

In silico Docking Study of Some Coumarin Derivatives as Potential Inhibitors on Different Dengue Viral Proteins

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In this study, the binding efficiency of 10 coumarin derivatives with some selected proteins from Dengue virus through in silico method was done. By virtual screening and docking results, we have found that the hybrid derivative between coumarin and isatin has the most convenient binding activity for the seven selected proteins.

Keywords: coumarin derivatives, molecular docking, dengue virus

Coumarin (benzopyran-2-one) is an oxygen heterocyclic compound that has a bicyclic structure with lactone carbonyl group, firstly isolated in 1820 by Vogel.

Coumarin is found in many plants, notably in high concentration in the tonka bean (*Dipteryx odorata*). It also occurs in vanilla grass (*Anthoxanthum odoratum*), sweet woodruff (*Galium odoratum*), sweet grass (*Hierochloa odorata*) and sweet-clover (genus *Melilotus*) [1].

Coumarins represent a class of compounds with a broad spectrum of biological activities: anticancer, anti-HIV, antimicrobial, antithrombotic, antiviral, antioxidant and antiinflammatory [2, 3]. Coumarin ring can be considered as a privileged scaffold and an ideal framework for the design of compounds that can interact with different targets [4-6].

On the other side, isatin is a versatile chemical skeleton able to participate to a broad range of synthetic reactions, being an important molecule in synthetic medicinal chemistry. Recently, isatin and its derivatives have gained great attention due to its biological potential like antibacterial, antifungal, antimalarial, antiviral, anti-HIV, anti-mycobacterial, anticancer [7-9].

Dengue virus (DENV) is a member of the Flaviviridae family. Dengue virus is an enveloped RNA virus containing a positive-sense and single strand genome. Upon DENV entry into the target cells, the RNA genome is translated to a single polyprotein associated with the host endoplasmic reticulum. The polyprotein is cleaved by host and viral proteases into three structural proteins (capsid, premembrane and envelope) and seven non-structure proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [10-14].

Currently, there is no vaccine or antiviral treatment for dengue fever. The patients are only recommended to drink plenty of fluids or take medicines like acetaminophen to avoid dehydration from vomiting and high fever.

Thus, it is very important to develop new antiviral substances against dengue viruses.

Chemical synthesis is a very important tool to insert new chemical groups in order to obtain new derivatives with improved profile from biological effect point of view.

Impressed by the important biologically active profile of coumarin and isatin derivatives and as a part of our interest in the synthesis and screening of potentially bioactive compounds, we performed the evaluation of anti Dengue potential of some coumarin derivatives, previously synthesized, via molecular docking approach.

Experimental part

Synthesis of coumarin derivatives

The synthesis of the target compounds was reported previously [15].

Preparation of Dengue viral proteins

The protein data bank (PDB) was used to obtain the three-dimensional structure of the macromolecule [16-19]. The structures are downloaded and saved either in mmCIF or PDB format. Proteins of dengue virus were used for this study. The 3D structure of all the fourteen proteins were downloaded from PDB and saved in PDB format. The downloaded proteins were viewed in Py-Mol viewer.

Preparation of ligands

Ten synthesized ligands with coumarin ring, were selected for this study (Table I). Ligands were constructed using ChemSketch. The constructed ligands were optimized to add the hydrogen bonds and the obtained structures were saved in mol for docking analysis and named as A, B and C respectively.

Docking study

Docking studies were conducting using iGEMDOCK software. The proteins and the ligands were loaded and the out path was set. Standard docking parameters were used for docking (population size=200, generations =70 and Number of solutions =2). The docking process was initiated. After the docking process, the best docking pose for the individual ligands can be obtained for all the seven dengue viral proteins.

Results and discussions

Two acetohydrazide and eight Schiff bases with coumarin skeleton were selected (Table 1).

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Table 1
STRUCTURE AND NAME OF THE INVESTIGATED COUMARINES

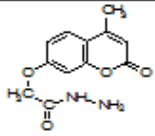
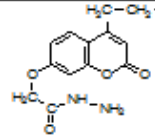
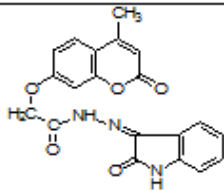
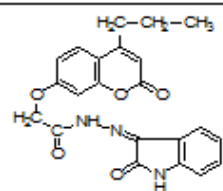
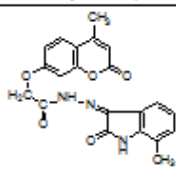
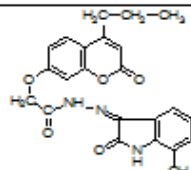
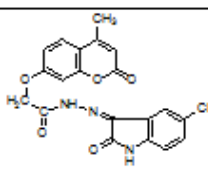
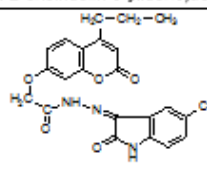
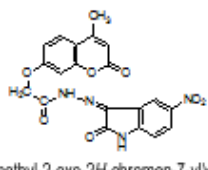
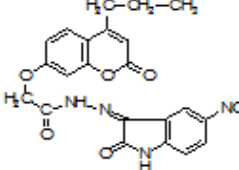
Comp.	Structure/Name	Comp.	Structure/Name
Ia	 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetohydrazide	Ib	 2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)acetohydrazide
IIa	 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(2-oxindolin-3-ylidene)acetohydrazide	IIb	 2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(2-oxindolin-3-ylidene)acetohydrazide
IIIa	 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(7-methyl-2-oxindolin-3-ylidene)acetohydrazide	IIIb	 2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(7-methyl-2-oxindolin-3-ylidene)acetohydrazide
IVa	 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(5-chloro-2-oxindolin-3-ylidene)acetohydrazide	IVb	 2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(5-chloro-2-oxindolin-3-ylidene)acetohydrazide
Va	 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(5-nitro-2-oxindolin-3-ylidene)acetohydrazide	Vb	 2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(5-nitro-2-oxindolin-3-ylidene)acetohydrazide

Table 2
THE TOTAL BINDING ENERGY (kcal/mol) PROFILE FOR DENGUE VIRUS PROTEINS WITH THE INVESTIGATED LIGANDS

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS2B-NS3 protease protein	NS3 helicase protein	NS5 protein
Ia	-92.49	-94.29	-101.06	-84.19	-91.87	-102.76	-98.44
Ib	-100.96	-86.23	-100.24	-79.62	-102.33	-97.91	-100.42
IIa	-105.19	99.93	-122.25	-85.94	-101.13	-119.08	-113.15
IIb	-106.87	-105.14	-111.42	-96.99	-105.23	-116.59	-109.35
IIIa	-100.53	-108.95	-107.46	-87.76	-101.33	-118.23	-107.29
IIIb	-102.21	-101.88	-117.23	-99.59	-107	-114.38	-122.59
IVa	-111.22	-105.84	-116.3	-87.72	-103.08	-108.87	-117.93
IVb	-117.07	-92.5	-110.28	-99.18	-106.81	-118.5	-111.61
Va	-102.92	-109.01	-125.79	-100.59	-98.07	-114.47	-128.93
Vb	-111.12	-113.61	-135.55	-124.34	-104.22	-144.65	-129.78

From docking study, we listed binding affinity of 10 compounds based on ligand binding energy (Tables 2 and 3). The lower energy scores represent better target protein-ligand binding affinity compared to higher energy score.

Visualization of hydrogen bond interaction between the compounds **I-V** and various target proteins were shown in Table 4.

The interactions of the designed compounds with the amino acids in the active site of proteins are listed in Table 5.

A close-up view of the profile for Dengue virus selected protein with the investigated ligands is shown in the figures 1-7.

The 3D structure coordinates of seven proteins of Dengue virus are optimized and 10 coumarin derivatives were used for these proteins via molecular docking approach. Their total binding energy was calculated using iGEMDOCK. Evaluations of binding conformation of 10 compounds with Dengue viral proteins are performed using

Table 3
VAN DER WAALS FORCE (kcal/mol)

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS2B-NS3 protease protein	NS3 helicase protein	NS5 protein
Ia	-80.91	-63.93	-72.28	-36.39	-67.58	-65.43	-75.07
Ib	-67.83	-54.99	-65.04	-64.12	-70.23	-71.74	-66.97
IIa	-88.92	-77.07	-107.53	-78.84	-86.55	-95.53	-91.97
IIb	-75.78	-82.86	-88.29	-91.93	-91.78	-98.94	-82.44
IIIa	-93.68	-88.14	-85.59	-77.26	-90.98	-102.4	-88.7
IIIb	-79.04	-83.33	-100.58	-92.59	-78.73	-88.25	-100.64
IVa	-98.11	-82.38	-96.87	-80.72	-85.67	-91.5	-92.72
IVb	-97.18	-83.33	-94.85	-87.26	-97.41	-87.29	-94.4
Va	-86.3	-74.43	-103.26	-79.58	-76.66	-85.96	-95.57
Vb	-76.72	-74.8	-111.79	-105.78	-85.11	-113.81	-98.68

Table 4
H-BOND PROFILE FOR DENGUE VIRUS PROTEINS WITH THE INVESTIGATED LIGANDS

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS2B-NS3 protease protein	NS3 helicase protein	NS5 protein
Ia	H-S	H-M	H-M H-S	H-M H-S	H-M	H-M H-S	H-M H-S
Ib	H-M H-S	H-M H-S	H-M H-S	H-M	H-M H-S	H-M H-S	H-M H-S
IIa	H-S	H-M H-S	H-M H-S H-M H-S	H-M H-S	H-M H-S	H-M H-S	H-M H-S
IIb	H-M H-S	H-M H-S	H-M H-S	H-M	H-M H-S	H-S	H-M H-S
IIIa	H-S	H-M H-S	H-M H-S	H-M	H-M H-S	H-S	H-M H-S
IIIb	H-M H-S	H-M H-S	H-M H-S	H-M	H-M H-S	H-S	H-M H-S
IVa	H-M H-S	H-M H-S	H-M H-S	H-M	H-M H-S	H-M	H-M H-S
IVb	H-M H-S	H-M H-S	H-M	H-M	H-S	H-M H-S	H-M H-S
Va	H-M H-S	H-M H-S	H-S	H-M H-S	H-M H-S	H-M H-S	H-M H-S
Vb	H-M H-S	H-M H-S	H-M H-S	H-M H-S	H-M H-S	H-M H-S	H-M H-S

Table 5
AMINO-ACID POSITION/PROFILE FOR DENGUE VIRUS PROTEINS WITH THE INVESTIGATED LIGANDS

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS2B-NS3 protease protein	NS3 helicase protein	NS5 protein
Ia	ARG(41)/-11.6	ILE(630)/-10.4	LYS(227)/-3.5 SER(228) SER(228)/-9.4	PHE(21)/-7 ARG(21)/-3.3	GLY(87)/-6.9	GLY(420)/-7 TYR(354)/-7.1	VAL(255)/-6.3 THR(39)/-5
Ib	SER(24)/-5.4 THR(25)/-12	ASN(663)/-7 TYR(605)/-5.8	TRP(210)/-7 SER(315) ARG(314)/-5.8	PHE(21)/-7	GLY(87)/-7 TRP(83)/-3.5	ALA(419)/-7 ASN(416)/-3.5	GLU(40)/-3.9 THR(39)/-9.5
IIa	ARG(41)/-10.3	SER(633)/-7 SER(633)/-3.5	SER(233)/-3.5 ILE(242) ASN(255)/-3.5 LYS(227)	GLY(24)/-3.4 ARG(18)/-3.5	THR(53)/-7 ARG(54)/-3.5	HYS(287)/-3.5 HYS(485)/-6.3	ILE(691)/-2.6 ASP(690)/-9.2
IIb	SER(24)/-4.4 ARG(22)/-9.7	SER(633)/-7 SER(633)/-5	GLY(235)/-3.5 THR(264)/-7.5	ALA(10)/-3.5	TRP(69)/-3.5 ARG(54)/-6.9	ARG(418)/-9.4	SER(661)/-7 SER(661)/-6.7
IIIa	ARG(41)/-6.9	ILE(616)/-9.6 ARG(619)/-5	SER(228)/-3.5 ARG(336)/-7	GLY(24)/-7	LYS(73)/-6.8 THR(120)/-3.5	HYS(487)/-6.3	ASP(663)/-3.5 ASP(663)/-2.5 ARG(729)
IIIb	ILE(59)/-6 THR(25)/-5	ASN(663)/-7 THR(579)/-3.7	PHE(217)/-4 GLU(274)/-3.5	GLY(3)/-3.5 THR(7)	GLY(151)/-3.5 ASP(75)/-8.4	HYS(485)/-7.2	GLN(693)/-3.5 PRO(692) THR(51)/-9.4
IVa	LEU(44)/-3.5 ARG(41)/-6.9	SER(633)/-4.7 SER(633)/-4.3	SER(228)/-3.5 TRP(232)/-6.4	VAL(17)/-3.5 ARG(18)	LEU(74)/-4.8 ARG(60)/-7	ALA(602)/-6.7	GLY(81)/-7.4 LYS(105)/-4.6
IVb	ARG(22)/-6.5 THR(25)/-6.6	ILE(631)/-4.8 SER(632)/-2.5	ASP(180)/-11.1	GLY(3)/-7	ARG(54)/-9.4	GLN(327)/-3.5 ASP(192)/-11.7	ILE(691)/-3.5 HIS(52)/-5.3 THR(51)
Va	THR(62)/-3.9 THR(620)/-7.4	GLY(628)/-9.5 LYS(625)/-3.3	ARG(294)/-12.9	LYS(16)/-6.4 THR(20)/-8.3	PHE(130)/-8.8 SER(135)/-7.9	LYS(388)/-8.3 SER(386)/-2.5	ILE(691)/-7 THR(51)/-3.7
Vb	PRO(61)/-7 ARG(22)/-6.3	THR(579)/-9 THR(579)/-6	TYR(260)/-4.5 SER(315)/-7.3	GLY(3)/-3.5 ASP(1)/-11.8	GLY(151)/-3.5 TYR(150)/-6.9	ARG(463)/-4.7 ASP(470)/-8.5	GLN(693)/-8.9 HIS(51)/-8

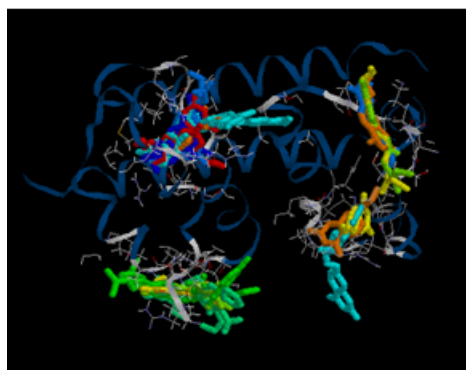


Fig. 1. Interaction of all compounds with capsid protein

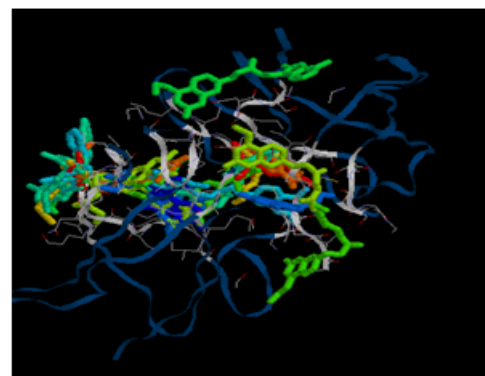


Fig. 2. Interaction of compounds with envelope protein

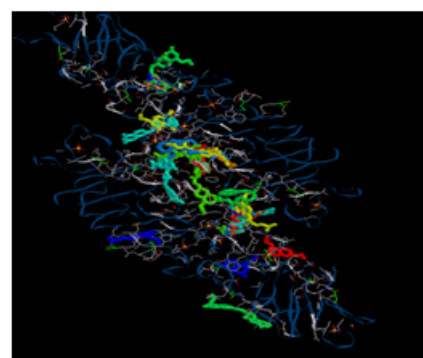


Fig. 3. Interaction of compounds with NS1 protein

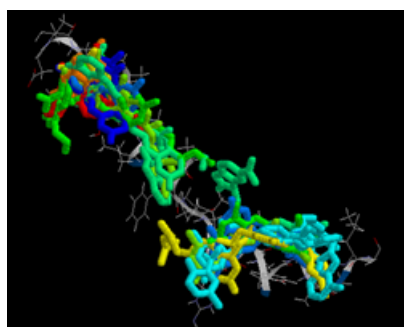


Fig. 4. Interaction of compounds with trans-membrane domain of NS2A protein



Fig. 5. Interaction of compounds with trans-membrane domain of NS2B-NS3 protease protein

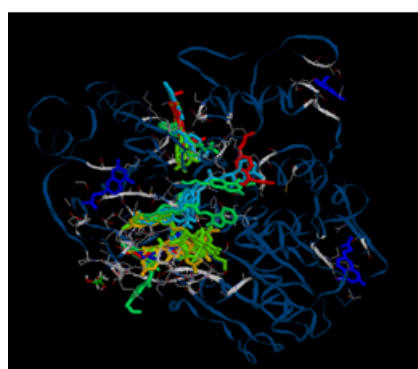


Fig. 6. Interaction of compounds with trans-membrane domain of NS5 protein

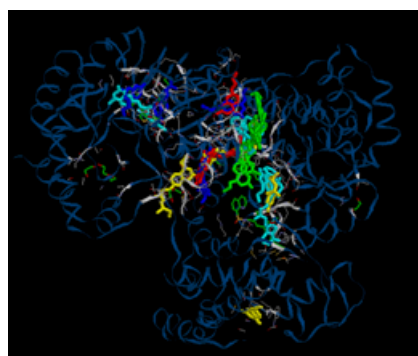


Fig. 7. Interaction of compounds with trans-membrane domain of NS3 helicase protein

iGEMDOCK. From docking study, we listed binding affinity of 10 compounds based on ligand binding energy (Table 2). The binding pose for each ligand molecule into the capsid dengue viral protein is analyzed and the one having lowest ligand binding energy with these proteins among the different poses are generated. The lower energy scores represent better protein-ligand target binding affinity compared to higher energy score.

Dengue virus envelope protein mediates the binding of the virus to the host cell surface receptors. Thus, interference of this interaction would be a potential avenue for antiviral therapy. A small hydrophobic channel has been identified in the DENV envelope protein and this site has been identified as a target for small-molecule inhibitors. The compound **Vb** was found to have the lowest ligand binding energy (-113.61 kcal/mol) compared to other analogs for envelope protein.

NS3 helicase is an essential enzyme for viral replication and has been identified as a potential drug target. The enzyme shows both ATP-hydrolysis activity as well as RNA duplex unwinding activity. 2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)-N'-(5-nitro-2-oxoindolin-3-ylidene)acetohydrazide (**Vb**) showed notably strong docking with DENV NS3 helicase (total binding energy is -144.65 kcal/mol).

NS2B-NS3 is a trypsin-like serine protease that cleaves the dengue protein into individual peptides necessary for viral replication. DENV protease has been identified as a primary target for the development of dengue antiviral drugs. The compound **IVb** has the lowest total binding energy (-106.81 kcal/mol); the binding site of DENV NS2B-NS3 protease involves the binding site of ARG(54).

Among the investigated derivatives, compound **IVb** is found to have lower ligand binding energy, compared to other analogs for capsid protein (binding energy value is -117.07 kcal/mol) and NS2B-NS3 protease protein (binding energy value = -106.81 kcal/mol). Evaluations of the results showed that, the compound **IVb**, 2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)-N-(5-chloro-2-oxoindolin-3-ylidene)acetohydrazide, forms two hydrogen bonds at positions ARG(22) and THR(25), with bond energies, -6.5 and -6.6 kcal/mol respectively. We further analyzed the docked pose for finding the binding mode of compound **IVb** in to capsid protein to validate the reasonable binding conformations.

The compound **Vb** was found to have lower ligand binding energy than other analogs for envelope protein,

NS1 protein, transmembrane domain of NS2A protein, NS3 helicase protein and NS5 protein.

Evaluations of the results in NS3 helicase protein interaction showed that, the compound **Vb**, (2-(4-propyl-2-oxo-2H-chromen-7-yloxy)-N'-(5-nitro-2-oxoindolin-3-ylidene) acetohydrazide), forms two hydrogen bonds at positions ARG(463) and ASP(470), with bond energies, -4.7 and -8.5 kcal/mol respectively.

Regarding to transmembrane domain of NS2A protein interaction, evaluations of the results showed that, also the compound **Vb** forms two hydrogen bonds at positions GLY(3), ASP(1), with bond energies, -3.5 and -11.8 kcal/mol respectively.

We further analyzed the docked pose for finding the binding mode of compound **Vb** in to transmembrane domain of NS2A protein, NS3 helicase protein, NS5 protein to validate the reasonable binding conformations.

Conclusions

Our molecular docking studies explored the possible binding modes of 10 compounds with coumarin ring with seven Dengue virus proteins. It revealed that all the investigated compounds show medium affinity for all the proteins. Especially the compound **Vb** (2-((4-propyl-2-oxo-2H-chromen-7-yl)oxy)-N-(5-chloro-2-oxoindolin-3-ylidene)acetohydrazide) shows best results compared to other compounds. Comparing the binding energy and the binding site residues, we found that all compounds differ either in their binding modes or with the binding site residues for hydrogen bond formation. The conclusion drawn from our virtual screening and docking result was that the compound **Vb** has highest binding affinity with most of the proteins and it can be used as an effective target compound for Dengue virus. Though, there are many reports on the *in vitro* analysis of these compounds and its antioxidant properties, but there are no *in silico* studies that predict the binding and active regions especially with these proteins. Our study is probably the first such attempt to predict the binding site. However, validation of our results through *in vivo* and *in vitro* experiments along with animal models will enlighten hope for the future development of more potent drugs for the treating Dengue.

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