides of N^4-alkoxycarbonylsulfanilic acid and esters of N^4-alkoxycarbonylsulfanilamino acids was established.

EXPERIMENTAL CHEMICAL PART

The TLC was carried out on Silufol UV-254 plates (Czechoslovakia) with propanol–water (7:3) mobile phase and detection by UV light, iodine, and ninhydrin. The IR spectra were run on the UR-20 spectrometer (GDR) in mineral oil (sodium chloride and lithium fluoride prism).

N-Isopropyl-N^4-methoxycarbonylsulfanilamide (IIh). A solution of 0.02 mole of isopropylamine in ether is added dropwise to an ether solution of 0.01 mole of N-methoxycarbonylsulfanilyl chloride, with the temperature of the reaction mixture maintained at −5 to −10°C. The mixture is left to stand overnight at room temperature. The precipitate is filtered, washed with ether (3 × 50 ml), and then with water, and recrystallized from aqueous acetone. The physicoanalytical data of II are listed in Table 1.

N^4-hippurysulfanilamide (IIIb). A 0.03 mole portion of V is added at −5 to −7°C to a solution of 0.03 mole of hippuric acid and 0.03 mole of triethylamine in 50 ml of dry THF. The mixture is stirred for 15-20 min, and then 0.03 mole of VI to 20 ml of dry THF is added dropwise. The mixture is then stirred at room temperature for 3 h, and the solvent is evaporated. To the residue, 50 ml of water are added, and the mixture is extracted by ethyl acetate (3 × 50 ml). The extract is washed with 0.1 N hydrochloric acid, a 5% solution of sodium bicarbonate, and water, and dried over sodium sulfate. After distillation of the solvent, the precipitate is recrystallized from aqueous acetone. Other compounds III were obtained similarly (Table 2).

N-Sulfanilyl-N-carbethoxymethylacetamine (IVe). A 0.01 mole portion of ethyl chloroacetate is added to room temperature, with stirring, to a solution of 0.01 mole of VII in 50 ml of alcohol or water. The reaction mixture is stirred for 4 h and left to stand overnight. The precipitate is filtered, washed with ether (3 × 50 ml), and then with water, and recrystallized from acetone. The physicoanalytical data of compounds IV are listed in Table 2.

EXPERIMENTAL BIOLOGICAL PART

The acute toxicity of the compounds obtained was studied with a single oral administration in experiments on nonpedigree white mice of both sexes, weighing 18–20 g each. The compounds are nontoxic; in a dose of 2500 mg/kg they did not induce any changes in the behavior and state of the animals; in higher doses they were not studied.

TABLE 1. N^4-Alkoxycarbonylsulfanilamides (IIa-p)

Compound	Yield, %	mp, °C	R_f	Found				Empirical formula	Calculated			
				C	H	N	S		C	H	N	S
IIa	79,1	119	0,91	42,45	5,51	7,50	9,01	$C_{13}H_{15}Cl_2N_2O_4S$	42,28	4,91	7,58	8,68
IIb	81,5	139	0,80	47,09	6,51	8,82	9,52	$C_{13}H_{20}N_2O_4S$	46,97	6,06	8,42	9,64
IIc	83,0	169	0,80	43,32	5,50	10,51	11,50	$C_{10}H_{14}N_2O_5S$	43,73	5,14	10,21	11,31
IId	87,0	148	0,89	54,19	6,20	8,68	10,01	$C_{14}H_{20}N_2O_4S$	53,82	6,45	8,96	10,26
IIe	89,3	151	0,89	55,29	6,57	8,60	10,64	$C_{15}H_{22}N_2O_4S$	55,19	6,79	8,58	9,82
IIf	83,0	170	0,86	49,03	6,41	7,51	9,81	$C_{13}H_{18}N_2O_5S$	49,67	5,77	8,92	10,19
IIg	78,0	123—25	0,35	51,90	6,04	10,94	8,20	$C_{16}H_{22}N_2O_5S$	52,16	6,02	12,40	8,70
IIh	87,0	217	0,80	48,50	6,14	10,37	11,25	$C_{11}H_{16}N_2O_4S$	48,51	5,92	10,28	11,77
IIi	89,7	194	0,97	49,90	6,10	9,35	11,02	$C_{12}H_{18}N_2O_4S$	50,30	6,30	9,70	11,10
IIj	85,5	142	0,97	53,40	6,17	8,41	10,10	$C_{14}H_{22}N_2O_4S$	53,10	7,04	8,90	10,20
IIk	88,6	226	0,94	54,45	7,63	9,01	10,12	$C_{15}H_{24}N_2O_4S$	54,85	7,36	8,52	9,76
III	78,6	105	0,98	50,05	6,23	7,95	8,08	$C_{15}H_{22}N_2O_5S$	50,28	6,14	7,82	8,92
IIm	75,7	167	0,98	59,03	6,20	6,60	6,99	$C_{22}H_{28}N_2O_5S$	58,91	6,29	6,24	7,11
IIn	68,2	114—16	0,99	50,40	6,08	6,95	7,80	$C_{17}H_{24}N_2O_5S$	49,06	5,80	6,70	7,70
IIo	65,8	200	0,95	47,36	5,70	6,95	7,61	$C_{16}H_{22}N_2O_5S$	47,76	5,47	6,90	7,96
IIp	71,3	163	0,96	48,91	6,02	6,03	6,90	$C_{18}H_{26}N_2O_5S$	50,22	6,08	6,50	7,40

TABLE 2. N^4-Acylglycylsulfanilamides (IIIa-e) and N-Sulfanilyl-N-carbalkoxyalkylacetamides (IVa-e)

Compound	Yield, %	mp, °C	R_f	Found				Empirical formula	Calculated			
				C	H	N	S		C	H	N	S
IIIa	52	228	0,81	44,58	5,11	15,70	12,00	$C_{10}H_{13}N_3O_4S$	44,27	4,83	15,48	11,81
IIIb	43	252—4	0,88	54,17	5,10	12,81	10,09	$C_{15}H_{15}N_3O_4S$	54,04	4,53	12,60	9,61
IIIc	51,7	297	0,79	52,42	4,68	11,70	8,70	$C_{18}H_{17}O_5N_3S$	52,88	4,71	11,56	8,82
IIId	48	208—10	0,89	53,91	5,31	11,45	8,93	$C_{17}H_{19}O_5N_3S$	54,09	5,07	11,19	8,49
IIIe	56	232—4	0,88	58,55	5,71	10,42	8,71	$C_{19}H_{23}O_5N_3S$	58,29	5,92	10,73	8,19
IVa	85,8	255	0,85	45,03	5,11	11,01	11,17	$C_{10}H_{12}N_2O_5S$	44,27	4,42	10,28	11,17
IVb	91,5	231	0,82	46,41	4,83	10,26	10,84	$C_{11}H_{14}N_2O_5S$	46,14	4,92	9,78	11,20
IVc	80,3	203—04	0,81	44,27	4,62	10,50	11,82	$C_{10}H_{12}N_2O_5S$	44,27	4,42	10,28	11,77
IVd	87,5	172	0,64	46,29	4,55	9,81	10,89	$C_{11}H_{14}N_2O_5S$	46,14	4,92	9,78	11,20
IVe	89,7	185	0,63	46,41	4,83	10,26	10,84	$C_{12}H_{16}N_2O_5S$	47,99	5,36	9,32	10,67

TABLE 3. Chemotherapeutic Action of Compounds II-IV *in Staphylococcus* Infection of Mice, Induced by *Staphylococcus aureus*, Strain 4-0

Compound	One-time dose per os, mg/kg	Number of animals	Survived	Died	Total lifetime of animals		
					abs., days	% of control	*P*
IIa-g*	1500, 2000	10	0	10	0/100	0	—
IIh	1500	10	5	5	50/100	50	0,001
IIi	1500	10	6	4	60/100	60	0,001
IIj	1500	10	4	6	40/100	40	0,01
IIk	1500	10	6	4	60/100	60	0,001
III	2000	10	4	6	42/100	42	0, 01
IIm	2000	10	5	5	50/100	50	0,001
IIn	2000	10	6	4	60/100	60	0,001
IIo	2000	10	4	6	40/100	40	0,01
IIp	2000	10	5	5	50/100	50	0,001
IIIa	1500	10	3	7	31/100	31	0,05
IIIб	1500	10	5	5	50/100	50	0,001
IIIc-e	1500	10	0	10	0/100	0	—
IVa-e*	1500, 2000	10	0	10	0/100	0	—
Norsulfazole	1500	30	16	14	164/300	54,7	0,001
Control		30	0	30	0/300	0	—

*Results are given on examination of each of the compounds in the group, in doses of 1500-2000 mg/kg.

Note. In numerator — number of mice in given group, in denominator — maximally possible number of mice-days during observation for 10 days.

The chemotherapeutic activity was studied on a model of a *Staphylococcus* generalized infection of white mice [12]. The compounds studied were administered orally in one single dose of 1500 and 2000 mg/kg, simultaneously with intraperitoneal infection by one lethal dose of a virulent strain of *Staphylococcus aureus*. Under similar experimental conditions we also

studied powdered norsulfazole, series No. 134079. The reliability of the differences in the rate of survival in the group of experimental and control animals was found according to an alternative form, taking into account the reaction with calculation of the χ^2 criterion. A total of 560 white mice were used in the experiments.

The results of the therapeutic action of the compounds in *Staphylococcus* infection of the white mice, induced by the 4-0 strain, are listed in Table 3. Of compounds II, the derivatives of isopropylamine, IIh-m, and amino acids, IIl-p, have therapeutic activity. They, like norsulfazole, increase the total life of the animals by 40-60%.

Sulfanilamides IIIa, b also have this activity. The introduction of an alkoxyl group into the p-position of the phenyl residue of IIIc-e leads to a loss of activity. The therapeutic action of derivatives IIh-p and IIIa, b used in the same doses (1500-2000 mg/kg) is also retained in the infection of mice by other strains of *Staphylococcus* (Smith or 91) giving up to 50% survival of the infected animals, while compounds IIa-g, IIIc-e, and IV do not have this property.

Thus, a marked chemotherapeutic activity of N-isopropyl-N^4-alkoxycarbonylsulfanilamides, esters of N^4-alkoxycarbonylsulfanilamino acids, and N^4-benzoylglycylsulfanilamide was discovered. Further studies on the series of amino acid derivatives of sulfanilic acid are promising in the search for active chemotherapeutic agents.

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