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Design and synthesis of 6-oxo-1,4,5,6-tetrahydropyrimidine-5-carboxylate derivatives as neuraminidase inhibitors

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ABSTRACT

A series of 6-oxo-1,4,5,6-tetrahydropyrimidine-5-carboxylate derivatives were prepared to evaluate their ability of inhibiting neuraminidase (NA) of influenza A virus. All the compounds were synthesized in good yields starting from aldehyde by using a suitable synthetic strategy, which showed moderate inhibitory activity against influenza A NA. Compound **6g** exhibited the strongest inhibitory activity against influenza virus A NA (IC_{50} = 17.64 μM), which indicated pyrimidine ring could be used as a core structure to design novel influenza NA inhibitors.

1. Introduction

The influenza virus, avian influenza A (H5N1) virus in particular, is a great threat to human health potentially causing serious public health and economic problems [1]. Person to person transmission is very rare, however genetic reassortment resulting in new virulent subtype of avian influenza virus has made the prospect of a new pandemic particularly alarming [2–5]. Influenza viruses contain two major membrane glycoproteins, hemagglutinin (HA) and neuraminidase (NA). NA is one of the surface glycoproteins of the influenza virus, which catalyzes the release of newly formed virions from infected cells, and is considered as an important target for anti-influenza drugs design [6–8].

Until now three neuraminidase inhibitors have been approved for the treatment of influenza infections: zanamivir[1], oseltamivir[2] and peramivir[3] (Fig. 1) [9]. Zanamivir[1] and Peramivir[3] are rarely used because of their low bioavailability and rapid elimination. Currently oseltamivir[2] is the only orally available drug effective against influenza virus which is world widely used [10–12]. However, the generation and circulation of oseltamivir-resistant seasonal influenza mutants such as H5N1 avian influenza, require the development of new NAIs to fight against potential human influenza pandemic [13–15].

According to the “air plane” model of NA active site (Fig. 2) proposed by Wang et al. [16], there are ten conserved residues and four water molecules in the NA active site, which is divided into four binding sites. Site 1 consists of Arg118, Arg292, and Arg371 which are positively charged to interact − with the carboxylic acid of − NA inhibitors. Site 2 consists of Glu119, Glu227 and Asp151 forming a negatively charged area to interact with base groups such as guanidine or amino. Site 3 is a hydrophobic pocket formed by the side chains of Trp178 and Ile222, which mainly interacts with acetyl group. Sited 4 consists of Glu276 and Glu277, which bind to hydrophobic side chains.

Pyrimidine is an important heterocyclic compound, which shows a wide variety of pharmacological properties. Many pyrimidine derivatives have been reported to have the properties of anti-HCV (hepatitis C virus), anti-bacterial, and anti-cancer. For example, 2-amino-6-hydroxy-3H-pyrimidine-4-one has been demonstrated to be a wide spectrum anti-bacterial reagent [17–19]. This inspired us to explore novel pyrimidine derivatives as NA inhibitors.

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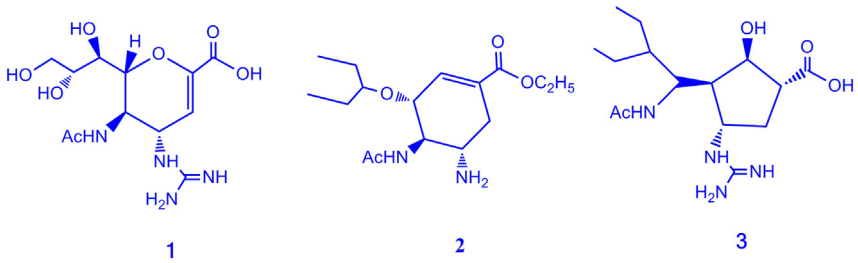

Fig. 1. The chemical structure of three NA inhibitors (NAIs) approved by FDA: zanamivir, oseltamivir, peramivir.

Based on the structure–activity relationship (SAR) of NA we devised and synthesized two series of pyrimidine analogs, 2-amino-6-oxo-1,4,5,6-tetrahydropyrimidine-5-carboxylate (**5a**–**5p**) and 2-acetamino-6-oxo-1,4,5,6-tetrahydropyrimidine-5-carboxy late (**6a**–**6r**). All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, and MS spectrometry. The overall strategies for the synthesis of **5a**–**5p** and **6a**–**6r** are outlined in Scheme 1. The NA inhibitory activity was evaluated in vitro.

2. Chemistry

The procedure of synthesizing compounds **6a**–**6r** was outlined as Scheme 1: A simple procedure that achieves a high compound yield. Piperidine and acetic acid were used as a catalyst and toluene as a water removed reagent. Diethyl malonate reacted with different aldehydes under the refluxing condition, affording compound **4**. This reaction was favorable due to the withdraw-electron group in the benzene ring. The desired product **5**, with yield of 61%–84%, was obtained by treating compound **4** with guanidine hydrochloride in the presence of sodium ethoxide under the condition of refluxing ethanol for 10 h. The cyclization appears to be initiated via Michael addition of guanidine to a double bond and then to complete the cyclization with an ester group. Substituents on the phenyl ring influenced the trans-cis relationship between the 5-proton and the 6-proton of the dihydropyrimidone skeleton, the ratio of trans/cis increased from ortho substitution to para (only trans) illustrated by the ^{1}H NMR characterization. Differentiation between the signals of trans/cis is clear (trans $J_{5,6}$ = 10.8 Hz, cis $J_{5,6}$ = 4.0 Hz). Usually, a para-substituted and small group in ortho compound was obtained stereo selectively as the trans isomer. The stereo selectively of all the compounds were trans isomer [28]. Compound **6** was produced in excellent yield through compound **5** further reacting with acetic anhydride in dichloromethane at room temperature, using DMAP as catalyst.

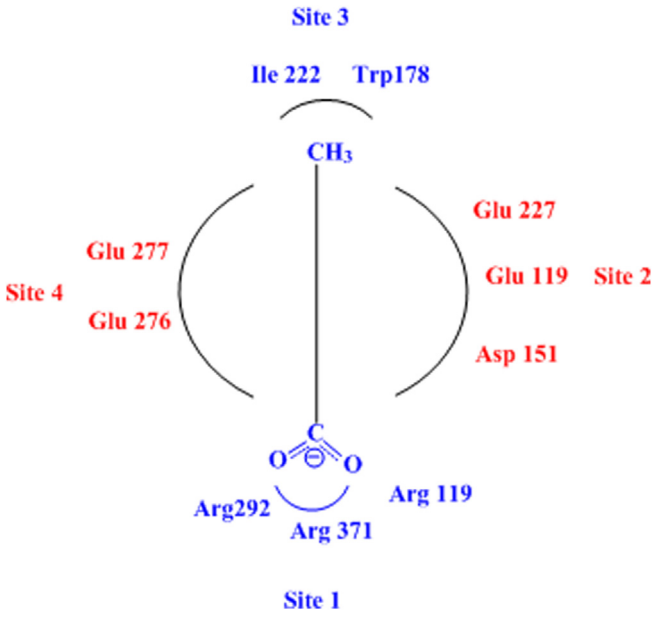

Fig. 2. "Air plane' model of the NA active site".

3. Results and discussion

The neuraminidase inhibitory activities of target compounds were evaluated in vitro. As depicted in Table 1, thirty four compounds displayed the NA inhibitory activity with IC_{50} value from 17.64 to more than 200 μM. Compound **6c**, **6d**, **6e** and **6g** exhibited better inhibitory activities (IC_{50} = 17.64–36.67 μM) than others (Table 1). Compound **6g** (IC_{50} = 17.64 μM) had the best performance. In Table 1, we show that the compound **6** series with acetamide group in C-2 had better inhibitory activities than the series of compound **5** with amino group. According to SAR acetamide group possesses stronger affinity than the amino group when binding to the hydrophobic pocket.

In order to better understand the activity of this series of compounds we used 3D models to simulate the interactions between compound **6g** and N1 (PDB entry code: 2HU0) in an open form based on the docking stimulation (Fig. 3). As mentioned before, the active site of NA can be divided into four different binding areas, which are the potential targets for designing NA inhibitors. Fig. 3 indicates that compound **6g** adopts a similar binding mode in active site as zanamivir or oseltamivir. The carboxyl group at C-5 forms tight salt-bridge with three arginine residues consisting of Arg118, Arg292 and Arg371. The carbonyl group at C-4 also interacts with two amino residues (Arg371 and Arg292) by hydrogen-bond. Acetamide group in C-2 can bind with a small hydrophobic region that was formed from the side chains of Trp178 and Ile222 adjacent to Arg152. The benzene ring with different substituent groups at C-6 binds with active sites through hydrophobic bond.

Compounds **6e**, **6g** and **6o** had better inhibitory activities than **6l**, **6h** and **6j**. This may be attributed to the ortho position substituent group of benzene which can occupy the hydrophobic pocket of active site better. Comparing compound **6b**–**6e**, we find that F and Cl in benzene ring have little effects on the NA inhibitory activity. Compound **6m** with acetamide group at C-2 has better inhibitory activity than **6q** with propionamide group, since the hydrophobic cavity is narrow and there is no space for large groups to fit into this area. Compound **6a**, **6f** and **6n** do not have good activities because these compounds have no potent hydrophobic interaction with proteins. Compared to oseltamivir, the anti-virus activity of compound **6g** without base group at C-6 decreased dramatically. This suggests the base group plays an important role.