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Henngai Phom
Department of Chemistry, St. Joseph's College (A) Jakhama, Kohima, Nagaland, India

Somi Seipainao
Department of Chemistry, St. Joseph's College (A) Jakhama, Kohima, Nagaland, India

Corresponding Author:
Henngai Phom
Department of Chemistry, St. Joseph's College (A) Jakhama, Kohima, Nagaland, India

Potential inhibitor for dengue virus protease (NS2B-NS3): Computer-aided drug discovery

Henngai Phom and Somi Seipainao

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Abstract
In the paper in silico method has been used to investigate potential inhibitor for Dengue virus. As silymarin has exhibited potent antiviral activity against dengue virus, the molecule has been taken as a structural template to obtain 204 similar structures that were download from PubChem. Docking studies reveals that ligand 57 (PubChem ID: 45278380) and 201 (Pubchem ID: 162475710) can act as potential inhibitor for Dengue protease, as they exhibit good binding affinity of the target protein (PDB ID:2 fom) and are involved in a number of hydrogen bonding interactions with key amino acids of the catalytic triad.

Keywords: *In-silico*, drug, dengue, silymarin

Introduction
The concept of "*in-silico*" emerged during late 20th century refers to processes or experiments that are performed using computer simulations. In the context of drug discovery, *in-silico* drug design involves using computational models and technologies to identify potential drug candidates [1]. Unlike traditional trial-and-error methods that rely on testing chemicals on animals, in-silico drug design starts with a deep understanding of specific chemical interactions within the body or target organism. Researchers tailor combinations of molecules to create treatments that match desired profiles.

The dengue viruses belong to the genus Flavivirus within the family Flaviviridae. Alongside the dengue virus, this genus includes several other viruses transmitted by mosquitoes and ticks, which are responsible for various human diseases. Notable members of the Flavivirus group include yellow fever, West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis virus [2]. In 1943, scientists Ren Kimura and Susumu Hotta made the initial isolation of the dengue virus. They conducted their research using blood samples from patients during the 1943 dengue epidemic in Nagasaki, Japan. A year later, independently, scientists Albert B. Sabin and Walter Schlesinger also isolated the same virus, which is now referred to as dengue virus 1 (DEN-1) [3]. Dengue virus is a significant emerging pathogen, affecting approximately one-third of the global population. Through comparative analysis of gene sequences, researchers have gained insights into its origin, spread, and genetic diversity. The primary mode of dengue virus transmission is through the bites of infected female mosquitoes, particularly the Aedes aegypti mosquito. While there is no specific antiviral medication for dengue, supportive care is essential.

The dengue virus has a roughly spherical structure with a diameter of approximately 50 nm (1 nm being one millionth of 1 mm). The dengue virus genome consists of a single-stranded positive-sense RNA. The genome consists of ten genes that are translated into a single polyprotein that encodes for three structural proteins, namely, capsid (C), pre membrane (prM/M), envelope(E), and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Figure 1) [4].

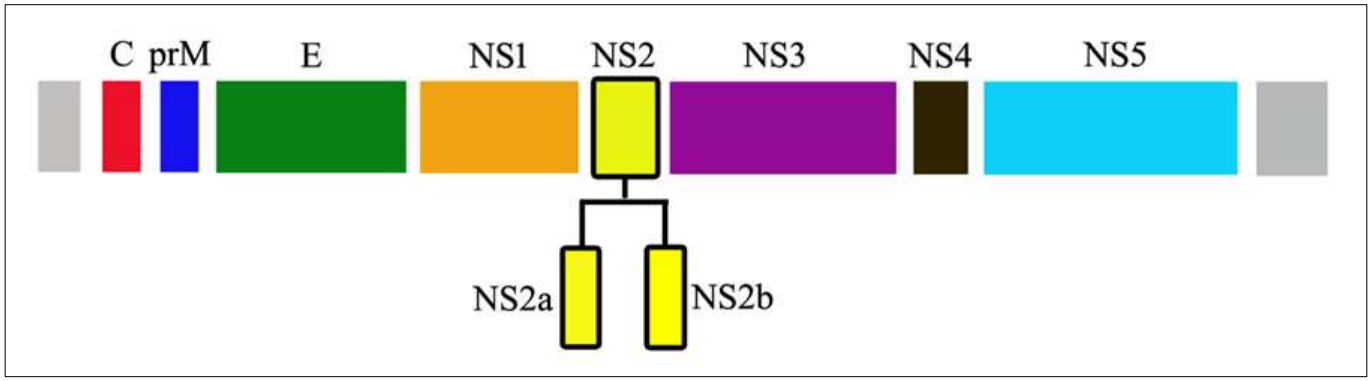
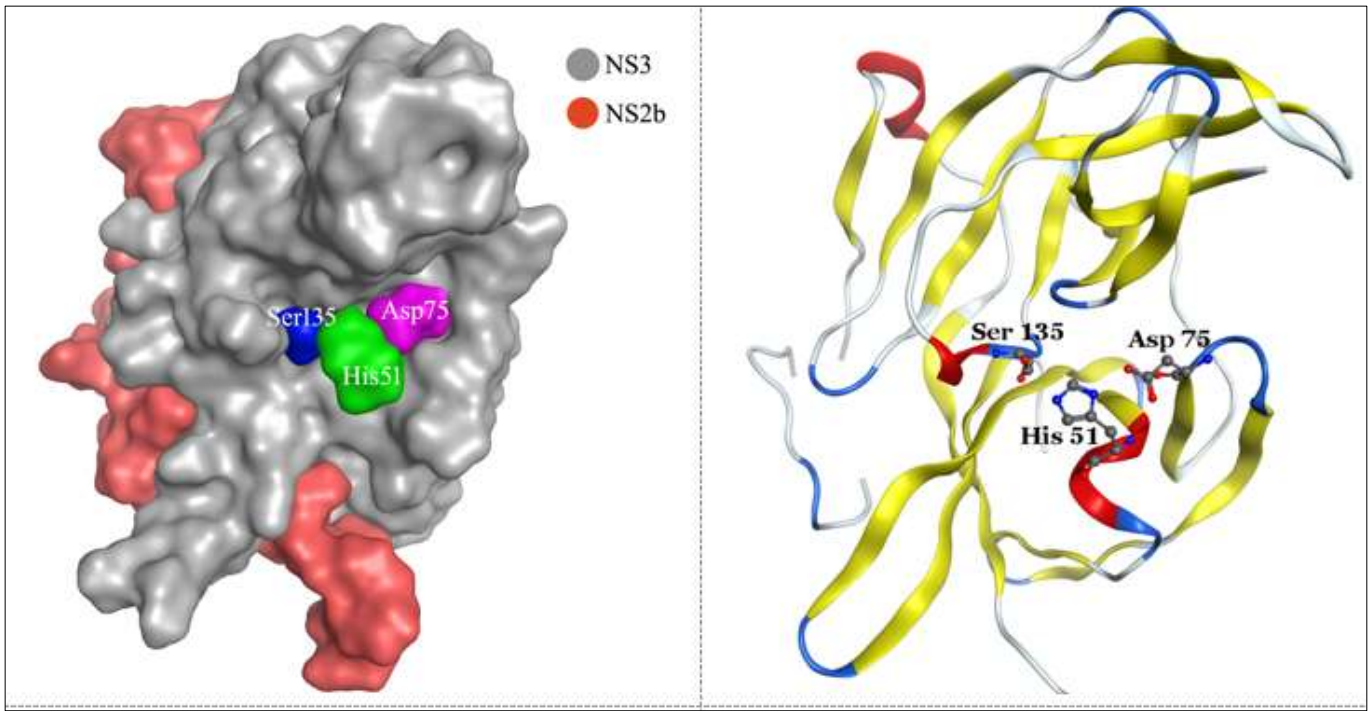


Fig 1: Structural components of dengue protease

NS3 protein is a multifunctional protein that can act as a trypsin-like serine protease, ATPase, RTPase, and RNA helicase. The N-terminus of NS3 (residues 1–180) is considered as the protease domain, while the rest of the protein (residues 180–618; the C-terminus) functions as the RNA helicase [5]. Like any other trypsin-like serine protease, NS3pro has a catalytic triad which is made of HIS51, ASP75, and SER135 residues (Figure 2) [6]. NS3pro was proven to require a co-factor; NS2b, for its functionality, where NS2b provides structural stability for NS3pro and permits protein folding for a successful proteolytic activity (figure 2). This NS2b/NS3pro is responsible for the cleavage of multiple proteins at the junctions of C-prM, NS2a-NS2b, NS2b-NS3, NS3-NS4a, and NS4b-NS5 [7]. Therefore, NS2b/NS3pro is a crucial enzyme complex for the proteolytic and cleavage activities of the translated polyprotein during the virus life cycle [8].



(A) **(B)**

Fig 2: Protein structure of NS2b-NS3 with the catalytic triad. (A) Protein surface with different colours depicting the NS2b and NS3 regions, catalytic triad surface in different colours. (B) Ribbon depiction of the protein structure with ball and stick model for the catalytic triad.

Materials and Methods

ADME Screening

The ligands were screened for druglikeness using Lipinski Rule of five [9] which predicts physiocochemical properties related to a drug's pharmacokinetics i.e. absorption, distribution, metabolism and excretion. Swiss ADME, a web-based program was used for ADME screening [10].

Molecular Docking

Based on a research paper Silymarin has been chosen as the template for the ligands, due to its antiviral activity against dengue virus [11]. All substructure with regard to silymarin were download from PubChem [12] and chosen for the computational study. A total of 205 compounds were selected for the *insilico* study. Energy minimization and file conversion to pdbqt were performed pyrx using autodock vina [13]. Protein structure with PDB ID: 2FOM was selected as the target protein and download from RCSB protein data bank [14]. Hetero atoms and waters were deleted from the system and energy minimization and file conversion to pdbqt were perform in pyrx. Docking studies were performed using Autodock Vina implemented in Pyrx. A site-specific docking was performed where a grid box was set around the catalytic triad i.e., His51, Asp75 and Ser135.

Results and Discussion

ADME Screening for drug-likeness

After completion of the ADME screening it was observed that all the ligands violate one or more of Lipinski rule of five. The ADME screening results of all the ligands are shown in supplementary table 1. The ligands have masses ranging from 482 to 692 Daltons, and 80% of the ligands have masses higher than 500 Dalton. The number of hydrogen bond donors vary from 0-8 with most ligands having 5 or below. The number of hydrogen bond acceptors ranges from 10-16, with most ligands having around 10 hydrogen acceptors. With regard to LogP value all the ligands except 7 have lower values than 5, and even among the 7 ligands the highest LogP value is 5.53. Accept for the slightly higher mass, as there were no serious violations to the rule, all the ligands were selected for the docking study.

Molecular Docking Analysis

There were 205 ligands used for docking and the docking scores are listed in supplementary table 1. The Top six binding ligands were selected as the top ligands with the PubChem ID: 124929904, 7564646, 45278380, 162475710, 7564637 and 145966569. The binding score and the Hydrogen bonding interactions of the ligands with the active site residues are shown in table 1. The reference ligand used is Silymarin with PubChem ID: 5213.

Table 1: Docking score and amino acids interacting with top six ligands and reference ligand

Sl. No.	Ligand No.	PubChem ID	Docking Score (Kcal/mol)	Hydrogen Bond
1.	156	124929904	-8.6756	Val52, Gly153, His51, Tyr150
2.	22	7564646	-8.4990	Asn152
3.	57	45278380	-8.3626	Gly153, His51, Tyr150
4.	201	162475710	-8.3501	His51, Asp75, Gly153, Val72
5.	20	7564637	-8.2447	Asn152
6.	182	145966569	-8.1451	Gly153, Trp50
7.	1 (ref)	5213	-6.3568	Trp50, Asn152

Ligand 156 (PubChem ID: 124929904) has the binding affinity of -8.6756 kcal/mol. It interacts with the amino acids Val52, Ile36, Gly153, His51, Gly151, Tyr150 in the active site. There is a conventional hydrogen bonding with His51, which is an amino acid of the catalytic triad (figure 3 A and B). There is an unfavourable interaction with the residue Ser131.

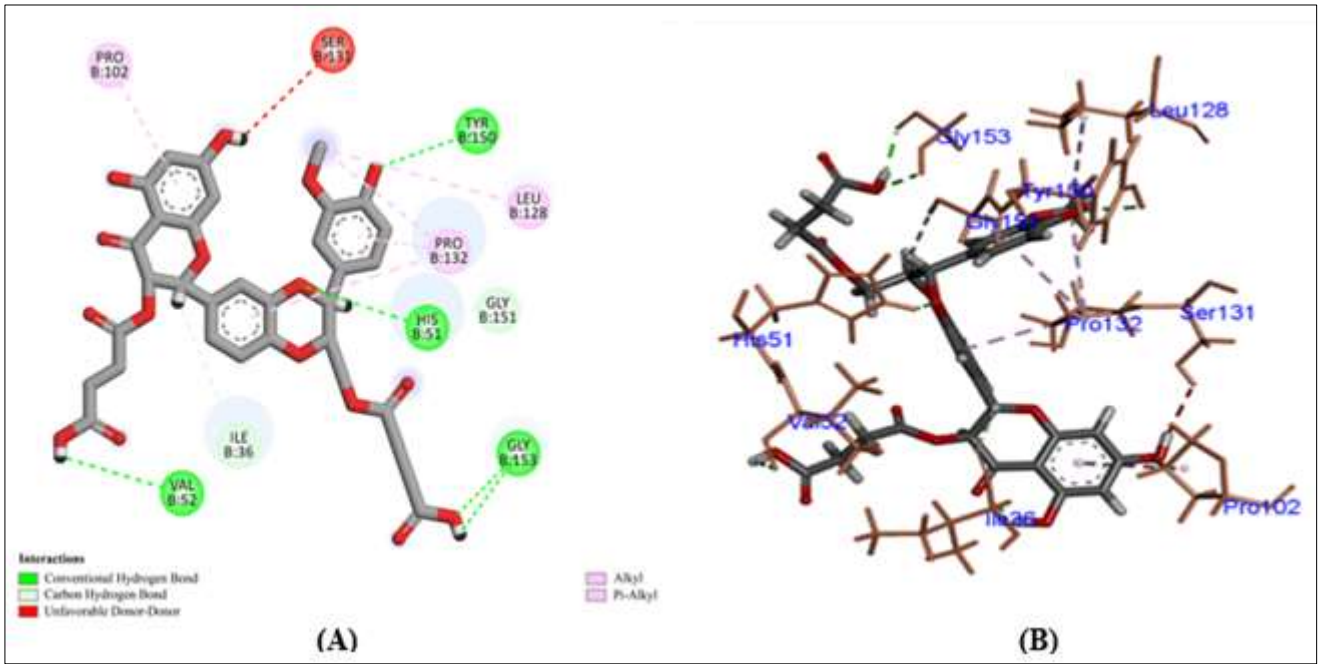


Fig 3: Image of ligand 156 interacting with target protein (2fom) (A) 2D image of interactions (B) 3D image of interactions

The ligand 22 (PubChem ID: 7564646) has a binding affinity of -8.4990kcal/mol. It is involved in conventional hydrogen bonding with Asn152. It is not involved in any interact with the amino acids of the catalytic triad (figure 4A and B).

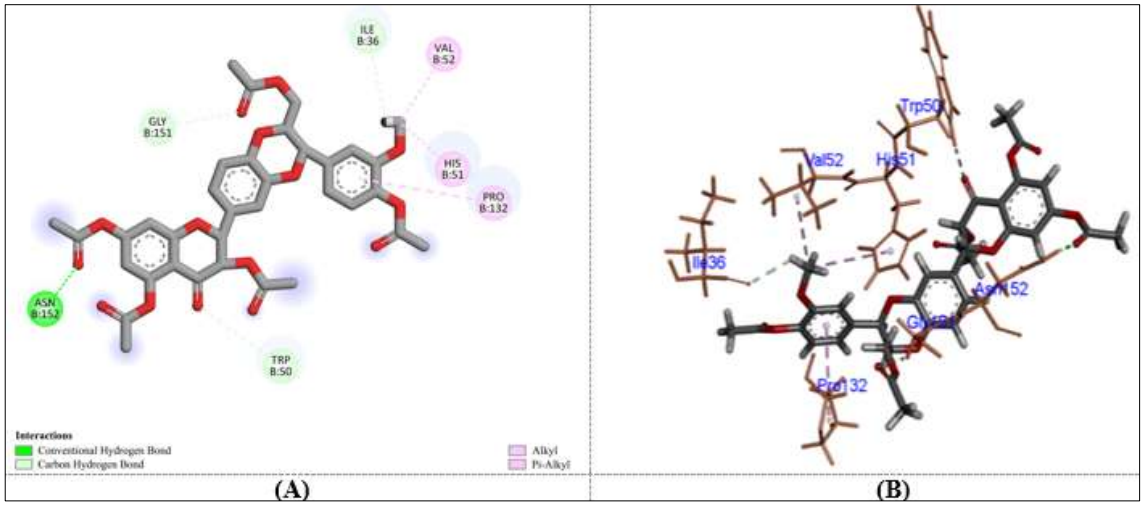


Fig 4: Image of ligand 22 interaction with target protein (2fom) (A) 2D image of interactions (B) 3D image of interactions

The ligand 57 (PubChem ID: 45278380) has the binding affinity of -8.3626kcal/mol. It undergoes conventional hydrogen bonding with His51, Tyr150 and Gly153, and carbon hydrogen bond with Pro132, Ser135 and Gly151. The ligand interacts with all the amino acid residues of the catalytic triad (Figure 5A and B).

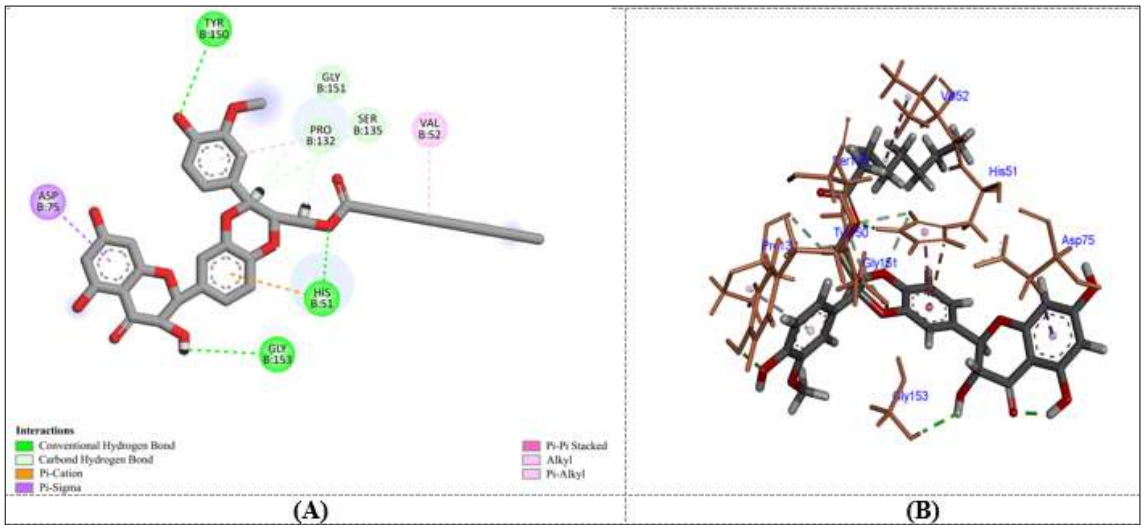


Fig 5: Image of ligand 57 interaction with target protein (2fom) (A) 2D image of interactions (B) 3D image of interactions

The ligand 201(PubChem ID: 162475710) has a binding affinity of -8.3501kcal/mol. It undergoes conventional hydrogen bonding with His51 and asp75, which are an amino acid of the catalytic triad (Figure 6A and B). It is also involved in conventional hydrogen bonding with Val72 and Gly153, and conventional hydrogen interaction with Trp50, Gly151 and Asn152.

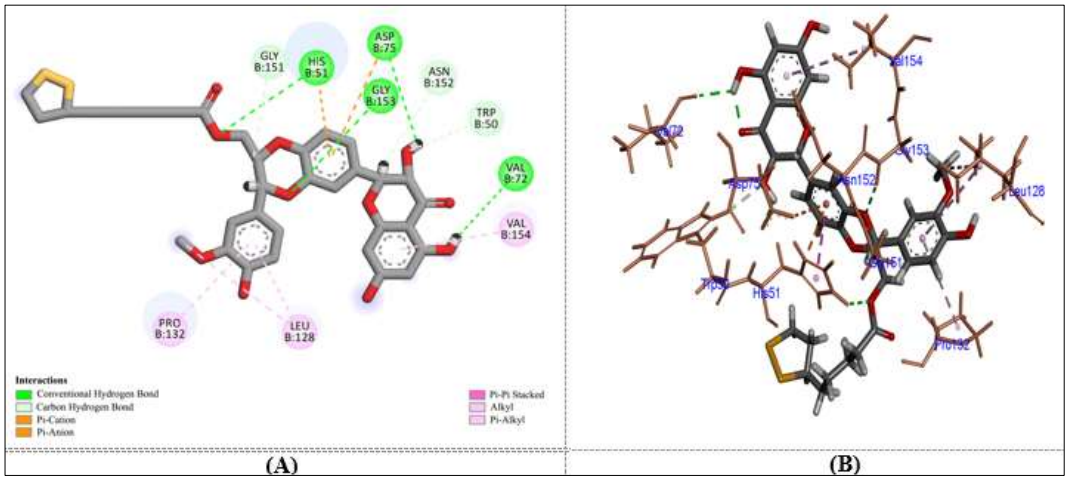


Fig 6: Image of ligand 201 interaction with target protein (2fom) (A) 2D image of interactions (B) 3D image of interactions

Ligand 20 (PubChem ID: 7564637) has a binding affinity of -8.2447kcal/mol. It is involved in conventional hydrogen bonding with Asn152, carbon hydrogen bonding in Trp50, His51 and Ile36 (Figure 7A and B) where His51 is an amino acid residue of the catalytic triad.

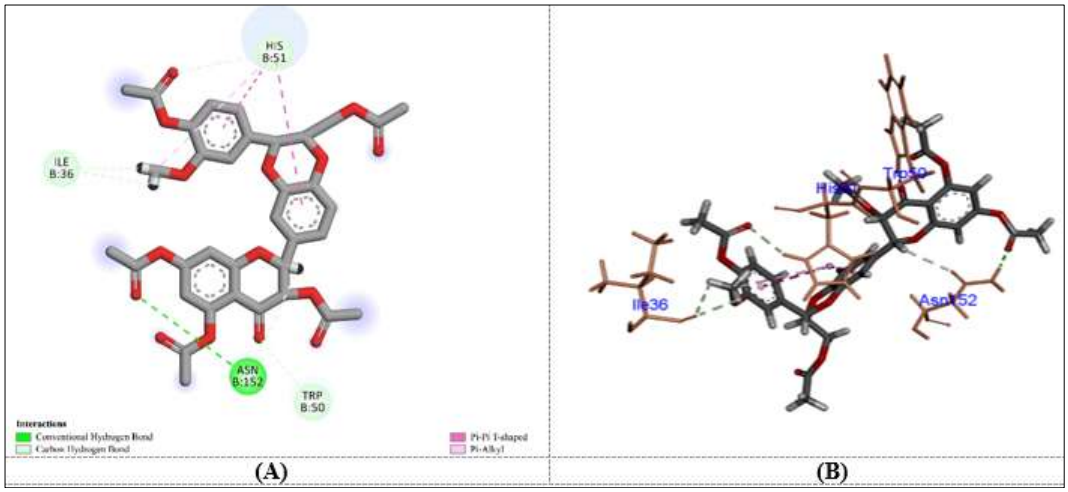


Fig 7: Image of ligand 20 interaction with target protein (2 Fom) (A) 2D image of interactions (B) 3D image of interactions

The ligand 182 (PubChem ID: 145966569) has the binding affinity of -8.1451kcal/mol. It is involved in conventional hydrogen bonding with Gly153 and Trp50, and carbon hydrogen bond interactions with His51, Asn152, Asp75, Val72, Lys73, Thr53 at the active site. His51 and asp75 are an amino acid of the catalytic triad (Figure 8A and B).

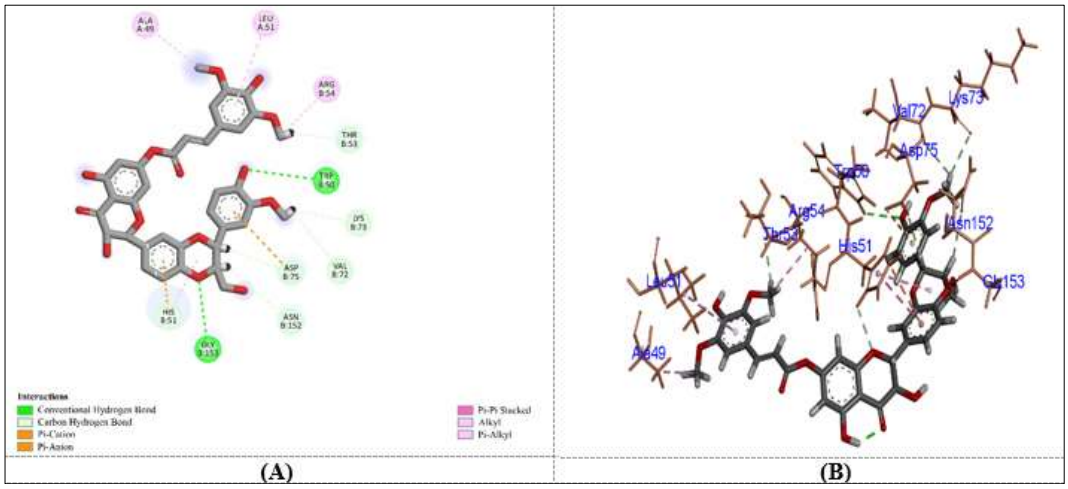


Fig 8: Image of ligand 182 interaction with target protein (2fom) (A) 2D image of interactions (B) 3D image of interactions

The reference ligand (PubChem ID: 5213) has a binding affinity of -6.3568 kcal/mol. The ligand has hydrogen bonding interactions with Trp50 and Asn152. The ligand doesn't interact with any of the amino acids of the catalytic triad (Figure 9A and B).

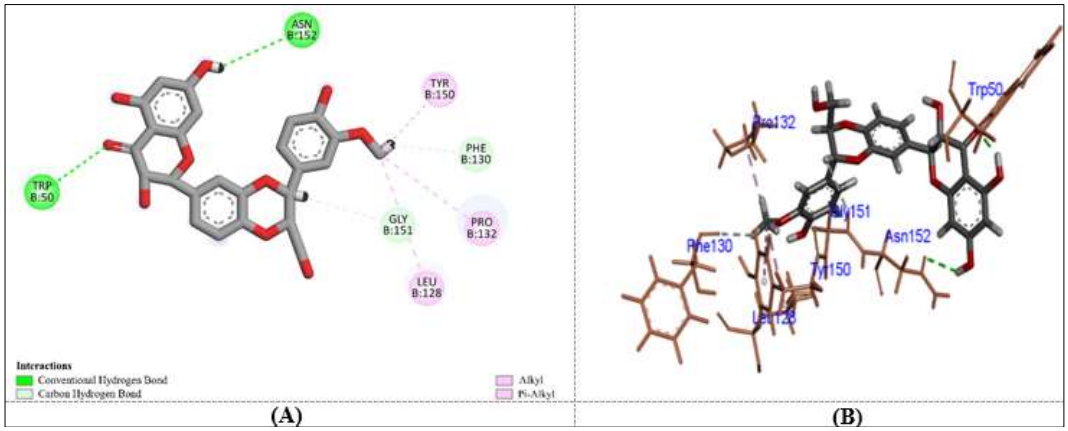


Fig 9: Image of reference ligand interaction with target protein (2fom) (A) 2D image of interactions (B) 3D image of interactions

Among the top 6 ligands, the ligand 57 (PubChem ID: 45278380) can be considered as the most potent inhibitor of dengue protease as it interacts with all the amino acids of the catalytic triad. Ligand 201 (Pubchem ID: 162475710) can also be considered as a potential antiviral drug against dengue as it is involved in hydrogen bonding with His51 and Asp75, which are amino acids of the catalytic triad. Although ligand 156 (PubChem ID: 124929904) has four hydrogen bonding interactions it can be ignored as it also involved in unfavorable interaction with Ser131.

Conclusion

In this study, *in silico* computational methods has been used to find potential inhibitors for dengue protease (NS2B-NS3). Based on Silymarin as template, 204 ligands with structural similarities were selected and downloaded from PubChem. All ligands were subjected to screening for drug-likeness with Lipinski rule of five. The interactions of the top six binding ligands including the reference ligand was studied. After protein-ligand interaction analysis ligand 57 (PubChem ID: 45278380) and 201 (Pubchem ID: 162475710) has been handpicked as potential inhibitor for Dengue protease, as they exhibit good number of hydrogen bonding interactions with key amino acids of the catalytic triad.

Further studies can be performed using different antiviral drugs as templates and more advance studies like Molecular Dynamics simulations can be performed on the studied complexes.

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Supplementary Table 1. List of 205 selected ligand with ADME screening indicators and docking scores

Comp	Pubchem ID	Mass (500 dalt)	H bond donor <5	H bond acceptor <10	LOGP (<5)	Docking score	comp	Pubchem ID	Mass (500 dalt)	H bond donor <5	H bond acceptor <10	LOGP (<5)	Docking score
1	5213	482.22	5	10	1.58	-6.3568	33	15813684	644.17	8	15	-O.12	-7.9840
2	31553	482.44	5	10	1.51	-6.3485	34	15981462	512.46	5	11	1.56	-6.6053
3	150709	682.15	5	16	1.85	-7.9078	35	16211710	482.44	5	10	1.50	-6.0076
4	161822	682.15	5	16	1.85	-7.8433	36	24086320	482.44	5	10	1.54	-6.3793
5	187901	482.12	5	10	2.01	-6.0520	37	24832061	482.44	5	10	1.54	-6.5770
6	313326	482.44	5	10	1.58	-6.2992	38	38988805	482.44	5	10	1.56	-6.5296
7	1548994	482.44	5	10	1.59	-6.4047	39	40428694	482.44	5	10	1.57	-6.4369

8	1549163	482.44	5	10	1.50	-6.1897	40	40768299	482.44	5	10	1.61	-6.9596
9	3086637	482.44	5	10	1.51	-6.3771	41	44147684	482.44	5	10	1.57	-6.1930
10	3116164	692.17	0	15	2.43	-8.1047	42	44159294	616.16	4	12	4.09	-7.9256
11	5748849	482.44	5	10	1.59	-6.3694	43	44159809	526.49	5	11	1.90	-6.6535
12	6604604	482.44	5	10	1.59	-6.6000	44	44199363	510.15	3	10	2.36	-6.3820
13	6610285	482.44	5	10	1.58	-6.0019	45	44235721	596.15	4	13	3.02	-7.1486
14	6995499	482.44	5	10	1.54	-6.3686	46	44514500	596.15	4	13	3.02	-7.3163
15	6999760	482.44	5	10	1.61	-6.1946	47	44514501	596.15	4	13	3.02	-6.7132
16	7073226	482.44	5	10	1.57	-6.6255	48	44514785	596.15	4	13	3.02	-7.4704
17	7073227	482.44	5	10	1.56	-6.5771	49	44515969	596.15	4	13	3.02	-6.8537
18	7073228	482.44	5	10	1.62	-6.3138	50	44611660	562.18	3	10	3.72	-7.2515
19	7564629	692.17	0	15	2.43	-7.8274	51	44625891	568.16	4	12	2.33	-7.1682
20	7564637	692.17	0	15	2.43	-8.2447	52	45101465	646.14	6	15	3.64	-7.7263
21	7564641	692.17	0	15	2.43	-8.1194	53	45101466	676.18	4	14	3.62	-8.0390
22	7564646	692.17	0	15	2.43	-8.4990	54	45278060	552.16	4	11	2.91	-6.5227
23	10174739	524.17	2	10	2.47	-6.5507	55	45278061	608.23	4	11	4.48	-7.2486
24	10436837	524.17	2	10	2.47	-6.5635	56	45278379	552.16	4	11	3.68	-7.5491
25	10699372	644.17	8	15	-0.12	-6.9491	57	45278380	608.23	4	11	5.04	-8.3626
26	10841883	644.17	8	15	-0.12	-7.0225	58	45933924	482.44	5	10	1.54	-6.2600
27	11093488	644.17	8	15	-0.22	-6.5678	59	46202617	682.15	5	16	1.85	-7.7316
28	11994508	482.44	5	10	1.51	-6.4229	60	46227026	616.16	4	12	4.09	-7.1462
29	12442777	482.44	5	10	1.58	-6.5260	61	46227027	631.13	4	14	4.03	-8.0296
30	12442778	482.44	5	10	1.57	-6.2278	62	46227028	586.15	4	11	4.06	-6.9752
31	13091820	682.15	5	16	1.85	-8.0528	63	46227029	676.12	4	17	3.88	-7.6019
32	13091822	582.14	5	13	1.86	-7.3077	64	46227030	626.18	4	11	4.42	-7.4683
65	46227031	646.12	4	11	4.68	-6.9224	102	71524028	496.46	4	10	1.97	-6.4770
66	46227032	690.07	4	11	4.87	-7.4986	103	71524029	510.15	3	10	2.36	-6.6194
67	46227033	620.11	4	11	4.61	-7.8231	104	71587964	682.15	5	16	1.85	-7.4966
68	46227034	646.17	4	13	3.76	-7.6274	105	71590100	595.21	4	12	2.22	-7.4893
69	46227035	630.14	4	13	4.02	-7.9406	106	71590101	682.15	5	16	1.85	-7.8182
70	46227036	601.16	6	12	3.57	-7.0208	107	71752198	482.44	5	10	1.51	-5.9406
71	46397421	692.17	0	15	2.43	-7.7799	108	73330896	595.21	4	12	2.22	-7.2353
72	46870829	558.15	5	10	3.09	-6.8416	109	73330897	593.23	4	11	3.46	-7.2728
73	46870830	522.15	5	10	3.12	-6.9287	110	73330898	581.23	4	11	2.92	-6.8342
74	46870915	561.33	5	10	2.29	-6.1627	111	73330899	553.19	4	11	2.32	-6.7786
75	46870917	516.88	5	10	2.19	-6.2110	112	73331042	609.22	4	12	2.48	-7.0236
76	46870918	608.33	5	10	2.29	-6.8295	113	73331043	607.24	4	11	3.65	-7.3249
77	46881100	622.24	4	11	5.48	-7.9966	114	78097726	496.46	4	10	1.97	-6.7266
78	49763974	542.14	5	12	1.67	-6.4731	115	89611863	514.43	7	12	0.92	-6.4638
79	49783777	526.49	5	11	1.81	-7.0831	116	89869556	670.24	4	11	5.53	-7.4538
80	49783850	595.21	4	12	2.02	-7.0421	117	89869559	670.24	4	11	5.53	-7.4413
81	49802140	496.46	5	10	1.94	-6.2207	118	89869564	496.46	4	10	2.08	-6.2362
82	49806110	646.12	4	11	4.68	-7.9414	173	135020967	482.44	5	10	1.54	-5,9060
83	51347369	512.46	5	11	1.66	-6.5293	174	135020968	650.16	1	14	1.88	-7.1037
84	53233446	676.12	4	17	3.88	-7.8411	119	89869566	659.16	5	14	3.21	-7.7420
85	53240612	556.16	5	12	2.25	-6.5154	120	89869587	602.14	5	12	4.25	-7.7071
86	53241527	512.46	5	11	1.56	-6.4218	121	89869617	496.46	4	10	2.08	-6.2739
87	53241995	631.13	4	14	4.03	-6.8015	122	89869621	644.15	4	13	3.56	-7.5053
88	56589623	634.13	7	14	2.95	-7.1286	123	89888477	496.46	4	10	2.12	-6.4322
89	56589624	634.13	7	14	2.95	-7.1733	124	89888488	602.14	5	12	4.25	-7.0271
90	56595327	634.13	7	14	3.16	-7.4563	125	89888489	644.15	4	13	3.56	-7.7276
91	56595328	634.13	7	14	2.90	-7.3962	126	89888508	670.24	4	11	5.53	-7.7241
92	56595453	634.13	7	14	2.82	-7.3918	127	89888509	670.24	4	11	5.53	-7.1676
93	56595717	634.13	7	14	2.95	-7.6981	128	89888510	670.24	4	11	5.53	-8.0079
94	56595718	634.13	7	14	2.95	-7.3372	129	89888516	659.16	5	14	3.21	-6.7841
95	59926410	524.17	2	10	2.47	-7.0563	130	90135786	562.50	5	13	1.30	-6.9212
96	60150399	498.44	6	11	1.32	-6.5073	131	90918774	675.20	4	14	2.10	-8.0026
97	60155089	582.14	5	13	1.93	-6.9296	132	92906836	692.17	0	15	2.43	-7.2355
98	70681231	562.50	5	13	1.19	-6.7500	133	92906838	692.17	0	15	2.43	-7.3635
99	71119844	496.46	5	10	2	-6.4283	134	100093242	692.17	0	15	2.43	-7.6529
100	71524005	538.18	1	10	2.63	-6.9870	135	100880017	692.17	0	15	2.43	-7.7766
101	71524006	524.17	2	10	2.99	-6.6974	136	101092314	658.15	8	16	0.06	-7.1067
139	101500553	644.17	8	15	-0.12	-7.9619	137	101092315	658.15	8	16	-0.06	-7.3711
140	101542502	524.47	4	11	2.09	-6.1256	138	101092316	658.15	8	16	-0.35	-7.2121
141	101542503	524.47	4	11	2.12	-6.6083	175	143393162	496.46	5	10	1.95	-6.3246
142	101720469	644.17	8	15	-0.12	-7.0668	176	145053512	496.46	5	10	1.89	-6.6458
143	101720470	644.17	8	15	-0.12	-7.3892	177	145053519	524.17	4	10	3.24	-6.1856
144	101720471	644.17	8	15	-0.12	-6.9546	178	145053534	525.16	4	11	0.66	-7.0253

145	101840019	496.46	4	10	1.90	-6.3869	179	145964664	632.15	5	13	3.06	-6.7755
146	101840020	496.46	4	10	2.17	-6.8178	180	145965502	690.19	5	14	3.12	-7.7116
147	102106873	562.50	5	13	1.21	-6.6662	181	145965974	612.16	4	11	4.06	-7.0118
148	102328121	482.44	5	10	1.59	-5.9637	182	145966569	688.18	5	14	3.54	-8.1451
149	102368691	524.47	4	11	2.13	-6.6833	183	145966993	660.18	5	13	2.88	-6.8526
150	102368692	524.47	4	11	2.15	-6.4287	184	145968691	658.17	5	13	3.28	-6.9049
151	117827168	496.46	4	10	2.08	-6.9343	185	145968934	658.17	5	13	3.28	-7.6956
152	122164557	482.44	5	10	1.51	-6.0457	186	145973511	658.17	4	13	4.14	-6.8834
153	124304713	482.44	5	10	1.60	-6.3625	187	145973715	686.20	4	13	4.02	-7.3047
154	124776031	692.17	0	15	2.43	-7.8223	188	145973787	586.15	4	11	3.87	-7.0512
155	124776032	692.17	0	15	2.43	-7.8084	189	145975101	684.18	4	13	4.14	-7.5335
156	124929904	682.15	5	16	1.85	-8.6756	190	145975144	612.16	4	11	3.90	-7.3364
157	124929905	682.15	5	16	1.85	-7.9649	191	145978145	658.17	5	13	3.28	-7.0875
158	124929907	682.15	5	16	1.85	-7.7213	192	145978575	662.16	5	14	3.33	-7.5523
159	127027401	566.22	4	10	4.38	-6.8020	193	146270554	526.15	4	11	2.03	-6.4874
160	127027402	580.23	4	10	4.48	-7.3437	194	146270576	561.33	5	10	2.20	-6.6639
161	127027403	572.17	4	10	3.70	-6.8364	195	146270577	498.44	6	11	1.28	-7.2245
162	127042299	552.20	4	10	3.95	-6.9112	196	155093814	584.14	4	11	3.35	-7.0449
163	127042935	496.46	4	10	1.97	-6.4490	197	156079985	482.44	5	10	1.59	-6.3831
164	127042936	510.15	4	10	2.65	-6.5389	198	156240286	512.46	5	11	1.67	-6.4811
165	127042937	524.17	4	10	3.11	-6.6907	199	156623371	482.44	5	10	1.59	-6.1135
166	127042938	538.18	4	10	3.49	-6.7768	200	162460144	498.44	6	11	1.18	-6.5799
167	129852421	482.44	5	10	1.59	-6.2574	201	162475710	670.15	4	11	4.37	-8.3501
168	130287825	482.44	5	10	1.59	-6.1493	202	162706948	482.44	5	10	1.59	-6.1562
169	132042707	562.42	6	13	0.90	-6.9098	203	163096546	528.46	6	12	1.41	-6.2900
170	133554171	482.44	5	10	1.57	-6.6363	204	163096547	528.46	6	12	1.27	-6.7443
171	134816768	562.42	6	13	0.90	-6.8203	205	163096548	528.46	6	12	1.28	-6.4118
172	134817040	658.19	7	15	0.38	-6.8840							