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Research paper

Design and synthesis of 1,2,3-triazole-containing *N*-acyl zanamivir analogs as potent neuraminidase inhibitors

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ABSTRACT

The design of potent metabolically stable neuraminidase (NA) inhibitors represents an attractive approach for treating influenza virus infection. In this study, we describe the exploitation of the 150-cavity in the active site of group 1 NA for the design, synthesis, and in vitro evaluation of new triazole-containing *N*-acyl derivatives related to Zanamivir. Inhibition studies with influenza virus NAs of group 1 (H1N1) and group 2 (H3N2) revealed that several of them are good inhibitors, with IC_{50} values in the low nanomolar (2.3 nM–31 nM) range. Substituents that form stable van der Waals interaction with the 150-cavity residues play crucial roles in NA inhibition as demonstrated by the potency of **6a** (H1N1 IC_{50} = 2.3 nM, and H3N2 IC_{50} = 2.9 nM). Docking studies indicated that the cyclohexane-substituted triazole ring extended toward the hydrophobic region in the active site of group 1 NA in open form. The high potency observed for inhibitor **6a** may be attributable to the highly favorable hydrophobic interactions in this region.

1. Introduction

Influenza, more commonly referred to as flu, is a respiratory infection caused by RNA viruses of the family *Orthomyxoviridae* (influenza viruses). Influenza is one of the most widespread pandemic human diseases because of its rapid and global outbreaks. Despite significant advances in our understanding of the molecular and cellular aspects of influenza, acute infections caused by the influenza virus occur repeatedly. The outbreaks of avian influenza H5N1 in 1997 and 2003 [1], the spread of H1N1 in 2011, and the emergence of H7N9 in 2013 have all heightened public awareness of global influenza pandemics [2]. Millions of people are adversely affected by the disease, and a pandemic of influenza has posed a continuing threat to public health worldwide. Although vaccinations are important for epidemic management, they may be inadequate in the case of influenza because of the high mutability of the virus and its propensity for transmission of genetic material between subtypes. Thus, there is a demand for development of a drug that can resist mutation of virus and block spreading of the virus.

Many microorganisms, such as the influenza virus, express a neuraminidase (NA) enzyme, a sialidase that cleaves terminal sialic acid linkages in cell surface glycoconjugates and facilitates an essential step in virus propagation. The spread of viral infection could be reduced by inhibition of this catalytic process, which prevents the release of nascent virions from the cell surface and their self-aggregation. Viral NAs are therefore an attractive therapeutic target for anti-flu treatment and development of new antiviral drugs [3]. Tamiflu (Oseltamivir phosphate, **1**) [4], and Zanamivir (Relenza, **2**) [5] are the only two NA inhibitors that are currently available as antiviral drugs for treating influenza infections. In addition, two other NA inhibitors, namely peramivir (**3**) [6] and laninamivir (**4**) [7], have been recently approved as anti-influenza agents (Fig. 1). Despite their tremendous success, these inhibitors have limitations: **2** suffers from low oral bioavailability and rapid renal clearance, and Tamiflu (**1**) exhibits resistance to viral mutation [8,9].

On the basis of sequence analysis, the nine known NA serotypes of influenza A viruses are classified into two phylogenetic groups:

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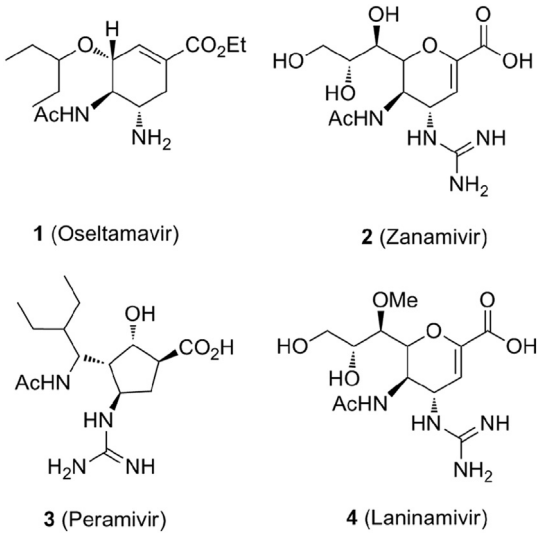

Fig. 1. Tamiflu (**1**), Zanamivir (**2**), and structure of other known NA inhibitors (anti-influenza drugs) in current use or under clinical development.

group 1 consists of subtypes N1, N4, N5, and N8, whereas group 2 contains subtypes N2, N3, N6, N7, and N9 [10]. The most common human influenza virus subtypes are N1 and N2, more specifically, the recent pandemic H1N1 and H5N1 viruses belong to the former subtype. X-ray crystallographic study of several NAs determined that the active site is highly conserved among all serotypes of influenza A virus and influenza B virus [11]. However, in group 1 NAs, a flexible "150-loop" composed of amino acid residues 147–150 and a cavity (the 150-cavity) adjacent to the active site is present, whereas group 2 NAs are devoid of the cavity. In addition, the open conformation of group 1 subtypes adopts a closed form upon substrate (or inhibitor) binding, whereas this loop is always closed in group 2 NAs. The identification of the 150-cavity has led to the discovery of several highly potent inhibitors with increased specificity from the exploitation of additional interactions in this region [12].

Based on the crystal structures of group 1 NAs, several new antivirals resembling a modified Zanamivir skeleton have been developed to target the 150-cavity [13–17]. Fang et al. [14], incorporated various substituents at the C-4 guanidine moiety of Zanamivir by employing N-alkylation, and the resulting derivatives only showed moderate inhibitory activity at the micromolar level against H1N1 NAs. Von Itzstein et al. demonstrated that inhibitors bearing extended substituents at the C-3 position of a sialic acid derivative with a (*p*-tolyl) allyl group lock the flexible 150-loop in the open form of group-1 NAs as determined by X-ray crystallography [15]. Although inhibitors synthesized in this manner exhibit selective inhibition of group-1 NAs, their inhibitory activity remains at approximately the 10 μM level due to the lack of a guanidine group at the C-4 position. Very recently, a C-1 modified Zanamivir derivative with an elongated carboxamide containing a 3-fluorophenyl substituent was reported to produce IC_{50} values of 90 nM against group-1 H1N1 NAs. However, the most notable among the C-1 modified congeners was reported by Wong et al., who discovered that replacement of the C-1 carboxyl group of **2** with a phosphonate group made it more potent compared to Zanamivir against the NAs of avian and human influenza viruses, including **1**-resistant strains [17].

Previously, we reported on exploitation of the 150-cavity in the active site against NAs for the design of new acylguanidine derivatives related to Zanamivir [18]. The most active inhibitor (Fig. 2, compound **5**) exhibited IC_{50} values as low as 20 nM and 25 nM for

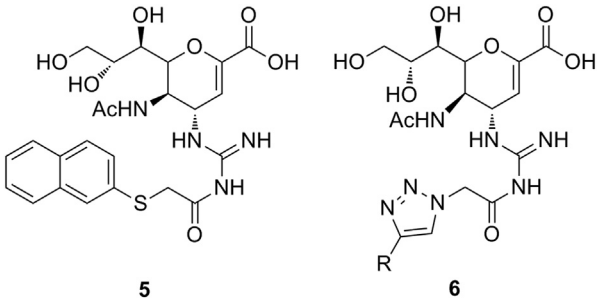

Fig. 2. The structure of a previously reported *N*-acylguanidine analog of Zanamivir (**5**) and the next generation Zanamivir analog **6**.

the N1 and N2 strains of influenza A, respectively. In an attempt to design small molecule inhibitors with improved cellular activity exploiting this unique interaction, we further explored NA inhibitors with *N*-acyl 1,2,3-triazole substituents at the C-4 guanidino moiety of **2** and incorporated functionality to improve inhibition potency. The 1,2,3-triazoles function as rigid linking units mimicking the electronic features of an amide bond. Additionally, triazoles are more stable under hydrolytic and oxidative/reductive conditions when compared to amides. Although triazole modified Zanamivir as neuraminidase inhibitors was reported [13a,13b], to the best of our knowledge, the 1,2,3-triazoles have never been exploited to functionalize the C-4 guanidin $-NH_2$ group in Zanamivir (**2**). The triazole unit in the Zanamivir analogs (**6**) is also expected to be projected into the hydrophobic region of the 150 cavity. Herein, we report the design and synthesis of next-generation Zanamivir analogs (**6**), their anti-influenza activity evaluation in vitro against both group 1 (H1N1) and group 2 (H3N2) subtype NAs, and molecular dynamics (MD) studies of them in complex with NA enzymes.

2. Results and discussion

2.1. Chemistry

Our 2nd generation NA inhibitors contain the Zanamivir core structure and incorporate a hydrophobic substituent appended at the C-4 guanidino moiety. To functionalize the C-4 guanidino group, we selected chemistry that would introduce a common functional group that provides rapid access to different target compounds. We assumed that introduction of an azide functionality would provide opportunities to incorporate new entities through Cu(I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC) [19]. Based on these considerations, we prepared various 1,4-substituted 1,2,3-triazole derivatives as potential 150-cavity binders essentially from one key intermediate.

As shown in Scheme 1, the strategy utilizes the guanidinylation of amine **12** [20] with mono Boc-protected guanidinylating reagent **11a**. For a typical reaction, **11a** was synthesized in 75% yield from azido carboxylic acid **8a** [21] through amide bond formation with **9** and subsequent reaction with $(Boc)_2O$ in the presence of NaH in THF using a method analogous to one reported previously by us [18]. Then, guanidinylation of the amine functional group in **12** with **11a** under slightly basic conditions in THF furnished azide **13a** in 90% yield. The reaction of azide **13a** with phenyl acetylene provided triazole derivative **14a** in the presence of CuI (1 eq) at room temperature. We note that CuAAC is highly regioselective and only a single regioisomer was produced. The formation of a triazole was verified by the appearance of a singlet ^1H NMR signal at ~8.3 ppm (in CD_3OD) for 1,4-substituted 1,2,3-triazole hydrogen. Removal of the Boc group in **14a** with trifluoroacetic acid (TFA) in CH_2Cl_2