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INSILICO SCREENING OF BENZYLIDINE DERIVATIVES OF PYRAZOLONE AS ANTI VIRAL SCAFFOLD

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ABSTRACT

Flavivirus belonging to the flaviviridae family is an important human pathogen, especially in the tropical and subtropical parts of the world, causing considerable morbidity and mortality. They are transmitted by arthropods, especially mosquitoes. It is the peek time when we need to develop an antiviral agent targeting the viral replication. The incidence of vector borne diseases has grown rapidly and human inhabitants are at risk of becoming infected by virus. No specific therapeutic agent has yet been proved for the treatment or prevention of dengue virus. The present study by in silico method focus on exploring the inhibitors of the NS5 RNA dependent RNA polymerase enzyme. The ns5 protein plays as important key enzyme because of the presence of its N-terminal methyltransferase (MTase) and C-terminal RNA-dependent-RNA polymerase (RdRp) domains which are the main targets of antivirals. Design and synthesis of noval compounds have become an important aspect in the field of drug discovery. Hence we have made an attempt to design computationally drugs against dengue virus using pyrazolone derivatives.

Key words : NS5 Protein, Pyrazolone, Anti Viral, PDBCode: 4C11and Molecular Docking.

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INTRODUCTION

The pyrazolone derivatives are organic compounds used as intermediates for synthesizing pharmaceuticals. It is an important nitrogen containing five-membered heterocyclic compound. The pyrazolone function is quite stable and has inspired chemists to utilize this stable fragment in bioactive moieties to synthesis new compounds possessing biological activities [1].

Pyrazolone nucleus has been shown to possess high biologically activities such as tranquillizing [2], muscle relaxant, antibacterial [3], psychoanaleptic, anticonvulsant, analgesic [4], antihypertensive [5], antidepressant, antioxidant activities, antitumor.

After the work of Fischer and Knovenagel in the late 19th century, the reaction of α, β-unsaturated aldehydes and ketones with hydrazine became one of the general methods for the preparation of 2-pyrazolones [6]. The chemistry of pyrazolones began in1883 when Knorr reported the first pyrazolone derivative. The reaction of phenyl hydrazine and ethyl acetoacetate resulted in novel structure identified in 1887 as 1-phenyl-3-methyl-5-pyrazolone. The Knorr pyrazole synthesis is the reaction of hydrazine with 1,3-dicarbonyl compounds to provide the pyrazolone ring system. Pyrazolone is a membered lactam ring containing two nitrogen and ketone group in its ring. The prototype molecules, antipyrine was synthesized for clinical use in 1883 [7].

The process of drug discovery is very complex and requires an interdisciplinary effort to design effective and commercially feasible drugs. The objective of drug

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design is to find a chemical compound that can fit into a specific cavity on a protein target both geometrically and chemically. After passing the animal tests and human clinical trials, this compound becomes a drug available to patients.

Pyrazolone derivatives are an important class of heterocyclic compounds that occur in many drugs and synthetic products . These compounds exhibit remarkable analgesic, antitubercular, antifungal, antibacterial, anti-inflammatory , antioxidant and antitumor activities. Due to their easier preparation and rich biological activity, pyrazolone framework plays an essential role and represents an interesting template for combinatorial and medicinal chemistry. [8].

Drug discovery process involves the identification of target, target validation, lead identification, lead optimization, synthesis, screening for its therapeutic efficacy. Once the testing is completed, drug development process will started prior to the clinical trials.[9]

The structural and non-structural proteins of DENV have become the major target of antiviral design. The structural proteins (capsid (C), premembrane (prM) and envelope (E)) play vital roles in viral formation and life cycle. While DENV non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) involve in genome replication, virion assembly and avoiding innate immune responses. Among DENV nonstructural proteins, NS5 is the largest (900 amino acid residues) and the most conserved protein in DENV (67% amino acids sequence identity among dengue serotypes) [10]. NS5 has also been an attractive target for antiviral development, as it is required for RNA capping and DENV genome replication.

MATERIALS AND METHODS

HCV (genus *Hepacivirus*) and DENV (genus Flavivirus) belong to the same viral family *Flaviviridae* sharing similar genome organization and replication strategies. Initially, research conducted on dengue virus (DENV) was the actual starting and inspiration point for HCV research, when it became known that HCV had a flavivirus-like genome. Presently and conversely, knowledge and strategies gained from the successful drug discovery and design process against HCV can now be translated back to the DENV research field.

Dengue virus (DENV) is responsible of worldwide arthropod borne viral infection, which globally represents a serious human health concern. DENV are single-stranded, positive sense RNA viruses belonging to the Flaviviridae family. The DENV family can be viewed as falling in four related, but antigenically distinct, DENV 1–4 serotypes. The carriers of DENV to humans are the mosquitoes Aedes aegypti and Aedes albopictus. DENV has a 10.7 kb, positive-sense RNA genome with 50- and 30-untranslated regions flanking a polyprotein that encodes three structural (C, prM/M and E) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins. prM and E structural proteins are the primary antigenic targets of the humoural immune response in humans [11-14].

Major steps involved in the mechanics of docking

Step 1:Preparation of protein

3d structure of the protein has been retrieved from protein data bank(PDB),after that retrieved structure has been pre –processed. The protein selected for the study is NS5 Protein(PDB code:4C11). This is a suitable format for autodock 1.5.6. Then water molecules has been removed from the cavity, stabilized the charges, merged the non polar hydrogens,according to the procedure. And finally saved as pdbqt.

Step 2: Active site prediction

After the preparation of protein, the active site of protein has been predicted using Q site finder. Mostly the water molecules and hetero atoms are removed that are present.

Step 3: Prepation of ligand

The designed ligands are sketched applying chemsketch tool. The ligands are selected based on the Lipinski rule of 5which helps to discern non drug and drug like scaffolds.It

For choice of a ligand allowing to the LIPINSKY'S RULE OF FIVE:

(1) Not more than five hydrogen bond donors
(2) Not more than ten hydrogen bond acceptors
(3) Molecular mass less than 500 Daltons
(4) LogP not over 5
(5) Not more than one violation of the above mentioned.

The ligands were build into the chemdraw ultra 8.0 version and saved in mol format which was further imported into PRODRG for the energy minimization and the energy minimized structure was saved in the pdb format which is compatible input file to the autodock. The imported file has given the partial atomic charges and set of torsion angles. The resultant structure is saved in *.pdbqt* format.

DOCKING METHODOLOGY

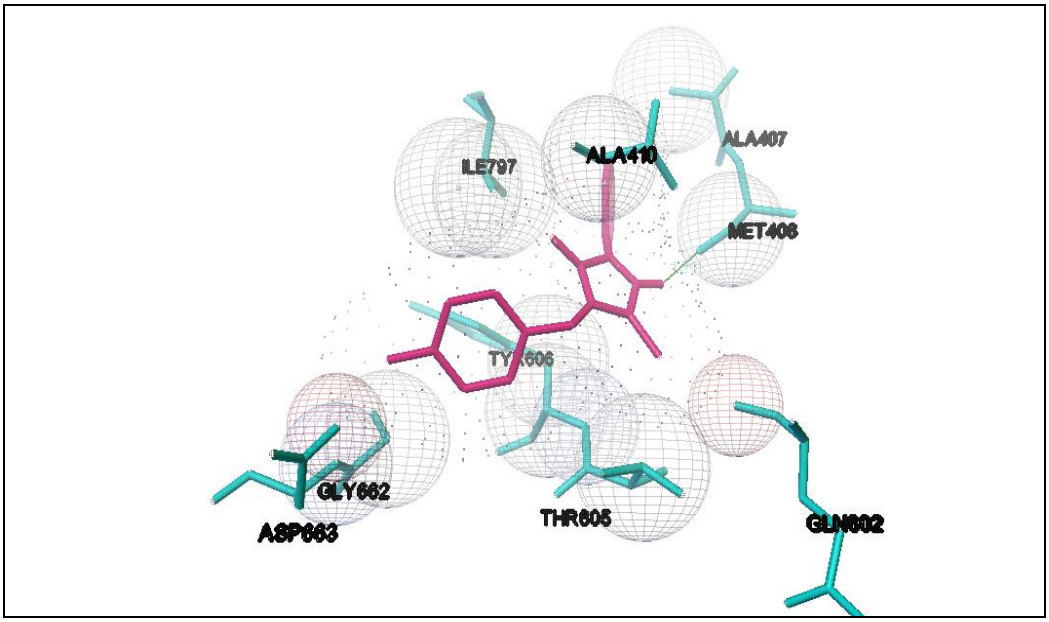
A usually smaller molecule which binds to a larger molecule such as an enzyme or protein initiates the replication process. On the basis of docking of anti-Dengue drugs with the receptor protein,(15). Autodock 1.5.6 was used as docking tool. All of the NS5 RdRp sculptured macromolecule structures were then subjected to an optimization procedure by adding polar hydrogens gasteiger charges and merging non polar hydrogens. Finally assigned AD4 type and save as pdbqt. The ligand molecule is optimized by giving appropriate torsions and saved as pdbqt. We perform docking with AutoDock employing a grid with dimensions of 60×60×60 points

Grid spacing of 0.375Å.the population size is 150 and no:of generations are 27000. The RMSD value is 2.0Å. Molecular simulations were given by lamarckian genetic algorithm. The confirmation with lowest energy are taken as best confirmation. (16).

RESULTS AND DISCUSSION

Screening of the designed ligands has been carried out using the autodock 1.5.6. previously, many studies have been done by the insilico using the NS5 PROTEIN as this the main target of antiviral activity. The structural proteins (capsid (C), premembrane (prM) and envelope (E)) play vital roles in viral formation and life cycle. Keeping in the view the pyrazolone has been gaining as an important scaffold for the many biological activities we designed molecules taking pyrazolone as the basic nucleus. Screening of commercially available cyclic peptides was performed to find potential inhibitors against two binding sites of NS5 methyltransferase (SAM site and RNA-cap site).

Fig 1. Docking pose of PC5



The values of predicted binding energy and docked energies are the sum of inter molecular energy and torsional energy and ligands internal docking energy respectively.

The low inhibition constant values indicate the efficacy of the ligand to stimulate the enzyme and proves its increased affinity towards the catalytic site of enzyme. The calculated binding energy ranges from -8.03 to -8.41 K cal /mol and inhibition constant ranges from 963.18nM to 1.13µM.

Table 1. Docking Score of Synthesized compounds

S. No	Code	Binding energy	Inhibition constant(nm)	No: of h bonds
1	PC1	-8.03	1.3*	0
2	PC2	-8.28	859.46	0
3	PC3	-8.22	949.24	0
4	PC4	-8.21	963.18	2
5	PC5	-8.41	682.54	1
6	PC6	-8.12	1.12*	0
7	STD	-5.69	66.94*	3

*= µM(Micromol)

Among the designed analogs 5- methyl-4- (4-methylbenzylidene)-2- phenyl-2,4-dihydro- 3H-pyrazol- 3-one (PC5) , 4- (4-chlorobenzylidene)- 5-methyl-2- phenyl-2,4- dihydro-3H-pyrazol-3- one (PC2) showed good binding energy of -8.41K cal /mol & -8.28K cal /mol & inhibition constant of 682.54 nM and 859.46 nM respectively.

CONCLUSION

The designed pyrazolone scaffolds (PC1-PC6) are being attached with the different aldehyde derivatives leads to the formation of benzylidene derivatives of pyrazolone. The scaffolds (PC5 & PC2) have shown good docking score against the NS5 protein. Here the computational values show the best score for the antiviral activity, thus further experimental work has to done to confirm their activity. On the other hand while revealing the docked scores both electron releasing group and electron withdrawing group has given the significant binding as well as inhibition constant.

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CONFLICT OF INTEREST

No interest

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