Designing Inhibitors for the SARS CoV Main Protease as Anti-SARS Drugs

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ABSTRACT   **Severe Acute Respiratory Syndrome (SARS) is a serious respiratory illness reported in parts of Asia and Canada. A novel coronavirus (CoV) has been isolated and identified as the cause of the SARS for which there is currently no effective treatment. Given the epidemic, the rapid development of efficacious antiviral drugs is needed. The key replicative enzyme SARS CoV main protease (Mpro) represents an attractive target for antiviral chemotherapy. Detailed structural study of the substrate binding cavity led to the generation of a 3-D pharmacophore. Subsets of chemical structures were extracted from the commercial databases by using the defined pharmacophore. Compound mapping to the pharmacophore were docked into the substrate-binding cavity and scored. The selected chemicals were assayed against the SARS CoV Mpro for their inhibitory activity. Three of the compounds showed significant inhibition of the SARS CoV Mpro at low micromolar concentration. This study provides potential lead compounds for specific SARS CoV protease inhibitors. It also signifies the utility of computational techniques for rapid discovery of inhibitors for novel targets.**

INTRODUCTION

One of the most significant events of 2003 was the "SARS Outbreak." It was a disease unknown to mankind, which spread rapidly from continent to continent, and amazed many by its quick transmissibility. By the end of the epidemic, SARS had affected 8442 individuals and had caused 916 deaths in 32 countries. Thus, SARS was purported to have a fatality rate as high as 11% (1). Moreover, the epidemic brought the world's economy to its knees as the economic cost of SARS amounted to US$30 billion dollars (2). Furthermore, three new confirmed and one new probable case of SARS were diagnosed in the month of December 2003. Thereafter a few new cases in China were reported in 2004. Currently, there are no treatments available to eradicate, stop or slow the virus's life cycle in an infected body; however, recently, scientists have found an antibody, which blocks the disease from attacking the human cell. This could be used as a preventative measure. The purpose of our project is to design non-peptidal inhibitors for the

Severe Acute Respiratory Syndrome Coronavirus' Main Protease (SARS CoV Mpro) as anti SARS drugs. Extensive research to identify targets for chemotherapy has led to the identification of protease inhibitors as an attractive approach. Using our designed non-peptidal inhibitors, we plan on blocking the active site of the main protease so that the virus is unable to replicate.

HYPOTHESIS

In one of the stages of viral replication, the SARS main protease enzyme cleaves functional proteins from the poly-protein needed for the viral replication. This action is necessary for the disease to spread inside the human body. To counter this process, biochemists have proposed inhibitors, which can stop up to 99% of the viral spread. Using the same concept, we hypothesized that the use of such inhibitors, to stop the highly contagious disease SARS, might be a viable therapeutic option. To design the inhibitors, we used modern structure base computational drug design strategy. This approach utilizes structural information of the substrate binding pocket of the target enzyme obtained from examining the available crystal structure of the target enzyme with the help of different drug designing

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software. Then small molecule inhibitors would be proposed by examining their interactions with the amino acid residues of the substrate-binding pocket of the target enzyme.

METHODOLOGY

A target enzyme, which is essential for viral survival, was identified and inhibitors to block the target enzyme function and thereby prevent the virus to grow were designed.

A detailed study of the structure of the protease was conducted and the crystal structure of SARS CoV Main protease bound with a substrate like covalent inhibitor was used as the starting point for the modeling study. Structure manipulation and visualization were done in SYBYL 6.6 (Tripos Inc., St. Louis, MO). In this step, we analyzed properties of different amino acids that were present in the substrate binding pocket. Using the vital interactions between the substrate analog inhibitor and the binding pocket residues, we designed a pharmacophore. In the drug design process, a pharmacophore, is the minimum of structural features of a candidate drug that will allow binding to the target protein. Designed pharmacophores were used as a query to search the databases, which contain hundreds of thousands of commercially available drug-like-compounds. This was done by the program UNITY (a program provided with SYBYL). We searched three databases, namely the Interbioscreen (IBS), COMBILAB and National Cancer Institute (NCI). A list of compounds that fit the constraints of the pharmacophore was obtained through this search. We obtained 305 hits from IBS, a database of 195,988 compounds; NCI_2000, which consisted of 234,055 compounds and yielded 74 hits. Combilab, a database of 125,793 compounds, gave us 270 hits. In total, we had 649 hits from the 555,836 compounds searched. After finding a list of the compounds extracted from the commercially available databases, we ran them through the program FlexX (also included in SYBYL). This program docked every compound chosen into the binding pocket of the protease, and gave us a score depending on the interactions (include hydrogen bonding, ionic and hydrophobic) between the compound and the binding pocket residues. Visual estimation of the high scoring compounds provided by the FlexX were carried out to determine their similarity with the pharmacophore and their interactions with the binding pocket. Ultimately, 18 drug-like-compounds were picked and evaluated as potential inhibitors against the SARS main protease. Finally the proposed compounds were purchased. Unfortunately, we were only able to obtain 10 of the

18 compounds. These proposed compounds were evaluated as anti SARS compounds both by in vitro enzyme assay with purified SARS CoV main protease and in-vivo cellular assay using SARS CoV infected cells with all biosafety measures at a collaborating facility.

RESULTS AND DISCUSSION

Among these 10 compounds three compounds showed significant inhibitory activity against the SARS CoV main protease at low micromolar range. Activity against the virus is still pending.

CONCLUSION

By using this directed structure based computational drug design approach, only eighteen compounds were required to be purchased. These compounds were also able to yield inhibitors of SARS CoV Mpro more potent than the covalent substrate analog type inhibitor. This success highlights the value of utilizing computational drug design techniques in drug discovery process. Finally, this project generated a number of new lead compounds which will aid the development of anti-SARS drugs, which might be used for rapid control of future SARS outbreak in Canada and other parts of the world.

AKNOWLEDGEMENTS

REFERENCES

1   Communicable Disease Surveillance & Response (CSR) "Summary table of SARS cases by country, 1 November 2002 - 7 August 2003" Copyright 2003 World Health Organization, 15 August 2003. http://www.who.int/csr/sars/country/2003_08_15

2   Khan, Mehmood-Ul-Hassan "Global comparative study and Socio-economic implications of SARS" Copyright 2004 MMN International Inc. 24 January 2004 <http://usa.media monitors.net/content/view/full/4252/>

3   Yang H., Yang M., Ding Y., et al. The crystal structures of severe acute respiratory syndrome main protease and its complex with an inhibitor. Proceedings of the National Academy of Sciences of the United States of America (PNAS), 13190-13195, 2003.

4   Anand K., Ziebuhr J., Wadhwani P., Mesters J. R., Hilgenfeld 5 R.Coronavirus main protease (3CLpro) structure: Basis for design of anti-SARS drugs. Science, 300, 1763-1767. 2003.

5.   Thiel V., Ivanov K., Putics A., et al. Mechanism and enzymes involved in SARS coronavirus genome expression. Journal of General Virology, 84, 2305-2315. 2003.