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Research paper

Design and expeditious synthesis of organosilanes as potent antivirals targeting multidrug-resistant influenza A viruses

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ABSTRACT

The efficacy of current influenza vaccines and small molecule antiviral drugs is curtailed by the emerging of multidrug-resistant influenza viruses. As resistance to the only FDA-approved oral influenza antiviral, oseltamivir (Tamiflu), continues to rise, there is a clear need to develop the next-generation of antiviral drugs. Since more than 95% of current circulating influenza A viruses carry the S31N mutation in their M2 genes, the AM2-S31N mutant proton channel represents an attractive target for the development of broad-spectrum antivirals. In this study we report the design and synthesis of the first class of organosilanes that have potent antiviral activity against a panel of human clinical isolates of influenza A viruses, including viruses that are resistant to amantadine, oseltamivir, or both.

1. Introduction

The current countermeasures in preventing and treating influenza virus infection have limited efficacy [1]: despite the existence of vaccines and antiviral drugs, influenza virus infection accounts for approximately 36,000 deaths and millions of hospitalizations in the United States during the annual influenza epidemic [2–4]. In addition, influenza pandemics caused by emerging or re-emerging influenza viruses have more catastrophic impact as demonstrated by the 1918 Spanish influenza and the recent 2009 swine influenza [5,6]. Influenza vaccine remains the mainstay in prophylaxis of influenza virus infection [7]. However, it has to be reformulated each year to match the antigens of influenza viruses in the coming influenza season. As the manufacturing of influenza vaccines takes at least six months, influenza viruses might continue to mutate during this period, resulting in vaccine mismatch [8–10]. Moreover, influenza vaccines have little to no efficacy in young children, the elderly, and immunocompromised persons [11]. In addition to vaccines, there are two classes of anti-influenza drugs approved for the prevention and treatment of influenza virus infection (Fig. 1): M2 inhibitors, e.g., amantadine and rimantadine, that inhibit virus uncoating, and neuraminidase inhibitors, e.g., oseltamivir, zanamivir, and peramivir, that inhibit virus release [1]. Resistance to both classes of drugs, however, now necessitates the development of the next generation of anti-influenza drugs [12]. Amantadine and rimantadine are no longer recommended due to widespread drug resistance. Resistance to the only orally available drug, oseltamivir, continues to rise, and the 2008–2009 seasonal H1N1 strain was completely resistant to oseltamivir due to a H275Y mutation [12]. Influenza strains that are resistant to both classes of drugs have been reported [13,14]. Moreover, certain highly pathogenic avian influenza viruses such as H7N9 and H5N1, which have the potential to lead to the next influenza pandemic, are also resistant to oseltamivir [15,16]. Thus the next generation of antiviral drugs with broad-spectrum antiviral activity against both drug-sensitive and drug-resistant influenza strains is clearly needed [17,18].

In pursuing the next generation of influenza antivirals, we chose the AM2-S31N mutant as the drug target. AM2 forms a proton-selective channel in the viral membrane and plays important roles during the viral replication cycle [19]: in the early stage AM2 facilitates viral uncoating by acidifying the viral interior, which leads to the dissociation of viral ribonucleoproteins from the matrix protein M1; in the late stage of viral replication AM2 equilibrates the pH across the Golgi apparatus and prevents the premature

Abbreviations used: WT, wild type; DMEM, Dulbecco's modified eagle medium; MDCK, Madin–Darby Canine Kidney; TEVC, two-electrode voltage clamps.

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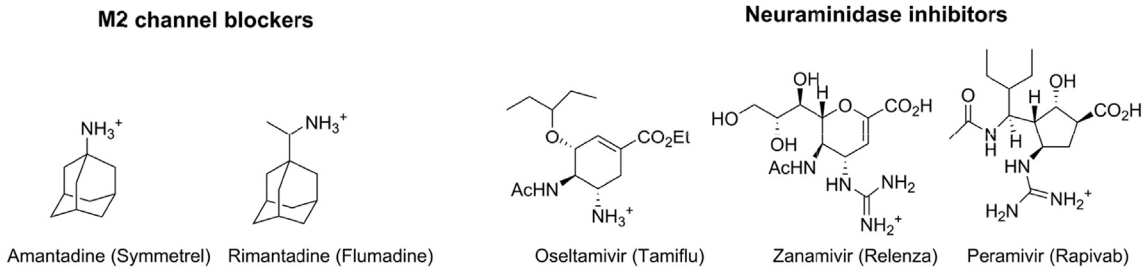

Fig. 1. FDA-approved influenza antivirals.

conformational change of hemagglutinin. More than 95% of currently circulating influenza A viruses carry the S31N mutant in their M2 genes, which renders them resistant to adamantanes [20]. The prevalence of this mutation thus makes AM2-S31N a desired target for drug design [21]. Propelled by structural and mechanistic understandings of AM2 proton conductance and drug inhibition [21,22], we successfully designed the first-in-class AM2-S31N inhibitors with both potent channel blockage and antiviral activity [21,23–26]. Encouraged by this progress, in this study we report the design and expeditious synthesis of organosilane-based AM2-S31N inhibitors. Silicon is a bioisostere of carbon, and the design of organosilane-based bioactive molecules has been hotly pursued [27,28]. Unique features of organosilanes include but are not limited to improved binding affinity, higher metabolism stability, and reduced cytotoxicity. More importantly, from the synthesis perspective, organosilanes are generally easier to synthesize than their carbon analogues. Furthermore, organosilanes have several unique properties that cannot be mimicked by their carbon analogues: (1) silicon can form stable silanediol to mimic the amide bond hydrolysis intermediate, and silanediols have been reported to be potent protease inhibitors (Fig. 2, compound **1**) [29]. (2) organosilanes can go beyond tetrahedral coordination to octahedral coordination (Fig. 2, compound **2**) and such compounds can be used as DNA chelators [30]. (3) Incorporation of silicone into rhodamine results in Si-rhodamine, which emits in the near infrared region (650–900 nm) (Fig. 2, compound **3**) [31]. Such fluorescence probes have been successfully applied for live cell and in vivo imaging [32].

2. Design rationale

In light of advantages of exploring organosilanes as bioactive molecules, we are interested in designing organosilanes as AM2-S31N inhibitors. Three criteria were taken into consideration when designing AM2-S31N inhibitors: (1) the designed organosilanes should meet the pharmacophore requirements of AM2-S31N inhibitors. The pharmacophore of AM2-S31N inhibitors consists of a hydrophobic scaffold such as adamantane, a positively charged ammonium linker, and an aromatic head group with a hydrophobic substitution (Fig. 3, compound **4**) [24–26]. (2) The designed organosilanes should be easy to synthesize by late-stage diversification. (3) Introduction of silicon to a drug molecule increases its hydrophobicity, which might lead to enhanced cellular cytotoxicity; thus a hydroxyl group should be added to the 3-position of adamantane to reduce the overall hydrophobicity and thus cellular cytotoxicity [24–26]. Taking these factors into consideration, the designed organosilanes **5** contain an adamantane cage, an ammonium methylene linker, and a para-substituted aromatic/heterocyclic head group. Such a design allows the introduction of diverse silicon substitutions starting from a common intermediate.

3. Chemistry

We then proceed to synthesize the designed organosilanes **5a**–**f** (Fig. 4). Reductive amination of amantadine **6a** or 3-amino-1-hydroxyadamantane **6b** with 4-bromobenzaldehyde **7a** or 5-bromo-2-pyridinecarbaldehyde **7b** gave the intermediates **8a**-**8c**. The yields range from 75% to 82%. In the next step, five equivalents of *n*-butyl lithium were used to generate the aryl lithium in the presence of a hydroxyl and an amine group. The resulting aryl lithium was then reacted with a variety of silyl chlorides to furnish the final products **5a**–**f**. The yields range from 62% to 78%.

4. Results and discussion

4.1. Channel blockage, antiviral efficacy, and cytotoxicity of organosilane-based AM2-S31N inhibitors

To validate the design hypothesis, the synthesized organosilanes were tested for their AM2-S31N channel blockage, antiviral activity, and cellular cytotoxicity by electrophysiological two-electrode voltage clamp (TEVC) assay, plaque assay, and neutral red assay, respectively (Table 1). Two compounds, **9** and **10**, which were the

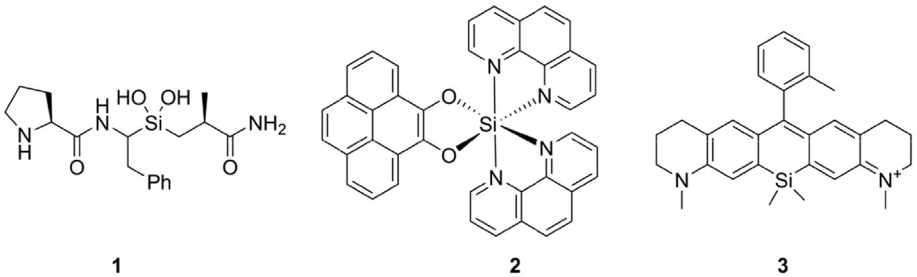

Fig. 2. Representative bioactive organosilanes. Compound **1** is a silanediol peptidomimetic and was designed as a serine protease inhibitor [29]. Compound **2** is an octahedral silicon complex and was designed as a DNA intercalator [30]. Compound **3** is a Si-rhodamine that emits in the near infrared region [31].