**ABSTRACT**

The recent outlook of Dengue viral infection as a global public health concern coupled with the reportage of resistance and lack of anti-dengue drugs calls for a concerted effort to find new leads. The study combined In-silico and in vitro approaches to identify novel potential synthetic small-molecule inhibitors targeting the DENV-2 NS2B-NS3 protease. The NS2B-NS3 protease enzyme in the dengue virus transmission pathway is required for the replication of the virus within the host cell. The lack of NS2B-NS3 protease homologue in the human host and its conserved nature among all dengue virus makes it a viable target for future anti-degue drugs. Initially, six inhibitors of dengue NS2B-NS3 protease with IC50 < 10 µM were used to generate a pharmacophore model with a score of 0.9143 using LigandScout. The validated model was used to screen a synthetic library of 65,345 compounds obtained from ChemDiv. Thirty compounds with pharmacophore fit scores above 55 were docked against the modelled three-dimensional structure of NS2B-NS3 protease using AutoDock Vina. Consequently, nine compounds with binding energies ranging from−7.5 to −8.7 kcal/mol were identified as potential hit molecules. Three compounds comprising STOCK6S-06667, STOCK6S-65928, and STOCK6S-65450 with respective binding energies of −8.7, −8.2, and −8.0 kcal/mol, were selected as plausible lead molecules. Molecular dynamics simulation studies and molecular mechanics Poisson–Boltzmann surface area calculations showed that the residues Asp75 and His51 were critical for ligand binding. The compounds were also predicted to have anti-dengue activity with reasonable pharmacological and toxicity profiles. When the anti-dengue activity of the three hits was evaluated in vitro against the dengue protease, mean half-maximal inhibitory concentrations (IC50) of 21.9 ± 1.5 µM (STOCK6S-5928), 23.5 ± 1.1 µM (STOCK6S-06667), and 118.3 ± 5.8 µM (STOCK6S-65450) were obtained. Furthermore, STOCK6S-5928 and STOCK6S-06667 inhibited the growth of dengue virus, with IC50 of 14.3 ± 2.0 µM and 18.1 ± 1.4 µM, respectively. The identified compounds could be optimised to develop potent anti-dengue therapeutic agents.

**KEYWORDS**: DENV-2; NS2B-NS3 protease; pharmacophore; molecular docking; molecular dynamics simulation; in vitro studies