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Research paper

Discovery of C-1 modified oseltamivir derivatives as potent influenza neuraminidase inhibitors

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ABSTRACT

Inspired by our initial discovery about a series of neuraminidase (NA) inhibitors targeting the 150-cavity, in present study, we designed, synthesized, and biologically tested a panel of novel oseltamivir derivatives with C-1 modification, targeting the 430-cavity, an additional binding site which widely and stably existed in both group-1 and group-2 NAs. Some of the synthesized compounds displayed robust anti-influenza potencies against H5N1 and H5N6 viruses. Among them, compound **8b** exerted the greatest inhibition, with IC_{50} values of 0.088 and 0.097 μM and EC_{50} values of 4.26 and 1.31 μM against H5N1 and H5N6 strains, respectively, which are similar to those of oseltamivir carboxylate (OSC). And its potency against mutant H5N1-H274Y NA was just 7-fold weaker than OSC. Molecular modeling revealed the elongated group at C-1 position being projected toward the 430-cavity. Notably, although compound **8b** was not sensitive toward H5N1 strain relative to OSC in the embryonated egg model, it displayed greater anti-influenza virus effect against H5N6 strain than OSC at the concentration of 10 mmol/L. Overall, this work provided unique insights in the discovery of potent inhibitors against both group-1 and group-2 NAs.

1. Introduction

Influenza virus is a respiratory pathogen which can give rise to substantial socio-economic loss and burden on medical treatment due to its rapid global spread and high mortality [1]. For example, the worldwide spread H1N1 influenza from the 2009 pandemic killed 300,000 people within only 18 months and its death toll appallingly peaked in younger persons [2], and H7N9 virus is a lethal avian influenza virus endemic to China that has evolved to infect humans since 2013 [3]. Moreover, it is known that the highly pathogenic avian influenza A (H5N1) virus, with 60% mortality, is also a great threat to humans owing to its potential propensity for acquiring human receptor specificity [4].

Currently, there are two classes of anti-influenza agents have been approved by the FDA, namely, M2 ion-channel blockers (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir, zanamivir, peramivir and laninamivir octanoate) (Fig. 1) [5]. In addition, Favipiravir (T-705), which inhibits the RNA-dependent RNA polymerase of multiple RNA viruses, was approved by Japan against influenza infection in 2011 [6]. The above mentioned agents all prevent infection by targeting conserved proteins which are functionally indispensable in lifecycle of influenza virus. However, the M2 ion-channel inhibitors are no longer recommended for treatment of influenza by Centers for Disease Control and Prevention due to their drug resistance and severe side effects on central nervous system, and Favipiravir is only used against influenza virus infections when other anti-influenza drugs are ineffective [7–9]. Thus, nowadays NA inhibitors are the most widely choice for anti-influenza treatment.

Neuraminidase, a surface glycoprotein responsible for cutting the connection between the hemagglutinin and the host cell to facilitate the release of viral progeny from host cells and promoting viral invasion in upper airways, is a promising target for anti-

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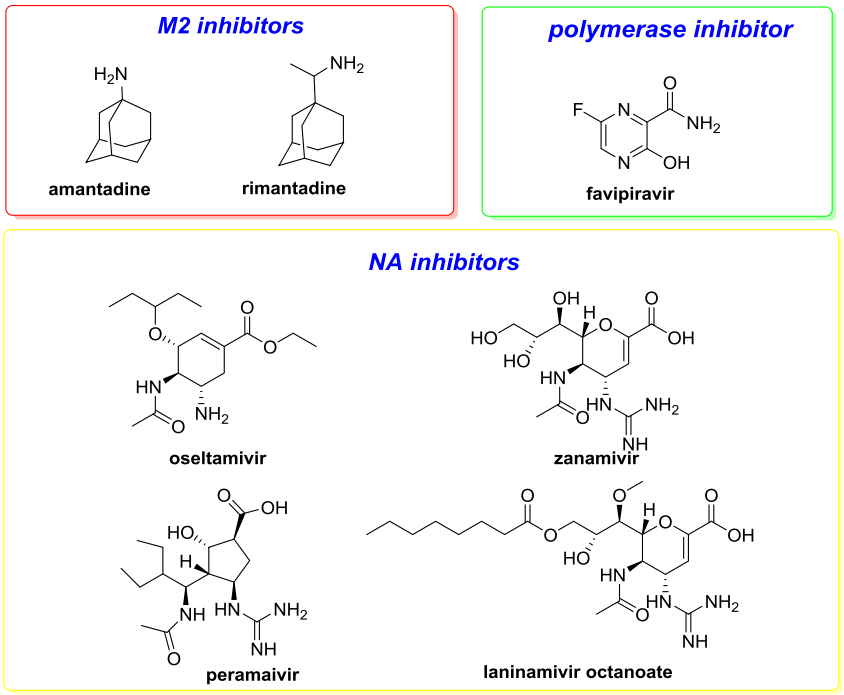

Fig. 1. Three classes of approved antiviral agents for the treatment of influenza virus infection.

influenza drug design. Currently, NA inhibitors have been developed as effective treatments for influenza A and B infections [10]. Among them, orally administered oseltamivir is the first choice and has been widely used since its approval in 1999. However the possibility of widespread resistance has been a concern for several years, for instance, oseltamivir-resistant influenza H1N1 viruses were disseminated worldwide in 2007–2009 [11]. In addition, viral strains that are resistant to the others NA inhibitors are also reported [12,13]. Consequently, it is imperative to develop novel NA inhibitors to overcome drug resistance and to treat influenza infection effectively.

There are two phylogenetically distinct groups in neuraminidases of influenza A viruses: group-1 (N1, N4, N5, and N8 subtypes) and group-2 (N2, N3, N6, N7, and N9 subtypes) [14]. Recently, two new pockets adjacent to the NA active site have been discovered and named as 150-cavity and 430-cavity [15,16]. These two cavities are directly connected with active site and have large molecular volumes, making them promising binding sites for inhibitor design. Interestingly, the X-ray crystallographic structures show that group-1 NAs have an opened 150-cavity adjacent to the active site, but the same structure have not be found in group-2 NAs (Fig. 2) [14]. Based on this finding, a very meaningful exploration on group-1-specific NA inhibitors through targeting the 150-cavity has been reported by our lab. We have identified a series of N-substituted oseltamivir derivatives with low-nanomolar activity against H5N1 NA and H5N1-H274Y NA, and it is worth noting that the most potent compounds **JMC20l** and **JMC32** have potency of N1 selectivity and showed an increase in inhibitory activity of about 10-fold relative to OSC on H5N1 and H5N1-H274Y mutant strains [17].

However, a recently study revealed that the 2009 H1N1 (group-1) influenza pandemic NA lacked the 150-cavity around its active site [18], implying that inhibitors targeted the 150-cavity may not be sufficient to combat all the subtype of group-1 NAs. Besides, in theory, the modified inhibitors targeting the 150-cavity cannot control group-2 NAs efficiently. Therefore, further optimization of oseltamivir is initiated with the aim of obtaining improved potency against all kinds of NA subtypes.

Inspired by our earlier studies, we focus our attention on 430-cavity, since it widely exists in a variety of subtypes, including group-1 and group-2, and could provide greater chemical space for further modification [19]. The crystal structure of oseltamivir carboxylate bounding with NA (N1 and N2) revealed that the C-1 carboxyl group was well exposed toward the newly discovered 430-cavity (Fig. 2) and could serve as the potential modification site for designing specific inhibitors to improve the antiviral efficacy, anti-resistance profiles and to react with various of NA subtypes. As far as we know, little work has been done focusing on C-1 modified oseltamivir derivatives with the exception of some ester prodrugs and isosteric groups of carboxyl [20–22]. Therefore, the exploration of this area is of great value and our proposed strategy is to extend the side chain of oseltamivir carboxylate by attaching additional groups of suitable shape, size, and/or hydrophobicity to fill the 430-cavity and to strengthen the affinity (Fig. 3). Specifically, by introducing various substituents into the scaffold of oseltamivir carboxylate, we have designed and synthesized 27 C-1 modified oseltamivir analogs and evaluated their biological activity.

2. Results and discussion

2.1. Chemistry

The target compounds **5a-5u** and **8a-8f** were prepared as outlined in Scheme 1. All derivatives were synthesized by well-established methods from commercially available oseltamivir phosphate (**1**) as the primary starting material. Reacting with Boc-anhydride in methanol and Et_3N to produce **2**, which was hydrolyzed by NaOH to give the key intermediate **3** [23]. In the presence of HATU, intermediate **3** reacted with different amines to afford **4a-4u**, and eventually the target compounds **5a-5u** were all prepared as hydrochlorides with HCl/ethyl acetate. As for the route to compounds **8a-8f**, the pre-steps were similar until **3** was treated with different ester-protected amino acids to obtain **6a-6f**, and then