SYNTHESIS AND TESTING OF ANTIVIRAL ACTIVITY OF AZAHETEROCYCLES WITH HYDROXYL-CONTAINING FRAGMENTS AS SUBSTITUENTS


Among acyclic nucleosides and their analogs are found the effective pharmaceutical compounds acyclovir and gancyclovir, as well as other compounds having high antiviral activity [5]. The antiviral activity of this type of compound is apparently due to the presence in its structure of hydroxyl groups with the potential to undergo phosphorylation in vivo. It is believed that this phosphorylation occurs to a greater degree in virus-infected than in uninfected cells. The triphosphates thus formed serve as active and selective inhibitors of viral-coded DNA polymerase, and are responsible for the antiviral activity of these compounds [6, 7].

From this point of view, it was of interest to study the antiviral activity of heterocyclic compounds not containing purine fragments, but having substituents with one or more alcoholic hydroxyl groups, which in principle could be phosphorylated in vivo. As objects of this type for study, in the present work we chose derivatives of 2-aminomethylene-4,5-dehydropyrrolidine-3(Ia-c), 2-aminomethyleneindolinone-3(IIa-c), pyrrolo[1,2-a]indole(IIIa-c), and pyrido[3,2:4',5']thieno[3,2-d]pyrimidine (IVa-d).

Synthesis of the above compounds was carried out via previously described enamines [2, 3], by their transamination, or by reaction of tricyclic chloroderviative V with different amines:

\[
\text{EtOOC} \quad \text{EtOOC} \\
H \quad H \\
\text{NMMe}_2 \quad \text{NMMe}_2
\]

\[
I \quad \text{a-c} \quad I \quad \text{a-c}
\]

\[
\text{H} \quad \text{H} \\
\text{NMMe}_2 \quad \text{NMMe}_2 \\
\text{N} \quad \text{N}
\]

\[
\text{a-c} \quad \text{a-c} \quad \text{J} \quad \text{J}
\]

\[
\text{R} = \text{CH}_2\text{CH}_2\text{OH}, \text{R}^1 = \text{H (Ia, IIa, IIIa, IVa)}; \text{R} = \text{CH}_3\text{CH(OH)}\text{CH}_2\text{OH}, \text{R}^1 = \text{H (Ib, IIb, IIIb, IVb)}; \text{R} = \text{CH}_2\text{[CH(OH)]}_4\text{CH}_2\text{OH}, \text{R}^1 = \text{CH}_3 (Ic, IIc); \text{R} = \text{CH}(_2\text{CH}_2\text{OH}), \text{R}^1 = \text{H (IVc)}; \text{R} = \text{R}^1 = \text{CH}_2\text{CH}_2\text{OH} (IVd).
\]

Transamination proceeded smoothly in all cases, with formation of the desired products I-III in high yields. The structures of compounds obtained were verified by mass spectral and elemental analysis. The usual procedure for synthesis was to briefly reflux the components in isopropyl alcohol; for pyrroloindoles IIIa and b, incubation at room temperature was sufficient. Using the secondary amine N-methylglucamine and in the case of pyrrolo[1,2-a]indoles, transamination was carried out by refluxing a solution of the reaction components in 2-propanol for 20 min. Physical constants, yields, and reaction conditions are given in Table 1.
### Table 1: Heterocyclic Derivatives of Aminoalcohols I-IV

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield, %</th>
<th>Mp, °C (solvent)</th>
<th>Medium and reaction temperature</th>
<th>Reaction time, h</th>
<th>Empirical formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>79</td>
<td>221 - 3 (DMFA - MeOH, 4:1)</td>
<td>2-propanol, reflux</td>
<td>1</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>Ib</td>
<td>85</td>
<td>206 - 8 (DMFA - MeOH, 4:1)</td>
<td>The same</td>
<td>1</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>Ic</td>
<td>93</td>
<td>202 - 5 (DMFA - MeOH</td>
<td>4:1)</td>
<td>2-propanol, reflux</td>
<td>2</td>
</tr>
<tr>
<td>IIIa</td>
<td>70</td>
<td>199 - 6 2-propanol</td>
<td></td>
<td>2</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>IIIb</td>
<td>93</td>
<td>210 - 2 (DMFA)</td>
<td>MeOH, 20°C</td>
<td></td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>IIIc</td>
<td>97</td>
<td>198 - 200 2-propanol</td>
<td>EtOH, 20°C</td>
<td>4</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>IVa</td>
<td>65</td>
<td>201 - 6 (EtOH)</td>
<td>Autoclave, 180°C</td>
<td>12</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>IVb</td>
<td>67</td>
<td>192 - 5 2-propanol</td>
<td>The same</td>
<td>12</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>IVc</td>
<td>81</td>
<td>173 - 4 2-propanol</td>
<td>&gt;</td>
<td>12</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
<tr>
<td>IVd</td>
<td>59</td>
<td>185 - 7 2-propanol</td>
<td></td>
<td>12</td>
<td>C₂₁H₃₅N₂O₅</td>
</tr>
</tbody>
</table>

Synthesis of compounds IVa-d requires considerably harsher conditions — to prepare them, chloroderivative V and the appropriate aminoalcohol were heated in an autoclave at 180°C for 12 h.

In accordance with the aims of the present work, the antiviral activity of the synthesized compound was studied; results obtained are summarized in the "Experimental (Biological)" section.

### Experimental (Chemical)

Mass spectra of compounds synthesized were obtained on a Varian MAT-112 spectrometer, at an ionizing voltage of 50 eV and ionization chamber temperature of 140°C. Melting temperatures were determined on a Boetius heating block.

**General Method for Synthesis of Hydroxyalkylaminomethylene Derivatives of 2-Methyl-3-ethoxycarbonylpyrrolin-2-one-4 (Ia-e), Indolinone-3 (IIa, b), and 3,9-Dioxopyrrolo[1,2-a]indole (IIIa-c).** A mixture of 10 mmoles of starting enamine, 15 mmoles of aminoalcohol, and 50-60 ml of appropriate solvent was incubated under conditions described in Table 1. The reaction mass was cooled, and the precipitate was filtered and washed with 2-propanol and ether.

**General Method for Synthesis of 4-Hydroxyalkylamino Derivatives of 9-Dimethylaminopyrido[3,2:4',5']-thieno[3,2-d]pyrimidine (IVa-d).** A mixture of 1.59 g (6 mmoles) chloroderivative V, 12 mmoles aminoalcohol, and 30 ml of absolute ethanol was heated in an autoclave at 180°C for 12 h. The reaction mass was evaporated under vacuum, and the residue was triturated with water and washed on a filter with 2-propanol.

Elemental analysis data corresponded to values calculated. Yields, solvents for crystallization, and melting temperatures are given in Table 1.

### Experimental (Biological)

In the experiments we used influenza A virus, strain A/Japan (H2N2). MDCK cells were grown on medium 199 with the addition of 10% fetal calf serum and 10 mM glutamine.

**Determination of Antiviral Activity of Compounds Synthesized by IEA in Cultured MDCK Cells.** MDCK cells were grown in 96-well plates (Costar) on medium 199, in the presence of 5% serum and 10 mM glutamine to a confluent monolayer. Cells were then washed free of serum with medium 199, and all test compounds were added to the cells at a final concentration of 10 μg/ml in medium 199 in the presence of trypsin (2 μg/ml). The experiments were then carried out as previously described [4]. Monoclonal antibodies to core proteins of type A influenza virus (NP and M) were generously provided by Dr. L. Kendal (Center for Infectious Disease Control, Atlanta, USA) and were used at a dilution of 1:4000.

As controls, we used wells not infected with virus. The usual control OD value was subtracted from the other ODs. For each dilution of virus, the mean OD₄₉₀ was found, percent decrease in OD with test compound for the first three viral dilutions was determined, and then the mean percent inhibition of OD₄₉₀ for these dilutions was determined.

Antiviral activity of compounds was studied against influenza virus A/Bethesda/63 (H2N2) in mouse influenza pneumonia, caused by intranasal infection with 10 LD₅₀ of virus, which caused the death of 80-90% of control (untreated)