AMINOALKANESULFONIC ACIDS AND DERIVATIVES: SYNTHESIS AND ANTIVIRAL ACTIVITY

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The identification of chemotherapeutic and chemoprophylactic drugs for influenzal infections is a pressing task for contemporary virology [1, 2]. Consequently, the search for antiviral drugs in many types of chemical compounds steadily becomes more intensive [4].

We here report the results of an examination of the antiviral activity in vitro of some aminosulfonic acids, as shown by their ability to retard the reproduction of influenza virus in surviving fragments of chick embryo chorioallantoic membrane (CAM), and by their ability to protect animals from death from experimental influenzal infection. The effects of aminosulfonic acid derivatives on immune response in infective immunopathology are examined.

Sulfomethylation of amino-compounds is known to reduce their toxicity and to increase their solubility, and varying the aldehyde used can markedly affect their activity [3]. The α-aminalkanesulfonic acids were obtained by reacting α-aminosulfinic acids with carbonyl compounds.

$$R_2N{-}S(=O){-}OH + R'C(=O)R'' \rightarrow R_2NCR'R''SO_3H$$

I—VII

NR_2=$N(C_2H_5)_2$ (I, IV, VI), $N(C_4H_9)_2$ (II, V, VII), morpholino (III);

R′=CH_3 (I—III); R″=C_2H_5 (I), CH_3 (II, III); R′+R″= $-(CH_2)_4-$ (IV, V), $-(CH_2)_5-$ (VI, VII)

The physicochemical properties of the α-aminoalkanesulfonic acids are given in Table 1.

Reaction of morpholinesulfinic acid with carbonyl compounds gave, not the acids, but their morpholides:

$$O(CH_2CH_2)NS(=O)OH + R'C(O)R''$$

$$\downarrow$$

$$O(CH_2CH_2)_2NCR'R''SO_2N(CH_2CH_2)_2O$$

VIII, IX

R′+R″= $-(CH_2)_4-$ (VIII), $-(CH_2)_5-$ (IX)

The physicochemical properties of the α-morpholinoalkanesulfonamides are given in Table 1.

EXPERIMENTAL (CHEMICAL)

The aminosulfinic acids were obtained from dimethyl sulfite and secondary amines.

2-N,N-Diethylamino-n-butane-2-sulfonic Acid Hydrate (I). To 4.5 g (0.033 mole) of N,N-diethylaminosulfinic acid was added 7.15 g (0.1 mole) of 2-butanone. The reaction was exothermic, and bulky crystals separated. The solid was filtered off, washed with ether, and recrystallized. Recrystallization from ethanol gave 6.03 g (81%) of 2-N,N-diethylamino-n-butane-2-sulfonic acid monohydrate, mp 78-79°C. IR spectrum (KBr): ν_s (SO_2) 1015 cm^{-1}, s; ν_{as} (SO_2) 1145 cm^{-1}, s. Empirical formula: $C_8H_{19}NO_3S \cdot H_2O$.

Compounds (II-IX) were obtained similarly.

The structures of the compounds were confirmed by IR and PMR spectroscopy. The elemental analyses were in agreement with the calculated values.

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TABLE 1. Physicochemical Properties of α-Aminomethanesulfonic Acids and α-Aminomethanesulfomorpholides

Compound	Yield, %	mp, °C	Empirical formula
I	81	78—79	$C_6H_{15}NO_3S \cdot H_2O$
II	87	81—82	$C_{11}H_{25}NO_3S \cdot H_2O$
III	93	91—93	$C_6H_{17}NO_3S \cdot H_2O$
IV	93	98—100	$C_8H_{19}NO_3S \cdot H_2O$
V	88	94—96	$C_{13}H_{27}NO_3S \cdot H_2O$
VI	80	119—121	$C_{10}H_{21}NO_3S \cdot H_2O$
VII	84	139—140	$C_{14}H_{29}NO_3S \cdot H_2O$
VIII	81	118—120	$C_{13}H_{24}N_2O_4S \cdot H_2O$
IX	92	143—145	$C_{14}H_{26}N_2O_4S \cdot 2H_2O$

TABLE 2. Antiviral Activity of Aminosulfonic Acids and Derivatives against Influenza Virus A/Leningrad 34/72 (H3N2)

Compound	MTD for CAM cells, μg/ml	Activity				Chemotherapeutic ratio
		maximum		minimum		
		No. of doses of virus	concentration of compound, μg/ml	No. of doses of virus	concentration of compound, μg/ml	
I	2000	100	250	1	125	16
II	2000	100	500	1	250	8
III	2000	100	500	1	125	32
IV	2000	100	500	1	250	8
V	500	10	250	1	15.6	32
VI	2000	100	250	1	31.2	64
VII	2000	100	250	1	250	8

TABLE 3. Protectant Activity of an Aminosulfonic Acid (VII) in Experimental Influenzal Infection in White Mice

Animal group	MTD, μg/ml	Dose, mg/kg		Mortality at day 7,%	Survival, %	Protection, %	Protectant index	Incubation period, days	Increase in lifespan, %
		single	course						
Test	2000	220	1100	12,5±6,9	87,5	64,2	66,7	9,5	12,5
Control	—	—	—	76,7±7,85	—	—	—	7,87	—

TABLE 4. Immunomodifying Activity of the Aminosulfonic Acid (VII)

Animal group	Dose, mg/kg	Mass of spleen, mg	Number of splenocytes, million per organ	Number of AFC, $\cdot 10^6$	Stimulation coefficient (No. of AFC in test group/number of AFC in controls)
Test	500	202,3±38,83	279,2±31,43	186,5±11,63	1,75
Control	—	197,3±9,98	171,6±13,34	105,3±14,4	—

EXPERIMENTAL (BIOLOGICAL)

The ability to retard the reproduction of influenza virus A/Leningrad 34/72 (H3N2) in surviving fragments of CAM was assessed by a standard method [4]. Antiviral activity was determined using several concentrations of the compounds. The highest working concentration was half the minimum toxic dose (Table 2). One, ten, and 100 hemagglutinating doses of the virus were used in the tests. The presence of virus was shown by hemagglutination with a 1% suspension of chicken erythrocytes. The test compounds were of low toxicity towards the CAM cells, being 2000 μg/ml for six of the compounds (I-IV, VI, VII), and 500 μg/ml for (V). Compounds (II-IV) and (VII) retarded the reproduction of virus in the cells infected with 100 doses of virus at a concentration of 500 μg/ml, and (I) and (VI) in a concentration of 250 μg/ml. The maximum dose of virus to be retarded by (V) was ten units. The greatest chemotherapeutic ratio (64) was shown by (VI). The value for (III) and (V) was 32, and for (I), 16. The lowest ratio, eight, was shown by (II), (IV), and (VII).

Measurement of the toxicity of the compounds towards warm-blooded animals (white mice) showed them to be of low toxicity. The minimum toxic dose of (VI) was 2200 mg/kg, and of (I, III, IV, VII), 1750 mg/kg.

The high antiviral activity of the compounds in vitro in conjunction with their low toxicity led to their being examined for antiviral activity in vivo. We examined the ability of compound (VII) to protect white mice from a lethal outcome in experimental infuenzal infection induced by the influenza virus A/Aichi 2/68 (H3N2), adapted to mouse lung.

The compound was administered to the mice in five doses, subcutaneously, 24 and 1 h before infection, and 24, 48, and 72 h after infection. Each dose of the compound was equal to 1/10 of the minimum toxic dose.

The test animals were infected by intranasal administration of 0.03 ml of allantois virus under light ether narcosis. The LD_{50} of the virus for mice was $10^{4 \cdot 5}$. The hemagglutinin titer of the virus was 1:2048.

The percentage mortality of the animals in the experimental groups was assessed when at least 50%, but not more than 80% of the animals had died in the controls. In accordance

with the method of measuring antiviral activity in vivo [3], the percentage mortality, survival, and protection were determined, together with the index of protection and increased lifespan.

Compound (VII) showed high protectant activity. The mortality in the experimental group was 12.5%, while that in the controls was 76.7%. The survival of the animals was 87.5%, protection 64.2, and protection index 66.7%. The lifespan of the animals was increased by 12.5%.

The immune-modifying activity of the compound (VII) was assessed in model humoral immune response, by the number of antibody-forming cells (AFC) in the spleen of the test animals [5].

The compounds were administered to the infected animals in a single subcutaneous dose of 500 mg/kg at the same time as immunization with ram erythrocytes ($8 \cdot 10^6$ cells/ml), and the numbers of AFC were measured after four days.

Administration of the compound resulted in a twofold increase in the weight of the spleen as compared with the controls, and a 1.5 times increase in the numbers of nucleated and antibody-forming cells in the organs as compared with the controls (Table 4).

These studies have shown that aminosulfonic acid derivatives retard reproduction of the influenza virus in tissue culture, i.e., they have a direct effect on the virus, protect the animals from a lethal outcome in experimental influenzal infection, and enhance the immune response to the administration of thymus-dependent antigen in infective immunopathology.

It would be of interest to extend studies of antiviral activity and examine the mode of action of α-aminosulfonic acids, and to study their immune-modifying properties in virus-induced immunopathology, and we intend to continue work in this area.

LITERATURE CITED

1. V. I. Votyakov, B. F. Semenov, and V. M. Zhdanov, Prospects for the Scientific Development of Antiviral Drugs [in Russian], Minsk (1978), pp. 3-13.
2. V. I. Votyakov, Problems and Prospects for the Study of Nucleosides, Bicycloheptane, Adamantane, and Other Antiviral Compounds [in Russian], Minsk (1982), pp. 3-16.
3. D. E. Gilbert, Sulfonation of Organic Compounds [in Russian], Moscow (1969).
4. V. I. Il'enko, Methods of Testing and Evaluation of Antiviral Activity of Chemical Compounds against Influenza Virus [in Russian], Leningrad (1977), pp. 12-15.
5. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).