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Research Article

In silico Prediction of Khayanolides as Antiviral Agents against Hepatitis B Virus

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Abstract

Increasing evidence reports the hepatoprotective effects of limonoids but their anti-hepatitis B properties were not yet demonstrated. In this study, we investigated the antiviral potential of four khayanolides against the hepatitis B virus (HBV) core and surface antigens using an in silico approach. Compounds were virtually screened against the followings viral targets : the Pre-S2 surface antigen (1WZ4), CryoEM structure of HBV core (3J2V), human HBV capsid (1QGT), the duck HBV capsid (6YGH) using the iGEMDOCK software, and the docking results were compared to that of Tenofovir. The druglikeness and pharamacokinetics profiles were assessed using SwissADME. The computational analysis revealed a higher binding affinity of khayanolide D with 1QGT (-104.34 vs -98.56 kcal/mol) and 3J2V (-100.92 vs 89.62 kcal/mol) as compared with Tenofovir, while khayanolide A showed the highest fitness score with 6YGH (-113.53 vs -98.21 kcal/mol) and khayanolide B a strong binding interaction with 1WZ4 (-92.24 vs 74.18 kcal/mol) compared with the standard drug. Among the screened khayanolides, khayanolide D is the only compound with high gastrointestinal absorption which meets most of the druglikeness rules (3/5). No interaction with all CYP isoforms was noted with khayanolide C, which also showed the lowest skin permeation value (-9.61 cm/s). These results suggest that khayanolides have strong anti-HBV potentials and khayanolide D might be used as a potent oral anti-HBV drug alone or in association with tenofovir in future pre-clinical or clinical studies.

Keywords : molecular docking, drug-likeness profile, khayanolides, HBV

1. Introduction

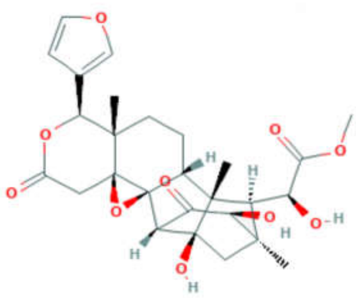
Hepatitis B is an inflammatory liver disease caused by the hepatitis B virus (HBV), a member of the Hepadnaviradeae family. HBV infection is regarded as a primary cause of cirrhosis and liver cancer as this disease is accountable of 50-80 % of clinically reported cases of hepatocellular carcinoma (Perz et al., 2006). The laboratory diagnosis is generally confirmed by the detection of the surface antigen (HbsAg) in the blood or serum of the suspected patients. According to current estimates, more than 240 million people are infected worldwide and about 786000 die each year (Locarnini et al., 2015). In Africa, hepatitis B is highly endemic with HBV infections rates ranging from 5-10 % and about 65 million people are chronically infected (Kramvis & Kew, 2007). A meta-analysis by Bigna et al. (2017) reported recently a pooled seroprevalence of 10.6% in Cameroon (Bigna et al., 2017).Vaccination is effective in preventing the spread of infection but only few people generally get diagnosed early and thereafter vaccinated. Recent evidence indicates that 1/12 healthcare workers in Cameroon are HBV-infected and 1/6 are fully vaccinated (Ndongo et al., 2019). The current antiviral therapies include the use of interferons and nucleoside analogs like Tenofovir or adefovir, but their use is still limited by resistant mutants and adverse effects (Mirandola et al., 2011). Previous evidence suggests that natural products might be an interesting source for the search of efficient anti-HBV drugs (Geng et al., 2018).

Limonoids are the class of modified triterpenoids that are found in plants'families such as Meliaceae, Cneoraceae, Ptaeroxylaceae, Rutaceae, and Simaroubaceae. Actually, about 1300 limonoids, exhibiting more than 35 different carbon frameworks have been reported on these families (Fang et al., 2011) with multiple and diverse biological activities including hepatoprotective effects. Recently, gedunin-type limonoids have been proposed as liver-protective drugs in D-galactosamine/lipopolysaccharide-induced hepato-toxicity thanks to their anti-inflammatory and antioxidant activities (Ninomiya et al., 2016). Mahmoud et al. (2014) have demonstrated that limonin mediates its hepatoprotective activities in D-galactosamine-induced liver injury through the down-regulation of Toll-like receptors. Rubescin E and limonoid TS3, two-havanensin-type limonoids isolated from *Trichilia rubescens* showed liver-protective effects by causing apoptosis of human hepatoma cell lines and interfering with the NF-kB activation (Lange et al., 2016). Similarly, these compounds were also found to protect mice from alcohol-liver injury by significantly reducing triglycerides and tumor-necrosis factor-α levels (Valansa et al., 2020). Recent studies showed that limonoids can exert antiviral activities. Vardhan and Sahoo, reported the anti-coronaviral activities of limonoids on SARS-CoV-2 through virtual screening (Vardhan & Sahoo, 2020). Khayanolides, the class of phragmalin-type limonoids previously described as *Khaya, Swietenia,* and mangrove genera were recently shown to possess anti-influenza A virus activities (Li et al., 2015). In fact, thaixylomolins I, K, and M, exhibited cytopathic effect against H1N1 viral isolates. However, the anti-hepatitis B activities of these compounds were not demonstrated so far. Herein, we report the inhibitory potential of four khayanolides (A, B, C, and D) against the hepatitis B virus (HBV) core and surface antigens through molecular docking studies.

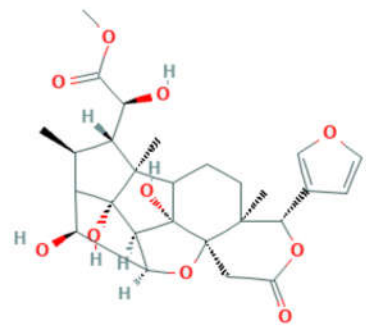
2. Material and Methods

The 3D structures of Tenofovir (reference compound ; CID : 464205) and Khayanolides A-D were obtained from the PubChem database (Figure 1) in SDF format (CIDs: 101069232 (Khayanolide A), 101069233 (Khayanolide B), 102375914 (Khayanolide C), and 101195134 (Khayanolide D)) Hydrogens were deleted and the files were then transformed into MDL MOL files with Open Babel. The 3D structures of viral proteins were retrieved from the protein data bank (PDB) repository. These include the Pre-S2 surface antigen (PDB ID : 1WZ4), CryoEM structure of HBV core (PDB ID: 3J2V), human HBV capsid (PDB ID: 1QGT), the duck HBV capsid (PBD ID: 6YGH). Docking was done against each PDB file using the iGEMDOCK software (version 2.1) provided by BioXGEM lab. The below parameters were used:

population size = 200, generation = 70, and number of solutions = 2. The postanalysis method helped us to visualize and determine drug interactions. The docking scores of the predicted poses were calculated as the total energy in the binding site. Fitness = VdW+ Hbond+Elec, with the vdW term refering to van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively. Fully description of the iGEMDOCK scoring function is presented by Yang and Shen (Yang & Shen, 2005). The pharmacokinetics and the druglikeness profiles were evaluated using SwissADME.



Khayanolide A



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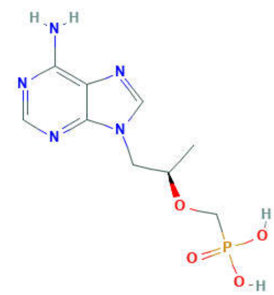
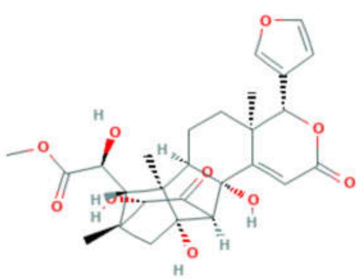
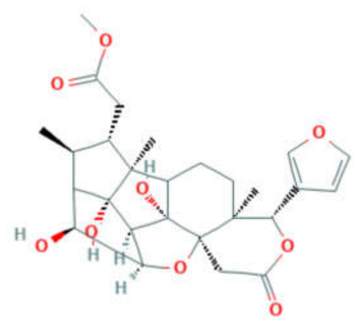


Tenefovir



Khayanolide C



Khayanolide D

Figure 1 : 2D structures of Khayanolides A-D and Tenofovir

3. Results and Discussion

3.1. Khayanolide D exhibited strong binding affinities with the human HBV capsid and the CryoEM structure of HBV core.

The docking scores of khayanolides and Tenofovir with the different HBV proteins are presented in Table 1. As shown in this table, khayanolide D (-104.34 kcal/mol), B (-103.77 kcal/mol), and C (-98.8 kcal/mol) demonstrated a higher fitness value with the human HBV capsid compared with Tenofovir (-98.56 kcal/mol). Ligand-protein interactions included both van der walls (vdW) and hydrogen (H-bond) interactions and were different for each ligand as no consensus amino acids were found between all the docked compounds. The docking poses of khayanolide D with 1QGT was stabilized by hydrogen bonds with Gly 10, Ala 11, Thr 12, Tyr 38, and Arg 39 while vdW bonding was formed with Gly 10, Leu 15, and Leu 115 (Figures 2a and 3a). Concerning the CryoEM structure of HBV core, all khayanolides compounds showed greater docking scores compared to tenofovir (-89.62 kcal/mol) but khayanolide D presented the top docking score (-100.92 kcal/mol) followed by khayanolide A (-96.63 kcal/mol). Unlike other compounds, tenofovir formed an electrostatic interaction with Arg 38, and its interaction profile is closer to that of khayanolide D (Figure 2b). Besides, no common amino acids were found in the 3JV2 binding site with the tested compounds. Khayanolide D was found to interact with Ser 21, Ser 26, and Arg 98 through H-bond, and Thr 128, Pro 129, Asp 22, Phe 24, Pro 25, Ser 26, Asn 90, and Arg 98 through vdW (Figure 3b). Previous computational modeling studies by Jia et al. reported similar findings using AT-130, a phenylpropenamide derivative, known to decrease HBV production by producing anormal capsids (Jia et al., 2015). In fact, AT-130 was found to interact with the HBV capsid through several amino acids including Pro 25, Thr 128, and Pro 129. This result may suggest the importance of these amioacids in virus inhibition and reveal the ability of khayanolide D to also inhibit HBV production.

Table 1: Docking scores of khayanolides and tenofovir with the different HBV proteins.

Ligand	Energy[a] (kcal/mol)	VDW[b] (kcal/mol)	Hbond[c] (kcal/mol)	Elec[d] (kcal/mol)
		Human HBV capsid (1QGT)		
Khayanolide A	-97.96	-75.81	-22.15	0
Khayanolide B	-103.77	-78.55	-25.22	0
Khayanolide C	-98.8	-76.14	-22.66	0
Khayanolide D	**-104.34**	-80.88	-23.46	0
Tenofovir	-98.56	-77.31	-21.25	0
		CryoEM structure of HBV core (3JV2)		
Khayanolide A	-96.63	-67.76	-28.87	0
Khayanolide B	-90.67	-57.25	-33.42	0
Khayanolide C	-93.51	-72.75	-20.76	0
Khayanolide D	**-100.92**	-72.75	-20.76	0
Tenofovir	-89.62	-58.28	-27.35	-3.99
		Duck HBV capsid (6YGH)		
Khayanolide A	**-113.53**	-84.2	-29.33	0
Khayanolide B	-105.92	-86.03	-19.89	0
Khayanolide C	-112.63	-83.8	-28.83	0
Khayanolide D	-108.67	-87.91	-20.77	0
Tenofovir	-98.21	-56.04	-36.44	-5.74
		HBV surface antigen (1WZ4)		
Khayanolide A	-84.46	-67.9	-16.56	0
Khayanolide B	**-92.24**	-69.79	-22.45	0
Khayanolide C	-80.28	-62.58	-17.7	0
Khayanolide D	-78.9	-61.45	-17.46	0
Tenofovir	-74.18	-55.67	-16.33	-2.18

a-Total interaction energy between the docked poses and the protein (kcal/mol); b-van der Waals interaction energy (kcal/mol);
c- hydrogen bonding energy (kcal/mol);

3.2. Khayanolide A showed strong binding affinities with the duck HBV capsid while Khayanolide B is the best ligand for the HBV surface antigen.

As far as the duck HBV capsid is concerned, all khayanolides presented higher binding scores with 6YGH in comparison to Tenofovir (-98.21 kcal/mol). Khayanolide A (-113.53 kcal/mol) and C (-112.63 kcal/mol) were those with the greatest binding affinities (Table 1) and their binding profiles were found closer each other (Figure 2c). Consensus amino acids were found between all khayanolides compounds in the 6YGH binding site and included H-bonds with Asp 24, Arg 28, Lys 31, and Leu 101, and vdW with Asp 24, Val 27, Arg 28, Lys 31, Glu 97, and Pro 100. This result is different from that found with 1QGT and may be explained by the different nature of the docked targets. The structural differences between duck HBV capsid and human HBV capsid could justify different binding modes of khayanolides on HBV capsids. With regard to HBV surface antigen, a higher binding score was noted with khayanolides compared to Tenofovir (-74.18

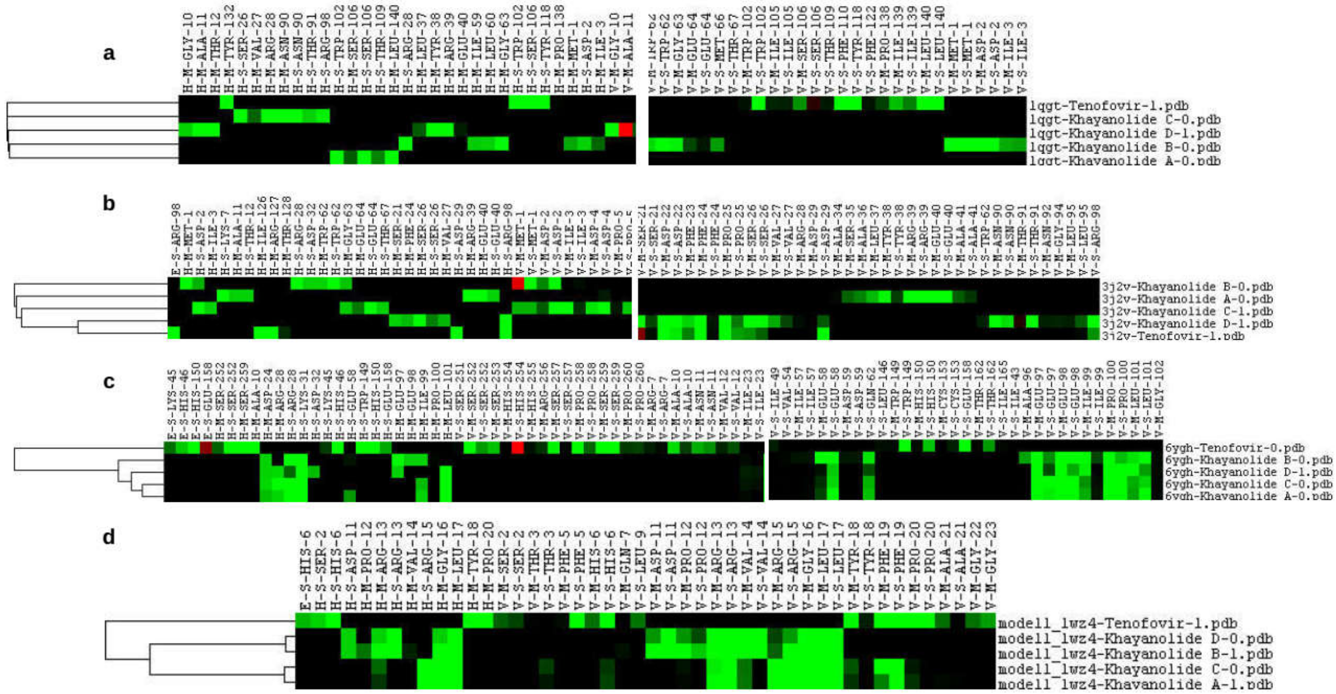


Figure 2 : Interaction profile clusters of Khayanolides with : (a) 1QGT, (b) 3J2V, (c) 6YGH, (d) 1WZK. H and V indicate hydrogen bonding and van der Waals interactions, respectively, while M and S correspond to the main chain or side chain of interacting residues. The interactions are colored in green when the energy is ≤1.5

kcal/mol). Khayanolide B, followed by A and C exhibited greater affinities with the binding scores of -92.24, -84.46, and -80.28 kcal/mol respectively. Gly 16, Leu 17, Arg 13, Pro 12 were identified as consensus amino acids in the interaction of khayanolides with this viral protein. Khayanolide B established H-bond with Asp 11, Arg 13, Gly 16, and Leu 17, while steric interactions were formed with Pro 12, Arg 13, Val 14, Arg 15, Gly 16, and Leu 17. As shown by figure 3d, khayanolides B and D share similar binding profiles distinct from those of A and C. Progress on disease pathogenesis underscored the role of HBV envelope proteins including the PreS2 domains in the morphogenesis, entry and multiplication of the virus (Urban et al., 2014). Thus, blocking this protein might be an interesting strategy to develop anti-HBV drugs. Previous investigations have reported high binding affinities of ZINC11882026, ZINC11882026, 00653293, and 19741044 with the large surface antigen of HBV (Mehmankhah et al., 2019). Interacting amino acids residues in the binding site were different from that found in this study and may be explained by the different nature of the molecule or the PDB file used.

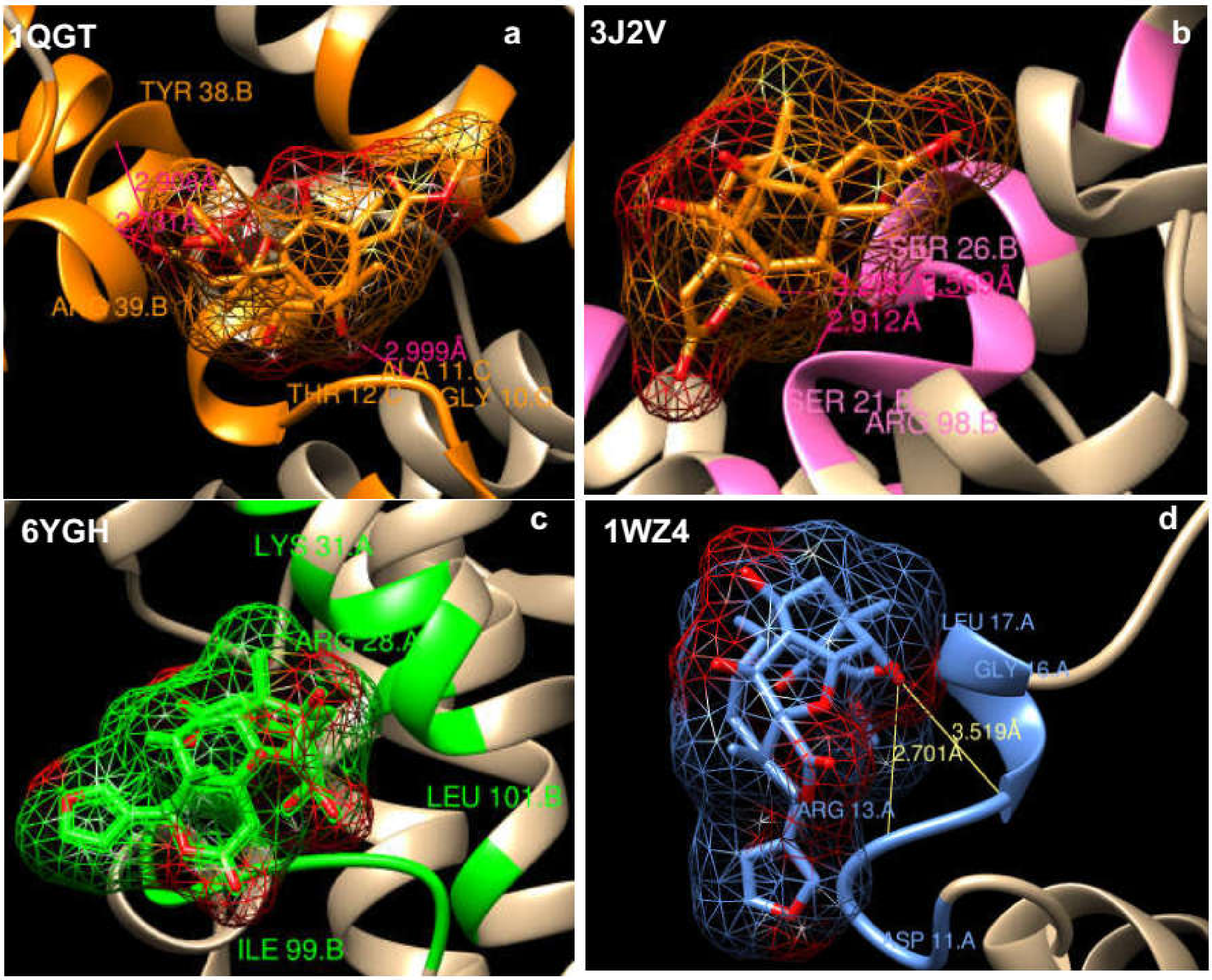


Figure 3: Docking poses of Khayanolide D (in orange) with 1QGT (a) and 3J2V (b), Khayanolide A (in green) with 6YGH (c), Khayanolide B with 1WZ4 (d). For each ligand-protein complex, the amino acid residues involved in hydrogen bonds (H-bond) are displayed as well as the distance of this H-bond whenever possible.

3.3. *In silico* ADMET and drug-likeness results

Table 2 highlights the predicted drug-likeness and pharmacokinetic profiles of the docked compounds. From this table, it emerges that among all khayanolides, only khayanolide D presented a high gastrointestinal absorption, similarly to Tenofovir. Also, this compound is the one that complied with most of the drug-likeness rules (3/5) including the Lipinski, Veber, and Muegge filters. None of these compounds were found able to cross the blood-brain barrier (BBB) and all showed their capacity to be used as substrates of the permeability glycoprotein (P-gp) unlike tenofovir, which suggests that they might influence their bioavailability or those of other drugs. Cytochrome P450 enzymes (CYPs) are subdivided into several isoforms and many drugs can be deactivated or activated by these enzymes. It has been suggested that inhibition of these isoforms might contribute to drug-drug interactions and cause undesirable effects (Daina et al., 2017). Khayanolide A was found to be a CyP2C19 inhibitor, while khayanolide B and D interfered with Cyp2D6.

Table 2 : Pharmacokinetic profile and drug-likeness properties

	Tenofovir	Khayanolide A	Khayanolide B	Khayanolide C	Khayanolide D
GI absorption	High	Low	Low	Low	High
BBB	No	No	No	No	No
P-gp substrate	No	Yes	Yes	Yes	Yes
Cyp1A2	Yes	No	No	No	No
Cyp2C19	No	Yes	No	No	No
Cyp2c9	No	No	No	No	No
Cyp2D6	No	Yes	Yes	No	Yes
Cyp 3A4	No	No	No	No	No
LogKp (cm/s)	-9.10	-9.30	-9.51	-9.62	-9.01
Lipinski	Yes	Yes (1 violation)	Yes (1 violation)	Yes (1 violation)	Yes (1 violation)
Ghose	No (2 violations)	No (1 violation)	No (2 violations)	No (1 violation)	No (1 violation)
Veber	Yes	No (1 violation)	No (1 violation)	No (1 violation)	Yes
Egan	Yes	No (1 violation)	No (1 violation)	No (1 violation)	No (1 violation)
Muegge	Yes	No (1 violation)	No (1 violation)	No (1 violation)	Yes (1 violation)
Bioavailability score	0.56	0.55	0.55	0.55	0.55

No interaction with all CYP isoforms was noted with khayanolide C. Concerning the skin permeation, khayanolide C has the greatest chances to cross the skin as the lowest LogKp value (-9.62 cm/s) was found with this compound, followed by khayanolide B (-9.51 cm/s), khayanolide A (-9.30 cm/s), tenofovir (-9.10 cm/s), and khayanolide D (-9.01 cm/s).

5. Conclusion

To conclude, limonoids in general and Khayanolides specifically offer a good alternative for the development of new anti-HBV drugs. Good binding affinities with the surface antigens and HBV capsids were found in this study and these interactions were better than that of Tenofovir. In vitro and In vivo studies with these compounds are necessary to validate and explore more deeply their antiviral activities.

Authors Contributions

BRGT and PDDC wrote, reviewed and edited the paper

Declaration of interests

The authors have no conflict of interests to declare.

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