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Molecular docking studies of phytochemicals from *Phyllanthus niruri* against Hepatitis B DNA Polymerase

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Abstract:
Hepatitis B virus (HBV) infection is the leading cause for liver disorders and can lead to hepatocellular carcinoma, cirrhosis and liver damage which in turn can cause death of patients. HBV DNA Polymerase is essential for HBV replication in the host and hence is used as one of the most potent pharmacological target for the inhibition of HBV. Chronic hepatitis B is currently treated with nucleotide analogues that suppress viral reverse transcriptase activity and most of them are reported to have viral resistance. Therefore, it is of interest to model HBV DNA polymerase to dock known phytochemicals. The present study focuses on homology modeling and molecular docking analysis of phytocompounds from the traditional antidote *Phyllanthus niruri* and other nucleoside analogues against HBV DNA Polymerase using the software Discovery studio 4.0. 3D structure of HBV DNA Polymerase was predicted based on previously reported alignment. Docking studies revealed that a few phytochemicals from *Phyllanthus niruri* had good interactions with HBV DNA Polymerase. These compounds had acceptable binding properties for further *in vitro* validation. Thus the study puts forth experimental validation for traditional antidote and these phytocompounds could be further promoted as potential lead molecule.

Keywords: Hepatitis B, *Phyllanthus niruri*, Phytochemicals, Homology modeling, Molecular Docking

Background

Hepatitis B virus (HBV) causes chronic hepatitis infection to over 350 million people worldwide and it is estimated that over 2 billion people have been exposed to HBV worldwide **[1, 2].** The risk of developing hepatocellular carcinoma associated with HBV is higher when compared to non- carriers worldwide and accounts for about 31% of cases **[3, 4].** HBV, small double-layered virus in the family hepadnaviridae, is a 42 nm partially double stranded circular DNA virus **[5].** Hepadnaviruses are known to exhibit limited tissue tropisms and host range, confined to their innate host and a few closely related species **[6].** Chronic hepatitis B is now treated with interferon-α-2a, interferon-α-2b, lamivudine and nucleotide analogue such as adefovir dipivoxil which all aim in suppressing viral replication thereby hindering the progression of disease **[7, 8].** Unfortunately currently available drugs have not shown beneficial effects on the treatment of chronic hepatitis B to a vast range of patients and are coupled with severe side effects. Moreover nucleoside or nucleotide analogues induce the suppression of viral replication during the course of treatment but have limited long term efficacy **[9].** Prolonged use of these drugs may lead to liver failure, acute infections and are also associated with a high rate of resistance to the drug **[10].**

Ayurvedic herbs and formulations have wide spectrum of therapeutical or biological activity that can be exploited in pharmaceutical drug discovery and drug design. Traditional medicines such as Ayurveda, Unani and Chinese are preferred for the treatment of chronic hepatitis B due to lesser side effects and low cost. Herbal extracts of the genus, Phyllanthus is

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composed of various combinations of secondary metabolites that have shown hepatoprotective effect, the most widely used ones are *Phyllanthus niruri* and *Phyllanthus amarus* **[11, 12].** Many active compounds were identified from the genus, Phyllanthus which possess anti HBV activities. Phytocompounds isolated from *Phyllanthus niruri* has shown inhibitory effect on endogenous DNA polymerase and surface antigen binding property thereby suppressing the replication of Hepatitis B virus *in vitro* **[13, 14].** *Phyllanthus niruri* extracts was also found to inhibit the replication of wood chuck hepatitis virus (WHV) *in vivo* and reduce the pathological effects of WHV in woodchucks (*Marmota monax*) **[15].**

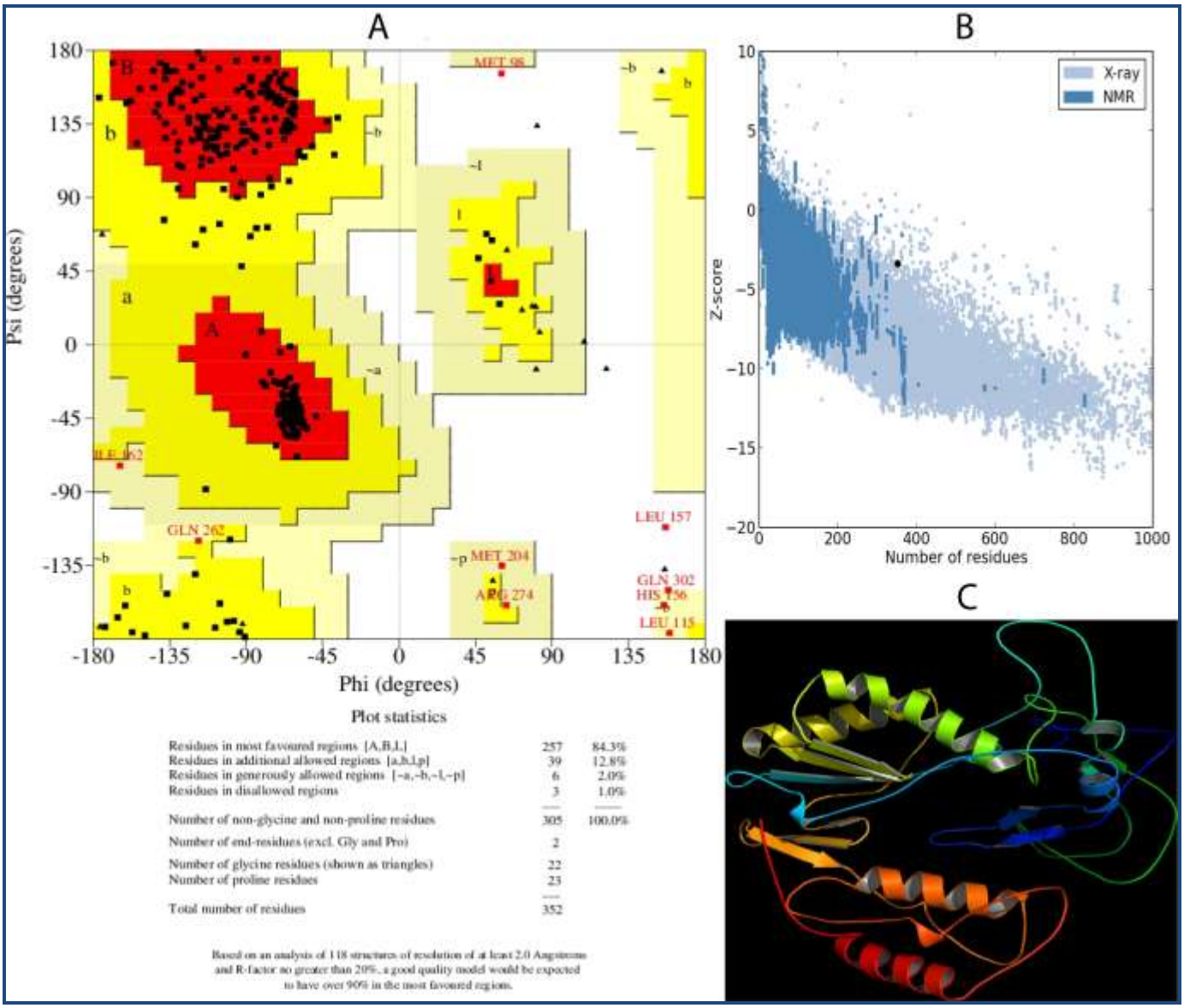

Figure 1: Homology modeling and validation of model: **A)** Validation using Ramachandran plot. Summary of the plot is as follows: Residues in favored regions- 257 (84.3%), Residues in additionally allowed regions- 39 (12.5%), Residues in generously allowed regions-6 (2.0%) and Residues in disallowed regions- 3 (1.0%); **B)** PROSA Z score for model. The Z score for the model was -3.39; **C)** Homology model of HBV-DP created using Pymol software.

DNA polymerase of HBV has been considered as a promising target for the treatment of HBV infections in the past few years. Targeting DNA polymerase can inhibit viral replication by affecting necessary cellular regulatory components that are coupled with HBV replication and viral nucleo capsid formation **[16].** The present study employs an *in silico* method to analyze the interaction of HBV DNA polymerase and phytochemicals from *phyllanthus niruri* using molecular docking studies.

Methodology:

Homology Modeling of HBV DNA polymerase

The X-ray structure of Hepatitis B virus DNA polymerase (HBV-DP) has not yet been successfully determined. HIV-1 reverse transcriptase (HIV -1 RT) shares ample structural similarity with HBV-DP to serve as a good template to predict the three dimensional structure **[17].** HIV-1 RT (template) (PDB ID: 1RTD) structure was obtained from protein data bank (http://www.rcsb.org/pdb). Sequence alignment of HBV-DP (target) and HIV -1 RT (template) provided by Daga *et al*; 2010

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was utilized for constructing the 3D structure of the target. 3D model of HBV-DP was generated from the aligned sequence using "Build homology model" protocol in Discovery Studio. A total of 20 models were generated. Out of generated models, one with least Discrete Optimized Protein Energy (DOPE) score was utilized for docking studies.

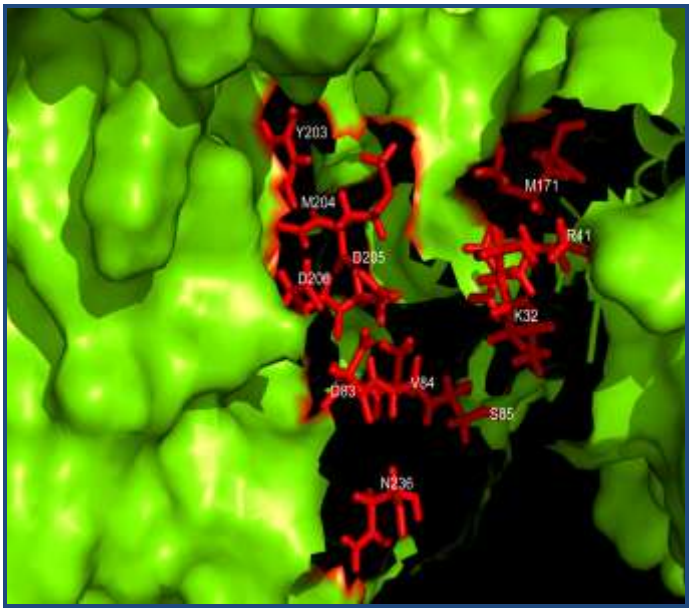

Figure 2: Residues involved in the binding cavity of HBV-DP. The figure shows the residues in the active site of the target protein (HBV-DP). The figure was made in Pymol software by implementing 'surface' and 'stick' representations and was colored accordingly

Protein Structure Validation and Active Site Prediction

Quality of generated model was assessed using "Verify Protein (Profiles-3D)" protocol of Discovery studio. Validation of the model was performed using Procheck **[18]** by analyzing Ramachandran plot. Structural evaluation and stereochemical analyses were performed using ProSA-web Z-scores (https://prosa.services.came.sbg.ac.at/prosa.php) **[19].** Energy minimization of the protein structure was performed by applying "prepare protein" protocol of DS. This protocol cleans the protein molecule by adding missing atoms, inserting missing loops, assigning charges and fixing CHARMm forcefields. Active site of HBV-DP was predicted using "Define and Edit Binding Site" protocol of Discovery studio.

Ligand Identification, Preparation and Screening

A total of 35 phytocompounds from *Phyllanthus niruri* was collected through literature survey. The 3D structure of 32 compounds was downloaded in .sdf format from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). 3D structure of 3 phytocompounds was generated using Marvin Sketch and exported in .sdf format for docking studies. All the compounds were then imported to DS and prepare ligands protocol was applied in order to add missing hydrogen bonds and energy minimization using CHARMm force fields. Prepared ligands were further filtered by applying Lipinski's properties such as Molecular weight, XLog P, number of hydrogen bond donors and acceptors.

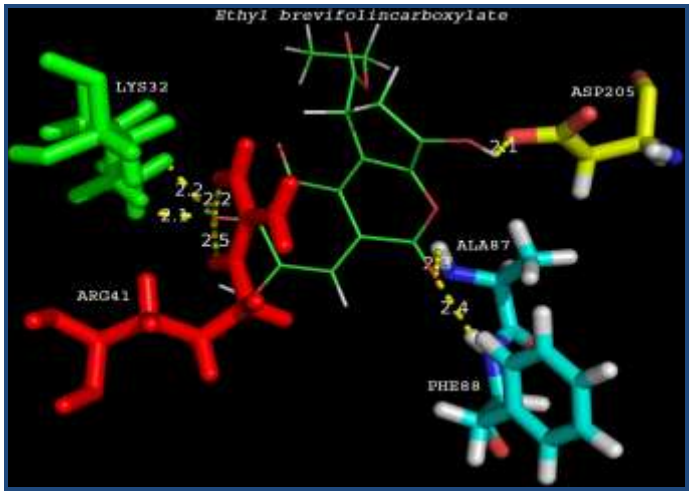

Figure 3: Ethyl brevifolincarboxylate docked to the active site residues in the protein. The figure shows the hydrogen bond distances between Ethyl brevifolincarboxylate and the active site residues (LYS32, ARG41, ALA87, PHE88 and ASP205).The distances are within 2.5Å distance which indicates strong binding.

Molecular Docking

Possible binding modes between the filtered ligands and HBV-DP model were studied by CDOCKER (CHARMm-based DOCKER) protocol incorporated within DS. The algorithm offers full ligand flexibly and employs CHARMm force fields. Ligand binding affinity was calculated using CDOCKER energy, CDOCKER Interaction energy, Hydrogen bonds, binding energies, protein energy and ligand protein complex energy.

Results & Discussion:

Homology Model of HBV-DP

Sequence alignment between HIV -1 RT and HBV -DP in Clustal format was imported to DS and homology modeling was performed. Out of 20 models generated, fifth model was selected for further studies based on minimum DOPE score (-32517.9). Verify model using profiles 3D was applied to the model and the verify score was observed as 85.87 that lie between expected high score of 160.379 and expected low score of 72.1706. The verified model was then validated using PROCHECK. 84.3% of residues were found in the most favorable regions, 12.8% in additional allowed region, 2% in generously allowed region and 1% residues in disallowed region of Ramachandran Plot **(Figure. 1A).** Protein structure analysis using PROSA showed a Z score of -3.39 **(Figure. 1B).** Selected model produced reliable results in protein verification steps and hence fourth one was chosen as the model for further docking analysis. The final structure of HBV-DP is given in **(Figure. 1C).**

Molecular Docking

The most critical requirement for interaction of HBV-DP protein and ligands was the proper orientation and conformation of ligands into the HBV-DP active site. The active site prediction protocol of DS produced 15 binding sites from which 1st active site grid box with dimensions of 52.074 x22.24 x32.556A° was used. Important amino acids present in active site of HBV- DP as previously reported is labeled in the structure and shown in **(Figure. 2).** The active site cleft contains

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amino acids LYS32, ARG41, AP83, VAL84, SER85, MET171, ASP203, MET204, ASP205, ASP206 and ASN236. 35 phytocompounds from *Phyllanthus niruri* generated 582 conformers of which 60 ligands passed Lipinski's rule. Docking simulations of HBV-DP active site and ligands were performed using the CDOCKER algorithm. The binding mode, hydrogen bond interactions and docking scores for 60 compounds identified through virtual screening were ranked based on the different scoring constraints. Based on CDOCKER energy and CDOCKER interaction energy scores, the binding energy (ΔGbindkacl/mol) for 11 inhibitors were calculated and represented in **Table 1 (see supplementary material).** The binding energies were observed in the range of -53 to -283. Phyllanthose (binding energy –132.3977 kcal/mol) showed maximum number of hydrogen bond interactions (9nos) with the target of which most of them (7 bonds) were with active site residues.

Ethyl brevifolincarboxylate (binding energy -195.409kcal/mol) and astragalin (binding energy -195.431kcal/mol) showed 7 hydrogen bonds with HBV-DP. Active site residues involved in hydrogen bond interactions of astragalin was less compared to ethyl brevifolincarboxylate. However the compound found to have the least binding energy (–283.757 kcal/mol) was quercitrin. The compound showed hydrogen bond interactions with Lys32, Asn36, Val84 and Asp205, residues in the binding pocket of HBV-DP. The second ranked compound based on binding energy was quercetin (-263.645) which showed hydrogen bond interactions with Lys32, Asn36 and Arg41. Comparative docking studies were performed using commercial nucleoside analogues such as lamivudine, tenofovir, telbivudine, and entecavir with the modeled protein. Result showed that analogue tenofovir ranked one among these 6 compounds with minimum binding energy of -225.652 kcal/mol and four hydrogen bonds **Table 2 (see supplementary material).** ADME-Toxicity for the top docking hits was predicted using ADMET descriptors of DS. ADME/Toxicity properties for compounds ranked on the basis of binding energies and number of hydrogen bonds were predicted using DS toxicity prediction module **Table 3 (see supplementary material).** All the phytocompounds except ethyl brevifolincarboxylate displayed hepatotoxicity, and nucleoside analogue tenofovir was found to be hepatotoxic in our study. Docking results showing interaction between active site residues of HBV-DP and ethyl brevifolincarboxylate was depicted in **Figure. 3.** Molecular docking studies using *Phyllanthus niruri* secondary metabolites and nucleoside analogues against the binding cavity of HBV-DP revealed that phytochemicals are having more favorable interactions with the target.

Conclusion:

Conventional medicines that are both safe and easily affordable have not yet been developed for the treatment of chronic Hepatitis B. *Phyllanthus niruri* is a medicinal herb used in traditional Indian medicine for the treatment of Hepatitis B. Therefore; it is of interest to relate molecular properties to its medicinal properties using molecular docking of the plant's phytochemicals with HBV DNA polymerase. In the present work we show the binding interactions of phytochemicals such as Ethyl brevifolincarboxylate, Tenofovir, Quercetin and Quercitrin from *Phyllanthus niruri* with the modeled structure of Hepatitis B Virus DNA polymerase using CDOCKER protocol in Discovery studio. *In silico* molecular docking studies clearly demonstrated binding activity of ligands with HBV-DP which warrants further studies for the development of potent inhibitors in the treatment of Hepatitis B. These results clearly indicate that the phytochemicals from *Phyllanthus niruri* have similar binding sites and better interactions with Hepatitis B Virus DNA polymerase compared to the nucleoside analogues at present utilized for treatment. Using a combination of *in silico* approaches such as homology modeling, virtual screening and molecular docking, we successfully validated *Phyllanthus niruri* phytochemicals as HBV-DP inhibitors. Hence we conclude that secondary metabolites from *Phyllanthus niruri* could be potential lead molecules against Hepatitis B which can be further evaluated through *in vivo* studies.

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Supplementary material:

Table 1: Ligands filtered based on binding energy

Ligand*	Hydrogen bond	Distance	Binding Energy (kcal/mol)
5280450	5280450:H48 - :D205:OD2	2.18563	-140.018
Linoleic Acid	5280450:H46 - :D205:OD2	1.84614	
5280459	K32:HZ1 - 5280459:O6	2.41274	-283.757
Quercitrin	N36:HD22 - 5280459:O8	2.10262	
	5280459:H46 - :D205:OD2	2.03763	
	5280459:H48 - :V84:O	1.99484	
	5280459:H48 - :D205:OD2	2.01413	
5281855	K32:HZ1 - 5281855:O5	1.72489	-263.645
Ellagic Acid	K32:HZ3 - 5281855:O5	2.45225	
	R41:HH21 - 5281855:O5	2.07841	
5282102	K32:HZ1 - 5282102:O10	2.20689	-195.431
Astragalin	K32:HZ2 - 5282102:O10	2.13703	
	N36:HD22 - 5282102:O9	2.1542	
	R41:HH12 - 5282102:O5	1.82705	
	A86:HN - 5282102:O7	2.19951	
	F88:HN - 5282102:O11	2.20532	
	5282102:H46 - :D83:OD2	2.02284	
5487248	K32:HZ1 - 5487248:O7	2.1618	-195.409
ethyl brevifolin-carboxylate	K32:HZ3 - 5487248:O7	2.09822	
	R41:HE - 5487248:O7	2.49362	
	R41:HH21 - 5487248:O7	2.24705	
	5487248:H34 - :D205:OD2	2.12902	
	A87:HN - 5487248:O6	2.32481	
	F88:HN - 5487248:O6	2.36227	
5491556	K32:HZ1 - 5491556:O8	2.38457	-86.0412
Nirphyllin	K32:HZ3 - 5491556:O7	2.24386	
44258681	W3:HE1 - 44258681:O11	1.93865	-224.768
Fisetin 4'-glucoside	K32:HZ1 - 44258681:O9	1.64135	
	K32:HZ1 - 44258681:O10	2.46022	
	R41:HH21 - 44258681:O9	1.76621	
	R41:HH12 - 44258681:O5	2.23136	
	44258681:H49 -:D205:OD1	2.09563	
5280343	K32:HZ1 - 5280343:O2	1.95249	-263.645
Quercetin	K32:HZ1 - 5280343:O4	2.299	
	K32:HZ3 - 5280343:O2	2.21915	
	N36:HD22 - 5280343:O5	2.10988	
	R41:HH21 - 5280343:O2	1.83142	
335928	W3:HE1 - 335928:O8	2.43148	-132.3977
Phyllanthose	K32:HZ1 - 335928:O9	1.76696	
	R41:HE - 335928:O2	2.41244	
	R41:HE - 335928:O6	2.44083	
	R41:HH21 - 335928:O2	2.48525	
	R41:HH21 - 335928:O9	2.09897	
	A87:HN - 335928:O3	2.07767	
	335928:H38 - :V84:O	1.80599	
	335928:H40 - :D205:OD2	2.03257	
358902	K32:HZ3 - 643684:O1	1.88201	-53.0451
Hypophyllanthin	K32:HZ2 - 643684:O1	2.4643	
	R41:HH12 - 643684:O2	2.03738	
	R41:HH22 - 643684:O2	1.95215	
643684	K32:HZ2 - 643684:O1	2.4643	-140.6709
Ricinoleic acid	K32:HZ3 - 643684:O1	1.88201	
	R41:HH22 - 643684:O2	1.95215	
	R41:HH12 - 643684:O2	2.03738	

*The ligands were taken from pubchem compound database

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Table 2: Interaction studies of commercial drugs

Ligand*	Hydrogen bond residues	H bond Distance (Å)	Binding energy (kcal/mol)
Entecavir 153941	153941:H33 - :D206:OD2	2.1014	-188.7924
	153941:H36 - :D206:OD1	2.14775	
	153941:H36 - :D206:OD2	2.15838	
Telbivudine 159269	R41:HE - 159269:O1	2.28517	-122.6785
Tenofovir 464205	R41:HE - 464205:O3	1.93362	-225.6516
	R41:HH12 - 464205:N8	2.12801	
	R41:HH21 - 464205:O3	2.20073	
	R41:HH21 - 464205:O5	1.8918	
Lamivudine 60825	R41:HE - 60825:O2	2.24648	-52.977

*The ligands were taken from pubchem compound database

Table 3: Comparative study of ADMET properties (desirable values given in parenthesis)

Ligand*	Aqueous Solubility (2-4)	Blood Brain Barrier (BBB) (2-4)	Hepatotoxicity (FALSE)	Intestinal Absorption (0-1)	Alog P (<4)
Phyllanthose 335928	5	4	TRUE	3	-2.762
Quercetin 5280343	3	4	TRUE	1	1.63
Quercitrin 5280459	3	4	TRUE	3	0.589
Astragalin 5282102	3	4	TRUE	3	-0.057
Ethyl brevifolincarboxylate 5487248	3	4	FALSE	1	0.653
Tenofovir 464205	4	4	TRUE	1	-0.911

*The ligands were taken from pubchem compound database
Note: Aqueous Solubility - Measure of the solubility of the drug in water.
Blood Brain Barrier – Measure of the drugs ability to cross the Blood Brain Barrier (Higher value indicates lesser penetration)
Hepatotoxicity – Indicate whether the drug would cause liver damage or not
Intestinal Absorption – Measure of the level of intestinal absorption of the drug
AlogP – Another measurement to indicate the level of solubility of the drug

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