# SYNTHESIS AND STUDY OF THE ANTIVIRAL ACTIVITY OF ENAMINOKETONES SUBSTITUTED ON NITROGEN WITH HYDROXYL-CONTAINING GROUPS

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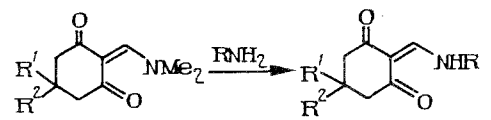
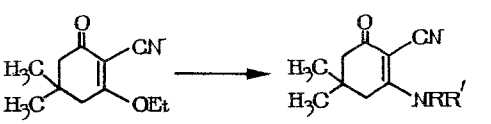
In a continuation of investigations on the synthesis and antiviral activity of compounds potentially capable of O-phosphorylation in the organism [5], the present work describes enaminoketones containing amino alcohol fragments as substituents. The presence of one or several hydroxylic alcohol groups provides marked antiviral activity of many acyclic nucleosides and their analogs [7]. It seemed of interest to examine the question of the possible manifestation of this activity based on the example of significantly more simple structures such as derivatives of cyclohexanone and similar systems. It should be noted that compounds of this type contain partially "hidden" polycarbonyl compounds, some of which are also antiviral agents [4].

The synthesis of the desired compounds was accomplished by transamination of previously known enaminoketones, derivatives of dimethylaminomethyl-cyclohexan-1,3-diones (1a-c) [8, 9], and 2-cyano-3-ethoxy-5,5-dimethyl-2-cyclohexen-1-one (II) [1]. The reaction, as a rule, proceeds easily and in high yield to give the corresponding enaminoalcohols (III, IV).

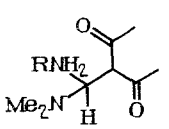


*I* a-c, *III* a-j



*II* *IV* a-f

The ease with which the transamination reaction proceeds for enaminocarbonyl (and especially enaminodicarbonyl) compounds is described in the literature [2] and requires the stabilization of the intermediate through substantial delocalization by the keto group of the negative charge arising by addition of the amine to the $\alpha$-position of the starting enamine.



Compound II also easily reacted with amines with the formation of enaminoketones IV. In this connection it should be noted that the ethoxymethylene derivative was significantly more easily transaminated by amines than enamines [3, 8].

From Table 1 it can be seen that the reaction of compound II with secondary enamines takes place significantly more slowly than with primary amines, which is a result, apparently, of the large steric requirement of the intermediate structure and the lower basicity of the secondary amine used. The structures of the products III and IV were confirmed by mass-spectral and elemental analysis data.

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TABLE 1. Reaction Conditions and Physicochemical Properties of the Synthesized Compounds

| Compound | R | $R^1$ | $R^2$ | Yield, % | mp, °C (solvent) | Medium and reaction temp., °C | Reaction time, h | Empirical formula |
|---|---|---|---|---|---|---|---|---|
| IIIa | $CH_2CH(OH)CH_2OH$ | H | H | 83 | 131—134 methanol | ethanol 78 °C | 2 | $C_{10}H_{15}NO_4$ |
| IIIb | $CH_2CH_2OH$ | H | H | 93 | 141—144 ethanol | ethanol 20 °C | 1 | $C_9H_{13}NO_3$ |
| IIIc | $CH_2CH(OH)CH_2OH$ | Me | Me | 97 | 176—178 ethanol | same | 1 | $C_{12}H_{19}NO_4$ |
| IIId | $CH_2CH_2OH$ | Me | Me | 95 | 142—144 ethanol | » » | 1 | $C_{11}H_{17}NO_3$ |
| IIIe | $CH_2CH_2CH_2OH$ | Me | Me | 78,3 | 144—147 ethanol | » » | 1 | $C_{12}H_{19}NO_3$ |
| IIIf | $CH(Et)CH_2OH$ | Me | Me | 73,5 | 81—82 benzene-petroleum ether | » » | 1 | $C_{13}H_{21}NO_3$ |
| IIIg | $CH_2CH_2OH$ | H | Ph | 80 | 156—157 ethanol | » » | 1,5 | $C_{15}H_{17}NO_3$ |
| IIIh | $CH_2CH_2CH_2OH$ | H | Ph | 89 | 130—132 ethanol | » » | 1,5 | $C_{16}H_{19}NO_3$ |
| IIIi | $CH_2CH(OH)CH_2OH$ | H | Ph | 90 | 134—136 ethanol | » » | 1,5 | $C_{16}H_{19}NO_4$ |
| IIIj | $CH(Et)CH_2OH$ | H | Ph | 94 | 130—132 ethanol | » » | 1,5 | $C_{17}H_{21}NO_3$ |
| IVa | $CH_2CH_2CH_2OH$ | H | — | 56 | 125—126 isopropanol | » » | 0,5 | $C_{12}H_{18}N_2O_2$ |
| IVb | $CH_2CH(OH)CH_2OH$ | H | — | 81 | 138—140 isopropanol | » » | 0,5 | $C_{12}H_{18}N_2O_3$ |
| IVc | $CH(Et)CH_2OH$ | H | — | 69 | 157—159 isopropanol | ethanol 78 °C | 0,5 | $C_{13}H_{20}N_2O_2$ |
| IVd | $CH_2CH_2OH$ | H | — | 80 | 159—161 isopropanol | ethanol 20 °C | 0,5 | $C_{11}H_{19}N_2O_2$ |
| IVe | $CH_2[CH(OH)]_4CH_2OH$ | Me | — | 88 | 159—161 methanol | ethanol 78 °C | 10 | $C_{16}H_{26}N_2O_6$ |
| IVf | $CH_2CH_2OH$ | $CH_2CH_2OH$ | — | 60 | 112 ethyl acetate-ethanol - 2:1) | same | 10 | $C_{13}H_{20}N_2O_3$ |

TABLE 2. Influence of Different Groups of Chemical Compounds on the Reproduction of Group A/Japan (H2N2) Virus in MDCK Cells *in vitro* (IFA method)

| Compound | Percent suppression of $OD_{490}$ in MDCK cells |
|---|---|
| IVa | n/a |
| IVb | n/a |
| IVc | n/a |
| IVd | n/a |
| IVe | n/a |
| IVf | n/a |
| IIIe | n/a |
| IIIg | n/a |
| IIIh | n/a |
| IIIi | 34 |
| IIIj | 36 |
| Arbidol | 90 |
| Remantadin | 80 |

**Note.** Here and in Table 4: n/a = inactive.

In accord with the above hypothesis on the possibility of O-phosphorylation of these compounds, the antiviral activity of the enaminoketones III and the cyanoaminoketones IV was studied *in vitro* and *in vivo*. The results of the tests are presented in the Biological section of the Experimental.

## EXPERIMENTAL (CHEMICAL)

The melting points were determined with a 'Boetius' hot stage. Elemental analysis data corresponded with the calculated values. Reaction conditions, solvents for crystallization, empirical formulas, and yields of the synthesized compounds are given in Table 1.

**General Method for the Synthesis of N-(Hydroxyalkyl)enamines of the 2-Methylenecyclohexan-1,3-dione and 2-Cyano-5,5-dimethyl-2-cyclohexen-1-one Series (IIIa-j and IVa-f).** A mixture of 10 mmoles of starting enamine, 11 mmoles of amino alcohol and 10-20 ml of absolute ethanol was maintained under conditions indicated in Table 1. The compounds obtained were isolated by known methods.

TABLE 3. Influence of Different Groups of Compounds on the Reproduction of Group A/Puerto Rico/8 (H1N1) Virus in MDCK Cells (IFA method)

| Compound | Percent suppression of $OD_{490}$ in MDCK cells |
|---|---|
| IIIa | 11 |
| IIIb | 27 |
| IIIc | 76 |
| IIId | 54 |
| Arbidol | 60 |
| Remantadin | 50 |

## EXPERIMENTAL (BIOLOGICAL)

**Materials and Methods**. The IFA method was used with viruses of group A, strain A/Puerto Rico/8 (H1N1) and strain A/Japan (H2N2). MDCK cells were incubated in medium 199 with the addition of 10% fetal calf serum and 10 mM globulin.

**Study of Antiviral Activity of the Synthesized Compounds by the IFA Method in MDCK Cell Culture**. MDCK cells were incubated in 96-cavity plates (Costar Company) in 199 medium in the presence of 5% serum and 10 mM glutamine to a complete monolayer. Then the cells were washed out of the serum with 199 medium, all test compounds were added to the cells in a final concentration of 10 $\mu$g/ml in 199 medium in the presence of trypsin (2 $\mu$g/ml). Further experiments were carried out as described earlier [6]. Monoclonal antibodies to the introduction of proteins of the virus group A (NP and M) were kindly made available by Doctor L. Kendal (Center for the Control of Infectious Diseases BOZ, Atlanta, USA) and were used in a dilution of 1:4000.

Plates not infected by virus were used as controls. The standard OD value for the control was found from the residual OD value. The mean of the $OD_{490}$ values, determined by the percent decrease in OD of the test compound for the first three cultures of the virus, was found for each virus culture and then the average percent inhibition of the $OD_{490}$ for these cultures was determined.

The antiviral activity of the compounds was studied by comparison of the group A/Bethesda/63 (H2N2) virus on mouse influenza pneumonia elicited by intranasal infection with 10 $LD_{50}$ of the virus, which elicited the death of 80-90% of the control (nontreated) animals. The studied materials were introduced *per os* in doses of 100 and 30 $\mu$g/kg daily once per day for 5 days. The activity of the compounds was calculated by the decrease in lethality of the treated animals compared with the controls.

The antiherpes activity of the compounds was studied in FEK cell culture against a herpes infection elicited by intranasal inoculation with simple herpes virus.

**Discussion of Results**. This study used the IFA method modified for screening chemical compounds for antiviral activity *in vitro*. The value of OD at $\lambda$ = 490 nm was directly proportional to the quantity of virus antigen expressed on the surface of the cells infected by virus, and the influence of the compounds on level of this expression thus allows a judgment on their effectiveness *in vitro*. As control, both here and earlier [6], the preparations remantadin and arbidol were used, the levels of action of which on the reproduction of the viruses of group A in cells was studied earlier. The experimental data are presented in Table 2, from which it can be seen that all derivatives of 2-cyano-2-cyclohexenone do not influence the OD value at $\lambda$ = 490 nm, i.e., did not show viral inhibitory action in MDCK cell cultures.

Among the derivatives of phenylcyclohexan-1,3-dione, of 5 compounds only 2 show inhibitory activity on the reproduction of the Group A/Japan virus in MDCK cells, which comprise in both cases approximately one third of the activity of arbidol.

Experiments with derivatives of cyclohexan-1,3-dione also were carried out using the group A/Puerto Rico/8 virus. Arbidol and remantadin in these experiments inhibited reproduction of group A/Puerto Rico/8 virus by 60 and 50%, respectively (Table 3). As indicated, high activity, similar to that of remantadin and arbidol, is shown by compound IIId, while compound IIIc exceeds the activity of arbidol and remantadin. The activity of compound IIIb is 45% of the activity of arbidol and 54% of the activity of remantadin, but compound IVa does not significantly inhibit the reproduction of group A/Puerto Rico/8 virus in MDCK cells. Comparison of the activity of compounds of this group with their chemical structures indicates that the presence of the dimethyl groups in the 5th position is essential for the presence of antiviral activity *in vitro*.

TABLE 4. Antiviral Activity of the Compounds against Influenza Pneumonia in Mice Elicited by A/Bethesda/63 (H2N2) Virus

| Compound | Decrease in animal mortality rate compared to controls, % |
|---|---|
| IIIa | n/a |
| IIIb | 20 |
| IIIc | 37 |
| IIId | 32 |
| IIIf | 30 |
| IIIi | n/a |
| IIIj | 30 |
| IVa | n/a |
| IVb | 23 |
| IVc | n/a |
| IVd | n/a |
| IVe | n/a |
| IVf | n/a |
| Arbidol | 70 |
| Remantadin | 60 |

Results of the study of the antiviral activity of the compounds against influenza pneumonia in mice are presented in Table 4. Of the 13 studied compounds, 6 have activity, decreasing the animal mortality rate by 20-37% compared with the controls. The activity of the compounds lies primarily in the derivatives of cyclohexan-1,3-dione. The most significant anti-influenza activity *in vivo* is shown by compound IIIc, which also has the highest activity in *in vitro* experiments with activity determination by the IFA method. However, against influenza pneumonia compound IIIc is inferior to the antiinfluenza activities of arbitol and remantidin.

The eight compounds IVa-c, IIIa-c, IIIe, IIIf were studied *in vitro* and *in vivo* against simple herpes virus with negative results.

In conclusion it can be said that none of the studied compounds exhibited antiherpes activity. Materials were found possessing activity against group A viruses with different antigen structures. High antiviral activity (on the level of the comparison compounds) in the IFA system is shown by compounds IIIc and IIId, which are also active against the influenza pneumonia, although inferior to arbidol and remantidin in this test. In consideration of these results, a further search for materials with antiinfluenza activity among the N-(hydroxyalkyl)enamines is advisable.

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