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Letter

Facile Synthesis of Influenza Virus Neuraminidase Inhibitors and Molecular Studies

Tae Woo Kim, Kang-Yeoun Jung*

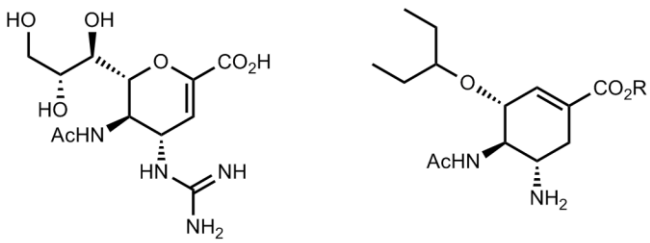
Department of Biochemical Engineering, College of Engineering, Gangnung-Wonju National University, Gangwon-do 25457, Republic of Korea

*Correspondence to KY Jung; kyjung@gwnu.ac.kr

Abstract: As a preliminary results, a small library of 1,2,3-triazole-linked D-fructose derivatives has been synthesized in good yield which have been screened for its influence on influenza neuraminidase inhibitory activity. The computational simulation by molecular docking procedure was conducted for the prediction of interactions between ligand and sialidase binding pocket.

Keywords: D-Tagatofuranoside; Sucrose; Epoxy phosphonate; Phosphonated sugar; Triazole derivatives

Influenza is a highly contagious, acute upper respiratory tract disease responsible for significant morbidity and mortality each year, particularly to the very young, elderly and immunosuppressed. In recent years, the worldwide spread of H5N1 avian influenza has raised concerns that this virus might acquire the ability to pass readily among humans and cause a pandemic. Protection through vaccination is limited due to the antigenic variation of the influenza virus. Two sialic acid recognizing proteins, hemagglutinin and sialidase, on the viral surface have been shown to be crucial in invasion and have provided many exciting opportunities for rational structure-based drug discovery of anti-influenza agents. To date the most successful structure-based anti-influenza drug discovery (oseltamivir, Tamiflu^{TM} and zanamivir, Relenza^{TM}) [Fig. 1] has arisen from targeting the sialidase function [1,2]. However, reports of the emergence of drug resistance make the development of new anti-influenza molecules a priority.



Zanamivir, Relenza^{TM} Oseltamivir, Tamiflu^{TM}

Fig. 1. Structures of influenza sialidase inhibitors.

Potent and selective inhibitors of influenza virus sialidase originating from a variety of scaffolds have been developed using structure-based methods and relying on crystallographic data, including: pyrrolidines [3], cyclopentanes [4], tetrahydrofurans [5] and benzenes [6]. The nine-carbon furanose **1** showed inhibition at micromolar levels which further led to the discovery of BCX-1812 (Peramivir) [7,8] possessing comparable sialidase activity to both Relenza and Oseltamivir against a number of influenza strains [9] and also showing excellent selectivity for influenza sialidase over mammalian, bacterial or other viral sialidases [10] [Fig. 2].

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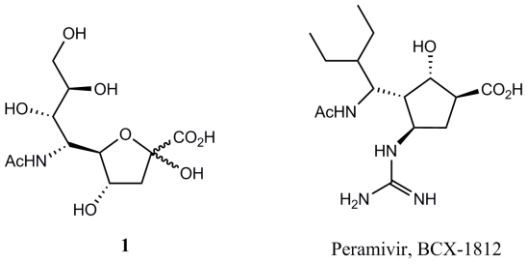


Fig. 2. Structures of influenza sialidase inhibitors.

This suggests that properly substituted pentacyclic compounds may also bind in the catalytic site of enzyme. Thus, we designed the inhibitor **2** choosing the furanosic ring scaffold that represents an economic and easily available ring system [Fig. 3].

Herein, we report the synthesis of fructofuranoside derivatives as highly versatile precursors of sialidase inhibitors. Our synthetic approach includes the possibility of selective modification at all position around the furanose scaffold, however, in this report we are interested in investigating the role of the C-4 position. We have also replaced monobasic carboxylate group with the dibasic phosphonate group to increase the charge-charge interaction with three highly conserved arginine residues in the active site of the ligand.

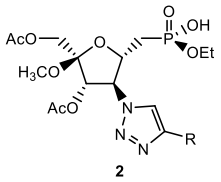
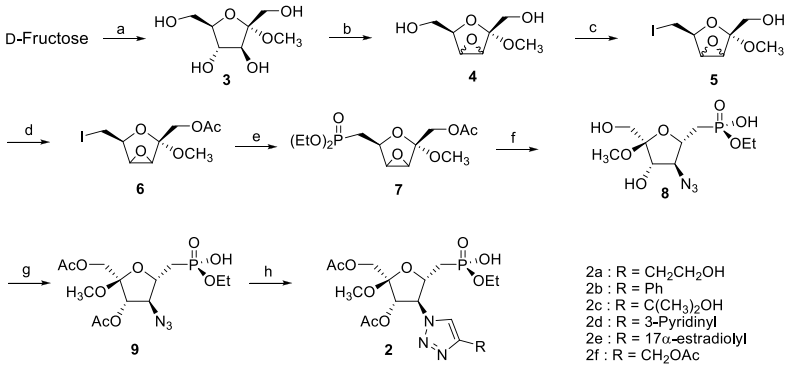


Fig. 3. Structures of anti-influenza molecules.

Similar to our previous report, a synthetic route for the preparation of sialyl nucleoside mimetic of the general structure **2** was developed using inexpensive the carbohydrate D-fructose as a starting material. Starting from the carbohydrate D-fructose, the methyl glycoside **3** was prepared via a Fischer type methodology which resulted in a mixture of α/β fructofuranoside and fructopyranoside, respectively [Scheme 1]. The tetrahydroxyl compound **3** obtained was then purified by performing column chromatography using 40% methanol in ethyl acetate to obtain the pure compound. Following modified Mitsunobu conditions [11,12], epoxide **4** was obtained as a mixture of stereoisomers in 9:1 ratio as determined from ^{1}H NMR data [13]. Regioselective iodination of the 6-hydroxy group in compound **4** gave compound **5** in 72% yield after purification. Subsequent acetylation afforded fully protected single isomer **6**, which was treated with freshly distilled $P(OEt)_3$ to obtain the corresponding 6-deoxy-6-diethoxy phosphonate **7**. For the synthesis of the core intermediate **7** we were able to achieve successively in a shorter way compared to the previously published work [14]



Scheme 1. a) AcCl, MeOH; b) PPh_3, DIAD, DMF, 0°C; c) I_2, Imidazole, Toluene, 75 – 80°C; d) Ac_2O, Pyridine; e) $P(OEt)_3$, reflux; f) NaN_3 (1.1 eq), DMF, overnight, 100°C; g) Ac_2O, Pyridine; h) Alkyne, $CuSO_4.H_2O$, sodium ascorbate, 2-propanol, H_2O, 40°C, 20 – 24h.

In this report, unlike the previous work [14], we used regioselective iodination directly from the 1,6-dihydroxy epoxide **4** in a shorter steps and reasonable yield, and also used triethylphosphite for the synthesis of more stable diethoxy phosphonate **7** instead of trimethylphosphite. Sodium azide triggered ring opening of the diethoxy epoxide **7** afforded hydroxy azide product [8]. Acetylation of dihydoxy azide **8** produced fully protected compound **9** which was followed by click reaction with a series of alkynes afforded a library of fructofuranoside derivatives **2a** – **2f**.

In silico docking experiment was carried out to study interactions between the seven analogs and sialidase. The three dimensional (3D) structure of neuraminidase used for the *in silico* docking experiments was 2HU0.pdb found in a protein data bank [15]. It consists of octamer, so that monomer A was selected for the docking. The *in silico* docking experiment was carried out on an Intel Core 2 Quad Q6600 (2.4 GHz) Linux PC with Sybyl 7.3 software (Tripos, St. Louis, MO) [16]. All compounds were docked into the protein well.

Influenza A virus neuraminidase consists of 449 amino acids. 2HU0.pdb contains residues between 62-232 and 234-447, and is composed of two tetramers. Chain A containing N1 of H5N1 was selected for the docking study. It had (3R,4R,5S)-4-(acetylamino)-5-amino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (Tamiflu^{TM}) as a ligand, so that the residues residing its binding site were determined using LigPlot analysis [17]: Arg371, Tyr347, Asn294, Arg292, Ser246, Glu277, Glu276, Arg152, Asp151, Trp178, Glu119, and Tyr406 [Fig. 4].

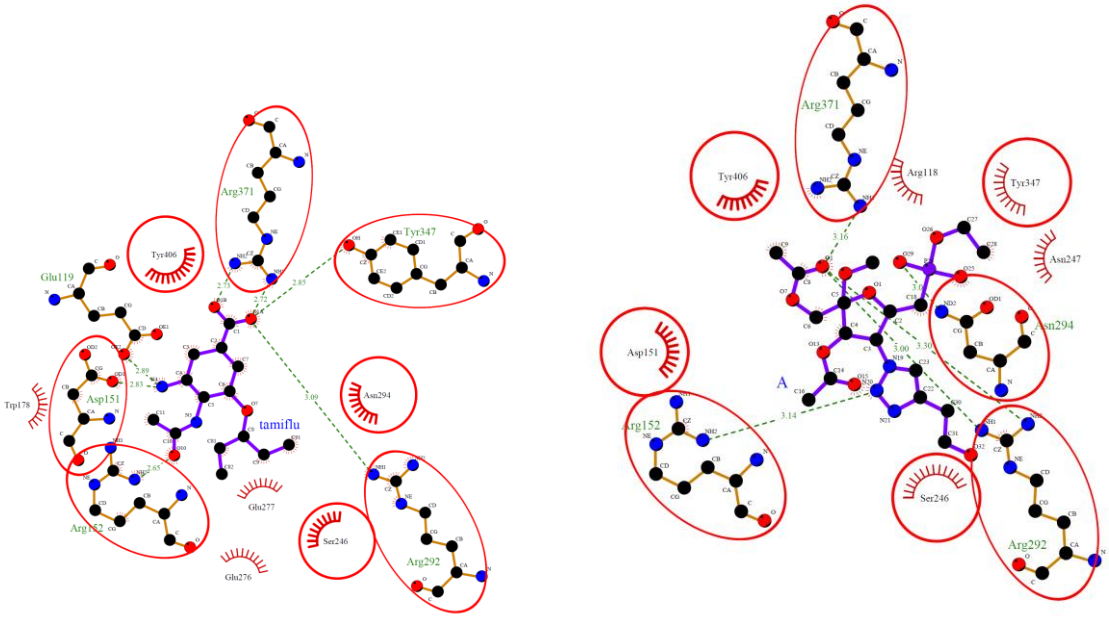


Fig. 4. Binding sites of (left) neuraminidase-tamiflu complex and (right) neuraminidase-compound **2a** complex analyzed using LigPlot [17]. Red circles denote identical residues interacting with tamiflu and compound **2a**, and green dot lines are H-bonds..

The FlexX program used for the docking study requires residues of the binding site, so that these residues were adopted. An apo-protein without a ligand was obtained by Sybyl program and energy minimization was performed.

The 3D structure of compound **2a** listed in Scheme 1 was determined using molecular modeling. From the docking experiments, 30 complexes of apo-protein neuraminidase and compound **2a** were generated and a complex showing the best docking score and docking pose was selected [18]. As shown in Fig. 4, 8 residues such as Arg152, Asp151, Tyr406, Arg371, Tyr347, Ser246, and Arg292 were observed in both neuraminidase-tamiflu complex and neuraminidase-compound **2a** complex, and 6 residues were involved in the hydrophobic interactions. However, while the former shows 7 hydrogen bonds, the later has 5 H-bonds [Fig. 5]. As a result, the binding condition of neuraminidase-compound **2a** complex can be weaker than that of neuraminidase-Tamiflu complex. Likewise, the docking experiments for compounds **2b** - **2f** were carried out and analyzed. All compounds listed in Scheme 1 showed weaker binding condition than Tamiflu.

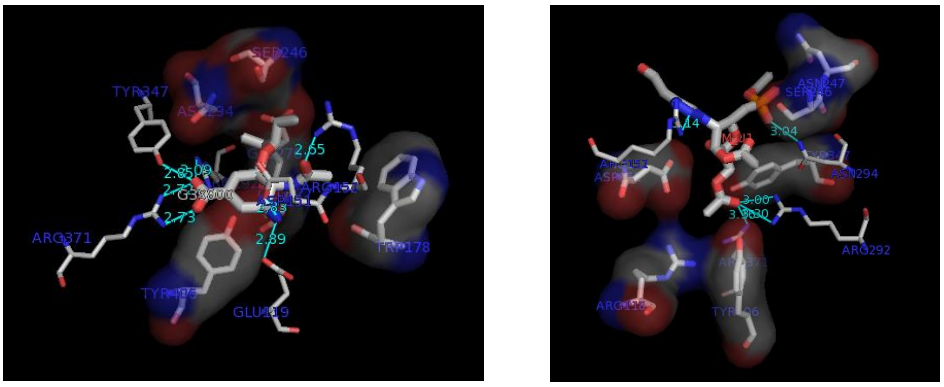


Fig. 5. The 3D images of (left) neuraminidase-tamiflu complex and (right) neuraminidase-compound **2a** complex generated using PyMol [The PyMOL Molecular Graphics System, Version 1.3; Schrödinger, LLC, Cambridge, MA].

In conclusion, we have developed of a series of potential sialidase inhibitors based on very inexpensive and easily available ring system, D-fructofuranoside scaffold. These precursors are highly versatile allowing modification around the ring and thus study their interaction with the sialidase active site. In this report, we explored the effect of the C-4 position and in addition, replaced the carboxylate group with a phosphonate group retaining a negative charge under physiological conditions. These analogs were then evaluated for their interaction with the sialidase binding pocket using in silico docking method. The docking results showed that hydrogen bonding and electrostatic interactions were highly correlated with the activities of neuraminidase inhibitors, followed by hydrophobic and steric factors. These data indicate that the compounds could be further explored for the design of viral sialidase selective inhibitors.

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Conflicts of Interest: The author has no conflict of interest related to this study to disclosure.

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