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Molecular docking studies of MBZM-N-IBT on non-structural protein targets of Dengue virus

Priyadarsee Mishra and Bharat B. Subudhi*

Drug Development and analysis laboratory, School of Pharmaceutical Sciences, Siksha O Anusandhan University, Ghatikia, Khandagiri, Bhubaneswar-751003

ABSTRACT

Dengue virus and Chikungunya virus share a common vector and their co-infection is being increasingly reported. Earlier we have demonstrated efficacy of MBZM-N-IBT against Chikungunya in vitro. Encouraged by this, in the present work we have investigated its potential against non-structural proteins targets of Dengue virus by in silico studies. Molecular docking data has revealed that MBZM-N-IBT has good potential to inhibit NS3 and NS5 proteins. However it has poor affinity for NS1. Considering the critical role of NS3 and NS5 in pathogenesis and progress of Dengue virus, MBZM-N-IBT can be expected to have good inhibition against it.

Key words: Dengue virus, molecular docking, antiviral.

INTRODUCTION

Dengue virus (DENV) has presence in every WHO region of the world and more than 125 countries are endemic to this [1]. DENV infection is borne by mosquito (*Aedes Aegypti*) causing acute febrile illness. The acute phase of this infection lasts for 1-2 weeks and about 5% of this infection leads to life threatening symptoms including internal hemorrhage and organ dysfunction. With cost to society in terms of lost of wages, reduced productivity, death and medical expenses, it has become a major health burden in tropical and subtropical regions of the world. Because of this disease burden, there has been wide research to develop vaccine or antiviral drugs against DENV. However, till date it has not been possible to develop clinically successful vaccine or antiviral against DENV.

DENV exists as antigenically distinct but co-circulating serotypes. The predominant serotype changes regularly (2-4 yeras) and may also co-circulate in a single region. Usually there is no cross neutralization between the serotypes. So antibodies developed by primary infection cannot neutralize subsequent infection by DENV. This may further enhance secondary infected DENV through cross reactivity because of the antibody dependent enhancement (ADE) phenomenon. Because of the same reason, it has not been possible to develop a vaccine against DENV. This is also a hurdle for development of antiviral against DENV.

Dengue fever, unless proceeds to severity, is usually self limiting. There is rapid decline in the viremia during natural course of infection which questions the utility of antiviral [2]. However, load of plasma viremia in case of dengue hemorrhagic fever and shock syndrome (DHF/DSS) is more than 10 times that of common dengue fever (DF) which indicates that increase in plasma viremia may lead to DHF/DSS [3]. So reduction in viremia in early infection during DF with an antiviral is required to prevent the infection progression to DHF/DSS. Besides in severe

dengue it will be essential to reduce the viremia by therapeutic application of antiviral. However, there is no drug available against DENV. In this scenario it is necessary to identify possible drug candidates against DENV.

One of the possible ways to identify drug candidates against DENV is to evaluate previously antiviral lead molecules against DENV. *In silico* approaches including molecular docking is an important tool to identify potential of leads against drug targets in DENV. We have earlier reported efficacy of 1-[(2-methylbenzimidazol-1-yl) methyl]-2-oxo-indolin-3-ylidene] amino] thiourea(MBZM-N-IBT) against Chikungunya virus [4]. Since CHIKV and DENV are borne by same vector, several cases of co-existence of DENV and CHIKV have also been reported [5]. In these cases, finding a single antiviral molecule which can act against CHIKV and DENV is ideally needed. Since we have shown effectiveness of MBZM-N-IBT against CHIKV, we are interested to see the potential of it against DENV targets.

The nonstructural proteins (nsps) of DENV are localized in the cytoplasm to form replication complexes which are involved in viral RNA synthesis [6]. Since nsps are usually responsible for virus replication, virion assembly and evasion of host immune response, they are considered as targets for development of antiviral [7]. Amongst them NS3 protease, NS3 helicase, NS5 methyltransferase and NS5 RNA-dependent RNA polymerase have been suggested to be therapeutic targets for specific antiviral searches against dengue [8-9]. In a recent study, NS1 has also been demonstrated to modulate virion production by interaction with structural proteins [10]. Hence, this can also be considered as a vital target for therapeutic intervention against DENV. With this background we are interested to find affinity of MBZM-N-IBT against these DENV targets.

MATERIALS AND METHODS

Optimization of target structure

The X-ray crystallographic structures of targets were recovered in PDB format from protein data bank. The structure of the target was visualized in discovery studio. Using this program the water molecules and hetero atoms were removed from the structure. The structure was further optimized by removing the polymeric chains. The geometry was optimized using the Argus Lab. Package.

Molecular docking studies.

The molecular docking study was carried out using the Auto Dock-Vina program [11-12]. The optimized PDB structure of targets were loaded and converted to target molecules. MBZM-N-IBT structure was optimized using the Argus Lab. Package and converted to ligand for the Auto Dock-Vina program. To validate the docking study decoys were employed. The structural components of MBZM-N-IBT including isatin, isatin-β-thiosemicarbazone (IBT) and 2-methyl benzimidazole (2-MBZM) were used as decoys. Known inhibitors of specific targets were used as positive controls in the study. The molecular binding affinity (Kcal/Mole) of the ligands obtained from the docking study were tabulated and analysed. The best fitting binding conformation was visualized using the PyMol**.**

RESULTS AND DISCUSSION

MBZM-N-IBT was earlier shown by us to inhibit CHIKV. It was shown to significantly inhibit non-structural proteins (nsp2) of CHIKV [4]. Since CHIKV and DENV have same mosquito vector species, there have been many reports of their co-infection. In this scenario, it is interesting to see, if MBZM-N-IBT can also have some potential binding affinity against therapeutic targets in DENV.

The nonstructural proteins of DENV are considered to be important targets for therapeutic intervention because of their critical role in replication, assembly and release of infectious virions [10]. NS1 of DENV plays distinct functions in immune evasion, pathogenesis and replication. It is critical to RNA replication. Its role in DENV replications is reported to be mediated by its interaction with structural proteins including envelope and precursor membrane, which makes it an important target for antiviral development against DENV [10]. There are no established inhibitors of NS1. So, antiviral which are known to inhibit other non-structural proteins of DENV were randomly taken as positive control. However these antiviral showed poor interactions with this target, justifying the fact that they have not been reported to inhibit NS1. The molecular docking studies have also shown that MBZM-N-IBT does not have very good affinity for this target (Table 1).

The NS3 protease-NS3 helicase is critically involved in the replication and polyprotein processing [6]. NS3 protease is responsible for cleavage at a number of sites, including NS2A-NS2B, NS2B-NS3, NS3-NS4A, and NS4B-NS5. Molecular docking studies have revealed that MBZM-N-IBT has good binding affinity for this target. Its binding affinity is similar to most of standard antiviral previously reported to significantly inhibit NS3. The most stable binding conformation of MBZM-N-IBT shows five polar interactions with GLY-151, ALA-163, GLY-153, VAL-154 and TYR-150 (Fig 1a). There is also non-polar interaction between phenyl group of TYR-150 and benzimidazole group of MBZM-N-IBT. Similarly, it also showed good affinity for NS3 helicase (PDB ID: 2JLR). This protein was co-crystallised with phosphor amino phosphonic acid-adenylate ester. Accordingly, phosphoaminophosphonic acid-adenylate ester was also taken as a positive control along with reported inhibitors of NS3 helicase. MBZM-N-IBT showed a binding affinity of -8.8 Kcal/mole, while phosphor amino phosphonic acid-adenylate ester showed binding affinity of -8.6 Kcal/mole (Table 1). The most stable conformation of MBZM-N-IBT shows four polar interaction with GLU-412, HIS-287, ASP-290 and CYS-428 respectively. Possible non-polar interaction can also be expected from the close proximity of phenyl group to PHE-288 (Fig 1b). It indicates that MBZM-N-IBT is very likely to interact with this target. This is also suggested by the fact that most of standard antiviral showed similar binding affinity.

The NS5 has a methyl transferase domain at its N terminus and an RNA-dependent RNA polymerase domain at its C-terminal end [6]. The RdRp domain is responsible for the replication of the positive-strand RNA genome. Accordingly, inhibitors of RdRp are expected to prevent viral replication. Molecular docking study shows that MBZM has better binding affinity for this target (PDB ID: 2J7U) as compared to the known inhibitors (Table 1). The most stable binding pose reveals three polar interactions with ASN-609, ASP-663 and SER-661. Non-polar interactions are also possible with TYR-606 and HIS-798 which are close to the ligand (Fig 1c).

The methyltransferase domain is involved in the mRNA capping process and is also a good therapeutic target [13]. Molecular docking studies with this target (PDB ID: 3P97) has shown very good binding affinity (-9.0 Kcal/mole) of MBZM-N-IBT (Table 1). Only very few compounds including “compound 10” has been reported to specifically inhibit this target [13]. Our studies have shown that MBZM-N-IBT has relatively better affinity for this target as compared to the known inhibitor. The most stable binding conformation shows five polar interactions with THR-104, GLY-81, CYS-82, GLY-83 and ASP-146 (Fig 1d). Possible non-polar interaction was also seen with HIS-110.These findings reveal that MBZM-N-IBT can be a potential lead compound against DENV. Keeping in view it in vitro inhibition of non-strctural proteins of CHIKV, it is also likely that it may inhibit DENV replication effectively. However further experimental validation is necessary to establish its effectiveness.

Table 1. Binding affinity of MBZM-N-IBT against DENV non-structural protein targets

Ligands	**NS1 (4OIG)**	**NS3 helicase (2JLR)**	**NS3 protease (2fom)**	**NS5 methyl-transferase (3P97)**	**NS5 RdRp (2J7U)**
MBZM-N-IBT	-9.0	-8.8	-7.7	-9.0	-7.9
IBT	-6.8	-6.7	-6.9	-6.8	-6.3
Isatin	-5.8	-5.8	-5.9	-5.8	-5.7
2-MBZM	-5.1	-5.2	-5.4	-5.1	-5.1
Compound 10	-8.2	-	-	-8.2	-
Ribavirin	-7.0	-7.2	-6.8	-7.0	-6.0
NITD107	-	-	-	-	-7.4
NITD008	-	-	-	-	-6.5
NT008	-	-7.5	-7.5	-	-6.5
Balapiravir	-	-8.5	-5.2	-	-6.6
ST610	-	-9.1	-7.4	-	-
Ivermectin	-	-10.6	-10.6	-	-
Alexidine	-	-	-6.3	-	-
ARDP0006	-	-	-7.1	-	-
Phospho aminophosphonic acid-adenylate ester	-	-8.6	-	-	-

Inhibitors of specific DENV targets were taken as positive controls in the molecular docking studies against specific non-structural protein targets.

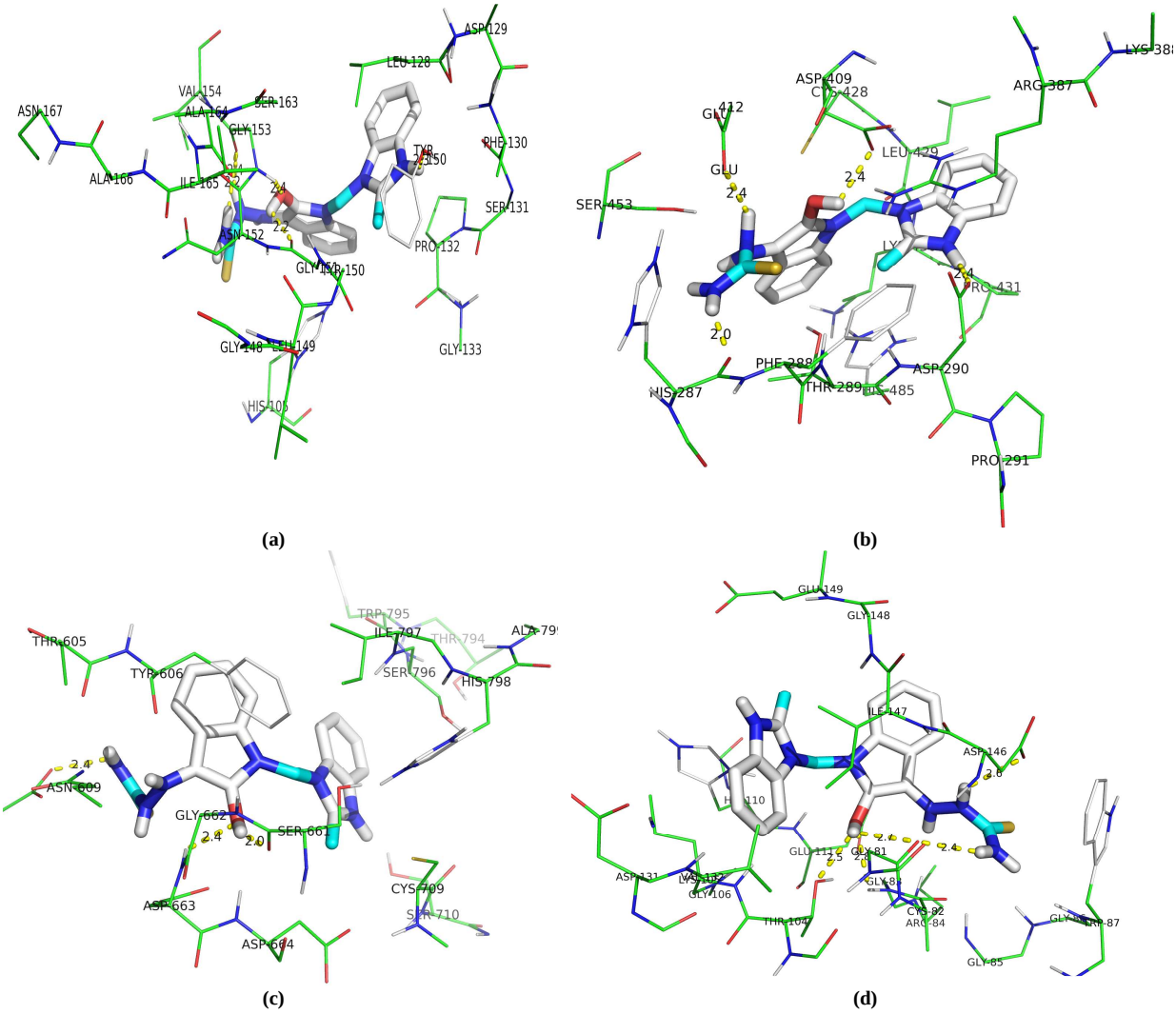

Fig 1. MBZM-N-IBT shows strong binding affinity for DENV non-structural proteins

The most stable conformation of MBZM-N-IBT showing interaction with, **(a)** NS3 protease of DENV(2FOM), **(b)** NS3 helicase of DENV (2JLR), **(c)** NS5 RNA dependent RNA polymerase (2J7U) and **(d)** NS5 methyl transferase of DENV(3P97) of DENV. MBZM-N-IBT was screened against the target protein in the AutoDock Vina open-source program for molecular docking and the best fit complex was visualised in the PyMOL viewer

CONCLUSION

Molecular docking study of MBZM-N-IBT against important therapeutic targets of DENV including NS1, NS3 protease, NS3 helicase, NS5 RNA dependent RNA polymerase and NS5 methyl transferase has suggested the potential of MBZM-N-IBT against DENV. Amongst these targets good binding affinity comparable to that of the known inhibitors of these targets were observed against all the targets, except against NS1. This indicates the potential of MBZM-N-IBT as a lead against DENV which needs further analysis and optimization.

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