Biomedical Data Mining

Extended Abstract

Computational screening of potent anti-dengue inhibitors against dengue NS2B/NS3 protease

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Abstract

Worldwide, the Dengue Virus (DENV) contamination has become a significant undermining medical problem. The World Health Organization (WHO) has announced 390 million individuals are getting influenced with DENV consistently. Despite the fact that there are some enemy of viral accessible in the market to diminish the seriousness of the illness, Still there is a need of medication to totally obstruct the infection replication and fixes the sickness. Consequently, it is of most extreme direness to receive imaginative methods to propel the medication disclosure measure.

In our investigation, we zeroed in on the distinguishing proof of inhibitors against DENV NS2B/NS3 protease complex. NS2B/NS3 protease goes about as a remedial objective in computational enemy of viral medication revelation. In view of the medication repurposing contemplates, a few enemy of viral were drilled down from the past investigations. Out of which, Bromocriptine was chosen as a source of perspective ligand, pharmacophore-based virtual screening is performed. The NS2B/NS3 protease was docked with drug mixtures of ZINC library. Each compound from the library is for all intents and purposes screened utilizing Argus lab dependent on its 3D pharmacophore highlight of the current ligand. Followed by this semi-adaptable docking examines were performed to foresee the best awaiting present alongside its limiting energy and IC50 esteem.

The potential lead compound is sifted dependent on the limiting energy. Further, the lead compound is improved utilizing platform jumping instrument. Sub-atomic powerful recreation examines were performed to uncover the conceivable method of its activity against the NS2B/NS3 protease of DENV. It is reasoned that information acquired through this examination would be of the great valve towards improving the revelation of NS2B/NS3 protease target explicit medication particles with the smallest expense and time.

Introduction

Dengue virus (DENV) of the Flaviviridae family, which likewise incorporates a few other human microbes like Zika virus (ZIKV), West Nile infection, Japanese encephalitis and yellow fever infections, is the most predominant human microorganisms communicated by Aedes mosquitoes with 3.6 billion individuals in danger, especially in tropical and subtropical districts. Every year more than 390 million human contaminations happen in ~110 nations including the southern US and Singapore, which prompts ~25,000 passings for the most part among kids.

DENV causes dengue fever, dengue haemorrhagic fever, and dengue stun disorder. In spite of extreme investigations, up until now, no advertised antiviral medication exists to adequately treat dengue related illnesses. The DENV genome is made out of a 11-kb single-abandoned positive sense RNA, which is converted into a huge polyprotein by the host-cell apparatus upon disease. The polyprotein of the Flaviviridae family should be in this manner prepared into 10 proteins, which incorporate three underlying proteins (capsid, film, and envelope) and seven nonstructural proteins (NS1, NS2A/B, NS3, NS4A/B, and NS5).

The underlying proteins establish the viral molecule while the nonstructural proteins are engaged with the

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replication of the RNA genome, virion gathering, and weakening of the host antiviral reaction, in this manner fundamental for replication of all flaviviruses. The right handling of the polyprotein is executed by have cell proteases including furin and signalaseas, just as an infection encoded NS2B-NS3 protease, which consequently has been set up as a significant objective for drug configuration to treat DENV and other flavivirus diseases. The dengue protease space comprises of the N-terminal piece of the NS3 protein embraces a chymotrypsin-like crease comprising of two β-barrels, each made out of six β-strands, with the reactant set of three (His51-Asp75-Ser135) situated at the separated between the two β-barrels.

Dissimilar to different proteases with a chymotrypsin-like crease, the flavivirus proteases including dengue one, moreover require a stretch of ∼40 amino acids from the cytosolic space of NS2B for its reactant work, in this way called a two-segment protease. While the protease areas receive profoundly comparable designs taking all things together gem structures, the NS2B cofactor was found to accept two unmistakable constructions, specifically, the idle or open structure in the unbound state just as dynamic or shut structure in complex with the substrate peptide by X-beam crystallography. Besides, ongoing NMR examines uncovered that in arrangement, the dengue NS2B-NS3 protease is extremely unique and goes through the trade between two compliances. Notwithstanding, the shut compliance is the significant structure even in the unbound state, which in this manner addresses the best model for structure-guided medication plans.

Curcumin inhibits the dengue NS2B-NS3 protease in a noncompetitive mode. (A) Crystal structures of the dengue NS2B-NS3 protease in the open (inactive) conformation in the unbound state (I); and in the closed (active) conformation in complex with a substrate peptide in sticks (II). The β-strand is colored in purple, helix in yellow and loop in brown for the NS2B cofactor, while the β-strand is in green, helix in cyan and loop in light blue for the NS3 protease domain. The catalytic triad His51-Asp75-Ser135 are displayed in spheres and labeled. (B) Chemical structure of curcumin and the inhibitory data used for fitting IC50 value for curcumin (I). Lineweaver–Burk plot for determining inhibitory constant (Ki) of curcumin on the dengue NS2B-NS3 protease (II). [S] is the substrate concentration; v is the initial reaction rate. The curves were generated by the program GraphPad Prism 7.0. The purple circle is used to indicate that the inhibition is noncompetitive, characteristic of the same Km but varying Vmax values in the presence of curcumin at different concentrations.

Previous efforts for drug development targeting the flaviviral NS2B-NS3 proteases revealed the major challenge in rational design of their active site inhibitors: their active sites are relatively flat. In this context, to respond to the urgency to fight ZIKV and DENV infection in Singapore, previously we conducted an intense attempt to screen inhibitors for the Zika and dengue NS2B-NS3 proteases from natural products isolated from edible plants. We successfully identified a natural phenol curcumin, or 1,7-bis(4-hydroxy-3 methoxyphenyl)-1,6-heptadiene-3,5-dione, with a significant inhibitory effect on both proteases. Further analysis of enzymatic kinetics revealed that curcumin inhibits the Zika NS2B-NS3 protease in a noncompetitive mode. In other words, Curcumin may act as an allosteric inhibitor for the NS2B-NS3 proteases.

Curcumin is of both fundamental and therapeutic interest because it has a significant inhibitory effect on the Zika NS2B-NS3 protease (IC50 of 3.45 μM and Ki of 2.61 μM) as we previously determined. Very recently, curcumin and its four analogues were also found to inhibit the dengue NS2B-NS3 protease as well as replicon replication in DENV-infected cells. Moreover, curcumin is isolated from a very popular food additive yellow ginger turmeric (Curcuma longa). A huge number of previous studies have shown that curcumin owns diversified biological and pharmaceutical activities. Including antitumoral, antimicrobial, anti-inflammatory, antioxidant,

antihepatotoxic, antihyperlipidemic, antiviral, and anti-Alzheimer's disease effects.

In this study, we aimed to understand the mechanism by which curcumin inhibits the dengue NS2B-NS3 protease with biochemical assay and biophysical methods including NMR spectroscopy and molecular dynamics (MD) simulations. For the first time, our NMR studies reveal that in contrast to the active-site inhibitors which act to reduce the dynamics of the dengue NS2B-NS3 protease, curcumin significantly increased the backbone dynamics of the dengue protease particularly on µs to ms time scale.

Nevertheless, we have successfully established the binding mode as derived from the NMR-derived constraints, showing that curcumin binds to a cavity of the dengue NS2B-NS3 protease which has no overlap with its active site. Further MD simulations reveal that the binding of curcumin leads to the disruption of the closed conformation which is essential for its catalytic activities. Altogether, our study provides a dynamic view of the mechanism by which curcumin allosterically inhibit the dengue NS2B-NS3 protease through mediating the equilibrium between the open and closed conformations. Therefore, the modulation of this conformational equilibrium might indeed represent a promising strategy to discover/design small molecules for allosterically inhibiting the flaviviral NS2B-NS3 proteases to treat flavivirus infections.

Materials & Methods

Previously, we have performed a pharmacophore features based virtual screening studies, which has led to the identification of ZINC92615064 compound as a potent NS2B/NS3 protease inhibitor and demonstrated its potential to act as anti-dengue drug-like compound using computational approaches. In this present study, the identified lead compound ZINC92615064 has been made to undergo scaffold hopping based novel library generation, and the resulted novel library of compounds has been virtually screened on to NS2B/NS3 protease towards identifying novel proprietary scaffold of compound which is acting as a potent inhibitor for the given drug target of NS2B/NS3.

Result & Conclusion

A total of 16,847 novel designed compounds library was generated using the scaffold hopping technology based on the structure of the lead compound ZINC92615064. Out of which, compound design no. 3718 has shown the best binding potential with a predicted IC50 value of 417.13 nM along with a permissible range of ADMET properties based on its descriptor values. This NS2B/NS3 protease in complex with compound 3718 was subjected to a rigorous molecular dynamic simulation study to further validate this complex thermodynamic stability, along with the aim to reveal the underlying molecular level interactions and potential mode of action.

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