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Structure based virtual screening of inhibitors for binding at the allosteric site of HCV RNA dependent RNA polymerase

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Abstract:
Hepatitis C Virus (HCV) infection is a serious cause of chronic liver disease worldwide with more than 170 million infected individuals at a risk of developing significant morbidity and mortality. There is no effective treatment or prevention till date for HCV infection. We describe the computed binding of 10 compounds to the allosteric binding site of RNA dependent RNA polymerase enzyme. These compounds were identified from the ZINC database through virtual screening followed by ADMET evaluation.

Background:

Hepatitis C Virus (HCV) causes "non-A non-B hepatitis" in humans. It is estimated that 170 million people has been infected worldwide by HCV **[1].** The virus is transmitted through contaminated blood, organ transplants and all other means of exposures that ranges from needle prick among drug abusers to unprotected sex **[2].** The acute infection will become chronic, leading to liver cirrhosis, hepatocelluar carcinoma and liver failure. There is no effective vaccine available to prevent the disease or a proper treatment regime to cure the infection **[3, 4].** Hence a large amount of resources are being utilized to discover a drug molecule which can clear the virus and cure the infection with minimum side effect and maximum efficacy. At present pegylated interferon, which generally targets the immune system and the purine analogue ribavarin are used against HCV. The combination of ribavarin and pegylated interferon is not specific to HCV infection and also associated with significant side effect **[5].** The HCV NS5B protein is a 65 kDa protein formed of the C-terminal 591 amino acids of the polyprotein. An RNA dependent RNA polymerase (RdRp) activity was predicted for the NS5B gene product due to the presence of the Gly-Asp-Asp motif (GDD) that is common to the reverse transcriptase and other viral polymerases **[5].** This was later confirmed *in vitro* by experiments using recombinant expressed NS5B protein **[6].** The active RdRp enzyme requires divalent metal ions and uses the RNA template strand to direct the synthesis of a complementary strand. Divalent manganese ions (Mn^{2+}) are preferred over magnesium ions (Mg^{2+}) for optimal enzyme activity, whereas zinc ions (Zn^{2+}) inhibit the RdRp activity **[7].**

HCV RdRp consists of 21 α-helices and 18 β-strands. The catalytic domain consists of 531 residues folded into characteristic fingers, palm and thumb subdomains. The NS5B adopts a unique globular shape due to extensive interactions between the fingers and thumb sub-domains that serve to encircle the active site of the enzyme **[8].**

HCV NS5B is an important target for therapeutic intervention aimed at inhibiting HCV replication. The rational approaches of drug designing which target NS5B have identified several classes of inhibitor molecules. But, a nucleoside analogue NM283 is the only inhibitor that has demonstratable levels of antiviral activity in human subjects **[9].** Chemically, NM283 is a 2'-C-methyl cytidine resistance to NM283 has been reported in sub-genomic replicons. Substitution of the amino acid serine at position 282 with threonine was seen to confer resistance to 2′-C-methyl ribonucleosides by efficient discrimination between the analogues and the natural substrates **[10].** Two non-nucleoside inhibitors of HCV NS5B, JTK 109 and JTK-003 are also under clinical trials. These are benzimidazole compounds and act as allosteric inhibitors of NS5B. Replacement of proline 495 with alanine or leucine is found to confer resistance against these compounds in sub genomic replicons **[11].** Several other compounds of the benzothiadiazine class are also being investigated. However, a cause for concern is the observation that the subgenomic mutants develop resistance against benzothiadiazines **[12].** The allosteric binding sites may be far away from the usual binding site of the protein, but binding of small molecules to these sites may alter the function of the protein **[13].** Crystallographic study by Di Macro *et al.* 2005 **[14]** revealed that an allosteric non-nucleoside inhibitor (NNI) binding site can be targeted for small molecule search. This allosteric binding site can lead a new path in discovering effective inhibitors against the polymerase enzyme. Here we describe the identification of 10 small molecules through molecular docking and ADME analysis. These show more binding properties than the existing inhibitor bound to the protein.

Methodology:

The 3D structure of HCV NS5B bounded with an allosteric inhibitor were retrieved from Protein databank (PDB), accession code 2BRK was found to bound to an allosteric inhibitor named compound 1. The ligand bounded with the protein was re-docked inorder to obtain binding affinity information. The active residues were identified as the neighbouring amino acids with in 10 Å distance from the bounded ligand.

Virtual screening using Zinc database

The ZINC database is a curate collection of commercially available chemical compounds prepared especially for virtual screening **[15].** ZINC is used by investigators (generally people with training as biologists or chemists) in pharmaceutical companies, biotech companies, and research universities. There are many subsets available in the Zinc database. We had selected leads subset containing 1,283,469 molecules which is ready to perform virtual screening.

Molecular Docking

Molecular docking was carried out using GOLD 4.1(Genetic Optimization of Ligand Docking) **[16].** The parameters used for genetic algorithm were population size - 100, selection pressure - 1.1, number of operations - 100,000, number of islands - 5, niche size - 2, migrate - 10, mutate - 95 and cross-over - 95. The default speed selection was used to avoid a potential reduction in docking accuracy. Fifty genetic algorithm runs with default parameter settings, were performed without early termination for both Gold score and Chem score. The whole protein complexes were used for Molecular Docking, run in order to obtain maximum accurate binding possibility. The best binding pose were determined after careful visualisation and scoring function.

ADME Prediction (Absobtion, Distribution, Metabolism, Excretion)

Predicting physiochemical properties of a chemical compound will always cut short the expensive experimental testing and hard labour. Molecular Docking studies revealed that 10 compounds from the Zinc small molecule databank have potential binding affinity towards HCV NS5B RdRp. Hence, the ADME predictions of these compounds were carried out using freely and commercially available web based ADME Boxes developed by Pharmaco Algorithms (http://pharmaalgorithms.com/webboxes/). It is a software module that calculates physiochemical properties, oral availability (human), human intestinal absorption, plasma bound distribution based on the chemical structure.

Discussion:

The HCV NS5B is the RNA dependent RNA polymerase responsible for replication of the viral genome and is a key target for the therapeutic intervention against HCV. The ADME/T characteristics of the compound reveal that it has high mutagenic potential which increases its toxicity. The LD50 studies also showed that it had to be administered at high dosages which would again increase its toxicity. It has low solubility characteristics in various pH and it is highly insoluble in water. Upon docking, Compound 1 showed a GOLD score and Chem score of 46.50 and 13.56. These drawbacks indicate the need for an effective drug that can replace this molecule. All the lead candidates that were shortlisted from small molecule databases had better docking scores than the known ligand **Table 1 (see supplementary material)**.

Lipoxin A4, Fluprostenol displayed excellent oral bioavailability and distribution characteristics. They had weak acidic character and obeyed Lipinski's Rule. They showed moderate to low solubility in various pH and water and do not act as P-gp substrates. They exhibited low probability of mutagenic potential and the LD50 studies suggested intravenous route of administration for mice and intraperitoneal route of administration for rats at low dosage forms.

Leukotriene E4 showed limited ability to cross the intestinal barrier by passive diffusion, hence affecting its oral bioavailability. It had good distribution characteristics and behaved as a weak base. It obeyed Lipinski's rule, had moderate to low solubilities in various buffers and water and does not act as a P-gp substrate. It showed moderate probability of acting as a mutagen thereby increasing its toxicity.

Lactacystin, Diethylnorspermine showed limited ability to cross the intestinal barrier by passive diffusion, hence affecting their oral bioavailability. They had low protein binding efficiency thereby affecting its distribution. They showed low probability of acting as P-gp substrates. They showed basic character, obeyed Lipinski's rule and had good solubilities in various buffers and water. They also displayed low probability of acting as a mutagen and were required to be delivered in low dosage inspite of the route of administration. The lead candidates **Thromboxane B2**, **Leukotriene B3**, **17-phenyl-trinor-pge2** and **Misoprostol free acid** displayed good oral bioavailability and distribution characteristics. They had strong acidic character and obeyed Lipinski's Rule. They showed moderate solubility in various pH while it was insoluble in water. They showed low probability of acting as a P-gp substrate. They exhibited low probability of mutagenic potential and the LD50 studies suggested intraperitoneal route of administration for mice and rats at low dosage forms for Thromboxane B2 and intravenous route for mice in the case of Leukotriene B3, 17-phenyl-trinor-pge2.

Lavendustin A shows high probability of undergoing first pass metabolism thereby affecting its oral bioavailability. It displayed good distribution characteristics and did not act as a P-gp substrate. It had both acidic and basic groups with a majority of the former. It obeyed Lipinski's rule and had low solubility in various buffers and water. It had low mutagenic potential and the LD50 studies showed that the preferred route of administration was intravenous in mice and intra-peritoneal in rats. It should be noted that these compounds are designed *in silico* and should be further tested *in vitro* and *in vivo* for toxicity and activity.

Competing interests:
The author(s) declare that they have no competing interests.

References:

[1] Choo QL *et al*. *J Hepatol*. 12002 **36**: 582 [PMID: 11983439]
[2] Orlando R & Lirussi F, *Infection*. 2007 **35**: 465 [PMID: 17906840]
[3] Fried MW *et al*. *N Engl J Med*. 2002 **347**: 975 [PMID: 12324553]
[4] Seeff LB & Hoofnagle JH, *Clin Liver Dis*. 2003 **7**: 261 [PMID: 12691470]
[5] Bernstein D *et al*. *Hepatology*. 2002 **35**: 704 [PMID: 11870387]
[6] Berdichevsky Y *et al*. *J Virol Methods*. 2003 **107**: 245 [PMID: 12505640]
[7] Miller RH & Purcell RH, *Proc Natl Acad Sci* U S A. 1990 **87**: 2057 [PMID: 2156259]
[8] Lohmann V *et al*. *Virology*. 1998 **249**: 108 [PMID: 9740782]
[9] Ishii K *et al*. *Hepatology*. 1999 **29**: 1227 [PMID: 10094969]
[10] Ishido S *et al*. *Biochem Biophys Res Commun*. 1998 **244**: 35 [PMID: 9514871]
[11] Migliaccio G *et al*. *J Biol Chem*. 2003 **278**: 49164 [PMID: 12966103]
[12] Tomei L *et al*. *J Virol*. 2003 **77**: 13225 [PMID: 14645579]
[13] Namboodiri S *et al*. *Syst Synth Biol*. 2011 **4**: 271 [PMID: 22132054]
[14] Di Marco S *et al*. *J Biol chem*. 2005 **280**: 29765 [PMID: 15955819]
[15] http://zinc.docking.org
[16] Jones G *et al*. *J Mol Biol*. 1997 **727**: 748 [PMID: 9126849]

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Supplementary material:

Table 1: Docking and post docking analysis of the lead compounds in comparison to compound 1 (solved by X-ray crystallography in complex)

SL NO.	LIGAND	Hydrogen bonding Residues	No. Of H bonds (distance <3 Å)	Close Contacts (<3 Å)	Distance From Close Contact (å)	Gold score	Chem score
1.	COMPOUND 1 (X-ray solved)	Arg 503	2 (1.666)	-	-	60.50	13.56
2.	LIPOXIN A4	Leu 492 Gly 493 Val 494 Arg 503 Trp 500	2 (2.482) 1 (2.262) 1 (1.927) 1 (2.284) 2 (2.202)	Val 494	2.647	81.50	21.70
3.	LEUKOTRIENE E4	Arg 503 Trp 500	4 (1.661) 1 (2.045)	Pro 495 Trp 500	2.735 2.371	77.06	18.36
4.	LEUKOTRIENE B3	Trp 500	1 (2.027)	Arg 503	2.735	74.08	22.98
5.	LACTACYSTIN	Arg 503 Trp 500	2 (2.228) 4 (1.875)	Pro 495	2.825	70.76	21.82
6.	THROMBOXANE B2	Arg 503 Trp 500	3 (1.694) 2 (1.570)	Arg 503	2.535	71.09	15.71
7.	17-PHENYL-TRINOR-PGE2	Arg 503 Trp 500	3 (1.727) 4 (1.970)	Pro495 Trp 500	2.587 2.489	71.28	21.20
8.	DIETHYLNORSPERMINE	Arg 503 Trp 500	3 (1.694) 1 (2.045)	Trp 500	2.857	72.91	18.91
9.	LAVENDUSTIN A	Trp 500 Arg 503	2 (2.728) 3 (1.695)	Arg 503 Trp 500	2.565 2.463	73.47	20.52
10.	FLUPROSTENOL	Arg 503 Trp 500	5 (2.246) 2 (1.640)	-	-	73.60	23.43
11.	MISOPROSTOLFREE ACID	Trp 500	3 (1.876)	Pro 495	2.569	73.76	17.36