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Original Article

Computational Studies and Molecular Dynamic Simulation to Design Lead Compounds for Hepatitis B Virus

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ABSTRACT

Objective/Background: A novel approach to develop anti-Hepatitis B Virus (HBV) by computational studies is proposed. **Methodology:** It used active compounds as the standard ligand. There are six parameters such as docking score, molecular weight, log P, Polarizability, Polar Surface (2D) and Molecular Surface (3D) that analyzed by software. **Result and Discussion:** The result of virtual screening can be used as a reference to calculate IC_{50} prediction of quinine and Gallic acid derivative compounds. Optimization of compounds structure geometry was using software Marvin Sketch 6.0.1. Meanwhile, virtual docking process to HBV capsid Y132A mutant (PDB ID: 5E0I) was using Autodock4, Auto dock Vina, and Plant. Result: The lowest IC_{50} prediction is gallic acid (64.1 μM) that had hydrogen and polar interaction for 20 ns. **Conclusion:** These computational studies not only shed light on understanding the IC_{50} prediction of the replication of the viral core protein inhibition, but also the stability of each interaction that inhibits of the viral core protein replication.

Key words: Amber, Docking, Galic Acid, Hepatitis B, Molecular Dynamic.

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INTRODUCTION

Hepatitis B virus (HBV) is a small, enveloped, partially double-stranded DNA virus that belongs to the Hepadnaviridae family. HBV is a disease of global concern that caused 1.34 million deaths in 2015, a number comparable to death caused by tuberculosis and higher than those caused by HBV (WHO Global Hepatitis Report, 2017). The number of deaths due to viral hepatitis is increasing over time. Currently, the available antiviral drugs include interferon-alpha (IFN-α) and nucleotide analogue inhibitors of HBV polymerase, among which lamivudine (3TC), telbivudine (LdT), entecavir (ETV), adefovir (ADV) and tenofovir (TDV) have been approved by the FDA (Figure 1).[1] HBV DNA polymerase (HDP) is interested object for treatment of HBV infections in the last few years. There are seven agents are approved for the treatment of chronic HBV. There are two biological products which have shown very good anti-HBV activity such as Interferon alfa-2b (IFN) and long-acting pegylated interferon (peginterferon) alfa-2a (PEG-IFN). However, there are some troubles for that product such as practicality, high cost, effectiveness for the treatment of chronic HBV. There are some publication that studied lead compound for inhibiting the replication of the viral core protein such as a QSAR study on bis (L-amino acid) ester prodrugs of 9-[2-(phosphonomethoxy)ethyl]adenine of anti-HBV agent.[1] Cannabinoid that found in cannabisplant was effective for HBV- virus treatment.[2] Virtual screening of *Cocculus hirsutus* compounds has been studied for anti-HBV by Samuel Thavamani *et al.*[3] that used GLID software.[4-6] Cluster Analysis and QSAR Study of Some Anti-hepatitis B Virus Agents Comprising 4-Aryl-6-chloro-quinolin-2-ones and 5-Aryl-7-chloro-1, 4-benzodiazepines.[7] Sheng Liu *et al.* created the docking and synthesis design of phenylpropanoid derivatives for anti – HBV.[8] Recently, a computational study is the best choice to reduce a cost and time for searching a lead compound. In this article, we report a computational study to predict the IC_{50} value and molecular dynamic simulation that can be verified the stability of the best compound for a lead compound to HBV.

MATERIALS AND METHODS

For the present study, the crystal structure of HBV capsid (PDB code 5E0I) were downloaded from the protein data bank (http://www.rcsb.org).9 A set of standard ligands (Table 1) that inhibit the replication of the viral core protein were taken from the literature.10 The ligands docked onto the active site of HBV core protein using Autodock 4.2,11 Autodock vina12 and Plats program.13 The best ligand result that be continued to molecular dynamic simulation by Amber 14.14

Selection of Standard Compounds

The compounds were selected from the literature.[10] The docking set consist derivates of bis (L-amino acid) ester prodrugs of 9-[2-(phosphonomethoxy)ethyl] adenine (PMEA), Lamivudine and Adefovir dipivoxil with IC_{50} value. To validate the simulation model, that compounds ware used as a test set (see Table 1). The IC_{50} values in units of mM were transformed in pIC_{50} ($logIC_{50}$) to give numerically more significant data.

Docking Studies

The 2D structures of ligand (standard and samples) were drawn by MarvinSketch 6.01 (Bennet, Clausen *et al.* 2009) and calculated the lowest energy. The best structure of HBV capsid (PDB code 5E0I).9 was obtained from the Protein Data Bank (http://www.rcsb.org). It has 1.95 A of resolution. To explore the specific contribution of docking score, molecular weight, log P, Polarizability, Polar Surface (2D) and Molecular Surface (3D) used Autodock 4.2, Autodock Vina, Plant and Marvin Sketch. Autodock 4.2, Autodock Vina and Plant were used

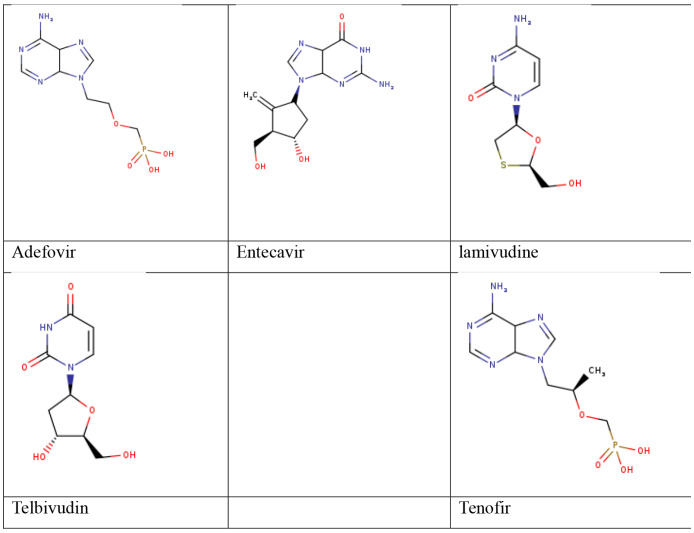


Figure 1: Active compound of HBV that have been approved by the FDA.

conformation calculating of each molecule. Physical properties of each molecule were optimized by Marvin Sketch.

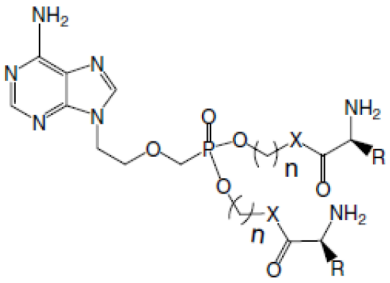
Statistic analysis

In the present study, five parameters (such as docking score, molecular weight, log P, Polarizability, Polar Surface (2D) and Molecular Surface (3D) of each compound simulation were evaluated using statistic program for getting the equation.

Molecular Dynamic Simulation

The simulation was carried out using the Amber 14 Molecular Dynamics package.[9] The system, prepared as solvated complex by carrying out a

Table 1: Structure and experimental anti-HBV activities of standard compounds.



Compound	R	X	n	IC_{50}	pIC_{50}
1	Methyl	O	2	1840	3.0
2	Isopropyl	O	2	2587	3.6
3	2-Methylpropyl	O	2	6636	3.7
4	Benzyl	O	2	2590	3.5
5	H	S	2	259	2.7
6	Methyl	S	2	2115	3.3
7	Isopropyl	S	2	28712	3.7
8	2-Methylpropyl	S	2	3409	4.0
9	benzyl	S	2	10984	4.1
10	Isopropyl	O	1	3378	3.8
11	2-Methylpropyl	O	1	8560	3.9
12	benzyl	O	1	15378	4.0
Adefovir dipivoxil	-	-	-	540	2.8
Lamivudine	-	-	-	230	2.3

Table 2: The docking score and physic properties of the standard.

Standard ID	Docking Score			Mr	logP	Polarizability	Polar Surface (2D)	Molecular Surface (3D)
	Autodock 4.2	Autodock vina	Plant					
1	-0.99	-4.60	-102.81	503.45	-1.77	47.41	220.64	728.46
2	-0.71	-5.20	-113.75	559.56	-0.02	54.71	222.26	853.16
3	0.69	-5.20	-116.54	587.62	0.82	58.36	222.26	920.41
4	-1.33	-5.60	-123.58	627.60	0.98	62.75	219.02	827.85
5	0.16	-4.80	-93.69	507.52	-1.91	48.48	203.80	689.74
6	-1.48	-4.90	-100.98	535.57	-0.86	52.13	203.80	747.17
7	-0.18	-5.00	-112.56	591.68	0.89	59.45	203.80	865.76
8	0.37	-5.20	-116.94	619.74	1.73	63.11	203.80	933.10
9	-1.99	-5.90	-123.34	659.72	1.89	67.52	202.18	892.20
10	-2.25	-5.10	-101.16	499.51	0.44	49.57	203.80	755.31
11	-0.84	-5.70	-106.25	527.56	1.28	53.23	203.80	817.84
12	-2.65	-6.30	-114.00	567.54	1.44	57.63	202.18	780.38
Adefovir dipivoxil	-2.43	-5.90	-100.24	501.48	3.06	48.00	166.98	764.47
Lamividine	-4.05	-5.00	-60.07	229.25	-1.10	21.35	88.15	273.28

Table 3: Data of PIC_{50} prediction for standard compound.

PIC_{50}	Autodock 4.2	Autodock vina	Plants
3.3	3.0	2.9	3.0
3.4	3.6	3.5	3.6
3.8	3.7	3.8	3.9
3.4	3.5	3.7	3.7
2.4	2.7	2.9	2.8
3.4	3.3	3.2	3.1
4.5	3.7	3.8	3.7
3.5	4.0	4.0	4.0
4.0	4.1	4.0	4.0
3.5	3.8	3.7	3.6
3.9	3.9	4.0	3.9
4.2	4.0	3.9	3.9
2.7	2.8	2.8	2.7
2.4	2.3	2.4	2.4
Multiple r	0.9	0.9	0.9

Table 4: Value of IC_{50} prediction calculation of compounds.

ID	Docking Score	Mr	LogP	Polarizability	Polar Surface (2D)	Molecular Surface (3D)	IC_{50} prediction (μM)
	Autodock 4.2						
Adefovir dipivoxil	-2.43	501.477	3.06	48	166.98	764.47	566.8
Lamivudine	-4.05	229.25	-1.1	21.35	88.15	273.28	220.6
cinchonidine	-6.18	294.398	2.67	35.87	37.56	408.31	112286.0
Cinchonine	-6.41	294.398	2.67	35.87	37.56	408.31	126388.2
Quinidine	-6.35	324.424	2.51	38.35	46.79	489.79	194958.2
Quinine	-6.46	324.424	2.51	38.35	46.79	489.79	205691.7
2,3,5-trihydroxybenzoic acid	-4.60	170.12	0.72	14.65	100.82	203.62	64.1
phenyl 3,4,5-trihydroxybenzoate	-5.85	246.218	2.72	24.34	86.99	316.13	423.7
4-methylphenyl 3,4,5-trihydroxybenzoate	-6.49	260.245	3.24	26.09	86.99	351.15	827.5
4-aminophenyl 3,4,5-trihydroxybenzoate	-5.42	261.233	1.9	25.49	113.01	333.86	473.1
5-(hydroxymethyl)benzene-1,2,3-triol	-4.57	156.137	0.3	14.68	80.93	210.72	246.7
3,4,5-trihydroxybenzamide	-4.91	169.236	-0.09	15.11	103.78	211.52	182.3
3,4,5-triaminobenzamide	-4.05	166.184	-1.66	16.61	121.15	220.8	1181.7
3,4,5-trihydroxybenzaldehide	-4.49	154.121	0.78	14.13	80.59	192.21	119.3

short minimization, 50 ps of heating, 50 ps of density equilibration with weak restraints on the complex followed by 500 ps of constant pressure equilibration at 300 K. The production phase of the simulation was run using the same conditions as the final phase of equilibration for 20 ns (production recording the coordinates every 10 ps). The simulation results were analyzed using the ptraj program in the Amber 14 package and VMD.

RESULT

Docking studies have been done on the compound structure of standard which is already successfully synthesized and known its activity as anti-HBV (see Table 1).

DISCUSSION

Docking Studies

Docking studies have been done on the compound structure of standard which is already successfully synthesized and known its activity as anti-HBV that will be a standard ligand for docking simulation on 5E0I using Autodock 4.2, Autodock vina and Plant. We will obtain docking score information (the lowest energy of ligand pose) and physic properties (Table 2). Statistical analysis has been done using a multivariate method and is obtained an equation that will be used to predict IC_{50} of the compound in Curcuma (**Eq 1, 2 and 3**).

pIC50 prediction = 1.061 – 0.220 Docking Score – 0.038 Molecular Weight – 0.182 log P + 0.329 Polarizability + 0.003 Polar Surface (2D) + 0.006 Molecular Surface (**Eq. 1**)

pIC50 prediction = 2.693 + 0.054 Docking Score – 0.038 Molecular weight + 0.059 log P + 0.346 Polarizability + 0.013 Polar Surface (2D) + 0.001 Molecular Surface (3D) (**Eq.2**)

pIC50 prediction = 1.715 – 0.026 Docking Score – 0.038 Molecular Weight – 0.039 log P + 0.317 Polarizability + 0.007 Polar Surface (2D) + 0.002 Molecular Surface (3D) (**Eq.3**)

Six parameters which are obtained from docking score, molecular weight, log P, Polarizability, Polar Surface (2D) and Molecular Surface (3D) are proven determining IC_{50} calculation (multiple r). The multiple results were shown in Table 3 (± 0.9) that given the correlation of six parameters. The IC_{50} prediction that calculated by three equations shown at Table 4.

The hydrogen bond and van der Waals interaction that given the lowest IC_{50} of 2, 3, 5-trihydroxybenzoic acid. 2, 3, 5-trihydroxybenzoic acid has six hydrogen bond interaction and five teen van der Waals interaction.

Molecular Dynamic Simulation

The relative stability of the whole model can be measured as the root mean square deviation (RMSD) of backbone atoms from the initial structure as a function of time. The model built with the ptraj program in the Amber 14 package was taken as the initial structure for comparison. The RMSD in Figure 2 was calculated for each MD snapshot (one every 10 ps) after the coordinates of the model were superimposed on the coordinates of the initial structure. The backbone RMSD values of the two systems tend to be convergent after 2 ns of simulation, with fluctuations of 4.5 Å and 4 Å approximately for the structures of the viral core protein. It means the system of molecular dynamic was stable during the simulation interaction.

The structures from before and after MD simulation are shown in Figure 3. Docking into the protein structure obtained after 20 ns of MD simulation produced smaller binding energy interaction poses than before MD simulation. The docking score that got after MD simulation shown at Table 5.

The Total energy of 2, 3, 5-trihydroxybenzoic acid after MD simulation was lower than before MD simulation, it means the structure of 2,3,5-trihydroxybenzoic acid was stable during simulation that can inhibit viral core protein replication.

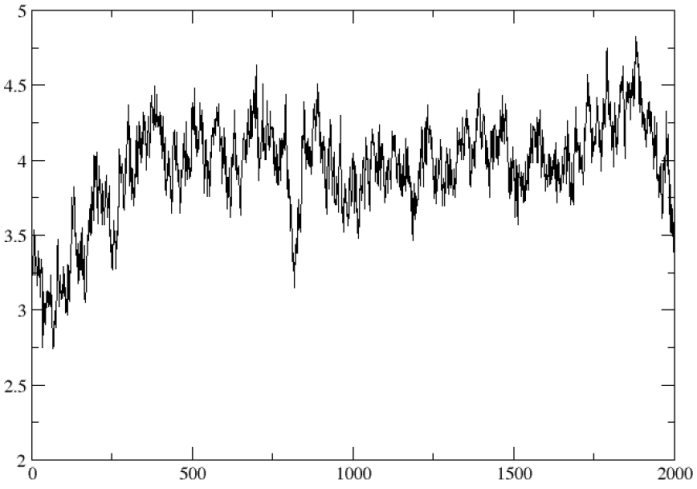


Figure 2: Backbone RMSD for 20 ns.

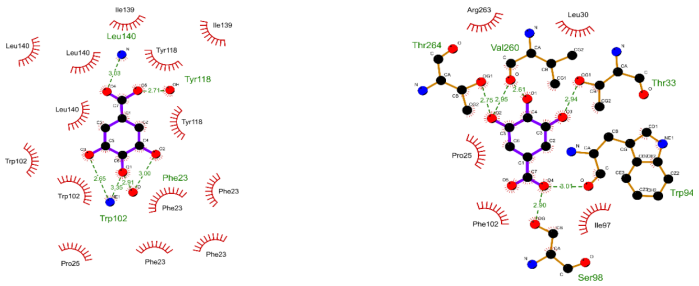


Figure 3: The interaction of 2, 3, 5-trihydroxybenzoic acid (a). before MD simulation (b). after MD simulation.

Table 5: The docking score of Value of 2, 3, 5-trihydroxybenzoic acid before and after MD simulation.

Compound	ΔG (kcal/mol) before MD simulation	ΔG (kcal/mol) after MD simulation
2,3,5-trihydroxybenzoic acid	-4.60	-5.50

CONCLUSION

In conclusion, these computational studies not only shed light on understanding the IC_{50} prediction of the replication of the viral core protein inhibition, but also the stability of each interaction that inhibits of the viral core protein replication.

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ABBREVIATIONS

HBV (Hepatitis B Virus), RMSD (root mean square deviation), MD (molecular dynamic)

CONFLICT OF INTEREST

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SUMMARY

A novel approach to develop anti-Hepatitis B Virus (HBV) by computational studies is proposed. It used 2D and 3D software interaction. The result of virtual screening can be used as a reference to calculate IC_{50} prediction of quinine and Gallic acid derivative compounds. The lowest IC_{50} prediction is gallic acid (64.1 μM) that had hydrogen and polar interaction for 20 ns. These computational studies not only shed light on understanding the IC_{50} prediction of the replication of the viral core

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