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Design of emodin derivative structures as HBV capsid assembly inhibitor .

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ABSTRACT

The core protein of hepatitis B virus (HBV) forms the capsid of viral particles and essential for viral genome DNA replication and maturation. It is an excellent target for the development of new, virus-selective, safe and effective antiviral agents to improve treatment options for hepatitis B disease due to drug resistance by reverse transcriptase. This study aimed to identify potential non-nucleoside analogs against hepatitis B virus replication from emodin derivatives. The design of the emodin derivatives structures was drawn using Marvin Sketch program. The macromolecule target was protein core of hepatitis B virus and downloaded from Protein Data Bank (PDB ID: 5E0I). Docking simulation and scoring were performed using CLC Drug Discovery Workbench program. Based on the results, it is shown that scores of emodin esterified were more negative than emodin that implies they were predicted more effective as an inhibitor of replication of HBV. Overall, this study provides a basis for further chemical design for finding potential substance to be developed and evaluated further as an anti-hepatitis B agent.

Keywords: core protein, hepatitis B virus, capsid assembly, emodin.

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INTRODUCTION

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health problem[1, 2]. It can cause chronic liver disease and chronic infection and puts people at high risk of death from cirrhosis of the liver and liver cancer. Approximately 360 million people worldwide are chronically infected with hepatitis B virus (HBV) and are at high risk of developing hepatocellular carcinoma (HCC)[2]. Current antiviral therapies control the progression of the disease, but fail to eliminate the virus. There is a need for improved therapies to combat chronic infections. One approach is to target virus assembly [3-8].

HBV is a small virus whose genome has only four open reading frames. The simplicity of the virion correlates with a complexity of functions for viral proteins. HBV core protein is a small (183 residue) protein that self-assembles to form the viral capsid. In an infected cell, it modulates almost every step of the viral lifecycle. It is bound to nuclear viral DNA and affects its epigenetics, correlates with RNA specificity, assembles specifically on a reverse transcriptase-viral RNA complex, participates in regulation of reverse transcription, signals completion of reverse transcription to support virus secretion, carries both nuclear localization signals and HBV surface antigen (HBsAg) binding sites [9]. The HBV core protein is a viral protein with no known related protein present in human cells. HBV core protein is, therefore, an excellent target for the development of new, virus-selective, safe and effective antiviral agents to improve treatment options for this disease. The HBV core protein consists of 183–185 amino acids that form an N-terminal (amino acids 1–149) capsid assembly domain and a C-terminal nucleic acid binding domain (amino acids 150–185). The viral capsid in infectious HBV particles is formed from 120 copies of assembled core protein dimers enclosing the viral DNA. Small molecules that target the HBV core protein assembly domain can disrupt functional HBV capsid assembly and can be potent inhibitors of HBV replication [10].

Emodin (1,3,8-trihydroxy-6-methyl-9,10-anthraquinone) is derived from herbal medicine and has a weak inhibitory effect on HBV replication but persistent, both in vitro and in vivo. Emodin could inhibit the secretion of HBsAg and HBeAg in the cultured cells. Emodin inhibits the production of HBV DNA dose dependently with The 50 % inhibitory concentration (IC50) are 0.21 g/L and the selectivity index (CC50/IC50) was 1.95 [11]. Emodin and Astragalus polysaccharide (APS) significantly reduced serum HBV DNA content of transgenic mouse model, although this effect was weaker than that observed with lamivudine. Interestingly, the reduction in serum HBV DNA content in the emodin and APS group lasted longer, compared with the lamivudine group [12].

Due to its activity, emodin is chosen as a lead compound for development a new anti hepatitis B agent because the structure shows a useful pharmacological activity and can act as the starting point for drug design. Furthermore, emodin has a high affinity for phospholipid membranes and can pass cellular membranes easily due to its higher phospholipid/water partition coefficient [13]. Emodin need further structural modification to increase its efficacy and to decrease its toxicity for further clinical application. In this study, we designed several compounds of emodin derivatives, which have the possibility to prepared in suitable synthesis condition. Then, we carried out docking simulations of emodin derivatives into HBV core protein to calculate their binding energies.

MATERIALS AND METHODS

The design of the compound ligand done using Marvin Sketch program. Docking studies were performed by CLC Drug Discovery Workbench installed on a single machine running on Intel CoreTM 2 i5-3470 processor with 8GB RAM with Windows 32-bit as the operating system.

Protein preparation

The X-ray crystallographic structure of protein core of hepatitis B virus complexed with 5J6 was retrieved from Protein Data Bank (PDB ID: 5E0I) [10]. This structure was saved as a standard PDB file and input into the CLCbio Drug Discovery Workbench program.

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Preparation and Design of ligands

Ligands were designed by considering of synthesis methods and from molecule library as well emodin reaction. The substitution of fuctional groups to emodin can showed at Table 1. Ligands that have drawn two-dimensional structure is then converted to 3-D and energy minimization is done in order to obtain the structure of the most stable conformation and save as output format .mol2. These molecules were saved in molecule table in CLC Drug Discovery Workbench library.

Docking Simulation

Basic set up like protein preparation, ligand preparation, detecting cavities, receptor grid generation, and targeted ligand docking were performed on CLC Drug Discovery Workbench program. Docking wizard was customized by using a default MolDock optimizer algorithm with 100 numbers of runs.

Validation of methods done by extracting ligands already present in proteins and performed the docking process. Programs that are able to return poses below a preselected Root Mean Square Deviation (RMSD) value from the known conformation (usually 1.5 or 2 Å depending on ligand size) are considered to have performed successfully [14]. Further, ligands present in the molecule table are replaced with a compound and its derivatives emodin by performing alignment and docking simulations performed for each of these compounds.

The docking parameters include population size 50, maximum iterations 2000, scaling factor 0.50, crossover rate 0.90 and RMSD thresholds for similar cluster poses were set at 1.00. CLC Drug Discovery Workbench uses a standard precision mode to determine the favorable binding poses, which detects various flexible ligand conformations while holding protein as a rigid structure during docking. Emodin derivatives were docked into the active site recognized in the macromolecule cavity by replacement the ligand reference. Maximum of 10 poses for each conformation was generated by using default parameter of CLC Drug Discovery Workbench. Docking studies were carried out to predict the binding affinities based on scoring functions. On the basis of hydrogen interaction and docking score, the best-ranked compounds were selected [15].

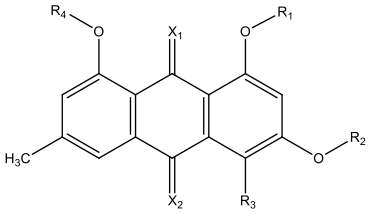

Table 1. The substitution of functional groups of emodin

Compound	X_1	X_2	R_1	R_2	R_3	R_4
EMD	O	O	H	H	H	H
EMD-1	O	O	H	CH_3	H	H
EMD-2	O	O	CH_3	CH_3	H	H
EMD-3	O	O	CH_3	CH_3	H	CH_3
EMD-4	O	O	H	CH_3	H	CH_2Br
EMD-5	O	O	H	CH_2CH_3	H	H
EMD-6	O	O	H	$(CH_2)_2CH_3$	H	H
EMD-7	O	O	CH_2CH_3	CH_2CH_3	H	CH_2CH_3
EMD-8	O	O	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	H	$(CH_2)_2CH_3$
EMD-9	O	O	H	COCH3	H	H
EMD-10	O	O	H	$CO(CH_2)_2CH_3$	H	H
EMD-11	O	O	H	$COCH(CH_3)_2$	H	H
EMD-12	O	O	H	$COCH_2CH(CH_3)_2$	H	H
EMD-13	O	O	H	$COCHCH_3CH(CH_3)_2$	H	H
EMD-14	O	O	H	CCH_3COCH_3	H	H
EMD-15	O	O	H	$COCHCH_3CH_2CH_3$	H	H
EMD-16	O	O	H	$CON(CH_3)_2$	H	H

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EMD-17	O	O	H	$CCOCH_3(CH_2)_2CH_3$	H	H
EMD-18	O	O	H	COC_2H_5	H	H
EMD-19	O	O	H	$COC_5H_4CH_3$ (ortho)	H	H
EMD-20	O	O	H	$COC_5H_4CH_3$(meta)	H	H
EMD-21	O	O	H	$COC_5H_4CH_3$(para)	H	H
EMD-22	O	O	H	H	$N(CH_3)_2$	H
EMD-23	O	NH	H	H	H	H
EMD-24	O	NOH	H	H	H	H
EMD-25	O	NCH_3	H	H	H	H
EMD-26	O	NNH_2	H	H	H	H
EMD-27	NH	NH	H	H	H	H
EMD-28	NNH_2	NNH_2	H	H	H	H
EMD-29	NOH	NOH	H	$CNOHC_5H_4CH_3$(ortho)	H	H
EMD-30	NCH_3	NCH_3	H	H	H	H
EMD-31	NOH	NOH	H	H	H	H
EMD-32	NOH	NOH	H	$CNOHC_5H_4CH_3$(meta)	H	H
EMD-33	O	NNH_2	H	H	H	H

RESULTS AND DISCUSSION

The most popular strategy in drug design is the synthesis of derivatives or analogs of exiting active compounds by various chemical transformation for which an increase in potency, a better spesific activity profile, improve safety or a comfort formulation. In the process of new drug design, virtual screening is a useful way as part of the molecular-aided drug design (computer aided drug design) to identify potential ligands. One method used in the screening process is to use structure-based search is molecular docking. The main purpose tethering molecule is to understand and predict how drugs work at a molecular level by looking at drug interactions with receptors. Computational approaches in docking simulation of small molecules into the structure of macromolecular targets and then scoring of their potential complementarily to binding sites are widely used in hit identification and lead optimization [16].

Emodin, as a lead, has a variety of functional group present in the structure so the potential binding interaction that is possible with a target binding site of the core protein of hepatitis B virus. The structure of emodin can be found at Figure 1.

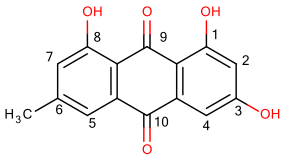

Figure 1. Chemical structure of emodin

This study has been conducted docking simulation of the emodin and several derivatives into target core protein (HBc) of the hepatitis B virus using CLC Drug Discovery Workbench program. First, docking simulation is performed to ligand 5J6 (NVR- 010-001-E2) on the bond side of 5E0I chain A with radius / dimensions: 13 Å shows RMSD value of 0.80 and affinity energy as -54.87. The NVR-010–001-E2 compound lies at the floor of the binding surface and anchors to the protein via a hydrogen bond between the side chain of Trp102. The redocking position of ligand can be shown in Figure 2. So that it can be said that the condition of the simulation is eligible validation and can be used for ligand-protein docking simulations.

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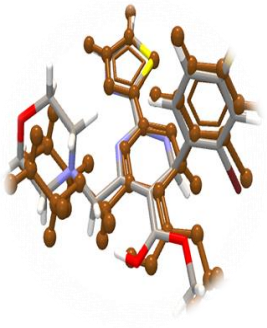

Figure 2: Redocking position of ligand NVR-010–001-E2

There have been designed the structure of emodin derivatives which can be prepared via reaction with some suitable chemical reagents. Emodin and its derivatives stuctures are tethered to the protein 5E0I at the same binding site previously and then it was performed analysis of the scores and interactions that occur between the ligand-protein. The docking scores of emodin and derivatives into the HBV core protein were obtained as shown in Table 2. Interactions between emodin and derivatives indicated by hydrogen bonding and steric interaction between the ligand with the amino acid residues in the HBV core protein. Emodin interacts with HBV protein core amino acid residue Trp 102, Leu 140, Pro 138 and Tyr 118. It is known that Pro-138 is essential for keeping an assembly competent conformation of virus capsid [17].

Results showed that scores of emodin modified were more negative than emodin that implies they were predicted more effective as an inhibitor of replication of HBV. The changes substituents on emodin structure will have a significant effect on hydrophobic and steric properties as well as better interaction with target.

Table 2. Docking score of emodin derivatives into HBV core protein (PDB ID : 5E0I)

Compound	Score	HBond Score	Steric interaction score
EMD	-46.15	-9.49	-36.66
EMD-33	-67.39	-10.00	-63.71
EMD-8	-66.76	-9.09	-62.57
EMD-7	-62.82	-6.00	-60.92
EMD-12	-61.86	-12.52	-50.91
EMD-13	-60.58	-4.00	-47.71
EMD-20	-60.54	-4.00	-58.01
EMD-16	-59.90	-8.77	-51.38
EMD-15	-59.86	-14.13	-57.39
EMD-19	-59.15	-7.00	-54.56
EMD-11	-57.78	-10.47	-48.56
EMD-17	-57.66	-11.49	-49.41
EMD-21	-57.29	-7.36	-53.82
EMD-10	-57.08	-6.86	-54.04
EMD-6	-54.37	-7.82	-48.20
EMD-18	-53.28	-0.03	-55.18
EMD-4	-53.00	-9.06	-50.45
EMD-32	-52.91	-12.79	-51.49
EMD-14	-52.39	-10.68	-43.98
EMD-3	-52.15	-5.56	-49.85
EMD-9	-51.58	-6.54	-46.56
EMD-5	-51.21	-8.13	-43.62
EMD-2	-49.39	-7.97	-43.39
EMD-26	-48.48	-12.48	-36.00

EMD-25	-48.08	-7.26	-40.82
EMD-24	-47.94	-8.00	-39.94
EMD-1	-47.15	-6.94	-41.30
EMD-27	-46.68	-9.69	-36.99
EMD-23	-45.73	-8.26	-37.46
EMD-22	-45.24	-5.24	-45.38
EMD-28	-44.99	-12.00	-32.99
EMD-29	-43.31	-7.22	-46.08
EMD-30	-42.57	-6.96	-35.61
EMD-31	-41.32	-6.89	-34.44

The original RO5 Lipinski deals with orally active compounds and defines four simple physicochemical parameter ranges (MWT= 500, log P = 5, H-bond donors = 5, H bond acceptors = 10) associated with 90% of orally active drugs that have achieved phase II clinical status. These physicochemical parameters are associated with acceptable aqueous solubility and intestinal permeability and comprise the first steps in oral bioavailability. The RO5 was deliberately created to be a conservative predictor in an era where medicinal and combinatorial chemistry produced too many compounds with very poor physicochemical properties [18].

Unfortunately, the three best score compounds have Lipinski violation which calculated value of LogP is over 5 as shown in Tabel 3. Therefore, the modification becomes the ester group such as EMD-12 and EMD13 can be an option where the outcomes score is more negative without violation of Lipinski rules. The 1.8-hydroxy groups of emodin are adjacent to the 9-carbonyl group so will form two intramolecular hydrogen bonds. while the 3-hydroxy group is far from 9-and 10-carbonyl group, with the result that its reaction activity is more active than the 1-and 8-hydroxy groups. Accordingly, emodin esterified has a chance to be developed further by synthesis and activities testing as inhibitors of HBV replication.

Table 3. Physicochemical properties and RO5 Lipinski violation of emodin and its derivatives

Compound	Weight	Hydrogen Donors	Hydrogen Acceptor	LogP	RO5 Violations
EMD	270.24	3	5	5.26	1
EMD-33	496.67	1	6	10.46	1
EMD-8	396.48	0	5	5.82	1
EMD-7	354.40	0	5	6.78	1
EMD-12	354.36	2	6	4.08	0
EMD-13	368.38	2	6	4.66	0
EMD-20	388.37	2	6	7.38	1
EMD-16	341.32	2	7	2.83	0
EMD-15	354.36	2	6	4.22	0
EMD-19	388.37	2	6	7.38	1
EMD-11	340.33	2	6	3.86	0

CONCLUSIONS

Based on the results we could predict the structure of emodin as a lead, which has potency to be developed and evaluated further as an anti-hepatitis B agent. Changes substituents on emodin structure will have a significant effect on hydrophobic and steric properties as well as biological activity. Emodin esterified have potency as small molecule non-nucleoside inhibitor candidate for hepatitis treatment via HBV core protein disruption mechanism.

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