treated to individually treated and placebo groups. The following values of the protection index were recorded: 34–41% for the combination of oseltamivir with 5 mg/kg rimantadine, 43–87% for oseltamivir with 7.5 mg/kg rimantadine, while the individual effects were 0–10% for oseltamivir, 0% for 5 mg/kg rimantadine and 18.7–29.6% for 7.5 mg/kg rimantadine. The mean survival time was lengthened by up to 6.9 days in the combination groups, up to 1.9 days in oseltamivir groups and to 3.2 days in rimantadine groups. The combination effect was characterized by three-dimensional method of Prichard and Shipman as synergistic. Lung virus titer in MDCK cells, lung index and consolidation score proved the high effectiveness of the combination of 5 mg/kg rimantadine and 0.05 mg/kg oseltamivir. At the peak of virus growth, 48–60 h post infection, the titer was 2.8 $\log_{10}$ 50% cell culture infectious dose ($CCID_{50}$) lower than in placebo control. In rimantadine and oseltamivir separately applied groups a decrease of only 1.1–1.4 $\log_{10}$ and 0.1–1.0 $\log_{10}$ $CCID_{50}$, respectively, was established. These data emphasize the high anti-influenza A potential of the combination.

Experiments on the effect of combinations of 10, 20 and 40 mg/kg/day rimantadine and 0.2, 0.4 and 0.8 mg/kg/day oseltamivir, and other rimantadine/oseltamivir doses' ratios (50:1 and 25:1) as well as on different delay schemes of drug application experiments are in progress.

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Phosphorodiamidate Morpholino Oligomer—Mediated Inhibition of Influenza A Virus in Mice

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Phosphorodiamidate morpholino oligomers (PMO) are single-stranded nucleic acid-like antisense agents that can reduce gene expression by sterically blocking complementary RNA sequence. PMO are water-soluble and nuclease resistant, and they readily achieve uptake into cells under standard conditions. Two PMO, were evaluated for their ability to inhibit influenza A viruses (H1N1) and (H3N2) replication in a murine model of infection. The PMO were designed to base pair with FLUAV RNA sequences that are highly conserved across viral subtypes and considered critical to the FLUAV biological-cycle, such as gene segment termini and mRNA translation start site regions. Several PMO previously shown to be highly efficacious in cell culture models of influenza infection were evaluated in a murine model of infection with influenza. Two PMO, one designed to target the AUG translation start site region of PB1 mRNA and the other the 3′-terminal region of nucleoprotein viral genome RNA, proved to be effective against influenza infection of mice reducing clinical signs (loss of body weight) and virus titers in the respiratory tract. These results, taken together with in vitro results, suggest these oligomers may represent a broad-spectrum therapeutic approach against a high percentage of known influenza A strains.

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Practical Synthesis of (−)-Carbocyclic Cytosine (Carbodine) and its In Vitro Antiviral Activity against Venezuelan Equine Encephalitis (VEE) Virus and Yellow Fever Virus

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Natural as well as synthetic carbocyclic nucleosides are well known for their interesting biological activities, including antitumor as well as antiviral activities against a wide variety of RNA and DNA viruses. The carbocyclic analogue of cytosine (carbodine **2**) was previously prepared as a racemic mixture and has been shown to possess significant antitumor (lymphoid leukemia L1210 in mice) and antiviral activities against human influenza type A virus, measles, vesicular stomatitis virus and herpes simplex viruses (HSV-1 and HSV-2). These interesting biological properties of carbodine, prompted us to synthesize enantiomerically pure (−)-carbodine (**2**) for biological evaluations. Herein, we report an efficient and practical synthesis of (−)-carbodine (**2**) as well as its antiviral activities against VEE and yellow fever virus (Fig. 1).

The key intermediate, chiral cyclopantanol **1**, was achieved from a chiral enone by a 1,4-addition reaction in a multi-gram scale. The cyclopentanol intermediate **1** was reacted with protected cytosine under Mitsunobu reaction conditions, however, it provided only O-alkylated product instead of the desired N-alkylated product. Therefore, the desired heterocycle, cytosine was constructed by the linear approach to afford the target nucleoside, (−)-carbodine (**2**) in a gram scale. The (−)-carbodine (**2**) showed potent antiviral activity against Venezuelan equine encephalitis virus (TC-83 virus strain, EC90 0.3 μM) with the high selective index >333 and yellow fever virus (17D virus strain, EC90 2.2 μM) in Vero cells.

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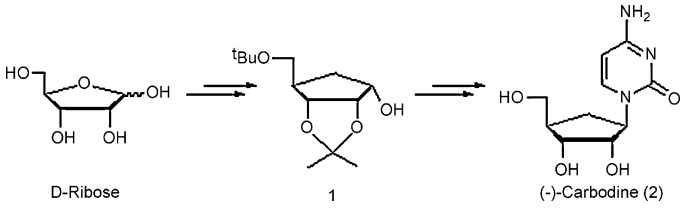

Fig. 1.

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