Discovery of multiple lead compounds as M2 inhibitors through the screening of a focused library of scaffold-hops postprint

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Abstract

The discovery of new anti-influenza drugs is urgent, considering the threat of so-called swine flu and Spanish flu. Although Adamantane derivatives are the only M2 inhibitors approved as anti-influenza A virus drugs, their use is limited in the US due to drug resistance. Herein, we report the rapid generation of multiple lead compounds as M2 inhibitors through screening of a focused library designed with a scaffold-hopping strategy based on Amantadine.

Full Text

Preamble

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Abstract

The threat of swine flu and Spanish flu makes the discovery of new anti-influenza drugs urgent. Although adamantane derivatives are the only M2 inhibitors approved as anti-influenza A drugs, their use is limited in the US due to drug resistance. Herein, we report that multiple lead compounds as M2 inhibitors were rapidly generated through the screening of a focused library designed with a scaffold-hopping strategy based on amantadine.

Introduction

The outbreak of new H1N1 influenza (swine flu) is spreading around the world, once again presenting a grave threat to human health. Although vaccination is the ideal way to prevent influenza viruses, the preparation of a new vaccine takes six months or more. Consequently, antiviral drugs represent the most important short-term resource for combating the disease. However, when examining our limited stocks, no more effective drugs are available.

The only existing anti-influenza A drugs are M2 inhibitors (amantadine and its derivative rimantadine) and NA inhibitors (zanamivir and oseltamivir). Even worse, amantadine and rimantadine have been limited for use in the treatment of influenza A in the US due to the rapid development of resistance. Additionally, there is growing concern that antineuraminidase-resistant viruses may emerge if these drugs are widely used. Given the threat of this deadly infectious disease and the very limited therapeutic options, there is an urgent need to discover new types of M2 inhibitors for the development of novel anti-influenza drugs. Although amantadine reached the market 40 years ago, all reported M2 inhibitors to date are adamantane derivatives (Figure 1 [Figure 1: see original paper]) with BL-1743 as the only exception, leaving many questions unanswered.

For many years, high-throughput screening (HTS) of corporate compound libraries has not fulfilled expectations. While this strategy still plays a key role in lead generation, there is growing interest in the design of focused libraries. Focused screening has emerged as a more rational approach that emphasizes quality rather than quantity. Although many publications have discussed trends and applications in drug discovery, there is a shortage of successful case studies, and most are limited to in silico approaches.

Thus, we designed and screened a focused compound library (<100 compounds) of scaffold-hops based on amantadine to generate new lead compounds in the M2 inhibitor class. Herein, we not only provide proof of concept that screening of a focused library is highly efficient, but also discovered multiple lead compounds to support antiviral drug discovery.

Results and Discussion

The mechanism of M2 inhibitors is to block the ion channel activity of the M2 protein of most influenza A viruses, thereby inhibiting viral replication through blockade of hydrogen ion flow. The amino group in amantadine is likely the pharmacophore necessary for blocking hydrogen ion flow, while the adamantyl group represents the chemotype. For unknown reasons, nearly all studies except for BL-1743 have focused on searching for new aminoadamantane derivatives, with less investigation conducted on the scaffold itself.

Our focused library design strategy was based simply on the structure-activity relationships of amantadine, keeping the primary amine constant while diversifying the scaffold. The scaffold covers different molecular properties with

emphasis on steric effects. As shown in Figure 2 [Figure 2: see original paper], this library contains linear amines, aromatic amines, monocyclic, bicyclic, and tricyclic amines sourced from major chemical companies. We rapidly assembled the primary amine library from commercial sources.

The library was promptly assayed in vitro using viral inhibition, cell-based assays, and patch clamp recordings. Among these 95 compounds, five compounds were identified for the first time as M2 inhibitors with activity comparable to amantadine in vitro.

Table 1 . Compounds as active as amantadine in the focused library (Chufang and Zhiyuan)

[Note: The original table content appears corrupted and contains fragmented chemical structures and data. The table reference marker is preserved as required.]

The activity data demonstrate that the M2 ion channel can accommodate some range of chemical space but requires a minimum steric functional group to block the channel. Both linear, monocyclic, and aromatic amines show no effect; however, substituted cyclohexylamine, some bicyclic, and tricyclic amines can exert inhibition comparable to amantadine. Expanding the size of amantadine by adding substituents on the ring, such as methyl or hydroxyl groups, results in loss of activity.

Although these scaffolds along with amantadine are not typically selected by medicinal chemists for drug development, the risk-versus-benefit equation may provide opportunities for these otherwise unfavorable scaffolds. These chemotypes can be used for drug discovery against acute and deadly infectious diseases. Thus, this study not only provides proof of concept that focused library screening is indeed practical for lead generation, but also discloses several new M2 inhibitors for scientists to discover new anti-influenza drugs. Drug discovery in academia may achieve good output through efficient use of focused library screening. As Louis Pasteur noted, "chance favors the prepared mind."

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Supplementary Information

Experimental Methods

Construction of Focused Library

All compounds are commercially available from Aldrich, Acros, and other sup-

pliers.

Viral Inhibition Assay (Chufang)
Protocol and data available.

Cell-Based Assay (Chufang)
Protocol and data available.

Electrophysiological Recordings by Patch Clamp (Zhiyuan)
M2-transformed HEK293T cells were used 24-48 hours after induction with 1 µg/ml tetracycline. Perforated whole-cell voltage-clamp recordings were carried out at room temperature (23-25°C) using an Axopatch 200B amplifier (Axon Instruments Inc., Union City, CA) as described previously (Li et al., 2004). Recording electrodes were pulled from 1.5 mM borosilicate pipettes (World Precision Instruments, Inc., Sarasota, FL) using a horizontal puller (Model P-87; Sutter Instrument Company, Novato, CA).

In the majority of experiments, the extracellular solution consisted of 150 mM NaCl, 1 mM MgCl , 1 mM CaCl , 10 mM glucose, and 10 mM HEPES, adjusted to pH 6.8, or 10 mM MES, adjusted to pH 5.5 and alternative pH values by the addition of either NaOH or HCl. The patch electrode had a resistance between 1.8 and 2.5 MΩ. The pipette tip was initially filled with amphotericin-free pipette solution containing 130 mM Cs-methanesulfonate, 24 mM CsCl, 1 mM CaCl , 1 mM MgCl , 10 mM HEPES, and 200 µg/ml amphotericin B. The pH of the intracellular solution was adjusted to 6.8 with CsOH. Salts and drugs were obtained from Sigma-Aldrich (St. Louis, MO) unless noted otherwise. The currents were filtered at 10 kHz with 16-bit accuracy using Macintosh G4 computers (Apple Computer, Cupertino, CA), ITC-16 analog-to-digital boards (Instrutech, Port Washington, NY), and external operations compiled in IGOR Pro (Wavemetrics, Lake Oswego, OR). Drug applications and changes in extracellular pH were performed using a commercially available automated fast solution exchange system (RSC-200 Rapid Solution Changer). All data are reported as mean ± SEM for n number of cells. Differences in antagonist inhibition were determined from statistical tests using IC values, and comparisons between two groups were made using Student' s t-test.

Note: Figure translations are in progress. See original paper for figures.