Bioorganic & Medicinal Chemistry Letters 24 (2014) 636–643

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Bifunctional thiosialosides inhibit influenza virus

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ARTICLE INFO

Article history:
Received 17 October 2013
Revised 23 November 2013
Accepted 27 November 2013
Available online 11 December 2013

Keywords:
Influenza virus
Sialic acid
Glycoconjugates
Inhibitors
Bifunctional

ABSTRACT

We have synthesized a panel of bivalent *S*-sialoside analogues, with modifications at the 4 position, as inhibitors of influenza virus. These first generation compounds show IC_{50} values ranging from low micromolar to high nanomolar in enzyme inhibition and plaque reduction assays with two intact viruses, Influenza H1N1 (A/California/07/2009) and H3N2 (A/Hongkong/8/68).

Influenza is an opportunistic pathogen that causes severe respiratory illnesses. The virus accounts for millions of infections worldwide each year, leading to significant morbidity and mortality. Senior citizens (over 65 years of age), young children, and individuals with underlying health problems are at increased risk for infection and subsequent secondary illnesses like pneumonia.[1] Along with vaccines, Oseltamivir®, Zanamivir®, and Premavir® (Fig. 1B–D) are among the frontline drugs used to fight the infection.[2] These FDA approved transition state analogs inhibit the activity of the viral surface enzyme, Neuraminidase (NA), from cleaving the residual *N*-acetyl neuraminic acid (or sialic acid, Figure 1A) present on the infected host cell, consequently arresting the virus progeny from escaping the cell. However, some strains which include emerging, highly virulent strains that can potentially cause pandemics, have started to exhibit resistance to some of these inhibitors. In one recent surveillance study, 100% of all patients had a resistant strain to Oseltamivir®,[3] and another study identified a strain that was resistant to both, Zanamivir® and Oseltamivir®.[4] These studies emphasize the need for vigilance and continued development of novel drugs. Recently, a new class of mechanism based anti-viral compounds against NA has been reported to show broad spectrum anti-viral activity against all strains in vitro and in animal models (Fig. 1E, F).[5,6] Unlike transition state analogs, these compounds are similar to natural substrate, with modifications at the 3 and 4 positions, which enhance the binding activity. All of these recent reports are based on the natural substrate (sialic acid) with structure based drug design leading to increased and highly specific inhibition.

A slightly different strategy of developing inhibitors follows Nature's method of using multivalency that target Hemagluttinin (HA), a surface glycoprotein that binds to cell surface sialic acid to facilitate viral entry and NA. HA and NA are excellent targets for inhibition, because labeling experiments have shown that an individual viral particle has approximately 200–300 copies of trimeric HA and 50–100 copies of tetrameric NA, leading to over 800 binding sites per virion.[7,8] Indeed, mucins, endogenous sialylated proteins released by respiratory epithelial cells, capture viral particles by virtue of their multiple sialic acid residues which bind to HA and NA, and flush them away by the natural process of sneezing and coughing.[9–11] A similar approach using glycopolymers and glycodendrimers with pendant sialic acids have been generated to capture the virus. It has been demonstrated that increase in the valency of the sialic acids increases the inhibitory effect significantly, from the micromolar IC_{50} value of a mono/di/tri saccharide, to the micromolar/submicromolar range.[12–14] These reports have focused on the architecture of the scaffolds, leaving the sialic acid unit unmodified.

In this Letter, we have increased the intrinsic binding affinity of a single sialic acid unit by introducing an amine/guanidine group at the 4 position of sialic acid. The basic amine/guanidine group fits perfectly into the binding pocket of viral NA as it interacts with the trio of amino acids present in the binding pocket.[2,15] We introduced sulfur at the anomeric center, which provides additional advantages. First, replacing the *O*-sialoside with an *S*-sialoside makes the ligand more robust as we and others have demonstrated that *S*-sialosides are not readily cleaved by the virus.[16,17] Second, the thiol group reacts with triflates and/or bromides present on multivalent scaffolds readily, thereby yielding rapid access to multivalent molecules. The combination of NA resistant sialosides

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http://dx.doi.org/10.1016/j.bmcl.2013.11.077

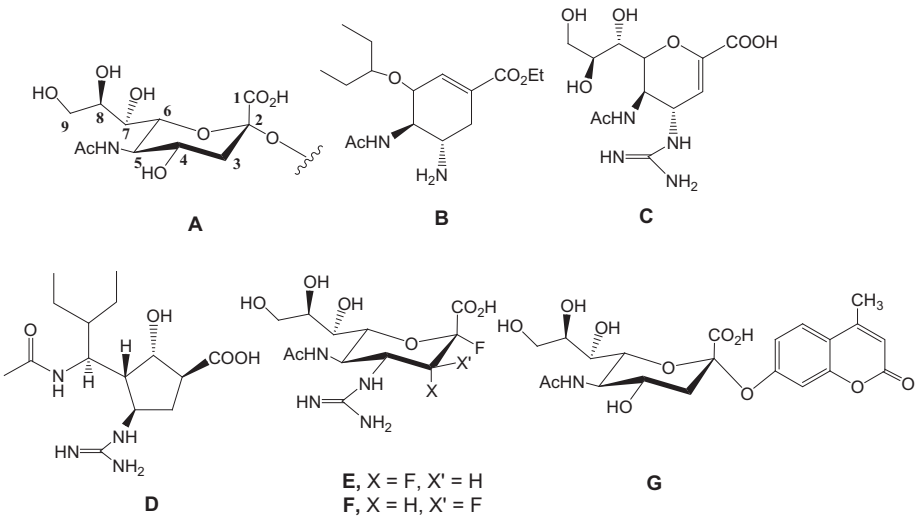
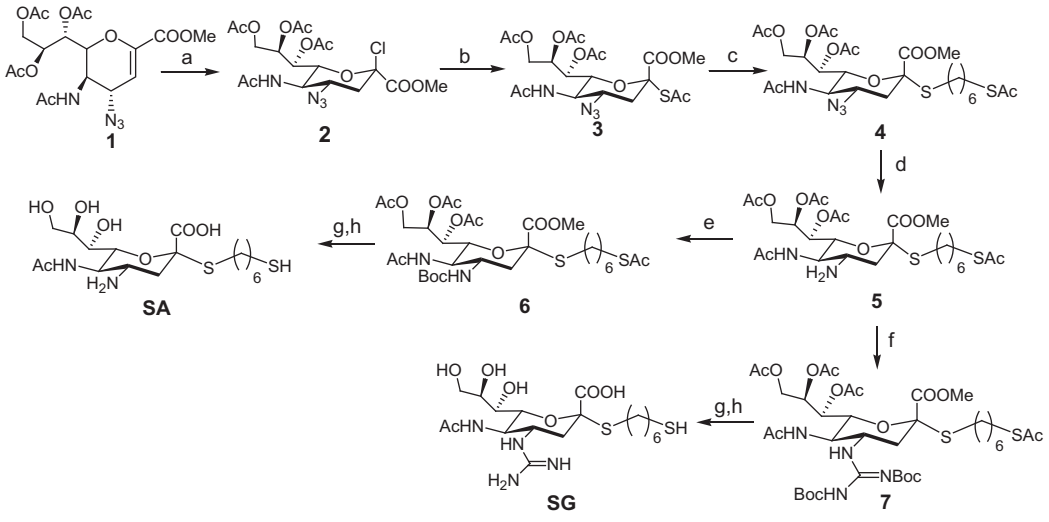


Figure 1. Structures of *N*-acetyl neuraminic acid (sialic acid) analogs. (A) Sialic acid with the numbering scheme. (B) Oseltamivir® (C) Zanamivir®, (D) Premavir® (E–F) Flourinated analogs of Sialic acid. (G) Fluorogenic substrate of sialic acid for obtaining IC_{50} values.

combined with the multivalent presentation provides easy access to a new class of influenza virus inhibitors. Using this approach, we constructed a panel of bivalent compounds, (Schemes 1 and 2) that are similar to the natural substrate and evaluated their inhibitory activities with two viral NAs, N1 and N2, and intact viral strains. These first generation compounds show micromolar to nanomolar inhibition and could be further elaborated into potential therapies for influenza.

The design and synthesis of the compounds is shown in Scheme 1. Starting with the known azido compound, **1**,[18] hydrogen chloride was added across the double bond to produce the β-chloride **2**, followed by SN_2 type replacement of the chloride by a thioacetate to introduce the sulfur moiety at the anomeric center in good yield. The α-anomeric conformation in **3** was confirmed by ^1H and ^{13}C NMR spectroscopies. Reaction of **3** with a hydrophobic six carbon spacer in the presence of diethylamine yielded **4**. Next, the azide group of **4** was reduced using triphenylphospine to yield the amine **5**, which was subsequently protected with a *t*-butylcarbonyl group or reacted with a suitable protected guanidinium group to yield **6** and **7**, respectively. Removal of ester moieties was conducted under basic hydrolysis conditions, followed by removal of the acid sensitive *t*-butylcarbonyl groups using trifluoroacetic acid to yield analogs of sialic acid with an amine (SA) or guanidine group (SG) at the 4 position. While not the focus of this report, we incorporated a thiol group at the terminus of the alkyl spacer in SA and SG as it provides facile conjugation to surfaces for capture of the virus or to a scaffold/protein to produce multivalent inhibitors.

We used a series of activated homobifunctional hydrophobic and hydrophilic linkers to produce the dimeric compounds, shown in Scheme 2. Different spacers were used because the bivalent molecules can interact with viral NA in three ways. Briefly, the bivalent molecules can crosslink adjacent NAs on the same tetramer or crosslink two NAs from two different NA tetramers on the same viral particle or crosslink NA's from two viral particles. The minimal distance between the active sites of the NAs in all three possibilities are ~16, 30, 50 Å, respectively.[19] Therefore, using a series of activated hydrophilic and hydrophobic spacers, we generated a



Scheme 1. Synthesis of **SA** and **SG**. Reagents and Conditions: (a) HCl (g), LiCl, CH_3CN, 6 days; (b) KSAc, TBAB (Tetrabutylammonium bisulfate), CH_2Cl_2/H_2O, overnight, 50% over 2 steps; (c) $TosOC_6H_{12}SAc$, DEA, DMF, 65%; (d) PPh_3, THF/H_2O, 12 h; (e) $(Boc)_2O$, TEA, THF, 60% over 2 steps (f) MeS-(C=NBoc)NHBoc, $HgCl_2$, TEA, CH_2Cl_2, 80% over 2 steps; (g) MeOH/NaOH (aq), 1 h; (h) TFA/DCM (1:1). 90% over two steps for SA and SG, respectively.