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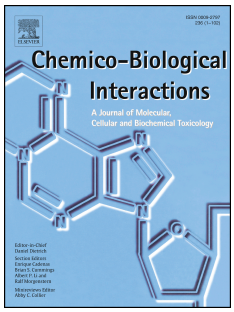

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PII: S0009-2797(16)30072-2

DOI: 10.1016/j.cbi.2016.03.011

Reference: CBI 7613

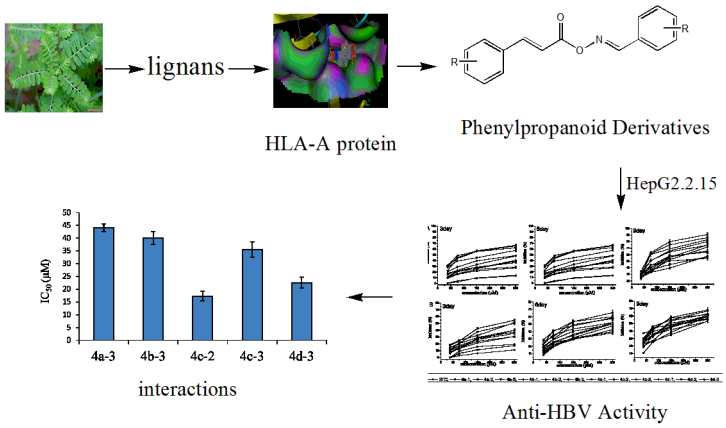
To appear in: *Chemico-Biological Interactions*

Received Date: 13 December 2015

Revised Date: 20 February 2016

Accepted Date: 4 March 2016

Please cite this article as: S. Liu, Y. Li, W. Wei, K. Wang, L. Wang, J. Wang, Design, synthesis, molecular docking studies and Anti-HBV activity of phenylpropanoid derivatives, *Chemico-Biological Interactions* (2016), doi: 10.1016/j.cbi.2016.03.011.



Design, Synthesis, Molecular Docking Studies and Anti-HBV Activity of Phenylpropanoid Derivatives

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Abstract: In this work, a series of phenylpropanoid derivatives were synthesized, and their anti-hepatitis B virus (HBV) activity was evaluated. Most of the synthesized derivatives showed effective anti-HBV activity. And compound 4d-3 showed the most effective anti-HBV activity, performing strong potent inhibitory not only on the secretion of HBsAg (IC_{50} =58.28μM, SI=23.26) and HBeAg (IC_{50} = 97.21 μM, SI=13.95), but also on the HBV DNA replication (IC_{50} = 42.28 μM, SI = 32.06). The structure-activity relationships (SARs) of the derivatives had been discussed, which were useful for developing phenylpropanoid derivatives as novel anti-HBV agents. Moreover, the docking study of all synthesized compounds inside the HLA-A protein (PDB ID: 3OX8) active site was carried out to explore the molecular interactions and a molecular target for activity and a modified assay method measuring the interaction between our derivatives and HBcAg was investigated, indicating that the HBV core protein might be their potential target for anti-HBV. This study identified a new class of potent non-nucleoside anti-HBV agents.

Keywords: Synthesis, Phenylpropanoid derivatives, Anti-HBV activity, Structure-activity relationships, Molecular docking

1. Introduction

Hepatitis B virus (HBV), a member of Hepadnaviridae family, is a human pathogen that causes acute and chronic hepatitisa and may lead to lifelong infection, cirrhosis, hepatocellular carcinoma, liver failure, or death [1,2]. HBV infects chronically over 350 million people globally with more than 1 million deaths annually [3]. Currently, therapies including immunomodulator, interferons (interferon-alpha and pegylated interferon), and nucleoside drugs (lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir) for treating HBV are still unsatisfactory [4,5]. Interferons is effective only in less than 30% of the chronic carriers and about 50% of interferons-treated patients experience recurrence of viremia after cessation of treatment [6,7]. Nucleoside drugs, whose major target is to inhibit the viral reverse transcriptase (RT)/DNA polymerase, are effective in suppressing the viral replication for extended periods, while drug resistance remains a critical issue due to the mutation of DNA polymerase [8,9]. Therefore, new classes of potent antiviral compounds with different antiviral targets and mechanisms seem highly desirable.

HBV contains a 3.2 kb partially double-stranded DNA genome with four open reading frames (ORFs) encoding four viral proteins including core protein (Cp), surface protein (S), polymerase (P), and X protein (X) [10]. Core protein mainly constitutes the nucleocapsid which harbors the viral DNA and polymerase [11]. It was reported that dominant negative core protein mutants, which could not support pregenomic RNA packaging and genome maturation, could effectively inhibit replication HBV [12]. Peptides that could inhibit the assembly of core proteins and intracellular single chain antibodies against HBV core protein could also inhibit HBV replication [13,14]. These observations suggest that the assembly

process of core protein could serve as a target for anti-HBV agents.

In our previous study, we found that lignans niranthin, nirtetralin, nirtetralin A and nirtetralin B isolated from *Phyllanthus niruri L.* exhibited significant anti-HBV activity [15-17]. We investigated the potential anti-HBV targets of the anti-HBV lignans with reverse docking approach using fifteen HBV related proteins and RNA including human leukocyte antigen HLA-A*02:03 (PDB ID: 3OX8), human leukocyte antigen HLAA*02:06 (PDB ID: 3OXR), human leukocyte antigen HLA-A*02:07 (PDB ID: 3OXS), hepatitis B virus preS1 protein (PDB ID: 3ZHF), hepatitis B virus preS2 surface antigen (PDB ID: 1WZ4), human hepatitis B virus surface antigen HzKR127 (PDB ID: 2EH8), human hepatitis B virus e-antigen (PDB ID: 3V6F, 3V6Z), Hepatitis B X-interacting protein HBXIP (PDB ID: 3MS6, 4WZR, 4WZW), HBV RNA polymerase (PDB ID: 2HN7), and human hepatitis B virus encapsidation signal (PDB ID: 2IXY, 2K5Z). The reverse docking results showed that HLA-A protein (PDB ID: 3OX8) may be the potential target for anti-HBV.

The HBV core 18-27 peptide (HBcAg18-27) was originally described as a human leukocyte antigen (HLA)-A epitope and detected in more than 90% of human leukocyte antigen (HLA)-A protein which was associated with severe liver inflammation in Chinese patients with chronic HBV infection [18]. The crystal structure of HLA-A protein (PDB ID: 3OX8) was investigated to be a fragment of HBV core protein and could serve as a target for anti-HBV agents [19].

In order to increase the activity of the lignans, we designed and synthesized series of analogues based on the reverse docking results and combination principle. HLA-A protein (PDB ID: 3OX8) was used as molecular targets for anti-HBV activity of the analogues in a

moe-docking technique. And the same structural fragments that the anti-HBV active lignans possess ((E)-3-(3,4-dimethoxyphenyl)acrylic acid, (E)-3-(3,4,5-trimethoxyphenyl)acrylic acid, (E)-3-(benzo[d][1,3]dioxol-5-yl)acrylic acid and (E)-3-(7-methoxybenzo[d][1,3]dioxol-5-yl) acrylic acid) were used as the main scaffold for the design and synthesis of novel compounds as potent anti-HBV agents. As a part of our continuous search for active anti-HBV leads from synthetic compounds, some phenyl acryloyl type oxime esters derivatives with heterocycle have been reported [20]. We found that the lignans were observed to exhibit low cytotoxicity and effective anti-HBV activity while the cytotoxicity increased after heterocycle were introduced into the derivatives. Then, the phenyl acryloyl type oxime esters derivatives also showed anti-HBV activity and our docking study shown that the oxime ester group could interact with the protein [20]. In the current studies, it was of interest to know how the oxime ester group maintained the anti-HBV activity by simplifying the structure of the active lignans with an oxime ester group.

In this paper, we reported the synthesis, SARs, molecular docking study, and anti-HBV activity of phenylpropanoid derivatives by modification of ester group heterocycle part with the structural fragment of the active lignans. Thus, the two fragments of the active lignans was connected and simplified by an oxime ester group. For further study, an assay was investigated to explore the interactions between the derivatives and the protein.

2. Results and Discussion

2.1. Chemistry

General synthesis for the intermediate and target compounds is depicted in Scheme 1. Substituted benzaldehyde was reacted with hydroxylamine hydrochloride in EtOH

in the presence of sodium acetate to yield oxime 1-3 in a good yield [21]. Intermediates 2a-d were prepared by Knoevenagel condensation of malonic acid and the aldehyde group of four benzaldehydes with yields of 80%-90% [22].The final oxime ester derivatives (4a-1~ 4a-3), (4b-1~ 4b-3), (4c-1~ 4c-3), (4d-1~ 4d-3) were obtained by reaction of oxime with cinnamoyl chloride 3a-d in the presence of TEA, which were obtained by reaction of substituted phenylacrylic acid 2a-d and thionyl chloride in DCM [23].

The structures of the newly synthesized compounds (4a-1~ 4a-3), (4b-1~ 4b-3), (4c-1~ 4c-3), (4d-1~ 4d-3) were characterized by ^{1}H NMR, ^{13}CNMR and MS data and their data are presented in the supplementary material. ^{1}H NMR spectra of the derivatives showed a singlet at about 8.35 ppm corresponding to N=CH proton. Two doublets at 6.29-6.47 and 7.57-7.80 ppm with J=15.57-15.93 Hz corresponding to trans hydrogens of CH=CH respectively. The singlet at 3.89-3.95 ppm attributed to O-CH_3 protons, and at 6.00-6.05 corresponding to OCH_2O protons. The chemical shifts of aromatic hydrogens of the phenyl ring appeared as multiplets in the region δ 6.74-7.25. ^{13}C NMR chemical shifts for title compounds were observed in their expected regions. ^{13}C NMR spectrum for the derivatives showed signals at 55.87-56.92, 101.52-102.35, 155.76-156.70 and 164.53-167.66 corresponding to CH_3, CH_2, C=N and C=O, respectively.

2.2. QSAR Study

The 3D structures of all the compounds were generated using the Built Optimum option of Hyperchem software (version 8.0), and subsequently energy minimized using MM+ force field. Then, the structures were fully optimized. Molecular descriptors were determined by

QSAR study, including logP, molar refractivity, surface area, volume, hydration energy and polarizability. And the results showed that all molecules had drug like properties (Table 1). All the compounds have the molecular weight ranging from 350 to 450 Da. The log P values of these compounds are superior to act as drug which is -0.14 to -3.39 and the molar refractivity is in the range of 101-121.

2.3. Molecular Docking

Molecular docking studies of phenylpropanoid derivatives were carried out using MOE 2008.10 as the docking software in order to rationalize the biological activity results and understand the various interactions between ligand and protein in the active site in detail. The crystal structure of HLA-A protein (PDB ID: 3OX8) was used for docking study and the 'Site Finder' tool of the program was used to search for its active site. We performed three docking procedures for each ligand and the best configuration of each of the ligand-receptor complexes was selected based on energetic grounds. The affinity scoring function δG was used to assess and rank the receptor-ligand complexes. The docking scores and the hydrogen bonding strength of all the molecules were shown in Table 2.

The synthesized series derivatives had dock score ranging from -14.6098 to -19.5089. Compound 4d-3 was showing the best least docking score of -19.5089 and the next best least docking score was found with 4b-3 followed by 4a-3. Four hydrogen bonds were present in the derivative 4b-3 and 4d-3, which was the highest among the series. Compound 4d-3 is found to be forming four hydrogen bonds of lengths 1.48, 1.55, 2.85 and 3.03 Å each with 15-OCH_3, 14-OCH_3, 13-OCH_3 in benzene ring of Asp53 and O-N in oxime ester group of Tyr27 respectively (Fig. 1). Compound 4b-3 also formed four hydrogen bonds of lengths 1.41,

1.65, 2.95 and 2.98 Å with 13, 14, 15-OCH_3 in benzene ring of Asp53 and O-N in oxime ester group of Tyr27 (Fig. 2). Compound 4a-3 formed three hydrogen bonds. In 4a-3, the 14, 15-OCH3 in benzene ring of Asp53 and O-N in oxime ester group of Tyr27 were with bond length of 2.61, 2.16 and 2.02 Å respectively (Fig. 3). The compounds 4d-3, 4b-3 and 4a-3 exhibited the best least docking score had good in vitro anti-HBV activity as well.

2.4. Anti-HBV Activity

All the newly synthesized derivatives were tested for their anti-HBV activity, namely inhibiting the secretion of HBsAg, and HBeAg in HepG 2.2.15 cells using lamivudine (3TC, a clinically popular anti-HBV agent) as a positive control. The anti-HBV activity of each compound was expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) to the secretion of HBsAg and HBeAg. And the cytotoxicity of each compound was expressed as the concentration of compound required to kill 50% (CC_{50}) of the HepG 2.2.15 cells. The selectivity index (SI), a major pharmaceutical parameter that estimates possible future clinical development, was determined as the ratio of CC_{50} to IC_{50}. The results of their anti-HBV activity and cytotoxicity were listed in Table 3.

The treatment of HBV-transfected HepG2.2.15 cells with various concentrations of drugs for 9 days exhibited a time-and dose-dependent inhibitory effect on the secretion of HBsAg and HBeAg (Fig. 4). In synthesized derivatives, all compounds showed better activity inhibiting the secretion of HBsAg than that of lamivudine. And eleven of twelve derivatives, with higher inhibitory activity against the secretion of HBeAg than lamivudine were obtained except for 4a-1. Moreover, all the twelve derivatives were observed analogical activities, but lower cytotoxicity than those with heterocycle [20]. Compound 4d-3 showed the most potent

anti-HBV activity, demonstrating potent inhibitory effect on the secretion of HBsAg (IC50 =58.28μM, SI=23.26) and HBeAg (IC50 = 97.21 μM, SI=13.95). Compound 4c-2 showed inhibitory potency to the secretion of HBsAg (IC_{50}=55.51 μM) and HBeAg (IC_{50}=109.34 μM), but appeared toxic (CC_{50}=381.35μM), which led to relatively low SI values (SI_{HBsAg}=6.87, SI_{HBeAg}=3.49). Compared to compound 4c-2, compound 4b-3 relatively low inhibitory potency to the secretion of HBsAg (IC_{50}=76.39 μM) and HBeAg (IC_{50}=145.774 μM), but weak toxic (CC_{50}=1554.70 μM) led to higher SI values (SI_{HBsAg}=20.35, SI_{HBeAg}=10.67).

Importantly, the most active compounds 4a-3, 4b-3, 4c-2, 4c-3 and 4d-3 with high activities against HBsAg and HBeAg were selected to investigate inhibition of HBV DNA replication using lamivudine as the reference drug. Compounds 4a-3, 4b-3, and 4d-3 exhibited anti-HBV activity with their IC_{50} values against HBV DNA replication of 151.57, 100.62, 42.28 μM, respectively. Compounds 4a-3, 4b-3, and 4d-3 displayed inhibiting not only HBsAg and HBeAg secretion but also HBV DNA replication, however, 3TC showed significantly activity against HBV DNA replication (IC_{50} = 6.86) while showed little inhibitory on HBsAg and HBeAg secretion.

2.5. Structure-Activity Relationship

The start reactants substituted benzaldehyde 1a-d, intermediates 2a-d and oximes 1-3 showed low suppressant properties on the HBV while most of the derivatives showed high potency activity against of the secretion of HBsAg and HBeAg as shown in Table 3. In the docking study, we also found that the O of O-N in the oxime ester group interacted with Tyr27 by hydrogen bond.

The phenylpropanoid part of the derivatives showed the similar structure-activity

relationship as our previous study [20]. The results also shown that the introduction of methoxy group to 5-C could enhance the anti-HBV activity and decrease cytotoxicity along with the high SI values, and the substituent of 3,4-dimethoxy by 3,4-methylenedioxy could increase activity and cytotoxicity along with relatively low SI values.

Derivatives 4a-1~3, 4b-1~3, 4c-1~3 and 4d-1~3 contained the same phenylpropanoid part respectively. Compound 4a-3 showed the best anti-HBsAg activity (IC_{50}=85.00 μM) and the next was 4a-1 (IC_{50}=128.86 μM), followed by 4a-2 (IC_{50}=222.19 μM), while the most potent anti-HBeAg activity was observed with 4a-3 (IC_{50}=146.24 μM), followed by 4a-2 (IC_{50}=196.15 μM), then 4a-1 (IC_{50}=290.28 μM). The order also applied to 4b-1~3, 4c-1~3 and 4d-1~3 except for 4c-2, which showed more effective on inhibiting HBsAg and HBeAg secretion than that of 4c-3. It suggested that the introduction of methoxy group to 13-C could enhance the anti-HBV activity, and the substituent of 13,14-dimethoxy by 13,14-methylenedioxy could decrease inhibition to the secretion of HBsAg and HBeAg. Compound 4a-1 showed cytotoxicity with CC_{50} values of 546.41 μM. The cytotoxicity of derivatives 4a-3 (CC_{50}=601.46 μM) decreased with methoxy group at 13-C. Compound 4a-2 (CC_{50}=582.83 μM) was observed with stronger cytotoxicity than 4a-3 with 13,14-methylenedioxy. Actually, all the derivatives obtained from oxime 3 did show weakest cytotoxicity, followed by that from oxime 2. It indicated that the introduction of methoxy group to 13-C could enhance the anti-HBV activity and decrease the cytotoxicity, and the substituent of 13,14-dimethoxy by 13,14-methylenedioxy could decrease the anti-HBV activity but increase cytotoxicity, leading to a low SI values.

According to the results mentioned above, SARs were summarized as followed: (1)

5-OCH_3-substituted compounds with methylenedioxy at 13,14-C could provide higher anti-HBV activity than other analogues. (2) 13,14,15-OCH_3-substituted compounds could provide higher anti-HBV activity than that with H-substituted or methylenedioxy-substituted.

2.6. The Interaction Between Derivatives and HBcAg

A modified enzyme-linked assay method using 96-well plates was used to explore the interactions between the derivatives and HBcAg. The most active compounds 4a-3, 4b-3, 4c-2, 4c-3 and 4d-3 were chosen as models to explore the interactions and the results were provided in Fig. 5. It was evident that there was a reduction of the OD values in a dose dependent way, which indicated that there would be an interaction between the compounds and HBcAg blocking the binding of HBcAg. Among them, compound 4c-2 showed the lowest IC_{50} value (17.25 μM) maybe for its cytotoxicity as mentioned above, and the next was compound 4d-3 (22.50 μM). It was compared to that of docking and in vitro anti-HBV activity results, and was analogous.

3. Conclusion

In conclusion, synthesis of phenylpropanoid derivatives and their potential anti-HBV activities was evaluated. Results observed in the present study clearly demonstrated that most of derivatives of the oximes ester phenylpropanoid family could exert interesting anti-HBV activities with the SI_{HBsAg} values from 2.14 to 23.26 and SI_{HBeAg} values from 1.88 to 13.95. Moreover, all the twelve derivatives were observed analogical activities, but lower cytotoxicity than those with heterocycle. Interestingly, compounds 4c-1, 4d-1, and 4d-2 displayed inhibiting not only HBsAg and HBeAg secretion but also HBV DNA replication, however, 3TC showed significantly activity against HBV DNA replication while showed little

inhibitory on HBsAg and HBeAg secretion. In addition, the docking study of all synthesized compounds inside the HLA-A protein (PDB ID: 3OX8) active site were carried out to explore the molecular interactions and a molecular target for activity, and indicated that the HBV core protein might be the potential target for anti-HBV. It was further proved by a modified assay method measuring the interaction between our derivatives and HBcAg. The results of the biological activities screened were consistent with the docking results and measurement of the interaction, indicating that the anti-HBV effect of the prepared compounds may exert its anti-HBV activity by inhibiting HBcAg. This study identified a new class of potent anti-HBV agents and offered valuable information for seeking non-nucleoside anti-HBV drug candidates.

4. Materials and methods

4.1. General

Melting points were determined using electrothermal melting point apparatus WRX-4 (Shanghai, China) and were uncorrected. MS spectra were run on a Finnigan LCQ Deca XP MAX mass spectrometer (Thermo Fisher, San Jose, CA, USA) equipped with an ESI source and an ion trap analyzer in the positive ion mode/in the negative ion. NMR spectra were recorded on Bruker AM 400 MHz ($^{1}H/^{13}C$, 400 MHz/100 MHz) or Bruker DRX 500 MHz ($^{1}H/^{13}C$, 500 MHz/125 MHz) spectrometer (Bruker, Bremerhaven, Germany) and chemical shifts were quoted in δ as parts per million (ppm) downfield with tetramethylsilane (TMS) as internal reference. Coupling constants, J, are expressed in hertz (Hz). Column chromatography (CC): silica gel (200 - 300 mesh; Qingdao Makall Group Co., Ltd; Qingdao; China). All reactions were monitored using thinlayer chromatography (TLC) on silica gel

plates. Yields refer to isolated pure products and were not maximized. On the basis of NMR and HPLC (Thermo Fisher UItiMate 3000, USA) data, all final compounds reported in the manuscript were >95% pure.

4.2. Chemistry (Scheme 1)

The chemical synthesis and further characterization were available in the supplementary material.

4.3. Molecular Docking Studies

The ligand study was carried out by HyperChem software, a sophisticated molecular modeling environment that uniting with quantum chemical calculations, dynamics, and molecular mechanics [24].Three-dimensional structures were constructed and optimized for all the molecules, and then QSAR descriptors were studied, which is a powerful lead optimization tool that can quantitatively relate variations in biological activity to changes in molecular properties.

The molecular docking study was performed using MOE 2008.10 to understand the ligand-protein interactions in detail. The target compounds were built using the builder interface of the MOE program and subjected to energy minimization. The crystal structure of human leukocyte antigen (HLA-A) protein (PDB ID: 3OX8) was retrieved from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) [25].The edited crystal structure after removing water molecules was imported into MOE and chain A was considered for docking process as the protein is a dimer consisting of A and B chains. The structure is protonated,

polar hydrogens were added and energy minimization was carried out till the gradient convergence 0.05 kcal/mol was reached to get the stabilized conformation. The active site was correlated with 'Site Finder' module of MOE to define the docking site for the ligands. Docking procedure was followed using the standard protocol implemented in MOE 2008.10 and the geometry of resulting complexes was studied using the MOE's Pose Viewer utility.

4.4. Pharmacology

4.4.1. Cells and Cell culture

HepG2.2.15 (clonal cells derived fromhuman hepatoma cell line G2) cells were provided by the Chinese Academy of Medical Sciences (P.R. China) and maintained in MEM medium supplemented with 10% fetal bovine serum and 380 μg/mLof G418, 50 u/mL of kanamycin, and 0.03% L-glutamine at 37 ^{o}C in a 5% CO_2 atmosphere with 100% humidity.

4.4.2. Drug Treatment

HepG 2.2.15 cells were seeded at a density of 1×10^5 cells/mL (200 μL/well) in 96-well plates and maintained at 37 ^{o}C for 24 h prior to extract addition, followed by treatment with various concentrations of drugs. Lamivudine (3TC) was served as the positive control. Cells were refed with drug-containing fresh medium every 3 d for up to 9 d in time-dependent experiment. Medium was taken at third day of treatment (T3), the sixth day of treatment (T6) and the ninth day of treatment (T9), and stored at -20 ^{o}C until analysis. The IC_{50} and selected index (SI) of each compound were calculated, respectively.

4.4.3. Cell Toxicity

Logarithmically growing cells were seeded in 96-well culture plates at a density of 1×10^5 cells/mL (200 μL/well). They were cultured for 24 h and then treated with various concentrations of drugs. OD values were read at 450 nm after 9days and the percent of cell death was calculated and the cells were refed with drug-containing fresh medium every 3 d for up to 9 d. After drug treatment, the cytotoxicity was measured using the MTT assay [26,27].

4.4.4. Determination of HBsAg and HBeAg

The levels of HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) were simultaneously detected using ELISA kits (Rongsheng Biotechnology Co. Ltd, Shanghai, China) according to the manufacturer's instructions.

4.4.5. Determination of HBV replication

Inhibitory activity against HBV was determined by a real-time fluorescence quantitative PCR (FQ-PCR) according to our previous description [12]. Briefly, 2.0 μL of HBV DNA was amplified in a 25 mL of mixture containing 12.5 μL 2 × SYBR Green Master (ROX) and 2 primers specific for HBV: a forward primer (5′-AAC CAT TGA AGC AAT CAC TAG AC-3′) and a reverse primer (5′- ATC TAT GGT GGC TGC TCG AAC TA -3′). The thermal program comprised of an initial denaturation at 95 ^{o}C for 10 min followed by 40 amplification cycles with each of the two following steps: 95 ^{o}C for 15 s and 60 ^{o}C for 1 min.

4.5. Measurement of the interaction between the derivatives and HBV core protein

HBcAg protein was purified by ultracentrifugation and gel filtration chromatography as described [28]. The interaction between the derivatives and HBV core protein was measured by ELISA with some modifications [28,29]. Briefly, 100 μL of HBcAg in 50mM sodium phosphate (pH 8.0) was incubated in 96-well plates overnight at 4 ^{o}C. After blocking the plate surface with 2% bovine serum albumin (Sigma, USA) (w/v) in PBS (50mM sodium phosphate, 0.15MNaCl, pH 7.4), various concentrations of derivatives were added and incubated for 20 min. The amount of HBcAg bound on the plate was measured using anti-HBcAb (Rongsheng Biotechnology Co. Ltd, Shanghai, China).

Acknowledgements

This work was financially supported by the Foundation of High level innovation team and outstanding scholar program in Guangxi collages, department of education of Guangxi Zhuang autonomous region (2015), National Natural Science Foundation of China (No. 81060261), Natural Science Foundation of Guangxi Province (2012GXNSFAA053021), and Department of Health of Guangxi Zhuang Autonomous Region (No. GZBZ14-19).

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Tables

Table 1. Molecular descriptors of derivatives from QSAR study.

Ligand	Molecular weight (Da)	LogP	Molar refractivity ($Å^3$)	Surface area ($Å^2$)	Volume ($Å^3$)	Hydration energy (Kcal/mol)	Polarizability ($Å^3$)
3TC	229.25	-0.55	55.14	525.55	606.87	48.20	21.71
4a-1	371.38	-1.40	109.06	852.16	1033.59	45.35	38.79
4a-2	385.37	-2.21	108.27	899.85	1036.63	39.22	38.65
4a-3	401.41	-2.40	115.43	906.26	1094.60	43.26	41.26
4b-1	401.41	-2.40	115.43	905.72	1081.70	43.13	41.26
4b-2	415.39	-3.20	114.65	953.39	1084.21	39.03	41.12
4b-3	431.44	-3.39	121.81	959.82	1142.71	43.04	43.73
4c-1	355.34	-1.21	101.90	862.83	957.70	41.00	36.18
4c-2	369.32	-2.02	101.11	910.52	961.08	36.87	36.04
4c-3	385.37	-2.21	108.27	917.00	1019.74	40.91	38.65
4d-1	385.37	-2.21	108.27	909.16	1012.94	39.92	38.65
4d-2	399.35	-3.01	107.49	956.84	1016.01	35.83	38.51
4d-3	415.39	-3.20	114.65	963.46	1075.15	39.83	41.12

Table 2. Docking score and bond interactions of synthesized compounds.

Ligand	S-score (kcal/mol)	No. of H-bonds	Distance (Å)	Amino acid involved	Molecular structure
3TC	-10.7074	2	2.03	TYR 27	O of -OH
			2.29	TYR 27	O of -COC-
4a-1	-16.1132	3	2.02	TYR 27	O of -ON
			2.61	ASP 53	O of 14 -OCH_3
			2.16	ASP 53	O of 15 -OCH_3
4a-2	-16.0508	3	2.03	TYR 27	O of -ON
			2.72	ASP 53	O of 14 -OCH_2-
			2.14	ASP 53	O of 15 -OCH_3
4a-3	-18.5917	3	2.02	TYR 27	O of -ON
			2.61	ASP 53	O of 14 -OCH_3
			2.16	ASP 53	O of 15 -OCH_3
4b-1	-17.6575	3	2.99	TYR 27	O of -ON
			1.65	ASP 53	O of 14 -OCH_3
			1.39	ASP 53	O of 15 -OCH_3
4b-2	-17.9895	3	2.99	TYR 27	O of -ON
			1.75	ASP 53	O of 14 -OCH_3
			1.39	ASP 53	O of 15 -OCH_3
4b-3	-18.9798	4	2.98	TYR 27	O of -ON
			2.95	ASP 53	O of 13 -OCH_3
			1.65	ASP 53	O of 14 -OCH_3
			1.41	ASP 53	O of 15 -OCH_3
4c-1	-14.6098	3	2.02	TYR 27	O of -ON
			2.55	ASP 53	O of 14 -OCH_3
			2.21	ASP 53	O of 15 -OCH_3
4c-2	-14.5949	3	2.00	TYR 27	O of -ON
			2.70	ASP 53	O of 14 -OCH_3
			2.18	ASP 53	O of 15 -OCH_3
4c-3	-17.2444	3	2.03	TYR 27	O of -ON
			2.54	ASP 53	O of 14 -OCH_3
			2.17	ASP 53	O of 15 -OCH_3
4d-1	-16.1088	3	3.02	TYR 27	O of -ON
			1.56	ASP 53	O of 14 -OCH_3
			1.50	ASP 53	O of 15 -OCH_3
4d-2	-17.1885	3	2.97	TYR 27	O of -ON
			1.72	ASP 53	O of 14 -OCH_3
			1.46	ASP 53	O of 15 -OCH_3
4d-3	-19.5089	4	3.03	TYR 27	O of -ON
			2.85	ASP 53	O of 13 -OCH_3
			1.55	ASP 53	O of 14 -OCH_3
			1.48	ASP 53	O of 15 -OCH_3

Table 3. Anti-HBV activity and cytotoxicity of the phenylpropanoid derivatives in vitro[a].

Compd	CC_{50}[b] (μM)	HBsAg[c]		HBeAg[d]		HBV DNA replication	
		IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]
1a	＞1500	-[g]	-	-	-	ND[h]	ND
1b	＞1500	-	-	-	-	ND	ND
1c	1289.31	-	-	-	-	ND	ND
1d	1075.75	-	-	-	-	ND	ND
2a	534.66	＞600	＜0.89	＞600	＜0.89	ND	ND
2b	834.13	＞600	＜1.39	＞600	＜1.39	ND	ND
2c	503.46	433.15	1.16	526.25	0.96	ND	ND
2d	667.25	410.96	1.62	476.75	1.40	ND	ND
1	402.02	387.17	1.04	469.11	0.86	ND	ND
2	475.21	479.80	1.01	519.47	0.91	ND	ND
3	562.19	353.56	1.59	431.23	1.30	ND	ND
4a-1	546.41	128.86	4.24	290.28	1.88	ND	ND
4a-2	582.83	222.19	2.62	196.15	2.97	ND	ND
4a-3	601.46	85.00	7.08	146.24	4.11	151.57	3.97
4b-1	599.10	124.50	4.81	243.86	2.47	ND	ND
4b-2	759.73	194.11	3.91	171.89	4.42	ND	ND
4b-3	1554.70	76.39	20.35	145.77	10.67	100.62	15.45
4c-1	255.99	119.53	2.14	210.97	1.21	ND	ND
4c-2	381.35	55.51	6.87	109.34	3.49	-	-
4c-3	532.67	69.22	7.70	125.91	4.23	-	-
4d-1	567.04	112.61	5.04	201.90	2.81	ND	ND
4d-2	626.14	167.47	3.74	166.03	3.77	ND	ND
4d-3	1355.64	58.28	23.26	97.21	13.95	42.28	32.06
3TC[i]	568.25	234.70	2.42	267.16	2.13	6.86	82.84

a. Values are means determined from at least two experiments.

b. CC_{50} is 50% cytotoxicity concentration in HepG2 2.2.15 cells.

c. HBsAg: hepatitis B surface antigen.

d. HBeAg, hepatitis B e antigen.

e. IC_{50} is 50% inhibitory concentration.

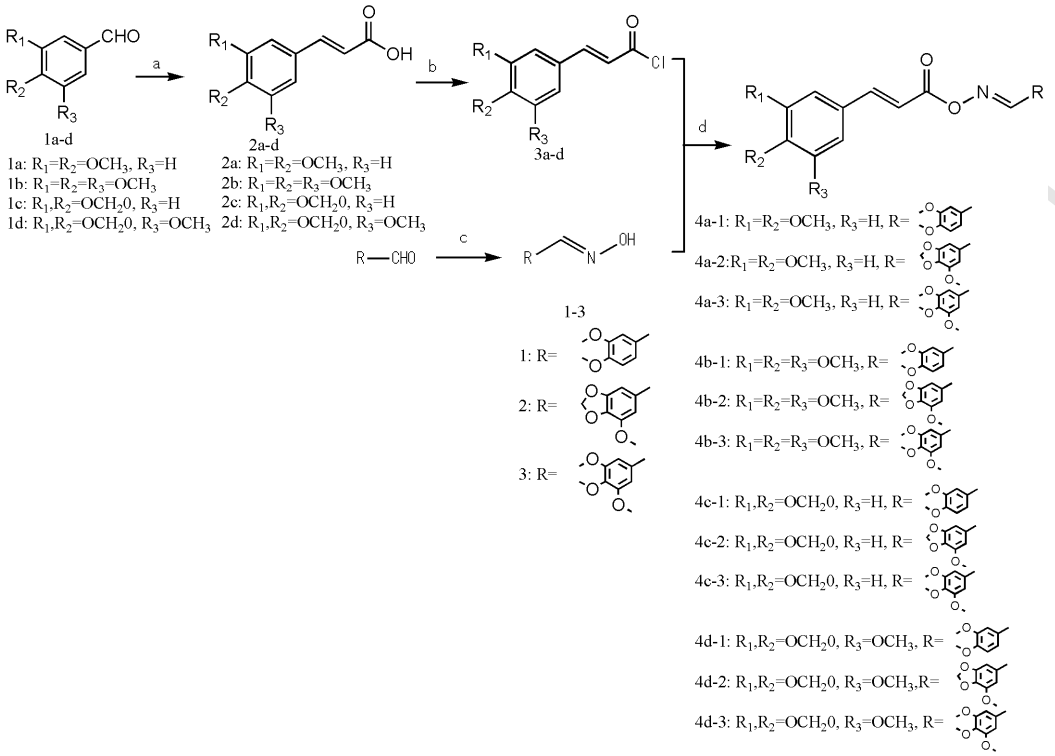
f. SI (selectivity index) = CC_{50}/IC_{50}.

g. Not active.

h. ND, Not determined.

i. Lamivudine (3TC) as the positive control.

Legend of figures



Scheme 1. Synthetic route to the series of compounds. Reagents and conditions: (a) $CH_2(COOH)_2$, piperidine, C_5H_5N, reflux, 4h, 80-90%; (b) $SOCl_2$, CH_2Cl_2, reflux, 5h, 95%; (c) H_2NOH-HCl, AcONa, EtOH, 60^oC, 1h, 98%; (d) Et_3N, CH_2Cl_2, rt, 12h, 50-70%.

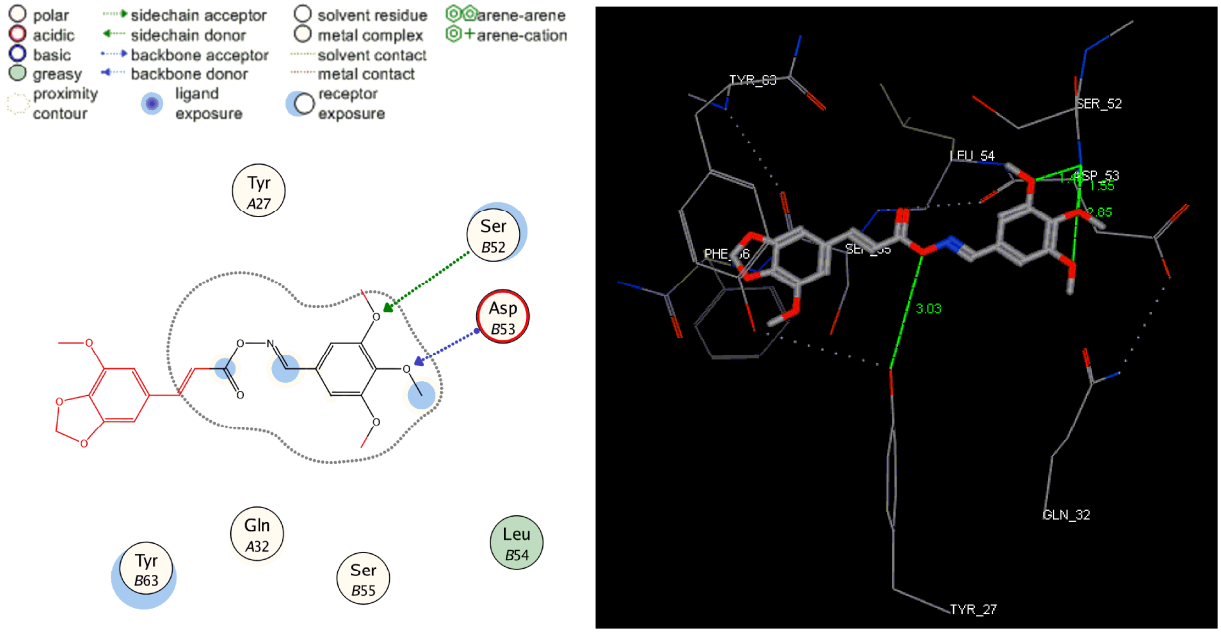


Figure 1. Ligand interaction and the binding mode of compound 4d-3 with HLA-A receptor.

The hydrogen bond formed colored in green.

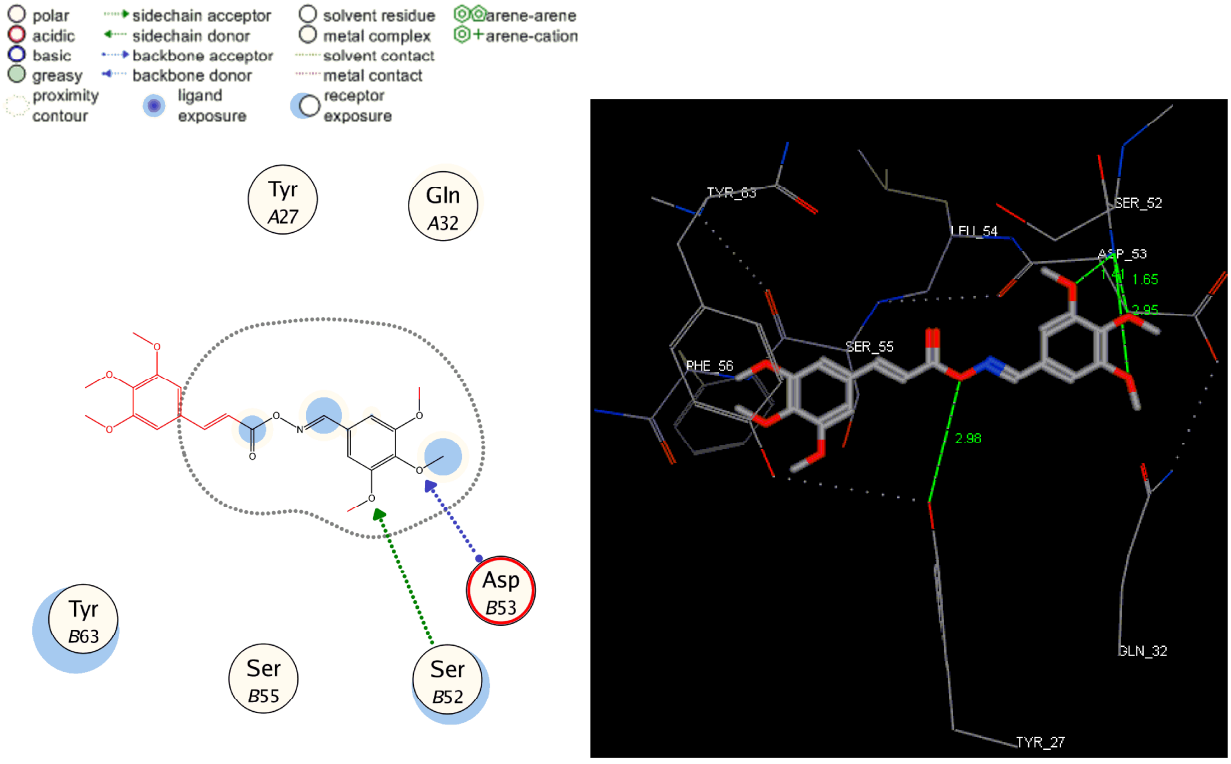


Figure 2. Ligand interaction and the binding mode of compound 4b-3 with HLA-A receptor.

The hydrogen bond formed colored in green.

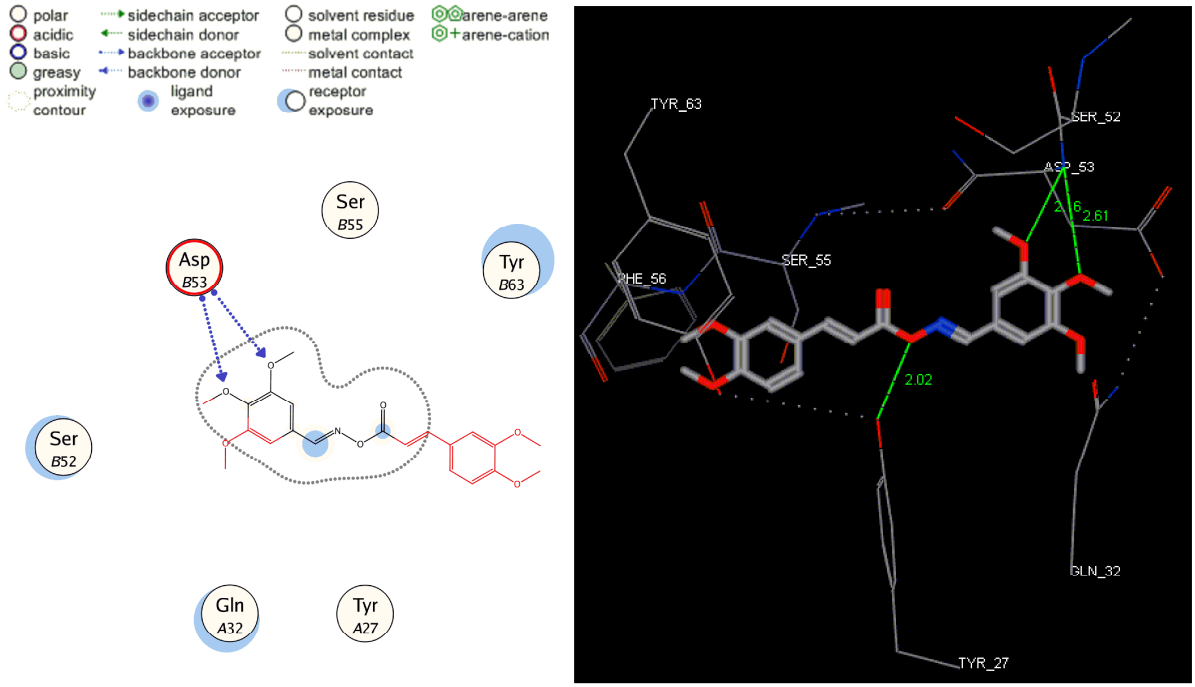


Figure 3. Ligand interaction and the binding mode of compound 4a-3 with HLA-A receptor.

The hydrogen bond formed colored in green.

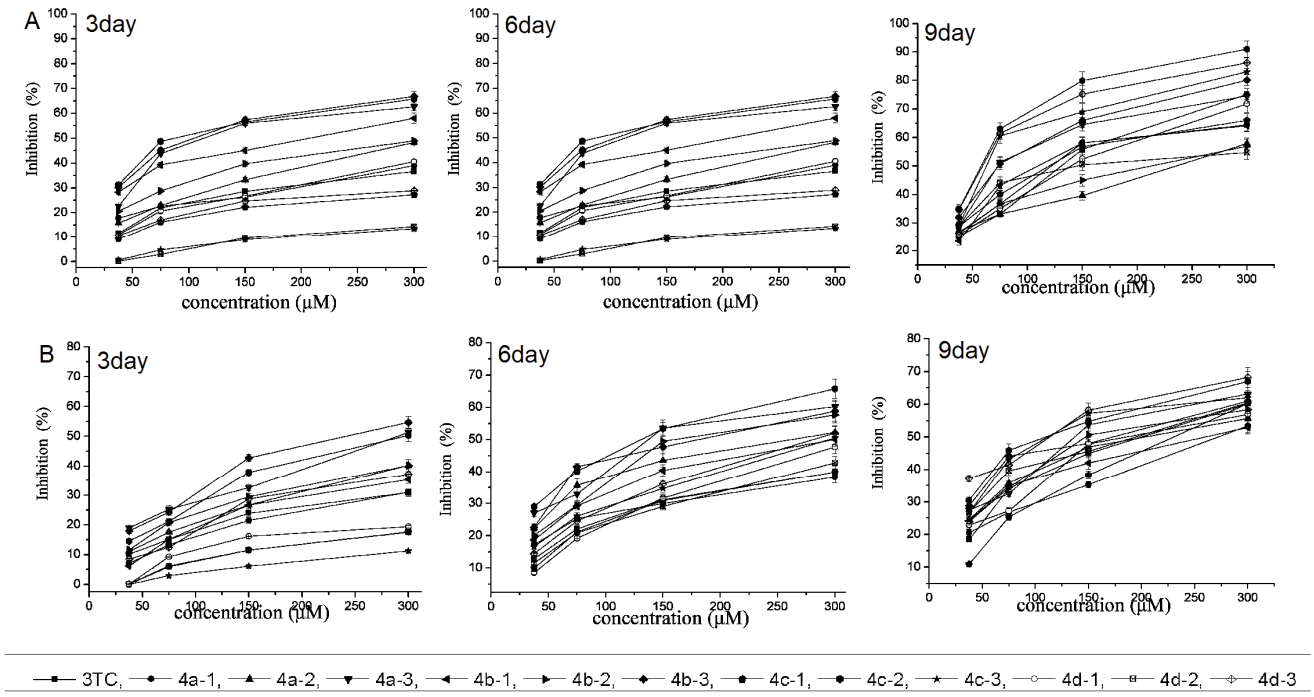


Figure 4. Inhibitory effect of the phenylpropanoid derivatives on secretion of HBsAg (A) and HBeAg (B) in the HepG2.2.15 cell line. Data were expressed as mean ± S.E. (*n* = 3).

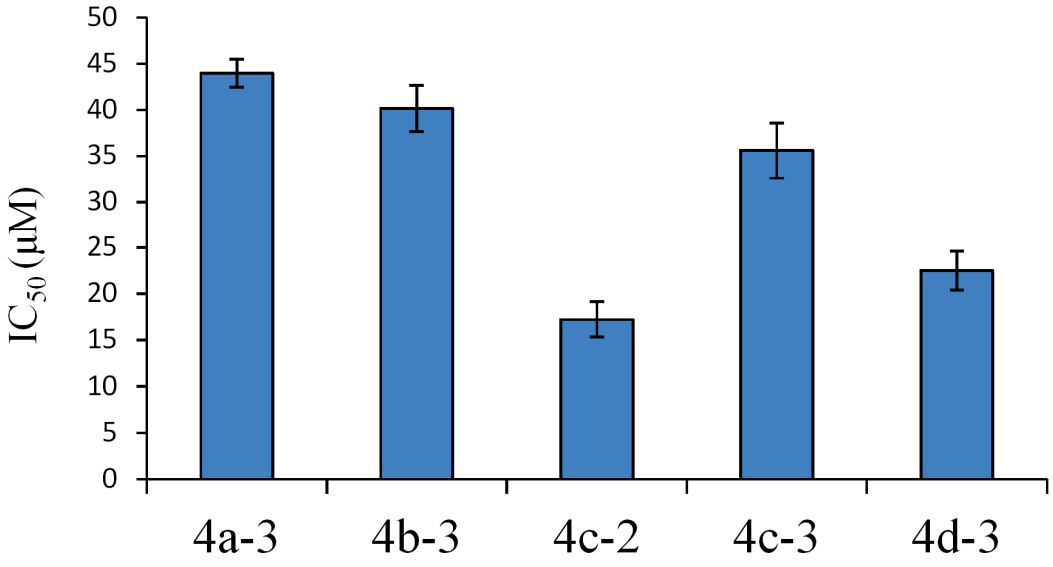


Figure 5. Results of the interaction between the derivatives selected from primary screening and HBcAg with the ELISA method.

Highlights

- Phenylpropanoid derivatives demonstrated potent anti-HBV activity.
- Phenylpropanoid derivatives had interaction with protein 3OX8.
- The anti-HBV effect of the phenylpropanoid derivatives may exert its anti-HBV activity by inhibiting HBcAg.