Influenza remains a major health problem for humans and animals. At present, four drugs are approved for influenza prophylaxis and treatment: amantadine and rimantadine act as the M2 ion channel blockers, whereas Tamiflu (the phosphate salt of oseltamivir ethyl ester) and Relenza (zanamivir) inhibit the activity of neuraminidase (NA). The NA inhibitors (NAIs) are designed to have (oxa)cyclohexene scaffolds to mimic the oxonium transition-state in the enzymatic cleavage of sialic acid. Tamiflu (1, shown in Scheme 1) is an orally administrated anti-influenza drug. On hydrolysis by hepatic esterases, the active carboxylate, oseltamivir (2, also known as GS4071), is exposed to interact with three arginine residues (Arg118, Arg292, and Arg372) in the active site of NA.

The phosphate group is generally used as a bioisostere of carboxylate in drug design. In comparison with the carboxylate−guanidinium ion pair, a phosphate ion exhibits stronger electrostatic interactions with the guanidinium ion. Our preliminary molecular docking experiments (Figure 1) using the known N1 crystal structure (PDB code: 2HU4) reveal that the putative phosphate inhibitor 3a indeed binds strongly with the triarginine residues of NA, in addition to other interactions exerted by the C3-pentyloxy, C4-acetamido, and C5-amino groups in the binding pocket similar to the NA−oseltamivir complex. Because the previously reported methods for the synthesis of oseltamivir/Tamiflu are not amenable to exchange of the C-1 carboxyl group to a phosphonate group, we thus explored a novel approach to the synthesis of both oseltamivir/Tamiflu and the phosphonate congener 3a using D-xylose as an appropriate chiral precursor (Scheme 1).

In brief, our present synthetic method is straightforward to culminate in an enantioselective synthesis of Tamiflu, oseltamivir, the phosphate congener, and the guanidine analogues with reasonably high yields (5.2−13.5%). An intramolecular Horner−Wadsworth−Emmons reaction was carried out to furnish the cyclohexene carboxylate 8a and phosphate 8b. On treatment with diphenylphosphoryl azide according to Mitsunobu’s method, the hydroxyl group in 8a/8b was successfully substituted by an azido group with the reversed configuration. The hazardous reagent of sodium azide was avoided in this procedure. This synthetic scheme allows late functionalization, which makes it attractive from a medicinal chemistry point of view.

The greater potencies of the phosphate congeners, 3 (namely Tamiphosphor) versus oseltamivir 2 and guanidine 13b versus 13a, were observed in the wild-type neuraminidases of H1N1 and H5N1 influenza viruses (Table 1). Both compounds 3 and 2 are significantly less potent toward the NAI resistant mutants of H274Y than the wild-type enzymes. Nevertheless, the phosphate compound 13b is an effective inhibitor that inhibits both mutant enzymes at low nM concentrations. Compounds 14a and 14b, which lack the

Consistent with our expectation, phosphonate 3 is a potent NA inhibitor and antiflu agent against influenza H1N1 virus with \( K_i \) and EC50 values of 0.15 and 4.67 nM (Table 2). In comparison, phosphonate 3 is more active than oseltamivir by 19- and 7-folds, respectively, in the NA inhibition and antiflu assays. The phosphonate 3 was further evaluated at multiple concentrations to determine the growth inhibition on the host MDCK cells. The deduced CC50 value of phosphonate 3 was 74 μM. The phosphonate 3, showing a high selectivity index of greater than 15800, is thus a potent antiviral agent against H1N1 virus with no toxicity to the host MDCK cells. By replacing the amino group in 3 with a guanidino group, the phosphonate 13b exhibits an enhanced NA inhibition (\( K_i = 0.06 \text{ nM} \)) and antiflu activity (EC50 = 0.09 nM). By analogy to the previous reports, the guanidinium group may exert strong electrostatic interactions with the residues of Glu119, Asp151, and Glu227.

Before a safe and effective vaccine is available to protect the possible pandemic avian flu, neuraminidase inhibitors are the only therapy we have. The recent reports on the drug resistant avian flu infections and the side effects in children receiving Tamiflu treatments suggest that new chemical identities for neuraminidase inhibitors are needed for our battle against the threat of the pandemic flu. The phosphonate congeners described in this study are significantly more potent than the carboxylic congeners against the wild-type neuraminidases of H1N1 and H5N1. In addition, compound 13b is an effective inhibitor at 19 nM for the H274Y mutant of a H5N1 neuraminidase. Because the high polarity of phosphonate and guanidinium groups may cause a problem of orally bioavailability, further investigation of formulation and use of prodrugs, for example, acyloxymethyl- and arylphosphonate esters, may eventually solve the problem in the drug development.

**Supporting Information Available**: Complete ref 3a and ref 7, experimental section, computer modeling of neuraminidase inhibition, 1H, 13C, and 31P NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

**References**


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