SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMINO ACID DERIVATIVES OF SULFANILIC ACID

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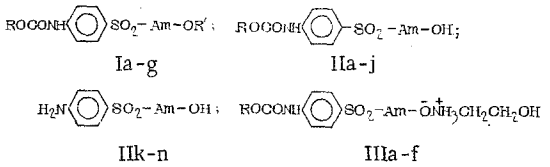
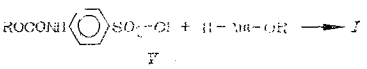
With the intention of looking for biologically active compounds in the series of amino acid analogs and of finding the relationships between chemical structure and biological activity, we have continued our earlier investigations [1, 2] by synthesizing the compounds of the general formula



Ia-g IIa-j

IIk-n IIIa-f

We prepared compounds Ia-f by the method of [2, 3], by the following scheme:



Ia:R = CH_3, Am = Gly, R′ = CH_3; Ib:R = C_2H_5, Am = Gly, R′ = C_4H_9;
Ic:R = CH_3, Am = L-Ala, R′ = CH_3; Id:R = C_2H_5, Am = *DL*-Ala, R′ = C_2H_5;
Ie:R = CH_3, Am = *D*-Val, R′ = CH_3; If:R = C_2H_5, Am = *D*-Val, R′ = C_2H_5;
Ig:R = C_2H_5, Am = *DL*-Val, R′ = C_2H_5.

Compounds IIa-*l* were prepared by interaction of N^4-alkoxycarbonylsulfanilyl chloride (IV) with the appropriate amino acid (V) in alkaline medium or in the presence of triethylamine (VI) [3]. The N^4-alkoxycarbonyl group was removed by the method of [2], by the scheme:

$$IV + H-Am-OH \xrightarrow{NaOH} IIa\text{-}i \xrightarrow{NaOH;\ HCl} IIj\text{-}l$$

IIa: R = CH_3, Am = Gly; IIb:R = CH_3, Am = *D*-Ala; IIc: R = CH_3, Am = *DL*-Ala;
IId: R = C_2H_5, Am = *DL*-Ala; IIe: R = CH_3, Am = *L*-Phe; IIf: R = C_2H_5,
Am = *D*-Val; IIg: R = C_2H_5, Am = *DL*-Leu; IIh: R = CH_3, Am = *L*-Trp;
IIi: R = CH_3, Am = *DL*-Trp; IIj: Am = *DL*-Ala; IIk:Am = *D*-Val;
II*l*: Am = *DL*-Leu.

Compounds IIa, b, c, h, and i were converted into the soluble monoethanolamine salts (IIIa-f) by reaction with monoethanolamine in absolute alcohol at room temperature.

$$II + H_2NCH_2CH_2OH \longrightarrow III\ (a\text{-}f)$$

IIIa: R = CH_3, Am = Gly; IIIb: R = CH_3, Am = Gly—Gly—Gly; IIIc: R = CH_3,
Am = *D*-Ala; IIId: R = CH_3, Am = *DL*-Ala; IIIe: R = CH_3, Am = *L*-Trp;
IIIf: R = CH_3, Am = *DL*-Trp.

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TABLE 1. Esters (I), Acids (II), and Monoethanolamine Salts (III) of Sulfanilyl Amino Acids

Compound	Yield, %	mp, °C	R_f	Found, %				Formula	Calculated, %			
				C	H	N	S		C	H	N	S
I	57,8	105	0,65	43,80	4,53	9,37	9,70	$C_{11}H_{14}N_2O_6S$	43,70	4,66	9,26	10,60
I	50,4	140	0,98	50,30	6,52	7,86	7,88	$C_{15}H_{22}N_2O_6S$	50,28	6,14	7,82	8,91
I	62,5	131	0,65	44,7	5,35	9,18	9,70	$C_{12}H_{16}N_2O_6S$	45,56	6,5	8,85	10,14
I	64,7	152—3	0,80	48,10	5,56	9,06	9,82	$C_{14}H_{20}N_2O_6S$	48,82	5,85	8,13	9,31
I	47,8	155	0,73	49,82	5,38	8,65	10,33	$C_{14}H_{20}N_2O_6S$	48,82	5,85	8,13	9,31
I	56,8	133—4	0,82	52,05	6,85	8,00	8,17	$C_{16}H_{24}N_2O_6S$	51,32	6,48	7,52	8,61
I	58,9	122—3	0,78	51,89	7,28	7,48	8,43	$C_{17}H_{26}N_2O_6S$	52,85	6,73	7,25	8,30
II	65,7	172—3	0,80	41,84	3,84	10,0	10,44	$C_{10}H_{12}N_2O_6S$	41,66	4,16	9,72	11,19
II	68,7	208—10	0,80	43,04	5,00	10,21	10,34	$C_{11}H_{14}N_2O_6S$	43,70	5,66	9,26	10,60
II	71,3	213—14	0,78	43,18	4,58	9,78	11,00	$C_{11}H_{14}N_2O_6S$	43,70	4,66	9,26	10,60
II	71,4	210—11	0,71	45,10	5,40	8,50	10,00	$C_{12}H_{16}N_2O_6S$	45,57	5,50	8,85	10,14
II	73,4	148	0,90	52,28	5,02	7,49	7,95	$C_{17}H_{17}N_2O_6S$	51,76	4,54	7,42	8,49
II	61,7	159—62	0,33	48,40	5,81	7,78	9,02	$C_{14}H_{20}N_2O_6S$	48,82	5,85	8,13	9,31
II	63,3	160	0,63	51,12	6,55	7,63	8,53	$C_{15}H_{22}N_2O_6S$	50,27	6,14	7,83	8,84
II	73,5	189	0,78	54,04	5,00	10,54	8,14	$C_{19}H_{18}N_3O_6S$	54,79	4,35	10,09	7,69
II	72,6	207	0,80	55,00	4,80	10,45	8,12	$C_{19}H_{18}N_3O_6S$	54,79	4,35	10,09	7,69
II	51,4	182—5	0,71	44,76	4,30	11,45	13,00	$C_9H_{12}N_2O_4S$	44,26	4,92	11,27	12,93
II	71,4	168—9	0,68	49,10	5,80	10,76	12,16	$C_{11}H_{16}N_2O_4S$	48,53	5,88	10,31	10,76
II	64,9	172—3	0,70	50,86	6,87	9,62	10,26	$C_{12}H_{18}N_2O_4S$	50,38	6,28	9,76	11,19
III	83,0	166—7	0,25	42,21	5,30	12,30	9,04	$C_{12}H_{19}N_3O_7S$	41,49	5,47	12,03	9,1
III	87,1	175—6	0,20	41,10	15,7	5,35	8,08	$C_{16}H_{25}N_5O_9S$	41,14	5,00	15,72	7,1
III	79,1	139	0,20	42,04	5,44	11,82	9,98	$C_{13}H_{21}N_3O_7S$	42,96	5,82	11,56	8,82
III	82,1	145	0,21	43,08	6,00	12,01	9,31	$C_{13}H_{21}N_3O_7S$	42,96	5,82	11,56	8,82
III	85,3	92	0,19	52,08	5,80	12,00	7,11	$C_{21}H_{26}N_4O_7S$	52,70	5,47	11,70	6,70
III	86,4	170	0,20	53,11	5,24	11,45	6,63	$C_{21}H_{26}N_4O_7S$	52,70	5,47	11,70	6,70

TABLE 2. Effect of D, DL-Alanine, and L, LD-Tryptophan Derivatives and Their Monoethanolamine Salts on Blood Glucose Content (M± m)

Compound	Blood glucose level, mg/100%			P
	before administration	after administration		
		100 mg/kg	250 mg/kg	
IIb	120±6,5	122±3,0	110±4,2	0,05
IIc	100±2,0	100±1,3	98±2,2	—
IIh	83±7,2	94±3,5	94±1,5	0,05
IIi	110±0,75	88±1,0	88±1,0	0,001
IIIc	75±1,4	64±2,0	62±0,7	0,001
IIId	104±1,1	94±4,0	102±1,3	—
IIIe	78±1,0	90±2,0	98±2,0	0,001
IIIf	71±1,4	95±32	104±2,5	0,001
Orabet	97±1,4	—	66±2,2	0,001

TABLE 3. Lethal and Mutagenic Effect of Sulfanilyl Derivatives of Glycine, Alanine, Valine, and Leucine (M ± m)

Compound	E. coli P=678 Thr			Actinomyces 222 lys		
	survival rate, %	encounter rate of revertants per 10^6 surviving cells		survival rate, %	encounter rate of revertants per 10^5 surviving spores	
		absolute	% of control		absolute	% of control
I	100±12	8±1,2	160	90±6,5	11±2	220
I	29±3,6	12,5±2,1	250	42±3,6	16±2,4	320
I	16±2,1	21±2,8	420	22±2,8	14±1,2	280
II	64±7,5	12±0,9	240	75±8,8	18±2,4	360
II	40±3,2	15±2,1	300	68±7,5	12±1,8	240
II	13±7	35±5,5	700	20±3,2	16±2,1	320
II	1±0,15	100±12	2000	8±0,7	42±6,2	840
II	1,2±0,1	160±20	3200	7,5±1,2	33±4,5	660
II	4,5±0,6	65±8,2	1300	11±1,5	28±3,4	560
III	75±8,2	7,5±0,85	150	100±13	8±1,2	160
III	21±2,4	22±3,2	440	26±3,4	25±4,2	5000
Urethane	89±9,5	15±2,2	300	92±8,4	9±1,4	180
Spontaneous mutation (control)	100	5±0,6	100	100	5±0,75	100

We verified the structures and compositions of the compounds from their IR and mass spectra, and elemental analyses, and their purity by TLC.

Identification was based on the presence of the characteristic absorption bands of the SO_2 group (1170 and 1230 cm^{-1}), CO ester group (1730 cm^{-1}), and free NH_2 and amide groups (1540, 3390, 3295, 3370, and 3320 cm^{-1}). The mass spectra of the compounds examined (Ia, f) contain the molecular ions and explicable fragment ions. Fragmentation involves stepwise loss of the side chain. Mass spectrum of IIa, m/e: 302 (80)$^{m+e}$, 270 (4), 243 (52), 215 (13), 214 (100), 213 (5), 150 (26), 91 (3), 77 (3), 64 (2), 59 (3), 43 (2). Mass spectrum of IIf: 372 (14)$^{m+e}$, 329 (4), 299 (100), 282 (2), 254 (8), 236 (14), 229 (14), 156 (6), 136 (14), 45 (16), 44 (17), 43 (22), 29 (16), 28 (16). The mass number appears in front of the brackets and the relative intensity inside them.

We examined the hypoglycemic, antibacterial, antitumor, and mutagenic activities of the synthetic compounds. Tests revealed that the compounds are nontoxic and that some of them have appreciable activity.

EXPERIMENTAL CHEMICAL PART

TLC was carried out on Silufol UV-254 plates (Czechoslovakia), mobile phase propanol—water (7:3), detection with UV and iodine vapor. The IR spectra were recorded on a UR-20

spectrometer (German Democratic Republic) in Vaseline oil (sodium chloride and lithium fluoride prisms). Mass spectra were recorded on an MX-1303 instrument with direct sample introduction into the ionization region at a temperature 10°C above the melting point, and energy of the ionizing electrons 40-45 eV. Compound I (Table 1) was prepared by the literature method [2, 3].

N^4-Methoxycarbonylsulfanilyl-N-glycine (IIa). A solution of glycine (0.05 mole) in 1 N sodium hydroxide (12.5 ml) was cooled to 0°C and 4 N sodium hydroxide (13.75 ml) and N^4-methoxycarbonylsulfanilyl chloride (0.05 mole) in tetrahydrofuran (30 ml) were added portionwise with stirring. Stirring was continued for another 5 h. The mixture was then extracted with ether and the aqueous layer was cautiously acidified with 0.5 N HCl with cooling. The precipitated crystalline IIa was filtered off, washed with water, and dried. The yield was 69.%, R_f 0.8, mp. 172-173°C. Found, %: C 41.84, H 3.84, N 10.00, S 10.44. $C_{10}H_{12}N_2O_6S$. Calculated, %: C 41.66, H 4.16, N 9,72, S 11.14. Compounds IIj-*l* were prepared by the method in the literature [2] (Table 1).

Monoethanolamine Salt of IIa (IIIa). Compound IIa (0.025 mole) was dissolved in absolute ethanol (50 ml) and freshly distilled monoethanolamine (0.025 mole) was added. The mixture was left at room temperature for 1-2 h. Compound III was precipitated with absolute ether, filtered off, washed with ether, and dried. The yield was 87.1%, R_f 0.25, mp 166-167°C. Found, %: C 42.21, H 5.30, N 12.30, S 9.04. $C_{12}H_{19}N_3O_7S$. Calculated, %: C 41.29, H 5.47, N 12.03, S 9.10.

The other compounds III were prepared by the same method (Table 1).

EXPERIMENTAL BIOLOGICAL PART

The hypoglycemic action was assayed with "Glyukoz" o-toluidine reagent. The preparations were administered to rats intraperitoneally in doses of 100 and 250 mg/kg. Blood samples were taken after decapitation of the test and control animals 2-2.5 h after administration [4]. The effect of each dose was examined on not less than 5 rats. The results are summarized in Table 2, which shows that compounds IIi and IIc had a hypoglycemic effect, reducing blood glucose content by 20 and 17% respectively. Compounds IIIe and IIIf had the opposite effect; they had marked hyperglycemic activity, elevating the blood glucose content by 20 and 33% respectively. Consequently, the DL-tryptophan derivative possessing hypoglycemic activity (IIi) acquires hyperglycemic activity on conversion to the monoethanolamine salt.

The antibacterial action of compounds Ib, d, f, g, and IId, g, j, and *l* was examined in Petri dishes with agar prepared on Hottinger's broth or on a synthetic medium. Testing was carried out against *Staphylococcus* and *Shigella dysenteriae*. The compounds had no marked antibacterial effect in these tests.

As the more active under *in vitro* conditions, compound Id was examined on generalized staphylococcal infection of white mice. In infection with strain 186 this compound, like Norsulfazole, which was used as control, prevented death in 40% of the animals when administered internally in a dose of 1000 or 1500 mg/kg. This compound in the same doses possessed the same activity in infection of mice with strain 4-0. In this case Norsulfazole prevented death in 70% of the mice. Consequently compound Id has fairly low chemotherapeutic activity *in vivo*.

The antitumor activity and acute toxicity of compounds IIb, c, h, and i and IIIc, d, e, and f were examined by the published method [5]. The test compounds proved to be slightly toxic or almost nontoxic. Their LD_{100} on single intraperitoneal administration to white noninbred mice varied from 1300 to 5000 mg/kg. The antitumor activity was assayed in rats with sarcoma 180 and Ehrlich ascites carcinoma (dose 1/10 of LD_{100}). Compound IIh revealed moderate antitumor activity against sarcoma 45, suppressing growth of the tumor in 42%. Compound IIIf had slightly higher growth-inhibiting action and also displayed the same activity toward sarcoma 180. None of the test compounds had any antitumor effect in the animals with Walker carcinosarcoma and Ehrlich ascites carcinoma.

The mutagenic activity was examined on the biochemical mutants, *E. coli* P = 678 and *Actinomyces rimosus* 222, on the basis of the frequency of encounter of inverse mutations from auxotrophic to prototrophic states at the loci responsible respectively for threonine and lysine synthesis [6].

The antimutagenic action of the compounds was examined on the same specimens and by the same methods. The effect of the substances was tested on spontaneous mutation in the bacteria and on UV-induced mutations [7, 8].

Our results for the mutagenic activity of the compounds, summarized in Table 3, reveal that, when tested in high equimolar concentrations — 100 mmole (*E. coli*) and 200 mmole (*Act. rimosus*) — the amino acid derivatives generally had weak mutagenic activity on prolonged treatment of the specimens (120 min), except for some compounds that, depending on their structure, had either appreciable (IIa) or fairly marked mutagenic activity (IIj, k, *l*). In the majority of cases the test substances had a slight lethal effect; *E. coli* was more sensitive to the action of the compounds. Urethane, which as the starting substance, was examined under the same conditions, had a very weak mutagenic effect. Examination of the antimutagenic effect of IIIa, which has no mutagenic activity, revealed that it had a protective effect in small doses (25 mmole, 10 min treatment): it reduced the spontaneous mutation in *E. coli* by 38% on average and increased the survival rate of these cells by 49%. It also reduced the number of UV-induced mutations in the test cultures, by 55% on average, and protected 31% of the UV-irradiated bacterial cells from death.

Thus our results show that compounds that have biological activity to some extent can be found in each of the areas examined here. Consequently the search for biologically active agents among amino acid derivatives of sulfanilic acid is of interest, and it should be carried out on a large scale and in several areas.

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SEARCHES FOR NONDEPOLARIZING SHORT-ACTION MYORELAXANTS

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Modern anesthesiology requires the creation of a rapid-acting myorelaxant, the effect of which would last for no more than 10 min. Such preparations may include ditilin and diadonium, but ditilin is a depolarizing myorelaxant [1], while diadonium is a nondepolarizing myorelaxant, but its activity and selectivity are low [2]. The short duration of the action of these preparations is associated with hydrolysis of the ester group by pseudocholinesterase [3]. Attempts to create short-action myorelaxants in the series of tetrazenes RN=NR (I) and (II) have not met with success.

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