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SYNTHESIS AND ANTIOXIDANT AND ANTI-INFLUENZA ACTIVITY OF AMINOMETHANESULFONIC ACIDS

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Aminomethanesulfonic acid (**I**) and its *N*-methyl- (**II**), *N*-(2-hydroxy)ethyl- (**III**), *N*-(*tert*-butyl)- (**IV**), *N*-benzyl- (**V**), and 4-(*N*-phenylaminomethyl)phenyl- (**VI**) derivatives (**V** and **VI** not previously reported) were synthesized and characterized by elemental analysis and IR and mass spectroscopy. The *in vitro* antioxidant activities of aminomethanesulfonic acids **I**-**VI** were found to be comparatively weak. Compounds **IV** and **V** suppressed statistically significantly reproduction of influenza virus strains A/Hong Kong/1/68 (H3N2) and A/PR/8/34 (H1N1) in chorioallantoic membrane tissue culture.

Keywords: aminomethanesulfonic acids, antioxidant activity, anti-influenza activity, A/Hong Kong/1/68 (H3N2), A/PR/8/34 (H1N1).

Aminoalkanesulfonic acids form a class of N- and S-containing organic compounds with important practical and medical applications that are interesting because of their specific physicochemical properties, in particular, the pK_a values of these acids are situated in the physiological pH range 6 – 8 [1 – 6]. Their antioxidant activity (AOA) protects erythrocytes from diabetes-induced changes of enzymatic and nonenzymatic processes, stabilizes cell membranes, and prevents them from being destroyed [7, 8]. The antiviral activity of recombinant human interferon was demonstrated to increase when combined with antioxidants, including taurine [9]. Furthermore, many antioxidants themselves can exhibit both *in vitro* and *in vivo* anti-influenza activity [10].

Known examples of antiviral activity of aminoalkanesulfonic acids (against bronchitis, vaccinia, mumps, herpes, and influenza) are limited to anecdotal publications [11 – 13]. This makes it critical to seek effective antiviral compounds with antioxidant properties among the new compounds of this class. The goal of the present work was to synthesize and study both the anti-influenza and antioxidant activity of aminoalkanesulfonic acids and their N-alkylated derivatives.

EXPERIMENTAL CHEMICAL PART

IR spectra were recorded from KBr pellets on a Spectrum BX II FT-IR system (PerkinElmer) in the range 4000 – 350 cm^{-1}. EI mass spectra were recorded on an MX-1321 instrument (direct sample introduction into the source, ionizing-electron energy 70 eV). FAB mass spectra were taken on a VG 7070 instrument (8-keV Ar-atom bombardment to desorb ions from a liquid *m*-nitrobenzyl alcohol matrix). Elemental analyses agreed with the empirical formulas.

AOA of aqueous solutions (10^{-3} M) of aminoalkanesulfonic acids and N-derivatives and typical antioxidants quercetin (Qu) and ascorbic acid (AA) [14] were determined *in vitro* by potentiometry using a Fe^{3+}/Fe^{2+} mediator [15] at 293 K. The limit of AOA determination by this method was 3.5×10^{-3} M [16].

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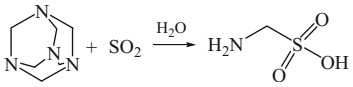
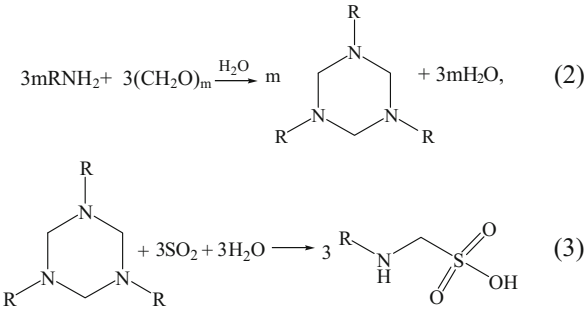
Aminomethanesulfonic acid (AMSA, I) was prepared using a one-step synthesis [17].



(1)

N-Derivatives of AMSA were prepared by two-step syntheses [18, 19].



(2)

(3)

R = CH_3 (**II**); $HOCH_2CH_2$ (**III**); $(CH_3)_3C$ (**IV**); $C_6H_5CH_2$ (**V**).

***N*-Methylaminomethanesulfonic acid (II).** An aqueous solution (25 mL) of methylamine (0.10 mol) was treated at ≤ 10°C with an equimolar amount of paraformaldehyde and left for 24 h. The resulting solution was purged with SO_2 until the pH ≤ 1.0 and left at room temperature until the water evaporated completely to produce a crystalline white product (12.32 g, ~100%), mp 167 – 168°C (dec.). IR spectrum, ν_{max}, cm^{-1}: 3172 (NH); 3060, 3027, 2973, 2896, 2818 (NH, CH); 2574, 2511, 2447, 2389 ($[NH_2]^+$); 1617, 1581 ($[NH_2]^+$); 1240, 1183 (SO_2); 1085, 1053, 1029 (SO_2); 541 (S-O). Mass spectrum FAB *m/z* (I_{rel}, %) (solution (**IV**) in 3-nitrobenzyl alcohol (154 (100)): $[M+H]^+$ 126(8), $[M-H]^+$ 124(5), $[M-SO_3–2H]^+$ 43(9).

***N*-(Hydroxyethyl)aminomethanesulfonic acid (III)** [18] and **N-(*tert*-butyl)aminomethanesulfonic acid (IV)** [19] were synthesized analogously.

***N*-Benzylaminomethanesulfonic acid (V).** Benzylamine (0.05 mol) was dissolved in H_2O (20 mL), left for 24 h, and mixed with paraformaldehyde to produce a heterogeneous mixture (yellow oily liquid over an aqueous solution) through which SO_2 was bubbled to afford a white crystalline solid (**V**, 10.0 g, ~100% in N and C), mp 144 – 145°C. IR spectrum, ν_{max}, cm^{-1}: 3440, 3174 (NH); 3044, 2940, 2847, 2819 (NH, CH); 2780, 2605, 2316 ($[NH_2]^+$); 1555 ($[NH_2]^+$); 1237, 1214 (SO_2); 1054, 1040, 1014 (SO_2); 589 (S-O). Mass spectrum EI *m/z* (I_{rel}, %): $[C_7H_7]^+$ 91 (100), $[C_6H_5]^+$ 77 (15), $[SO_2]^+$ 64 (50), 48 (21).

4-(*N*-Phenylaminomethyl)phenylaminomethanesulfonic acid (VI). A mixture of aniline (0.10 mol) and H_2O (20 mL) was cooled to 0°C, left for 5 h, and mixed with paraformaldehyde to produce a heterogeneous mixture (cream-colored solid over an aqueous solution) through which SO_2 was bubbled at =5°C to afford an amorphous yellow solid. IR spectrum, ν_{max}, cm^{-1}: 3380 (NH); 2969, 2935, 2875, 2816 (NH, CH); 1294, 1200 (SO_2); 1085, 1046 (SO_2); 531 (SO). Mass spectrum EI *m/z* (I_{rel}, %): $[M-SO_3+H]^+$ 213(15), $[M-SO_3-H]^+$ 211 (11), $[M-SO_3-CH_2+H]^+$ 199 (50), $[M-SO_3-CH_2-H]^+$ 197 (35), $[M-SO_3-CH_2-NH_2]^+$ 182 (13), $[M-SO_3-CH_2-NH_2-C_6H_4]^+$ 106 (27), $[C_6H_5NHNH_2]^+$ 106 (27), $[C_6H_5NH_2+H]^+$ 93 (37), $[C_6H_5]^+$ 77 (10), $[SO_2]^+$ 64 (100), 48 (76).

EXPERIMENTAL BIOLOGICAL PART

Virology studies used influenza virus strains A/Hong Kong/1/68 (H3N2) and A/PR/8/34 (H1N1).

Effective antiviral doses of the tested compounds against influenza virus were estimated by determining beforehand their toxicities for chorioallantoic membrane (CAM) tissue culture of 11 – 14-day chick embryos using the standard method [20]. The minimal toxic dose (MTD_{50}, M) was the compound concentration that killed 50% and more of CAM tissue culture fragments.

Anti-influenza activity of the compounds *in vitro* was studied using CAM tissue culture [20, 21], which is currently officially recommended for studying antiviral activity. This culture is considered closest to the level of the whole organism, i.e., chick embryo.

The log TID_{50} values (doses infecting 50% and more of CAM tissue fragments) were calculated in *in vitro* experiments by the Kerber method as modified by Ashmarin [22]:

$$\log TID_{50} = L - d(S - 0.5),$$

where *L* is the initial test dilution; *d*, difference between successive log dilutions; and *S*, sum of test-sample proportions giving positive results. The TID_{50} values *in vitro* experiments were calculated for test samples with compounds and for control samples. The antiviral activity parameter was the difference between the TID_{50} values of the control and test samples ($\Delta\log TID_{50}$).

TABLE 1. AOA of Aqueous Solutions of AMSA* **I-VI**

AOA	I	II	III	IV	V	VI
$AOA \cdot 10^2$, M	2.5 ± 0.2	2.5 ± 0.3	3.4 ± 0.4	4.4 ± 0.5	1.6 ± 0.2	2.5 ± 0.3
$\Delta AOA \cdot 10^2$, M	0.8 ± 0.1	1.6 ± 0.2	5.3 ± 0.6	1.6 ± 0.2	1.6 ± 0.1	3.9 ± 0.5

* $C = 10^{-3}$ M; $\Delta AOA = AOA_2 - AOA_{Qu}$; AOA_2 is the antioxidant activity of the AMSA—quercetin—H_2O system ($C_{acid} = C_{Qu} = 10^{-3}$ M).

The statistical significance of the antiviral activity of the compounds was determined using a nonparametric sign criterion for related sets [23].

The reference drug was Tamiflu (oseltamivir) [powder for preparation of suspension for internal use at 12 mg/mL in a 30-mL vial (F. Hoffmann La-Roche, Switzerland)] at a concentration of 410 μg/mL or 10^{-3} M.

RESULTS AND DISCUSSION

Table 1 contains results showing that AMSA and its *N*-alkyl derivatives exhibited comparatively low AOA as compared to AA and Qu (0.161×10^{-3} and 0.556×10^{-3} M, respectively). This agreed with the insignificant antioxidant properties of taurine that were reported earlier [6]. The observed difference was statistically significant ($p < 0.05$). Adding an equimolar amount of AMSA **I** – **VI** to an aqueous Qu solution led to a statistically significant ($p < 0.05$) increase of AOA. A statistically significant ($p < 0.05$) additive effect (ΔAOA = AOA) was observed for **V**; ΔAOA < AOA for **I**, **II**, and **IV**; and statistically significant ($p < 0.05$) synergy (ΔAOA > AOA) was observed for **III** and **VI**. Statistically significant differences in the AOA values were not found if **I-VI** were added to an aqueous solution of AA.

Toxicity of AMSA and its *N*-derivatives in CAM tissue culture showed that **I** – **V** at a dose of 10^{-2} M were cytotoxic to CAM tissue. Because the antiviral activity would be determined later using namely this model, dilutions of the compounds in glucose-gelatin growth medium at a dose of 10^{-3} M were used to study anti-influenza activity.

The tested compounds were dissolved beforehand in DMSO to a final concentration of 10 mg/mL. Then, dilutions in glucose-gelatin growth medium were prepared at a final concentration of 10^{-3} M.

Virus-containing liquid with a previously determined infectious titer was diluted in medium with (test) or without (control) compound. The virus content in the dilutions should be >100 TID_{50}. CAM fragments in polystyrene plates were infected with 10-fold dilutions of virus-containing material and incubated in a thermostat at 37°C for 24 h. Control and test samples were combined separately. The virus infection titer in them was determined. For this, CAM fragments in polystyrene plates were infected with 10-fold dilutions of these samples and thermostatted at 37°C for 48 h. The virus titer was determined from hemagglutination (HGA) results [20, 21].

Viricidal activity of the compounds against extracellular influenza virus was determined as follows. Virus-containing liquid was diluted to 10^{-4} in glucose-gelatin medium with (test) or without (control) compound at a certain concentration. Then, samples were stored at 4°C for 20 h and at 37°C for 2 h. The infection titer in them was determined as described above [20, 21].

Experimental results showed that **I** and **II** did not significantly suppress reproduction of influenza virus strain A/Hong Kong/1/68 (H3N2), reducing it only by 0.5 and 0.83 log TID_{50}, respectively, as compared to the control (Table 2). Compound **III** did not exhibit anti-influenza activity and increased virus reproduction in the test within error limits as compared to the control by 0.33 log TID_{50}. However, **IV** and **V** suppressed statistically significantly ($p < 0.05$) as compared to the control reproduction of influenza virus A/Hong Kong/1/68 (H3N2) by 1.5 and 4.08 log TID_{50}, respectively.

It is noteworthy that **V** reduced influenza virus reproduction at the Tamiflu level although at a lower concentration (201.25 μg/mL). The reference drug at a final concentration of 10^{-3} M suppressed statistically significantly ($p < 0.05$) as compared to the control virus reproduction by 4.07 log TID_{50}. Compounds **IV** and **V** were chosen for further detailed studies on influenza virus strain A/PR/8/34 (H1N1) considering the results.

TABLE 2. Antiviral Activity of AMSA **I-V** Against Influenza Viruses A/Hong Kong/1/68 (H3N2) and A/PR/8/34 (H1N1) in CAM Tissue Culture

Compound	Molar mass, g/mol	MTD_{50}, M	Δlog TID_{50}		
			1*	2**	3**
I	111.12	$> 10^{-3}$	0.50 ± 0.22	…	1.25 ± 0.26
II	125.15	$> 10^{-3}$	0.83 ± 0.12	…	0 ± 0.25
III	155.17	$> 10^{-3}$	– 0.33 ± 0.3	…	– 0.58 ± 0.5
IV	167.23	$> 10^{-3}$	1.50 ± 0.25#	2.17 ± 0.32#	– 0.25 ± 0.3
V	201.25	$> 10^{-3}$	4.08 ± 0.5#	1.67 ± 0.43#	0.67 ± 0.3
Tamiflu	410	$> 10^{-2}$	4.07 ± 0.52#	4.07 ± 0.5#	-0.17 ± 0.3

* Δlog TID_{50}(1) is average suppression of reproduction of virus strain A/Hong Kong/1/68 (H3N2); ** Δlog TID_{50}(2) is the average suppression of reproduction of virus strain A/PR/8/34 (H1N1); *** Δlog TIC_{50}(3) is the average effect on extracellular virus A/PR/8/34 (H1N1); # statistically significant vs. the control ($p < 0.05$).

Compound **V** suppressed to a greater extent reproduction of influenza virus A/Hong Kong/1/68 (H3N2) by 4.08 log TID_{50} although it was less active against influenza virus A/PR/8/34 (H1N1), suppressing its reproduction by only 1.67 log TID_{50}. Compound **IV**, which was less active against virus A/Hong Kong/1/68 (H3N2) and suppressed its reproduction by 1.50 log TID_{50}, exhibited more pronounced anti-influenza activity against influenza virus A/PR/8/34 (H1N1), 2.17 log TID_{50}. The reference drug suppressed statistically significantly ($p < 0.05$) as compared to the control reproduction of viruses A/PR/8/34 (H1N1) and A/Hong Kong/1/68 (H3N2) by 4.07 log TID_{50}. A statistically significant difference between the activities of the test compounds and reference drugs was not observed.

Table 2 presents results for the viricidal activity of **I** – **V** against influenza virus A/PR/8/34 (H1N1). It should be emphasized that **IV** and **V**, like the reference drug, did not affect extracellular virus A/PR/8/34 (H1N1). The difference of the control and test was within error limits. Compound **I** had little effect on extracellular virus.

Infection of cells by influenza virus is known to be associated with a change of their redox potential [24]. Therefore, several antioxidants are used to protect from infections, in particular, those caused by influenza virus [10]. Therefore, the anti-influenza activity of the studied AMSAs with antioxidant activity in addition may be related to reduction of the intracellular redox potential, as before [25].

Thus, it can be stated that the synthesized AMSAs under *in vitro* conditions demonstrated weak AOA that was markedly inferior to that of typical antioxidants, e.g., ascorbic acid and quercetin. Also, *N*-(2-hydroxy)ethyl and 4-(*N*-phenylaminomethyl)phenyl derivatives of AMSA enhanced the AOA of quercetin by 9.6 and 7.0%, respectively ($p < 0.05$). *N*-Benzylaminomethanesulfonic (**V**) and *N*-(*tert*-butyl)aminomethanesulfonic acids (**IV**) suppressed statistically significantly ($p < 0.05$) as compared to the control reproduction of influenza viruses A/Hong Kong/1/68 (H3N2) and A/PR/8/34 (H1N1). The results indicated that further detailed studies of the antiviral and antimicrobial properties of these compounds in *in vitro* models and their mechanisms of action are promising.

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