New Adamantane Derivatives Can Overcome Resistance of Influenza A(H1N1)pdm2009 and A(H3N2) Viruses to Remantadine

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New adamantane derivatives with amino acid residues and other bifunctional compounds were synthesized and their antiviral activity towards influenza A(H1N1)pdm and A(H3N2) viruses was studied. Some of these adamantane derivatives completely suppressed replication of remantadine-resistant influenza A virus strains.

Key Words: *adamantane derivatives; amino acids; remantadine; influenza A; resistance*

The known methods of influenza treatment and prevention include vaccination and drug therapy aimed at infection suppression. The drugs are effective for disease treatment and for its prevention in healthy individuals in contact with sick individuals.

Among modern anti-influenza drugs, remantadine and amantadine are most available. Both are adamantane compounds suppressing the function of influenza A protein M2.

This protein is formed by 4 subunits located in the protein membrane of the virus. The main function of M2 protein is selective transport of protons into the viral particle [7]. Normal functioning of the proton-conducting channel is the key event in the virus replication cycle. Protons transported from the host cell cytoplasm oxidize the medium inside the virion, thus triggering M1 protein deproteinization; after that, genetic material of influenza virus enters the cell nucleus for repeated copying of viral RNA. Blockade of M2 channel permeability for hydrogen ions prevents normal reproduction of influenza virus [4].

Studies by spectral methods showed that adamantane drugs are fixed in M2 channel via hydrogen bond between the carbocycle amino group and serine residue hydroxyl group in position 31 of the protein transmembrane domain [5].

Wide use of adamantane drugs for influenza treatment and prevention led to mutations in influenza virus genome, including position 31 of M2 protein transmembrane domain with substitution of serine residue for asparagine (Ser31Asn) [9]. This point restructuring of the virus ion channel made impossible fixation of amino-adamantanes in M2 channel. At present, 90% of the known remantadine-resistant influenza viruses carry this substitution that serves as a marker of remantadine-resistant strain [6].

Activity of adamantane drugs can be restored via introduction into the adamantane molecule of additional functional groups capable of binding to virus M2 channel proteins. These groups can be donated by amino acid and peptide residues. Being natural elements for the organism, they can form all types of intermolecular bonds, except covalent bonds, and can modulate hydrophobic/hydrophilic interactions of molecules.

MATERIALS AND METHODS

Adamantane amino acid derivatives were obtained via the formation of amide bond in the reaction between amino acid carboxyl group and adamantane carbocycle

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amino group or between adamantane-carbonic acid carboxyl group and amino acid amino group using classical methods of peptide chemistry [3]. The resultant compounds were identified by thin-layer chromatography and mass spectrometry.

Antiviral activity of the resultant compounds was evaluated on remantadine-resistant influenza A/IIV-Moscow/01/2009(H1N1)pdm strain (similar to the reference variant A/California/7/2009(H1N1)swl [2]) and A/Moscow/26/2009(H3N2) strain.

Antiviral activity of the synthesized compounds was evaluated in 96-well plates with MDCK monolayer. Adamantane derivatives in a concentration of 5 μg/ml were added to cell monolayer simultaneously with the infection. Remantadine hydrochloride (5 μg/ml) served as the reference preparation. Cellular IFA was carried as described previously [1]. The percentage of virus activity inhibition by the test compounds was evaluated by the formula:

$$100-\frac{OD_{exp}-OD_{cc}}{OD_{vc}-OD_{cc}}\times 100\%,$$

where OD is optical density at 492 nm, OD_{cc} is OD_{492} of cell control, and OD_{vc} is OD_{492} of virus control.

Toxicity of the test compounds was evaluated for concentrations of 5, 20, 40, and 80 μg/ml on MDCK monolayer in 96-well plates after incubation at 37°C. Cell monolayer was examined under a microscope. The maximum tolerated concentration was 50% compound concentration not producing toxic effect on the cells. Cytotoxicity was evaluated by the colorimetric method. The count of viable cells was evaluated by comparing staining intensity in the control and experimental wells after addition of neutral red on an automated spectrophotometer at λ=450 nm.

RESULTS

The synthesis of new adamantane derivatives led to creation of compounds containing almost all natural amino acid residues and some other bifunctional molecules. Only few synthesized adamantane derivatives almost completely inhibited replication of remantadine-resistant influenza A virus strains. The overwhelming majority of the compounds exhibited activity below 50%.

The most active compounds inhibiting influenza A viruses are presented in Table 1.

Analysis of the results of biological trials showed that the compounds with the amino group protected by tert-butylhydroxycarbonyl (Boc) group inhibited influenza A virus. These compounds were amino acids ornithine (compound 1) and sarcosine (compound 2); they exhibited no activity towards these viruses in a free state, but were active with blocked amino group. It can be hypothesized that the presence of a highly hydrophobic fragment of the protective group together with lipophilic carbocycle of adamantane created optimal structure for penetration into the membrane bilayer of virus envelope.

The compound containing histidine residue (compound 5) exhibited stable suppression of influenza A viruses replication. This positive effect could be due to the structure of the transmembrane region of the channel. Protons transported into M2 channel interact with histidine imidazole ring in position 37 and when 3 of 4 histidine residues are protonated channel conformation is changed and the protons enter the virion, after which the conformation is restored and the pore remains closed for hydrogen ions until the next surge [8]. Presumably, the presence of one more (5th) histidine residue in the channel pore enables competitive processes of imidazole ring protonation in the channel, which helps to overcome virus resistance.

Apart from derivatives containing amino acid residues, a compound with diamine was synthesized. An interesting peculiarity of 1,6-diaminohexane derivative with 1-adamantane-carboxylic acid (compound 4) is that active amino group was separated from adamantane carbocycle by 6 methylene moieties. This flexible methylene "tail" provided mobility of amino group. The compound suppressed replication of both influenza A virus strains by 70%.

Remantadine derivative with lipoic acid (compound 3) including cyclic disulfide exhibited significant (80-89%) suppression of both influenza A strains. The

TABLE 1. Inhibition of Virus Reproduction by Synthesized Adamantane Derivatives and Their Toxic Effects on MDCK Monolayer

No.	Compound	Activity, %		MTC, μg/ml
		A (H1N1) pdm	A (H3N2)	
1	Boc-Orn(Boc)-Rem	71	66	40
2	Boc-Sar-Rem	71	64	40
3	TOA-Rem	78	89	80
4	Ad-HDA	74	63	>80
5	H-His-Rem	91	94	40
6	Ad*-$(CH_2Ser\text{-}OMe)_2$	<95	<95	>80
	Remantadine	0	0	40

Note. Rem: remantadin carbocycle molecule residue; Ad: 1-adamantane-carbonic acid residue; Ad*: 1,3-adamantane-diacetic acid residue; TOA: thioctic (lipoic) acid residue; HAD: 1,6-hexanediamine residue. MTC: maximum tolerable concentration.

presence of a long carbohydrate backbone and sulfhydryl bonds could disorder not only proton transport in M2 protein channel, but also other membrane processes.

Compound 6 proved to be one of the most active (90-98%) amino acid derivative of adamantane acids. In addition, it was absolutely nontoxic for MDCK culture: the cells normally lived even at its concentration of 80 μg/ml, while inhibition was effective at 5 μg/ml.

These results indicate that adamantane carbocycle as a membranotropic carrier transports functionally active groups to M2 protein of influenza A(H1N1) pdm and A(H3N2) viruses. Such compounds as Boc-Orn(Boc)-Rem (1), Boc-Sar-Rem (2), TOA-Rem (3), H-His-Rem (5), Ad-$(CH_2$-Ser-OMe$)_2$ (6) can be of practical interest as promising nontoxic antiviral agents for influenza A viruses resistant to remantadine and amantadine.

REFERENCES

1. E. I. Burtseva, E. S. Shevchenko, I. A. Leneva, *et al.*, *Vopr. Virusol.*, No. 2, 24-29 (2007).
2. D. K. Lvov, E. I. Burtseva, A. G. Prilipov, *et al.*, *Ibid.*, No. 5, 10-14 (2009).
3. V. A. Shibnev, T. M. Garaev, M. P. Finogenova, *et al.*, *Ibid.*, **56**, No. 2, 36-39 (2011).
4. S. D. Cady, T. V. Mishanina, and M. Hong, *J. Mol. Biol.*, **385**, No. 4, 1127-1141 (2009).
5. F. Hu, W. Luo, and M. Hong, *Science*, **330**, 505-508 (2010).
6. C. Laohpongspaisan, E. Rungrotmongkol, P. Intharathep, *et al.*, *J. Chem. Inf. Model*, **49**, No. 4, 847-852 (2009).
7. W. Luo, R. Mani, and M. Hong, *J. Phys. Chem.*, **111**, No. 36, 10,825-10,832 (2007).
8. A. Okada, T. Miura, and H. Takeuchi, *Biochemistry*, **40**, No. 20, 6053-6060 (2001).
9. R. M. Pielak, J. R. Schnell, and J. J. Chou, *Proc. Natl. Acad. Sc. USA*, **106**, No. 18, 7379-7384 (2009).