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Synthesis and study of antiviral and anti-radical properties of aminophenol derivatives

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Abstract—A number of sterically-hindered *o*-aminophenol derivatives have been synthesized, and their antiviral activity in parallel with reactivity towards commonly encountered free-radical intermediates was investigated. Of the compounds tested, the highest activity in suppressing replication *of Herpes simplex* type 1 viruses was displayed by *N*-acyl and *N*-aryl derivatives of 4,6-di-*tert*-butyl-2-aminophenol, which were able to interact with organic free radicals and, at the same time, manifested low reactivity towards reactive oxygen species.

Although studies in the area of free-radical virology have started relatively recently, it has already been shown that the course of a number of viral infections, including rhino-, cytomegalo-, influenza, HIV, and various neuroviral infections, as well as chronic viral hepatitis,[1] is accompanied by generation of reactive oxygen species (ROS) and activation of lipid peroxidation (LPO) processes. Therefore, investigation of the possibility of using antioxidants for prevention and treatment of viral diseases is a trend in pharmacological research that is worthy of notice.[1] Nevertheless, the information available at present does not allow a relationship to be found between the effects produced by chemical compounds on free-radical processes and antiviral activity of these compounds. The complexity of the problem (i.e., finding out such relationships) is due to an ambiguous role of free-radical processes in the development of viral pathology. The reason is that the reactions involving ROS generally take place in a non-selective manner, so they can cause damage to both viruses and host cells. Thus, the question as to which compounds should be introduced into biosystems to modify the direction of free-radical reactions so that a predominantly antiviral effect is obtained, remains open. The search for such compounds and investigation of their properties is the main purpose of this study.

Some time ago, we have developed an antiviral product, Butaminophen®, based on a sterically-hindered *o*-aminophenol derivative, effective against herpetic injuries of various types.[2,3] Many aminophenol derivatives are known to be effective antioxidants[4,5] and to be capable of modifying the direction of various free-radical processes.[6]

We have synthesized a number of *o*-aminophenol derivatives and carried out investigations of their antiviral activity in parallel with their reactivity towards various free radicals—for the purpose of finding out interrelations between structures, antiviral and anti-radical properties of these compounds, which would be helpful for the search for new antiviral agents.

A general scheme depicting the synthetic pathways is shown in Figure 1.

Synthetic procedures used to prepare compounds (**1**–**9**) and other aminophenol derivatives are described in Refs. [7–10] Structures of all the compounds synthesized have been confirmed by ^{1}H NMR and mass spectrometry.

Antiviral properties of the synthesized compounds were studied in cell cultures infected with *Herpes simplex*

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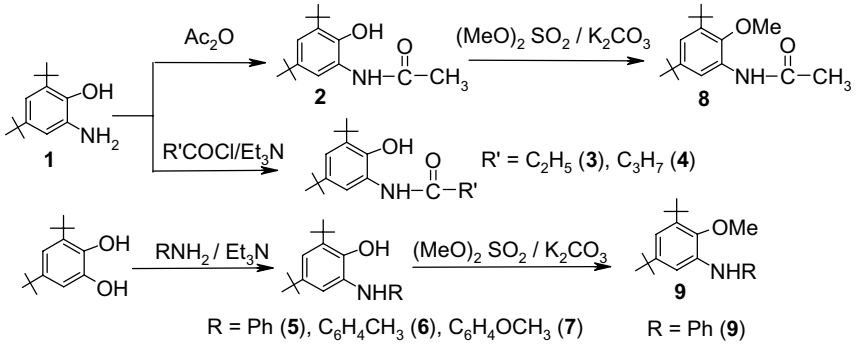


Figure 1. A general scheme depicting synthetic pathways.

virus of type I (HSV-1). The investigation was performed using the method based on the evaluation of cytopathic effects (CPE) produced by the virus in the cell culture of human rhabdomyosarcoma (RD) infected with HSV-1.[11] The compound tested was predissolved in 10% ethanol and then twofold dilutions in maintenance medium (medium 199, Sigma Chemical Co.) were prepared. Virus titer reduction in the presence of the test compounds as compared to control was taken as a criterion of antiviral activity. Based on the data obtained, concentrations of the test compounds that suppressed virus replication by 50% and 90% were calculated (EC_{50} and EC_{90}, respectively) (Table 1). Maximum non-toxic concentrations (MNTC) of the substances were determined in non-infected cell cultures after 72-h incubation. As seen from Table 1, the MNTC values are much higher than EC_{50} and EC_{90}.

The obtained results show that the aminophenol derivatives under study possess antiviral properties expressed to a variable degree, depending on the respective structures. The named properties are the most pronounced for those structures, in which one hydrogen atom in the amino group is replaced by an acyl (**2**–**4**) or an aryl (**5**–**7**) group. For structures, where free amino and hydroxyl groups are present (compound **1**), or where H atom in the hydroxyl group is replaced by a methyl group (compounds **8** and **9**), low antiviral activity is observed.

The reactivity of aminophenol derivatives towards ROS was investigated in a cellular model named 'the oxidant burst', which was induced in peritoneal macrophages of rats by particles of opsonized zymosan.[12] The oxidant burst in phagocytes provoked an effective production of ROS due to the activation of the NADPH-oxidase mechanism that generates superoxide radical anions ($\cdot O_2^-$).[13]

The intensity of ROS production was evaluated by the luminol-derived chemoluminescence method in the absence and in the presence of the test compounds. Effective inhibitory concentrations (EC_{50} and EC_{90}) of the compounds under study were measured, that is, the concentrations, at which the luminescence intensity decreased by 50% and 90%, respectively (see Table 2).

It follows from the obtained data that compound (**1**) reacts with ROS by two or three orders of magnitude more actively as compared with other aminophenol derivatives. Compounds (**2**–**4**, **6**, and **7**) and (**9**), taken at concentrations within the range studied, produced no effect on the intensity of chemoluminescence, while compound (**5**) decreased it slightly, evidencing the absence or low reactivity of the named compounds towards ROS. Similar results were obtained in our experiments performed in order to assess reactivity of aminophenol derivatives towards ROS generated using fermentative systems.[14]

The ability of the synthesized compounds to modify the probability of free-radical reactions involving *alkyl* (R•), *peroxyalkyl* (ROO•) and α-*hydroxyalkyl* (R•CHOH) radicals, was investigated using γ-radiation as initiator of various free-radical processes. Radical intermediates of such types are known to arise in the course of ROS-in-

Table 1. Antiviral properties and toxicity of the test compounds

Compound	MNTC (μM)	EC_{50} (I_{95})[a] (μM)	EC_{90} (I_{95})[a] (μM)
1	113.2	87.3 (214.9–35.3)	288.2 (709.9–117.2)
2	379.7	8.5 (10.5–6.9)	14.8 (18.2–12.1)
3	720.9	38.2 (41.3–35.3)	64.5 (69.6–59.5)
4	686.2	8.6 (10.3–7.2)	14.1 (17.2–11.7)
5	336.7	23.0 (56.4–9.4)	169.4 (316.2–90.9)
6	643.1	30.9 (37.0–25.7)	83.0 (99.4–69.1)
7	611.6	18.0 (22.4–14.5)	41.9 (52.3–33.6)
8	1444.0	798.0 (1053.8–604.3)	1960.5 (2588.8–1484.8)
9	722.0	255.2 (569.2–114.4)	623.8 (1373.9–283.3)

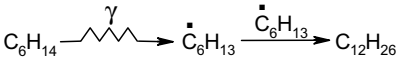
[a] I_{95} is confidence interval at 95% probability.

Table 2. Effective concentrations of aminophenols inhibiting the zymosan-stimulated production of ROS by macrophages

Test compound	Concentration range (μM)	EC_{50} (μM)	
1	0.001–10	0.06	0.65
2	0.001–10	No inhibition	
3	0.001–10	No inhibition	
4	0.001–10	No inhibition	
5	0.001–10	9.8	>10
6	0.001–10	No inhibition	
7	0.001–10	No inhibition	
9	0.001–10	No inhibition	

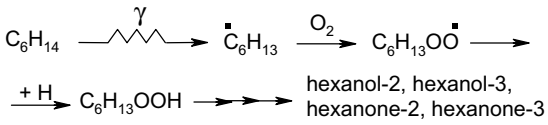
duced processes of oxidation and fragmentation occurring in cell membrane components.[15] γ-Irradiated hexane and ethanol have been selected as model systems because radiolysis of these substrates was extensively studied and its features are well known. The methods used for analysis of products formed in radiolysis of ethanol and hexane, as well as those employed for the determination of the respective yields, are described in detail in Ref. 16.

The main products obtained on radiolysis of hexane in the absence of oxygen are dodecanes of various structures formed by recombination of hexyl radicals on $^{\cdot}C_2$ and $^{\cdot}C_3$ atoms.[16]



Hence, by measuring the overall yield of dodecanes in the presence of additives, reactivity of the latter towards *alkyl* radicals can be evaluated (Table 3).

When γ-irradiation of hexane is performed in the presence of O_2, the formation of dodecanes does not occur because oxygen is an effective acceptor of alkyl radicals, transforming them into *peroxyalkyl* radicals ($ROO^{\cdot}$). From the latter, after further transformations, the corresponding alcohols and ketones are formed, hence, the main products to be expected on radiolysis of hexane in the presence of O_2 are hexanols and hexanones.[17]



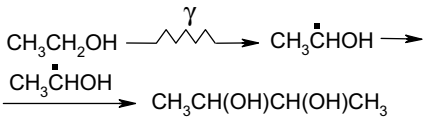
The overall yield of hexanols and hexanones ($\Sigma G(ox)$) in the presence of aminophenols depends on the effectiveness of interaction of the latter with $C_6H_{13}OO^{\cdot}$ radicals (Table 3).

Table 3. The effects of compounds (**1**–**9**) on yields (G, mol/J) of dodecanes $G(C_{12}H_{26})$, hexane oxidation products $\Sigma G(ox)$ and 2,3-butanediol G(2,3-BD)

Test compound ($C = 10^{-3}$ M)	$G(C_{12}H_{26})$	$\Sigma G(ox)$	G(2,3-BD)
—	0.48 ± 0.03	2.04 ± 0.11	1.29 ± 0.04
1	0.09 ± 0.01	1.46 ± 0.11	0.10 ± 0.01
2	0.27 ± 0.01	1.07 ± 0.09	0.72 ± 0.04
3	0.27 ± 0.02	1.07 ± 0.09	0.85 ± 0.06
4	0.32 ± 0.02	1.06 ± 0.08	0.76 ± 0.05
5	0.11 ± 0.01	1.36 ± 0.09	0.28 ± 0.01
6	0.09 ± 0.01	1.70 ± 0.13	0.31 ± 0.01
7	0.08 ± 0.01	0.87 ± 0.05	0.25 ± 0.02
8	0.46 ± 0.02	2.02 ± 0.14	1.28 ± 0.05
9	0.44 ± 0.02	1.99 ± 0.09	1.26 ± 0.07

Reactivity of the additives towards α-*hydroxyalkyl* radicals can be evaluated by studying the effects of additives on radiolysis of ethanol. This appears to be important because radicals of such kind are formed when ROS attack hydroxyl-containing biologically relevant substances, such as carbohydrates, lipids and nucleosides, and further transformations of these radicals result in fragmentation of the starting material.[18–23]

Radiolysis of ethanol has been studied in detail,[24] and its main product was found to be 2,3-butanediol (2,3-BD), formed by the reaction of α-hydroxyalkyl radicals:



Evaluation of yields of 2,3-butanediol in the presence of various additives makes it possible to judge about reactivity of the latter towards $CH_3{\cdot}CHOH$ radicals. The data obtained on this issue are presented in Table 3.

It follows from the data shown in Table 3 that compounds (**8**) and (**9**) produce virtually no effect on the probability of processes involving alkyl, peroxyalkyl and α-hydroxyalkyl radicals. Compounds (**1**–**7**) display the ability to inhibit, to a greater or lesser extent, the processes involving various organic free radicals.

While assessing the relationship between anti-radical and antiviral activities of the test compounds we proceeded from the following experimental facts. Compound (**1**) effectively interacted with both ROS (Table 2) and the organic radicals (Table 3), but manifested low antiviral activity (Table 1). Compounds (**2**–**7**), as well as compound (**9**), displayed low activity towards ROS (Table 2), however, unlike (**8**) and (**9**), reacted with organic radicals (Table 3) and manifested marked antiviral properties (Table 1). Hence, the obtained data indicate that those aminophenol derivatives, which interact with organic radicals while being indifferent towards ROS, display the highest activity as inhibitors of HSV replication.

The question why just such compounds manifest antiviral activity is open at the moment, and we shall try to find out an answer to it in our further studies.

The facts set forth above indicate that sterically hindered aminophenol derivatives possess antiviral properties and hence may be regarded as a novel class of antiviral agents. Of the compounds tested, *N*-acyl and *N*-aryl derivatives of 4,6-di-*tert*-butyl-2-aminophenol, which are able to interact with organic free radicals and, at the same time, display low reactivity towards reactive oxygen species, possess the most marked activity in suppressing replication of *Herpes simplex* type 1 viruses in cell cultures.

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