Background. Influenza pandemics occur when a new influenza strain emerges to which the human population has no immunity. The rapid spread of such a pandemic (H1N1) virus has put more human risk of infection and that raised the concern of a new pandemic. In addition, the virus has caused human fatalities in some cases. There is no vaccine against this virus. Moreover, appearance of neuraminidase inhibition (NA) resistant strain has been described as a neurotropic strain. Treatment is effective and could represent a new influenza virus. We identify and partially characterized infections of the NA-mediated entry process.

Methods. HA (H2) from Asian H5N1 (Qinghai strain) was used to generate pseudoviruses in 293T cells. Viral infectivity was measured in a high throughput screen (HTS) to identify small molecule inhibitors of HA (H5) mediated entry activity.

Results. We have so far screened approximately 40,000 discrete compounds and identified 176 primary hits (720 compounds). Primary hits were screened with pseudovirus vectors expressing an unglycosylated HA (H5) and IAV (H1N1) strain. They were evaluated for their potency and cytotoxicity with neuraminidase-resistent compounds. Only 16 of the primary hits specifically inhibited the HA mediated entry process. The final hits from this HTS were 6246. All the 360 compounds exhibited IC50 values of >750. The HA inhibitors can be represented as 6246 members each with limitations.

Conclusions. We have identified new compounds with potential to be developed as viral agents for the prevention and treatment of influenza infection. Compounds may also be used as molecular probes for the study of influenza virus entry into host cells.

INTRODUCTION

Influenza A viruses cause recurrent epidemics with substantial human morbidity and mortality, and are continually evolving. Swine influenza virus (IAV) pandemics occur when a new influenza strain emerges to which the human population has no immunity. New influenza strains emerge from the avian, fowl, and mammalian strains and are in constant evolution to cause influenza pandemics. The 1918-1919 influenza pandemic started at the height of World War I, which caused 20-40illion deaths worldwide. Asian H5N1 and the Hong Kong H5N1 influenza pandemics emerged by reassortment between the circulating human influenza virus and an avian H5N1 influenza virus. We are now faced with concerns regarding the emergence from the highly pathogenic avian influenza whooping cough (H5N1) virus and its potential to evolve into a pandemic strain. In addition, current spread of pandemic influenza A (H1N1) virus strains has significant implications for public health and the ability of government agencies to develop effective countermeasures against these strains. Since the first recorded direct human-to-human transmission of avian influenza A (H5N1) in 1997, World Health Organization (WHO) has received 465 confirmed H1N1 cases with 302 fatalities from 2003 to 2010. Vaccines, currently the primary strategy for protection against influenza infection, are only effective if they match the circulating virus strain. Since the rapid and dynamic evolution of the influenza virus cannot be predicted, a pandemic vaccine cannot be developed in advance and so new emerging strains will still be a problem as these inhibit influenza infections regardless of its genetic variations. However, the efficacies of the currently approved vaccines are lower because of the emergence of resistant strains, including the emergence of anti-influenza (Tamiflu) resistant variants of both human and avian influenza A H5N1 virus C5). These drug resistant viruses have highlighted the need for developing novel antiviral therapeutics with reduced resistance potential.

We report here the identification of a new class of influenza A virus inhibitors that target the viral envelope protein hemagglutinin (HA), HA mediate the binding and entry of the influenza virus into the host cell. Viral entry is a critical target for influenza inhibitors because inhibition of this step can block the propagation of viral infection, minimizing the chance for drug resistance to evolve and acquire drug resistance. Entry inhibitors have been developed for several important viruses and the TIPS has approved the use of the peptide-like entry inhibitor, enturain (5). Entry inhibitors (protease inhibitors have also been identified with the formation of the fusogen structure (HA). HA is a class A envelope protein, and mediates influenza virus entry through receptor binding and fusion of the virus with host cells. Like other class A envelope proteins, it also undergoes a series of conformational rearrangements during fusion. Our plan for developing new entry inhibitors is to target HA. We have performed a high throughput screen (HTS) to identify HA specific inhibitors. While HA displays significant conformation and substrate specificity, the HA receptor binding and domain remain highly conserved. Targeting these two highly conserved sites will inhibit influenza virus infection with a lower risk of resistance development.