Research Article

A Novel, Selective Inhibitor of Hepatitis C Virus Targeted against the Viral RNA-Dependent RNA-Polymerase

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ABSTRACT

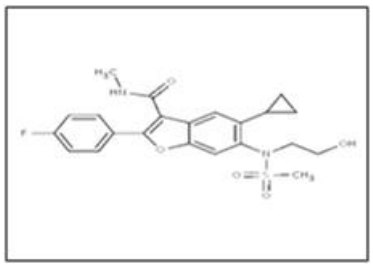
Hepatitis C virus, an enveloped positive – stranded RNA virus and the sole member of the genus Hepacivirus within the Flaviviridae. RNA dependent RNA polymerase (RdRp) plays an important role in the replication process, and thus is a validated target for antiviral drugs. The RNA polymerase Non-Structural protein 5B (NS5B) of HCV is a well-recognized and characterized drug target site. The pharmacophores were constructed and following the virtual screening was carried out for Nesbuvir and Filibuvir against RNA Dependent RNA Polymerase (Protein 3FQK). The interaction was screened by means of docking and best binding complex was loop docked to get the exact binding site which will inhibit the HCV. Therefore, from this study we proved that ZINC8765236 and ZINC08636466 showed the best result against HCV by binding RdRp.

Keywords: HCV, NS5B, Filibuvir, Hepatitis C, RNA-Dependent RNA Polymerase.

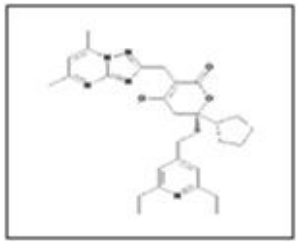
INTRODUCTION

Hepatitis is a medical condition which is determined by the inflammation of the liver, characterized by the presence of inflammatory cells in the tissue of the organ. Hepatitis can be acute or chronic depends on the duration of the cycle of infection. In case of acute it lasts less than six months, if not treated chronic condition may occur. Viral hepatitis is the most common cause of hepatitis worldwide. Viral hepatitis emerged as a major public health problem throughout the world, affecting several hundreds of millions of people.Hepatitis virus includes a range of unrelated and often highly unusual human pathogens.[1] Hepatitis virus is classified in different groups namely Hepatitis A, B, C, D, E and many.[2]

Hepatitis C Virus is an enveloped positive stranded RNA virus and the sole member of the genus Hepacivirus within the Flaviridae. According to the WHO report, around 3% of the world's population has been infected with HCV and more than 170 million people carries chronic HCV, leads to serious liver cirrhosis and/ or hepatocellular carcinoma (HCC).[3-4]The HCV virus has approximately 9.6kb of RNA genome which encodes a polyprotein of around 3,000 amino acids. This polyprotein is cleaved by viral and cellular proteases, generates the structural and non- structural proteins. The amino terminus of the polyprotein sequence contains structural proteins which include the core, the envelope glycoproteins (GPs) E1 and E2, and p7. The carboxyl terminus of the polyprotein contains NS protein, including NS2 through NS5.[5]Thenon-structural proteins are NS1/p7, NS2, NS3, NS4A, NS5B, NS5A and NS5B. E1 and E2 play important role in the entry of the virus into the host as they function as the outer surface proteins.[6] NS5B codes for the RNA-dependent RNA polymerase (RdRp).[7-8]



Nesbuvir



Filibuvir

Figure 1: Marketed drugs against HCV

Filibuvir**,** an oral drug being developed by Pfizer. It is a non-nucleoside inhibitor of the HCV NS5B RNA dependent RNA polymerase. Filibuvir demonstrated a good pharmacokinetic profile and oral bioavailability in preclinical animal trials. Filibuvir monotheorapy or in combination with pegylated IFN alpha 2a/ Ribavirin for up to 4 weeks significantly reduced the infection by the virus. Adverse events like SoC and placebo were observed.[9]

Nesbuvir, a novel selective non-structural protein 5B (NS5B) polymerase inhibitor against HCV. HCV- 796 reduced HCV RNA levels by 3 to 4 log (10) HCV copies/mug total RNA. The concentration of the compound that inhibited 50% of the HCV RNA level was 9 nM.[10]

Seven major genotypes and subtypes exist based on the sequence heterogeneity of the HCV genome; their distribution, transmission, and disease progression differ substantially. The distribution of genotypes 1 and 2 is global, whereas genotype 3 predominates in South-east Asia, genotype 4 in Africa and Egypt, genotype 5 in South Africa, and genotype 6 primarily in Hong Kong and Vietnam. Recently, the direct-acting antivirals(DAAs) boceprevir and telaprevir were approved for use in combination regimens with pegylated alphainterferon (PEG-IFN) and ribavirin(RBV), resulting in enhanced sustained virologic response(SVR) rates (66 to 75%) in genotype 1 treatment- naïve patients.[11-12] This is complicated three- times- daily dosing schedules and significant side effects such as anemia, adds further limitations to the well documentated tolerability issues associated with interferon- based regimens, result in higher discontinuation rates or dose reduction.[13]Therefore the development of additional direct antiviral agents with diverse resistance profiles is necessary, with the ultimate goal of developing all oral antiviral combinations that can achieve superior sustained virologic response (SVR) without the use of IFN or RBV. Thus, major efforts are under way to search additional novel inhibitors of HCV. And hence much focus is being placed on the viral polymerase NS5B as a target.[14]The limitations of suboptimal response shift research toward developing more specific targeted antiviral therapy against HCV (STAT-C). In 1999 the discovery of HCV's autonomous subgenomic replication system boosted knowledge of its replication cycle and a robust cell culture infection system JFH-1/HCVcc was established in 2005 which allowed researchers to study the HCV life cycle.[15]Current studies are focusing their interest against specific inhibitors of NS3/4A and NS5B.[16] These targeted therapeutics have advandced to phase II and III clinical trials.

This project will focus on finding novel drugs by means of insilico concept which has been proposed and successfully used for many targets and thus protein-ligand binding was carried out by Insilico concept. The main issue or interest of this project was to develop new novel drug against HCV. The pharmacophore based screening approach was selected wherein the pharmacophore scaffolds were subjected to structure based similarity search for anti- HCV to have an estimate of the binding affinity. These somewhat same scaffolds structure were then subjected to further cleansing by removing the duplicates structure followed by docking, which helped to study the binding sites of Protein- ligand. To make pharmacophore two anti-HCV were used Nesbuvir and Filibuvir.

MATERIALS AND METHODS

Target identification

Virus's mechanism for RNA synthesis needs RNA-dependent RNA-polymerase (RdRp) which interacts with nascent RNA to provide valuable insight for viruses. NS5B is aRdRp, essential for HCV replication, uses a viral positive RNA strand as its template. Further detailed information obtained from different databases like NCBI, Uniprot, Drug Bank, Therapeutic Targets Database regards target protein 3FQK. As per the information obtained from Uniprot P26663, we identify 3FQK has the property of RdRp.[17]

Binding site identification

HCV RNA replication occurs in the membrane bound replication complex that consists of various protein essential for replication. (Mutagenesis). Biochemical studies reveal that all the conserved functional motifs of NS5B are necessary for RdRP activity. PDBsum is the database that provides summary information about each experimentally determined structural model in the Protein Data Bank (PDB). PDBsum database was created at University College London in 1995.[18-19]To check the ligand binding site PDBSum (http://www.ebi.ac.uk/pdbsum/) database was used. Hepatitis C virus enters the host via multiple biding sites and can hence bind to several other molecules. HCV has high mutation rates because of the lack proofreading ability of its RNA-dependent RNA polymerase. Therefore, scientists are more concerned towards this binding site. Specifically NS5B is vigorously studied for the binding purpose with the novel drugs. Nesbuvir as well as Filibuvir acts against NS5B, hence we analyzed both the drugs against 3FQK which has the property of RNA dependent RNA polymerase.

Pharmacophore generation

Pharmacophore is defined as "an ensemble of steric and electronic features which is necessary to optimize the super molecule interaction with specific biological target structure and trigger its biological response - by IUPAC".[20] Pharmacophore represents following features – Hydrogen bond donor, Hydrogen bond acceptor, Aromatic, Positive charge, Negative charge, Ring aromatic. In our study we macromolecular approach implemented. The drugs were combined into a single mol2 file and submitted to the Pharmagist server to generate pharmacophores http://bioinfo3d.cs.tau.ac.il/pharma/index.html.This uses ligand-based pharmacophore detection. It aligns a set of drug- like molecule that can bind to the receptors and pharmacophore detection of ligand. Meanwhile proteins were searched with whom drugs will show interaction. Those proteins were searched in PDB and downloaded. After downloading the proteins were cleaned using Accelrycs Discovery Studio and water molecule as well as hetroatoms were deleted and further proceeded for virtual screening .[19]

Virtual screening using zincpharmer:

Chemical library is a database which holds information about collection of drug like compounds in SDF and MOL2 format. It also contains information regarding molecular weight, molecular formula, smiles, LogP, hydrogen bond donor, hydrogen bond acceptor, etc. in this project we

use ZINCPharmer. ZINCPharmer is a free database that contains millions of drug like molecule. Its search is based on fingerprint as wel as alignment based conformer. The best result was then uploaded in the ZINCPharmer server http://zincpharmer.csb.pitt.edu/. This server is a free pharmacophore search software for screening the purchasable subset of the ZINC database, it has features like it can identify pharmacophore features directly from structure and can identify the subset of ZINC database and gave the result of similar molecules to the pharmacophore uploaded. The best pharmacophore was then checked in different database like Zinc Drug Database, Zinc Natural Derivative and Zinc Product Derivative. For every best result these three criteria were chosen and then downloaded. The protein 3FQK, was downloaded and checked in PDBsum for the ligplot. Using ligplot co-ordinates where the ligand can bind was estimated and noted. The X, Y and Z axis were determined and the mean was calculated.

Knime

KNIME (The Konstanz Information Miner) is a data mining tool which is used for finding chemical diversity of drugs molecule using PubChem Substructure fingerprinting. It is an open- source workflow platform which supports a wide range of functionality and has an active chemoinformatics / bioinformatics community. This plugins is based on the Chemistry Development Kit (CDK). It wraps elements of the library's core functionality and expresses it to the user.

Docking

In this project we use Auto-Dock Vina for docking as it is one of the finest, fast, accurate, free software for multiple docking. It is a user friendly software that screens thousands of drugs using a simple protocol. This requires protein preparation as rigid and fixed. . AutoDockVina is a multiple docking open source software. Vina shows the possible binding sites and affinities of a ligand depending upon its complexity. The result of Vina was then analysed[11].The confirmation file that has information regarding XYZ co-ordinates and the ligand is docked with the receptor. All the sdf files of particular ligand were gathered and then docked with proteins. Protein3FQK was downloaded using PDBId and cleaned and converted using MGL Tool into PDBQT file format which is needed for docking. As the ligand were large in number, multiple docking was used which works in Auto Dock Vina. The required script and parameters were taken from http://autodock.sripts.edu/website. All the drugs from KNIME were docked with the protein in their binding site and further analyzed for the best score and energy.

Loop docking

The purpose behind the loop docking approach is based on the fact that conservative docking runs could remarkably improve the docking energy and orientation. Loop docking deals with the drugs with maximum conformation with minimum binding energy. Loop docking runs on an automated script. The automated loop docking will continue till threshold value is reached. The threshold value (d) governs the difference between the docking binding energy of the last run and the preceding one. When threshold value is 0.05 it is said to be appropriate. When this value is reached, the docking is turned off and best result is selected.

Lead optimisation

In addition to high biologic activity and selectivity for the target of interest, drug metabolism and pharmacokinetics (DMPK) properties that includes absorption, distribution, metabolism, excretion and the potential for toxicity (ADMET) in humans are critical for the success of any candidate therapeutic. There is considerable attention is given on improving the compound's in vivo DMPK/ ADMET properties without disturbing or altering its biologic activity. Computational tools are routinely used to filter large databases so that the compounds predicted to have poor DMPK/ADMET features may be avoided. The best filter to apply any compound database is the Lipinski's rule of five. These rules are (1) Molecular weight of 500 or less, (2) LogP coefficient less than 5, (3) 5 or fewer hydrogen- bond donor sites, (4) 2_5 or fewer hydrogen- bond accepting sites.[7]This set of rules suggests the necessary properties for the good oral bioavailability.

RESULTS AND DISCUSSION

Binding site identification

As described above, 3FQK acts as a protein responsible for the viral entry and as well as its replication, it was chosen as a primary protein binding site for the novel drugs. 3 FQK was searched in PDBSum for obtaining ligplot to get the co-ordinates detail where the drug has affinity to bind. The interaction of the binding sites is given in table 1.

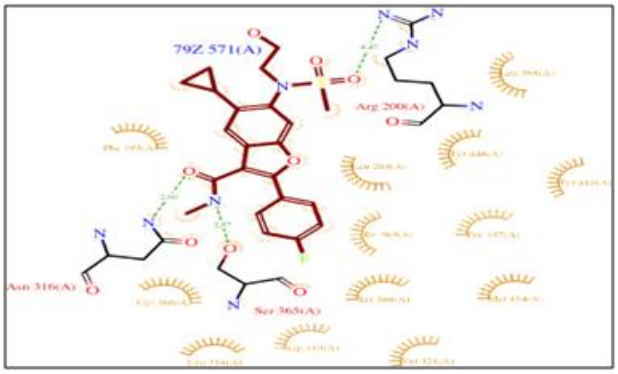


Figure 2: Ligplot of protein 3FQK

Table 1: Active sites for the anti- HCV

Anti-HCV	PDB ID	Amino acid
Nesbuvir, Filibuvir	3FQK	Asn 316(A) N
		Ser 365(A) O
		Arg 200(A) N

Knime analysis

The result obtained from Zincpharmer was in SDF file format. Thus a lot of hits or molecules were obtained. We presented these molecules to the some special features of KNIME-CDK workflow for the removal of duplicates. The ZINC molecules obtained later cleaned for Split Molecule File. And then these files all together proceed for docking with different targeted protein.[4]

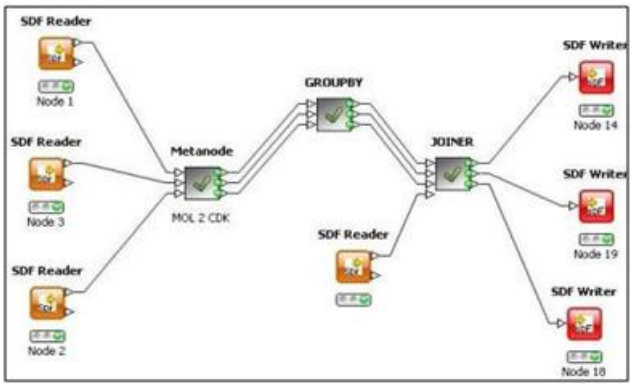


Figure 3: Knime workflow for combine drugs repeated in all the pharmacophore

Docking analysis

Initiallydocking was performed on all the chemically diverse ligands from ZINCPharmer with the Anti HCV, like Nesbuvir, Filibuvir, Setrobuvir and Tegobuvir. Out of many docked molecules approximately top 70 were analyzed from each set. Onlya few molecules showed the proper binding at Arg 200 (A). AutoDock and Cygwin plugin was performed to study molecular docking. And resultswere analyzed using MGL Tool of AutoDock. It was observed from docking studies that the ZINC00659092, ZINC04245683, ZINC0249543434, ZINC08636466 and ZINC08765236 were the top drugs result. These results were re-docked to confirm the stability of the conformation.

Table 2: protein ligand docking of best drugs based on binding affinity

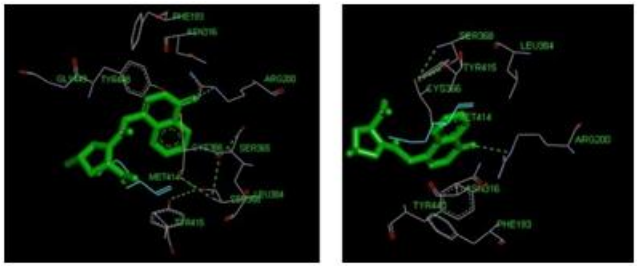
Molecule ID	Receptor	Affinity kcal/mol	Interaction
ZINC00659092	3 FQK	9.7	SER365: OG 1,ARG200: NH2 1, ARG200:NE 1
ZINC02495434	3 FQK	8	ARG200:NH1 1
ZINC04245683	3 FQK	8.1	ARG200:NH2 1,ARG200:NE 1
ZINC08636466	3 FQK	6.9	ARG200: NH2 1
ZINC08765236	3 FQK	6.8	ARG200:NE 1

Binding interaction of ligand and protein

The results obtained from loop docking was further analyzed for the ADMET prediction. This prediction shows good result and Bioavailability is astonishing. One by one the ZINC id's were submitted to the ADMETSAR server to get the information about the toxicity, Lipsinki rule of five, Non carcinogenic etc properties of the drug. These properties help to know whether the drug is having bioavailability nature and can it be use further. Out of five drugs, only two drugs followed the above mentioned properties. The result is shown in table 3, 4 and 5. The two molecules ZINC08765236 (4hydroxy-1-[2-(4-oxo-3H-phthalazin-1-yl)acetyl]-pyrolidine-2-carboxylic) and ZINC08636466(6,7-dihydroxy-N-phenyl-3-(4-pyrazol-1-ylphenyl)-2-thioxo-3a,4,5.6,7,7a-hexahydro-1H-benoimidazole-4) were the two who showed good result. The binding interactions was visualized in Figure 4.

Table 3: Loop docking result of selected drug

Zinc ID	Docking	Receptor	Affinity (kcal/mol)	Interaction
ZINC 08765236	Dock 1	3FQK	-7	Arg200:NE1
	Dock2	3FQK	-7	Arg200:NE1
	Dock 3	3FQK	-7	Arg200:NE1
	Dock4	3FQK	-7	Arg200:NE1
	Dock 5	3FQK	-6.8	Arg200:NE1
	Dock6	3FQK	-7	Arg200:NE1
	Dock7	3FQK	-6.8	Arg200:NE1
	Dock 8	3FQK	-7	Arg200:NE1
	Dock9	3FQK	-7	Arg200:NE1
	Dock10	3FQK	-7	Arg200:NE1
	Dock11	3FQK	-7	Arg200:NE1
	Dock12	3FQK	-7	Arg200:NE1
	Dock13	3FQK	-7	Arg200:NE1
	Dock14	3FQK	-7	Arg200:NE1
	Dock15	3FQK	-7	Arg200:NE1
ZINC08636466	DOCK1	3FQK	-6.9	Arg200:NH2
	DOCK2	3FQK	-7	Arg200:NH2
	DOCK3	3FQK	No interaction	
	DOCK4	3FQK	-6.7	Arg200:NH2
	DOCK5	3FQK	-6.9	Arg200:NH2
	DOCK6	3FQK	-7	Arg200:NH2
	DOCK7	3FQK	-6.9	Arg200:NH2
	DOCK8	3FQK	-6.9	Arg200:NH2
	DOCK9	3FQK	-6.8	Arg200:NH2
	DOCK10	3FQK	-6.9	Arg200:NH2
	DOCK11	3FQK	-6.9	Arg200:NH2
	DOCK12	3FQK	-7	Arg200:NH2
	DOCK13	3FQK	-6.7	Arg200:NH2
	DOCK14	3FQK	-6.9	Arg200:NH2
	DOCK15	3FQK	-6.9	Arg200:NH2



A B

Figure 4: (4A) shows the protein ligand interaction of ZINC08765236 (4B) shows the Protein ligand interaction of ZINC08636466

Table 4: bioavailability, drug likeness and toxic property of ZINC 08765236 and ZINC08636466

Properties	ZINC 08765236	ZINC08636466
Inhibitor	Week inhibitor	Week inhibitor
AMES Toxicity	Non-AMES toxic	Non-AMES toxic
Carcinogen	Non-Carcinogens	Non-Carcinogen
Biodegradation	Not ready biodegradable	Not ready biodegradable
MASS	316.289	449.525
LogP	0.68	1.85
LIPINSKI'S RUE	Yes	Yes
Bioavailability	Yes	Yes

CONCLUSION

The present studies say that there is no potent drug against HCV. The drugs which are available have few side effects as the dosage demands high intake for several times in a day. The insilico approach using PharmaGist, AutoDock and ADMET prediction can help out to find the potential inhibitor against Hepatitis C virus. The molecular docking is one of the powerful and trusted technique for identifying biological significance and exploring new drugs by screening several compounds. Here we targeted 3 FQK which is an RNA dependent RNA polymerase. The active sites of 3FQK were docked with Anti-HCV. The five compounds were finalized for the ADMET prediction. This prediction showed that compounds ZINC 08765236 and ZINC08636466 have the ability to act against HCV. As they have followed the Lipinski's rule as well as they are non- toxic and have property of Bioavailability. However, further in vivo experimentation is required for the validation of the drug. The rest three compounds ZINC00659092, ZINC04245683 and ZINC02495434 somehow failed to govern these properties, but if they will be optimize further as well as in depth structural and biological studies, can be used against HCV also.

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