


Article

Discovery of Oxime Ethers as Hepatitis B Virus (HBV) Inhibitors by Docking, Screening and In Vitro Investigation

Jie Tan ^{1,†}, Min Zhou ^{1,†}, Xinhua Cui ¹, Zhuocai Wei ¹ and Wanxing Wei ^{1,2,*} 

¹ College of Chemistry and Chemical Engineering, Guangxi University, Nanning 53004, China; yulitanjie@163.com (J.T.); eelmm@gxu.edu.cn (M.Z.); cuixinhua123@163.com (X.C.); liveupto@sina.cn (Z.W.)

² Guangxi Colleges and Universities Key Laboratory of Applied Chemistry Technology and Resource Development, Guangxi University, Nanning 53004, China

* Correspondence: wxwei@gxu.edu.cn; Tel./Fax: +86-771-323-3718

† These authors contributed equally to the work.

Received: 30 January 2018; Accepted: 9 March 2018; Published: 12 March 2018

Abstract: A series of oxime ethers with C₆-C₄ fragment was designed and virtually bioactively screened by docking with a target, then provided by a Friedel–Crafts reaction, esterification (or amidation), and oximation from *p*-substituted phenyl derivatives (Methylbenzene, Methoxybenzene, Chlorobenzene). Anti-hepatitis B virus (HBV) activities of all synthesized compounds were evaluated with HepG2.2.15 cells in vitro. Results showed that most of compounds exhibited low cytotoxicity on HepG2.2.15 cells and significant inhibition on the secretion of HBsAg and HBeAg. Among them, compound **5c-1** showed the most potent activity on inhibiting HBsAg secretion (IC₅₀ = 39.93 μM, SI = 28.51). Results of the bioactive screening showed that stronger the compounds bound to target human leukocyte antigen A protein in docking, the more active they were in anti-HBV activities in vitro.

Keywords: synthesis; oxime ethers derivatives; anti-HBV activity; molecular docking

1. Introduction

Hepatitis B virus (HBV) infection is a serious worldwide health problem, which causes acute and chronic hepatitis B, cirrhosis, hepatocellular carcinoma, and other hepatic diseases [1]. According to the World Health Organization, 240 million people in the world suffer chronic infection and develop into HBV carriers. About 0.78 million people die per year from HBV-related diseases [2]. The nucleoside analogs used in treatment of anti-HBV play the role of Trojan horse in the synthesis of HBV DNA and suppress replication of HBV [3–7], but they are not effective in eliminating the virus from patients. Meanwhile, HBV therapies with nucleoside analogs in the long term cause serious side effects and resistance [8–12]. For the improvement of HBV therapies, the structural modification of nucleoside analogs has focused and developed many derivatives [13–16]. Although more and more effective nucleoside analogs agents for treating HBV have been invented and developed, they only inhibit or stop replication of HBV DNA, and do not eliminate HBV cccDNA from patients. The cccDNA in patients will cause HBV DNA replication. Methods to stop and eliminate HBV DNA and cccDNA from HBV patients are still a great challenge for researchers. In searching for more effective anti-HBV agents, many significant anti-HBV non-nucleoside analogs from synthesized compounds [17–23] and natural products [24–26] have been found. Some were designed according to their interactions with receptor by simulating screen [27]. Researchers showed that HLA-A2 with the immunodominant HBcAg18–27 epitope (or HLA-A2.1-restricted CTL epitope) binds peptide of vaccine or of HBcAg to initiate a specific respond of T cell and resolve acute HBV infection [28–30]. Some other peptides

specifically bound to HBcAg18–27 epitope activate CTL response to prevent infection and eliminate HBV [31,32]. Therefore, this implies that other non-peptide compounds specifically interact with protein residue in HLA-A2.1-restricted CTL epitope may initiate CTL respond to prevent infection by HBV. A protein residue from HLA-A2.1-restricted CTL epitope (PDB ID 3OX8) with intensive interaction with non-nucleoside compounds displayed anti-HBV activities in docking investigations, and this protein residue (3OX8) has been used as a virtual target to design anti-HBV non-nucleoside compounds in our previous work. A series of oxime-containing compounds with a C₆-C₃ skeleton were designed, synthesized, and assayed for anti-HBV activities. Results showed that these designed compounds possessed significant anti-HBV activities [33–35]. On the basis of these results, we designed new oximes with a C₆-C₄ fragment and screened their anti-HBV activities to investigate the structure of the bioactivity relationship. Results of docking studies of these new oxime derivatives showed that N in the oxime group (-O-N=C) and O in the carbonyl group (-C=O) of these compounds interacted with amino acid residue by a hydrogen bond in active site of the HLA-Aprotein (HLA-A*02:03, PDB ID: 3OX8) [36]. This indicated the roles of oxime and carbonyl group in anti-HBV activity in theory.

2. Results and Discussion

2.1. Molecular Docking Study

Molecular docking studies of the oxime ester derivatives were carried out using MOE 2008.10 as 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. HLA-A protein (HLA-A*02:03, PDB ID: 3OX8) [36] was used for docking study. The “Site Finder” tool of this program was used to search for its active site. Three docking procedures for each ligand were performed 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 ligand–receptor complexes. The docking scores and the hydrogen bonding strength of all the molecules are shown in Table 1. These oxime ester derivatives had a dock score ranging from -12.0185 to -9.1113 , and all of them were involved in at least one hydrogen-bonding interaction with the active site of 3OX8 protein. Among them, N in the oxime group (-O-N=C) of 14 compounds interacted with amino acid residues by a hydrogen bond in the active site, which is similar to the reported works [33]. O in the carbonyl group (-C=O) of five compounds interacted with amino acid residues by a hydrogen bond in the active sites. This indicated in theory the roles of the oxime group and carbonyl group in anti-HBV activity. Bioactive results in vitro showed the compound **5c-1** had the most potent anti-HBV activity with IC₅₀ values of 39.93 μM for HBsAg, the next was **5a-1** (HBsAg IC₅₀ = 74.92 μM), followed by **3c-2** (HBsAg IC₅₀ = 94.71 μM , HBeAg IC₅₀ = 93.91 μM). Compounds **5c-1** (Figure 1) and **3c-2** (Figure 2) formed two hydrogen bonds in length 2.50 and 2.44 Å, 2.63 and 2.61 Å with O of O=C in amide group of Tyr27 and Tyr63, respectively (Figures 1 and 2), and compound **5a-1** formed only one hydrogen bond in length 2.40 Å with N of C=N in oxime group of Tyr26 (Figure 3).

Table 1. Docking results of synthesized compounds with 3OX8.

Compound	S-Score (kcal/mol)	Distance (Å)	Residue	Site of Actions
3a-1	-9.1113	2.72	Tyr27	N of -C=N-
3a-2	-9.7265	2.93	Tyr27	N of -C=N-
3b-1	-10.4709	2.94	Tyr26	N of -C=N-
3b-2	-11.2203	2.53	Tyr26	O of -C=O
		2.83	Tyr27	N of -C=N-

Table 1. Cont.

3c-1	-9.1497	3.09	Tyr63	N of -C=N-
3c-2	-10.0032	2.63 2.16	Tyr27 Tyr63	O of -C=O O of -C=O
4a-1	-9.9143	3.01	Tyr26	N of -C=N-
4a-2	-11.0692	2.94	Tyr63	O of -C=O
4b-1	-10.4782	2.82	Tyr26	N of -C=N-
4b-2	-10.8746	2.82	Tyr63	N of -C=N-
4c-1	-10.0261	3.01	Tyr27	N of -C=N-
4c-2	-10.3516	2.81 2.73	Tyr27 Tyr63	N of -C=N- N of -C=N-
5a-1	-9.9471	2.40	Tyr26	N of -C=N-
5a-2	-10.3332	1.96 2.52	Asp30 Tyr63	H of -N-H N of -C=N-
5b-1	-10.2883	2.75	Tyr63	N of -C=N-
5b-2	-12.0185	2.66	Tyr63	O of -C=O
5c-1	-9.9406	2.50 2.44	Tyr27 Tyr63	O of -C=O O of -C=O
5c-2	-11.2609	2.92	Tyr63	N of -C=N-

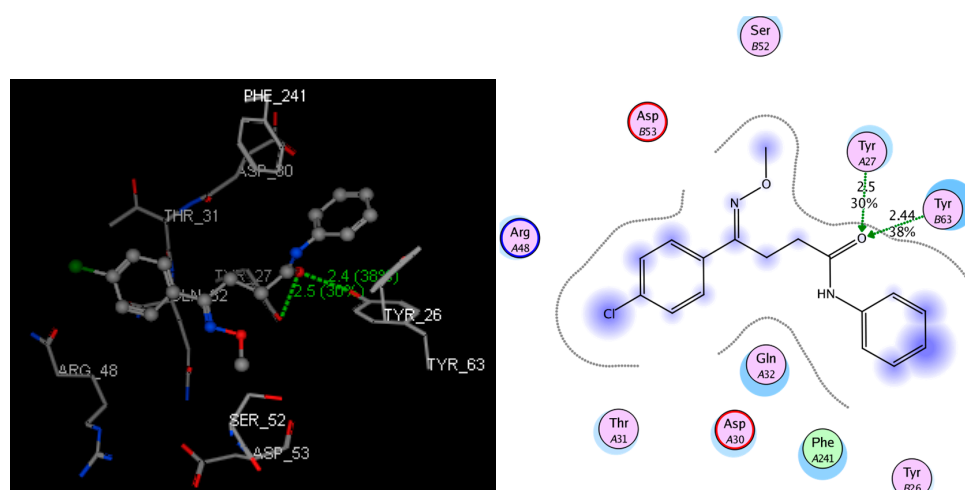


Figure 1. Binding mode of compound 5c-1 with 3OX8. The hydrogen bond formed is colored in green.

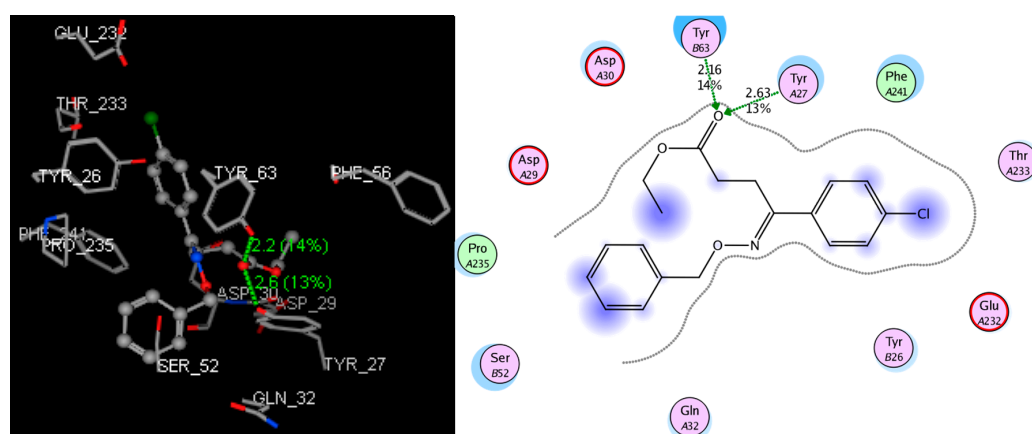


Figure 2. Binding mode of compound 3c-2 with 3OX8. The hydrogen bond formed is colored in green.

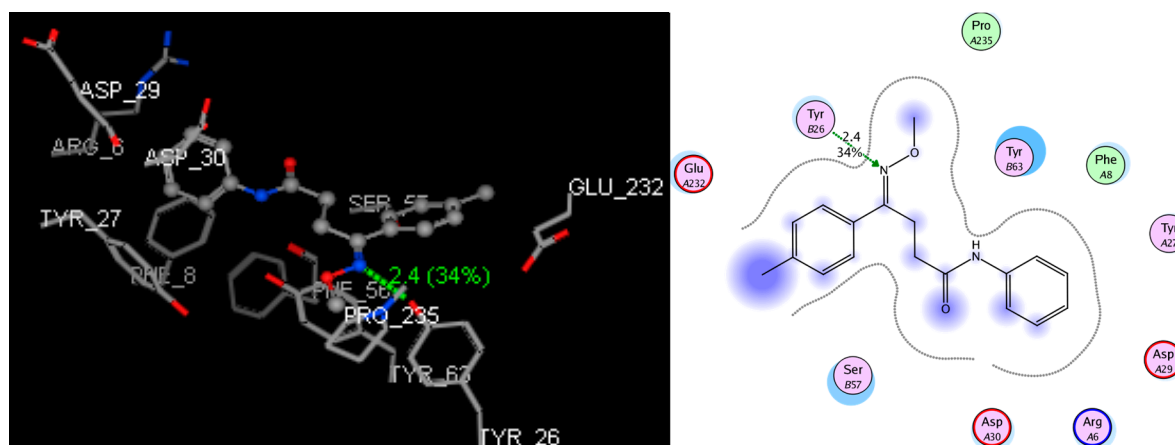


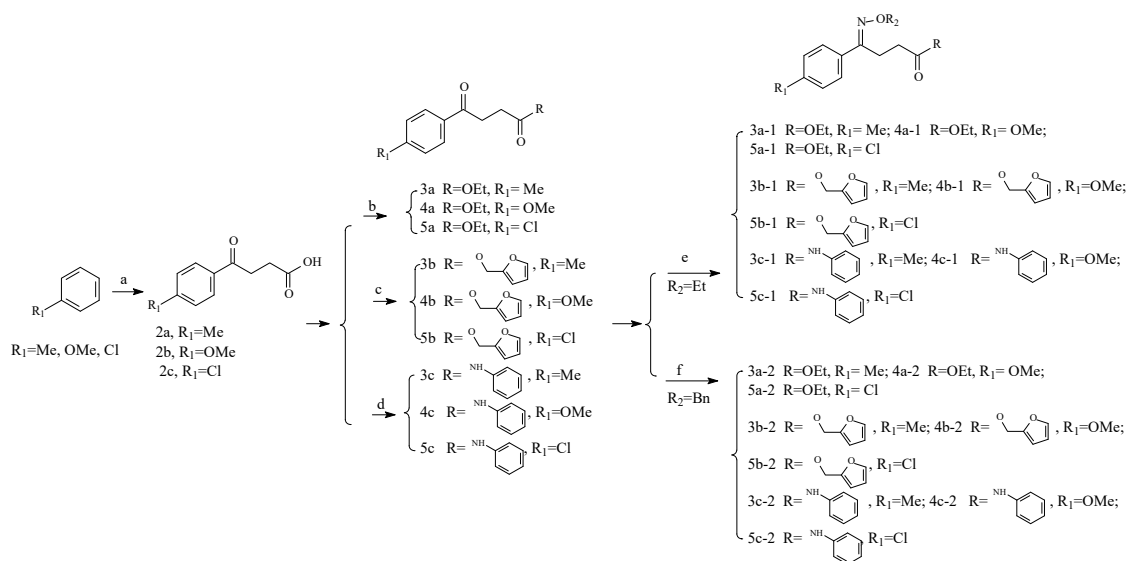
Figure 3. Binding mode of compound **5a-1** with 3OX8. The hydrogen bond formed is colored in green.

2.2. Chemistry

General synthesis of the intermediate and target compounds is depicted in Scheme 1. In the initial step, 3-arylpropionic acids (**2a**, **2b** and **2c**) were prepared by condensing substituted benzenes with succinic anhydride under Friedel–Crafts acylation reaction conditions [37]. Treatment of 3-arylpropionic acids (**2a**, **2b** and **2c**) with ethanol in the presence of *p*-toluenesulfonic acid provided ethyl 4-(4-substituted benzoyl)-4-oxobutanoate (**3a**, **3b** and **3c**) in 94–96% yield. Compounds **4a**, **4b**, **4c**, **5a**, **5b** and **5c** were formed by the reaction of 3-arylpropionic acids (**2a**, **2b** and **2c**) with furfuryl alcohol or aniline in the presence of *N,N*-dicyclohexylcarbodiimide and 4-dimethylamiprdine in THF with yields of 88–96% [38–43]. The intermediate compounds (**3a**, **3b**, **3c**, **4a**, **4b**, **4c**, **5a**, **5b** and **5c**) finally reacted with methoxylamine or hydroxylamine hydrochloride in pyridine and DCM to afford oxime ethers derivatives (**3a-1**, **3a-2**, **3a-3**; **3b-1**, **3b-2**, **3b-3**; **3c-1**, **3c-2**, **3c-3**; **4a-1**, **4a-2**, **4a-3**; **4b-1**, **4b-2**, **4b-3**; **4c-1**, **4b-2**, **4b-3**; **5a-1**, **5a-2**, **5a-3**; **5b-1**, **5b-2**, **5b-3**; and **5c-1**, **5c-2**, **5c-3**) as mixture of isomers. These isomers were easily separated by column chromatography. The major isomer of oxime ether compounds is presumed to have the *E* configuration [44]. The structures of the synthesized compounds were characterized by ^1H NMR, ^{13}C NMR and MS data and their data are presented in the experimental section. Signals at 2.28–2.35, 3.77–3.84, 3.91–3.98 and 5.17–5.26 ppm in ^1H NMR were related to CH_3 , OCH_3 , N-OCH_3 , N-OCH_2 protons, respectively. The chemical shifts of aromatic hydrogens of the phenyl ring appeared in the region δ 7.71–6.83. ^{13}C NMR spectra of the derivatives showed signals at about 155.61–157.93 and 170.16–172.80 ppm related to carbons in $\text{C}=\text{N}$ and $\text{C}=\text{O}$ respectively. The signals at 60.91–62.36 ppm attributed to carbons in N-OCH_3 , and 75.74–76.51 ppm to carbons in N-OCH_2 .

2.3. Anti-Hepatitis B Virus (HBV) Activity

All synthesized derivatives were assayed for their anti-HBV activities in vitro, which included inhibiting the secretion of HBsAg and HBeAg in HepG2.2.15 cells with lamivudine (**3TC**, a clinically popular anti-HBV agent) as a positive control. The anti-HBV activities of the compounds were expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) to the secretion of HBsAg and HBeAg. Some compounds in which the inhibition rate of the secretion of HBsAg or HBeAg did not reach 50% after **9d** would not have the IC_{50} value calculated (Figure 4A,B). The cytotoxicity of compounds was expressed as the concentration of compound required to kill 50% (TC_{50}) of the HepG2.2.15 cells. Parts of compounds were shown to be more active for inhibiting the secretion of HBsAg and had lower cytotoxicity than lamivudine. Eleven among the 18 derivatives displayed higher inhibitory activity against the secretion of HBsAg than lamivudine (Figure 5), and five among those derivatives demonstrated better inhibitory effect in the secretion of HBeAg than lamivudine (Figure 6). Among them, compounds **5c-1** and **5a-1** had the most potential as anti-HBV agents.



Scheme 1. Synthetic route of the series of compounds. Reagents and conditions: (a) succinic anhydride, AlCl_3/DCM , overnight, r.t.; (b) TsOH/EtOH , reflux, 6–8 h; (c) Furfuryl alcohol, DMAP, DCC/THF, 0.5 h 0 °C; 6–8 h, r.t.; (d) Aniline, DMAP, DCC/THF, 0.5 h 0 °C; 6–8 h, r.t.; (e) $\text{NH}_2\text{OCH}_3 \cdot \text{HCl}$, Pyridine/DCM, 70 °C, 6–7 h; (f) $\text{NH}_2\text{OCH}_2\text{C}_6\text{H}_5 \cdot \text{HCl}$, Pyridine/DCM, 70 °C, 6–7.

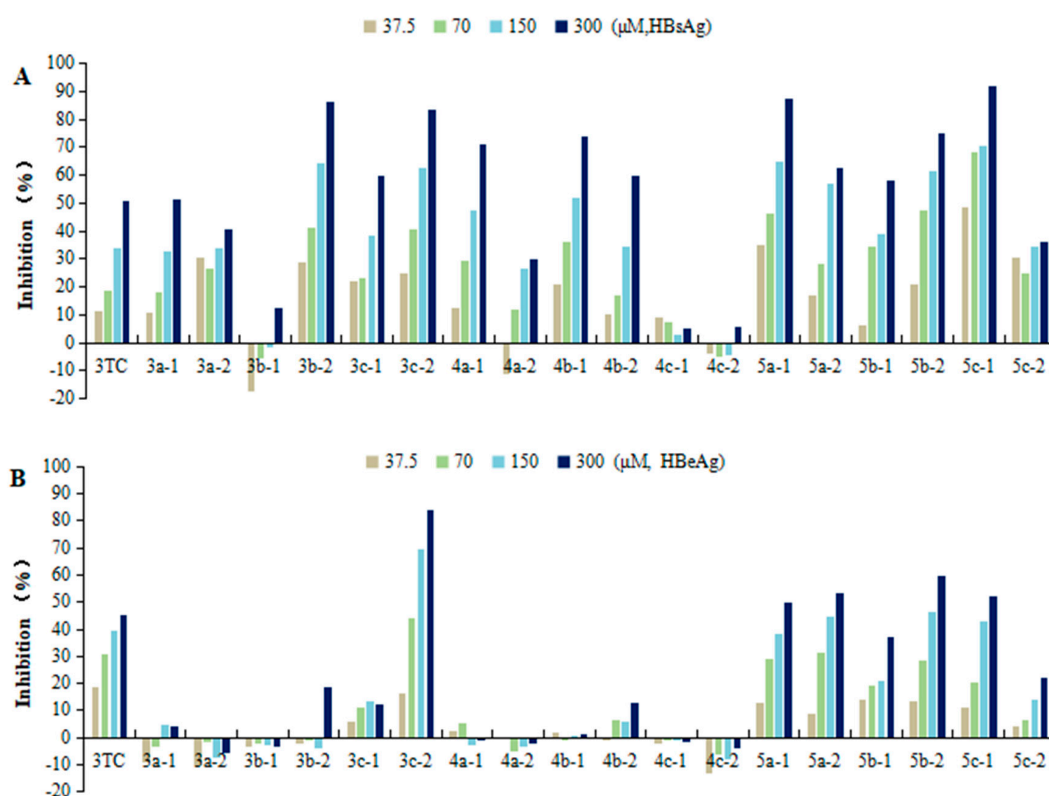


Figure 4. Inhibitory effects of the compounds on secretion of HBsAg (A) and HBeAg (B) in the HepG 2.2.15 cell line. Data were expressed as mean \pm S.D. ($n = 3$).

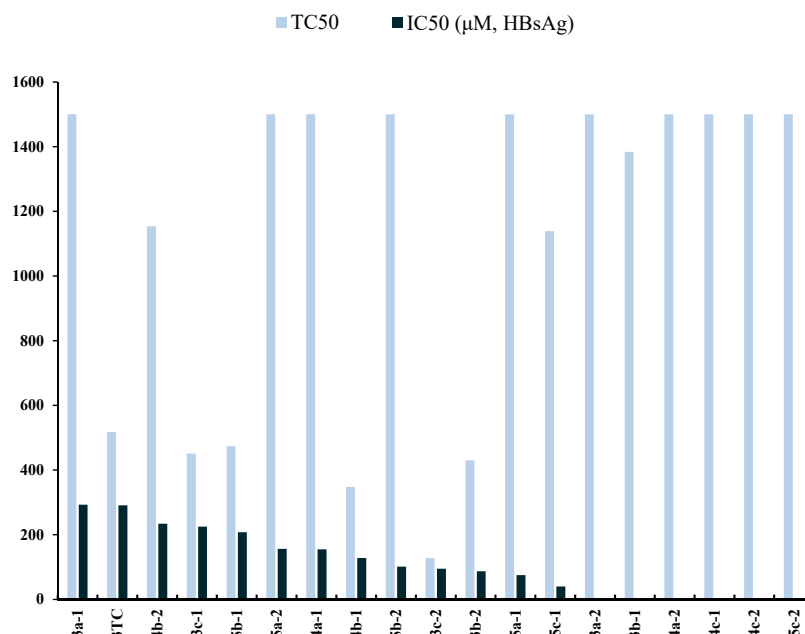


Figure 5. Cytotoxicity and inhibiting HBsAg activity of compounds in 9 days.

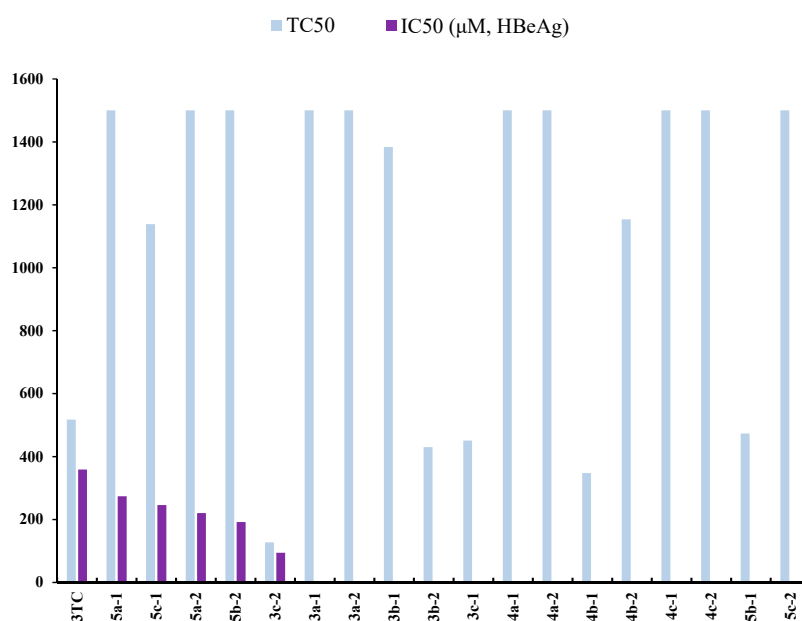


Figure 6. Cytotoxicity and inhibiting HBeAg activity of compounds in 9 days.

2.4. Structure–Activity Relationship (SAR)

Most of these compounds showed low cytotoxicity to HepG2.2.15 cells lines except the derivatives **3c-2** and **4b-1**. The inhibition ratio of part of the derivatives on HBeAg was less than 50% in the test concentration range. The anti-HBV activity of the derivatives was evaluated from their inhibition on the secretion of HBsAg. Compounds **5a-1**, **5a-2**, **5b-1**, **5b-2**, **5c-1** and **5c-1** with similar structures but different oxime ether groups showed different anti-HBV activity (Table 2). Compound **5a-1**, with IC_{50} values of 74.92 μ M and 273.87 μ M for HBsAg and HBeAg, respectively, was shown to be more effective at inhibiting HBsAg secretion but weaker at HBeAg secretion than that of compound **5a-2** (HBsAg IC_{50} = 156.27 μ M, HBeAg IC_{50} = 220.09 μ M). It was similar to the compounds **5b-1**, **5b-2**, **5c-1** and **5c-1**. Compound **5c-1** (HBeAg IC_{50} = 245.96 μ M) was shown to be more effective at inhibiting HBeAg

secretion than that of **5c-1** (HBeAg IC₅₀ = non). A series of derivative **4** also had similar behavior at inhibiting HBsAg secretion. With an electronic withdrawing group Cl, compound **5a-1** showed a better inhibitory effect on the secretion of HBsAg (IC₅₀ = 74.92 μM) than compound **3a-1** and **4a-1** (IC₅₀ = 292.73, 154.50 μM). Similar behaviors were shown in compounds **3a-2**, **4a-2** and **5a-2** (IC₅₀ = non, non, 156.27 μM) and **3c-1**, **4c-1** and **5c-1** (IC₅₀ = 224.82, non, 39.93 μM), respectively. This revealed the formation of an amide fragment increased bioactivity. Compared to compound **3b-1**, whose inhibitory activity could be neglected, compound **5b-1** had relatively high inhibitory potency to the secretion of HBsAg (IC₅₀ = 207.63 μM). An electronic donating group MeO in compound **4b-1** (with an IC₅₀ value of 127.68 μM for HBsAg) was shown to be more effective at inhibiting HBsAg secretion than compound **5b-1**, but appeared toxic (TC₅₀ = 347.55 μM, SI = 2.72), but the Me group in **3b-1** presented less bioactivity. As amides, in compounds **3c-1**, **4c-1** and **5c-1** an electronic withdrawing group Cl in **5c-1** presented a more significant inhibiting effect than compounds **3c-1** and **4c-1**. For compounds **3b-2**, **4b-2** and **5b-2**, a more electronic donating group MeO in **4b-2** showed less bioactivity, and compound **5b-2** showed better inhibition of HBsAg secretion (IC₅₀ = 101.19 μM) than compound **4b-2** (IC₅₀ = 233.60 μM), but weaker than **3b-2** (IC₅₀ = 86.85 μM). For amide compounds **3c-2**, **4c-2** and **5c-1**, only a mild electronic donating group Me in **3c-2** showed more bioactivity. In general, most of the compounds with R₂ = OMe were more bioactive than their compounds with R₂ = Bn.

Table 2. Inhibition on the secretion of HBsAg, HBeAg and cytotoxicity of compounds in 9 days.

Compound	TC ₅₀ ^a (μM)	HBsAg ^b		HBeAg ^c	
		IC ₅₀ ^d (μM)	SI ^e	IC ₅₀ ^d (μM)	SI ^e
3a-1	>1500	292.73	>5.12	- ^f	-
3a-2	>1500	-	-	-	-
3b-1	1383.74	-	-	-	-
3b-2	429.88	86.85	4.95	-	-
3c-1	450.67	224.82	2.00	-	-
3c-2	127.10	94.71	1.34	93.91	1.34
4a-1	>1500	154.50	>9.71	-	-
4a-2	>1500	-	-	-	-
4b-1	347.55	127.68	2.72	-	-
4b-2	1153.49	233.60	4.94	-	-
4c-1	>1500	-	-	-	-
4c-2	>1500	-	-	-	-
5a-1	>1500	74.92	>20.02	273.87	>5.48
5a-2	>1500	156.27	>9.60	220.09	>6.82
5b-1	473.25	207.63	2.28	-	-
5b-2	>1500	101.19	>14.82	191.58	>7.83
5c-1	1138.45	39.93	28.51	245.96	4.63
5c-1	>1500	-	-	-	-
3TC ^g	517.40	290.73	1.78	358.59	1.44

^a TC₅₀ is 50% cytotoxicity concentration in HepG 2.2. 15 cell; ^b HBsAg: hepatitis B surface antigen; ^c HBeAg: hepatitis B e antigen; ^d IC₅₀ is 50% inhibitory concentration; ^e SI (selectivity index) = TC₅₀/IC₅₀; ^f The inhibition ratio less than 50% in the test concentration range; ^g Lamivudine (3TC) as the positive control.

Most *N*-phenyl amide derivatives among all the compounds exhibited an inhibitory effect on HBeAg secretion. These results suggested that the introduction of *N*-phenyl amide shows relatively greater anti-HBV activity than the introduction of furan-2-ylmethyl or ethyl.

According to the results mentioned above, the SARs were summarized. The *N*-phenyl amide-substituted methoxy oxime ether derivatives possessed higher anti-HBV activity than other analogs.

3. Methods

3.1. Molecular Docking

The docking study was performed using the MOE 2008.10 to understand the ligand–receptor interactions in detail. The crystal structure of human leukocyte antigen (HLA-A) protein (PDB ID: 3OX8) [36] was retrieved from the Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>). Conformation of all the compounds was constructed in ChemDraw Ultra 7.0 and optimized in HyperChem 8.0.7 Software, and included hydrogen addition, 3D structure conversion, force-field optimization, and geometry optimization. The optimized ligands were built using the builder interface of the MOE program. The crystal structure was imported into MOE and chain A was considered for the docking process as the protein is a dimer consisting of A and B chains. The structure was protonated, unbound waters were deleted, polar hydrogens were added, and energy minimization was carried out. The active site was correlated with the ‘Site Finder’ module of MOE to define the docking site for the ligands [33–35]. The docking procedure was followed using the standard protocol implemented in MOE 2008.10 and the geometry of the resulting complexes was studied using the MOE’s Pose Viewer utility.

3.2. Synthesis Methods

3.2.1. Materials and Methods

Melting points were determined using a fully automatic melting point apparatus MP420 (Jinan, China) and were uncorrected. Mass spectrometry (MS) spectra were recorded on Thermo Scientific ITQ 1100 instrument (Thermo Fisher Scientific, Waltham, MA, USA), Varian CP 3800+ Saturn 2200 instrument (Agilent Technologies, Santa Clara, CA, USA). NMR spectra were recorded on a Bruker AV III HD 600 MHz ($^1\text{H}/^{13}\text{C}$, 600 MHz/150 MHz) spectrometer (Bruker, Corp, MA, USA) by using CDCl_3 , CD_3OH , or DMSO as solvent and TMS as an internal standard. All the chemicals were obtained from local suppliers and were used without further purification.

3.2.2. Chemistry

The general procedure (Scheme 1) for the preparation of compounds (**2a**, **2b** and **2c**): succinic anhydride (1 equiv, 10 mmol) was reacted with an appropriate aromatic compound (substituted benzene, 1 equiv, 10 mmol) in DCM (20 mL) in the presence of anhydrous aluminium chloride (1.5 equiv, 15 mmol). The reaction mixture was stirred under anhydrous conditions overnight at room temperature and then ice-cold diluted hydrochloric acid solution was added dropwise. A solid mass separated out which was filtered and purified by recrystallization to give **2a**, **2b** and **2c** [44].

The general procedure for the preparation of compounds (**3a**, **3b** and **3c**): a mixture of the appropriate acid **2a-c** (1 equiv, 10 mmol) and *p*-toluenesulfonic acid (0.4 equiv, 4 mmol) in EtOH (20 mL) was refluxed for 6–8 h and evaporated to remove EtOH. The residue was suspended in H_2O (30 mL) and extracted with EtOAc (2×50 mL) which was further purified by column chromatography on silica gel eluting with AcOEt–petroleum ether (1:4, *v/v*) to get **3a-c** as crystals. The general procedure for the preparation of compounds (**4a-c–5a-c**): an appropriate acid **2a-b** (1 equiv, 10 mmol), and 4-dimethylamioiprdine (0.02 equiv, 0.2 mmol) was added to a solution of the furfuryl alcohol or aniline (1 equiv, 10 mmol) in THF (20 mL). The mixture was stirred and cooled to 0 °C and then *N,N*-dicyclohexylcarbodiimide (DCC) (1.1 equiv, 11 mmol) was added over a 5-min period and the reaction was stirred under anhydrous conditions for 6–8 h at room temperature. The mixture was filtered and the filtrate was evaporated to yield a crude product which was finally purified by recrystallization to give **4a**, **4b**, **4c**, **5a**, **5b** and **5c** as crystals [38–43].

3-(4-Methylbenzoyl)propionic acid (**2a**). Yield 95%; m.p. 93.7–94.9 °C. ^1H NMR (600 MHz, MeOD) δ : 7.91–7.86 (m, 2H, H-6, 10), 7.29 (d, $J = 8.0$ Hz, 2H, H-7, 9), 3.30–3.25 (m, 2H, H-3), 2.70–2.66 (m, 2H, H-2), 2.39 (s, 3H, H-11).

3-(4-Methoxybenzoyl)propionic acid (**2b**). Yield 98%; m.p. 148.9~150.2 °C. ¹H NMR (600 MHz, MeOD) δ: 7.90–7.85 (m, 2H, H-6, 10), 6.92–6.87 (m, 2H, H-7, 9), 3.76 (s, 3H, H-11), 3.18–3.14 (m, 2H, H-3), 2.60–2.56 (m, 2H, H-2).

3-(4-Chlorobenzoyl)propionic acid (**2c**). Yield 90%; m.p. 130.2~131.5 °C. ¹H NMR (600 MHz, DMSO) δ: 12.16 (s, 1H, H-OH), 7.99 (dd, *J* = 8.5, 1.7 Hz, 2H, H-6, 10), 7.62–7.58 (m, 2H, H-7, 9), 3.26–3.22 (m, 2H, H-3), 2.58 (t, *J* = 6.2 Hz, 2H H-2).

Ethyl 4-(4-methylbenzoyl)-4-oxobutanoate (**3a**). Yield 96%; m.p. 66.6~68.0 °C. ¹H NMR (600 MHz, MeOD) δ: 7.88 (dd, *J* = 8.2, 1.7 Hz, 2H, H-6, 10), 7.29 (d, *J* = 6.8 Hz, 2H, H-7, 9), 4.11 (q, *J* = 7.1 Hz, 2H, H-1'), 3.29 (td, *J* = 6.5, 2.3 Hz, 2H, H-3), 2.70–2.66 (m, 2H, H-2), 2.39 (s, 3H, H-11), 1.23 (t, *J* = 7.1 Hz, 3H, H-2').

Ethyl 4-(4-methoxyphenyl)-4-oxobutanoate (**3b**). Yield 98%; m.p. 55.9~57.1 °C. ¹H NMR (600 MHz, MeOD) δ: 7.90–7.85 (m, 2H, H-6, 10), 6.92–6.87 (m, 2H, H-7, 9), 4.02 (q, *J* = 7.1 Hz, 2H, H-1'), 3.76 (s, 3H, H-11), 3.19–3.16 (m, 2H, H-3), 2.58 (dd, *J* = 7.3, 5.4 Hz, 2H, H-2), 1.13 (t, *J* = 7.1 Hz, 3H, H-2').

Ethyl 4-(4-chlorophenyl)-4-oxobutanoate (**3c**). Yield 94%; m.p. 55.5~56.5 °C. ¹H NMR (600 MHz, CDCl₃) δ: 7.93 (t, *J* = 5.4 Hz, 2H, H-6, 10), 7.44 (t, *J* = 5.4 Hz, 2H, H-7, 9), 4.16 (q, *J* = 7.1 Hz, 2H, H-1'), 3.27 (t, *J* = 6.6 Hz, 2H, H-3), 2.75 (t, *J* = 6.6 Hz, 2H, H-2), 1.27 (t, *J* = 7.1 Hz, 3H, H-2').

Furan-2-ylmethyl 4-(4-methylbenzoyl)-4-oxobutanoate (**4a**). Yield 95%, m.p. 79.6~80.5 °C; ¹H NMR (600 MHz, CDCl₃) δ: 7.87 (d, *J* = 8.2 Hz, 2H, H-6, 10), 7.41 (d, *J* = 1.1 Hz, 1H, H-5'), 7.25 (d, *J* = 8.1 Hz, 2H, H-7, 9), 6.40 (d, *J* = 3.2 Hz, 1H, H-3'), 6.36 (dd, *J* = 3.1, 1.9 Hz, 1H, H-4'), 5.09 (s, 2H, H-1'), 3.29 (t, *J* = 6.7 Hz, 2H, H-3), 2.78 (t, *J* = 6.7 Hz, 2H, H-2), 2.41 (s, 3H, H-11); ¹³C NMR (151 MHz, CDCl₃) δ: 197.56 (C-4), 172.66 (C-1), 149.45 (C-2'), 144.03 (C-8), 143.24 (C-5'), 134.07 (C-5), 129.29 (C-7, 9), 128.16 (C-6, 10), 110.63 (C-4'), 110.56 (C-3'), 58.28 (C-1'), 33.19 (C-3), 28.19 (C-2), 21.65 (C-11); ESIMS: *m/z* 273 [M + H]⁺, 290 [M + NH₄]⁺, 295 [M + Na]⁺, 567 [2M + Na]⁺, calc. for C₁₆H₁₆O₄ (272.2958).

Furan-2-ylmethyl 4-(4-methoxyphenyl)-4-oxobutanoate (**4b**). Yield 96%, m.p. 61.0~61.7 °C; ¹H NMR (600 MHz, CDCl₃) δ: 7.97–7.94 (m, 2H, H-6, 10), 7.43–7.40 (m, 1H, H-5'), 6.96–6.91 (m, 2H, H-7, 9), 6.40 (d, *J* = 3.2 Hz, 1H, H-3'), 6.36 (dd, *J* = 3.1, 1.9 Hz, 1H, H-4'), 5.09 (s, 2H, H-1'), 3.86 (s, 3H, H-11), 3.27 (t, *J* = 6.7 Hz, 2H, H-3), 2.78 (t, *J* = 6.7 Hz, 2H, H-2); ¹³C NMR (151 MHz, CDCl₃) δ: 196.44 (C-4), 172.72 (C-1), 163.59 (C-8), 149.46 (C-2'), 143.24 (C-5'), 130.31 (C-6, 10), 129.65 (C-5), 113.75 (C-7, 9), 110.62 (C-4'), 110.57 (C-3'), 58.27 (C-1'), 55.47 (C-11), 32.93 (C-3), 28.25 (C-2); ESIMS: *m/z* 289 [M + H]⁺, 311 [M + Na]⁺, calc. for C₁₆H₁₆O₅ (288.2952).

Furan-2-ylmethyl 4-(4-chlorophenyl)-4-oxobutanoate (**4c**). Yield 90%, m.p. 64.2~65.1 °C; ¹H NMR (600 MHz, CDCl₃) δ: 7.91 (d, *J* = 8.5 Hz, 2H, H-6, 10), 7.44 (d, *J* = 8.5 Hz, 2H, H-7, 9), 7.42 (s, 1H, H-5'), 6.41 (d, *J* = 3.2 Hz, 1H, H-3'), 6.36 (d, *J* = 1.8 Hz, 1H, H-4'), 5.09 (s, 2H, H-1'), 3.28 (t, *J* = 6.6 Hz, 2H, H-3), 2.80 (t, *J* = 6.6 Hz, 2H, H-2); ¹³C NMR (151 MHz, CDCl₃) δ: 196.76 (C-4), 172.43 (C-1), 149.34 (C-2'), 143.29 (C-5'), 139.70 (C-8), 134.86 (C-5), 129.47 (C-6, 10), 128.95 (C-7, 9), 110.70 (C-4'), 110.58 (C-3'), 58.35 (C-1'), 33.26 (C-3), 28.08 (C-2); ESIMS: *m/z* 293 [M + H]⁺, 310 [M + NH₄]⁺, 315 [M + Na]⁺, calc. for C₁₅H₁₃ClO₄ (292.7143).

4-(4-Methylbenzoyl)-4-oxo-N-phenylbutanamide (**5a**). Yield 90%, m.p. 147.5~148.5 °C; ¹H NMR (600 MHz, DMSO) δ: 10.04 (s, 1H, H-NH), 7.91 (d, *J* = 8.2 Hz, 2H, H-6, 10), 7.60 (t, *J* = 8.4 Hz, 2H, H-2', 6'), 7.34 (d, *J* = 7.1 Hz, 2H, H-7, 9), 7.29 (t, *J* = 7.9 Hz, 2H, H-3', 5'), 7.02 (t, *J* = 7.4 Hz, 1H, H-4'), 3.31 (t, *J* = 6.4 Hz, 2H, H-3), 2.72 (t, *J* = 6.3 Hz, 2H, H-2), 2.38 (s, 3H, H-11); ¹³C NMR (151 MHz, DMSO) δ: 198.80 (C-4), 170.84 (C-1), 143.94 (C-8), 139.83 (C-1'), 134.55 (C-5), 129.70 (C-7, 9), 129.11 (C-3', 5'), 128.43 (C-6, 10), 123.33 (C-4'), 119.35 (C-2', 6'), 33.46 (C-3), 30.76 (C-2), 21.61 (C-11); ESIMS: *m/z* 268 [M + H]⁺, calc. for C₁₇H₁₇NO₂ (267.3224).

4-(4-Methoxyphenyl)-4-oxo-N-phenylbutanamide (**5b**). Yield 93%, m.p. 113.3~114.2 °C; ¹H NMR (600 MHz, DMSO) δ: 10.01 (s, 1H, H-NH), 8.00–7.96 (m, 2H, H-6, 10), 7.59 (d, *J* = 7.6 Hz, 2H, H-2', 6'), 7.30–7.26 (m, 2H, H-3', 5'), 7.07–7.03 (m, 2H, H-7, 9), 7.01 (t, *J* = 7.4 Hz, 1H, H-4'), 3.85 (s, 3H, H-11), 3.28 (t,

$J = 6.5$ Hz, 2H, H-3), 2.70 (t, $J = 6.5$ Hz, 2H, H-2); ^{13}C NMR (151 MHz, DMSO) δ : 197.64 (C-4), 170.90 (C-1), 163.56 (C-8), 139.87 (C-1'), 130.64 (C-6, 10), 130.00 (C-5), 129.12 (C-3', 5'), 123.31 (C-4'), 119.34 (C-2', 6'), 114.34 (C-7, 9), 55.99 (C-11), 33.22 (C-3), 30.83 (C-2); ESIMS: m/z 284 $[\text{M} + \text{H}]^+$, calc. for $\text{C}_{17}\text{H}_{17}\text{NO}_3$ (283.3218).

4-(4-Chlorophenyl)-4-oxo-N-phenylbutanamide (5c). Yield 88%, m.p. 140.2–141.0 °C; ^1H NMR (600 MHz, DMSO) δ : 10.04 (s, 1H, H-NH), 8.03–8.00 (m, 2H, H-6, 10), 7.60 (dd, $J = 12.5, 5.5$ Hz, 4H, H-2', 6', 7, 9), 7.28 (t, $J = 7.9$ Hz, 2H, H-3', 5'), 7.02 (t, $J = 7.4$ Hz, 1H, H-4'), 3.33 (t, $J = 6.4$ Hz, 2H, H-3), 2.73 (t, $J = 6.4$ Hz, 2H, H-2); ^{13}C NMR (151 MHz, MeOD) δ : 198.08 (C-4), 171.77 (C-1), 139.13 (C-8), 138.58 (C-1'), 135.26 (C-5), 129.41 (C-6, 10), 128.56 (C-3', 5'), 128.37 (C-7, 9), 123.62 (C-4'), 119.76 (C-2', 6'), 33.17 (C-3), 30.05 (C-2); ESIMS: m/z 288 $[\text{M} + \text{H}]^+$, calc. for $\text{C}_{16}\text{H}_{14}\text{ClNO}_2$ (287.7409).

Ethyl (E)-4-(4-methylbenzoyl)-4-[(methoxy)imino]butanoate (3a-1). Yield 78%; ^1H NMR (600 MHz, MeOD) δ : 7.52 (dd, $J = 4.9, 3.3$ Hz, 2H, H-6, 10), 7.22–7.17 (m, 2H, H-7, 9), 4.07 (dd, $J = 12.7, 5.5$ Hz, 2H, H-1'), 3.95 (s, 3H, H-1''), 3.04–2.98 (m, 2H, H-3), 2.50 (ddd, $J = 7.8, 4.1, 1.5$ Hz, 2H, H-2), 2.34 (s, 3H, H-11), 1.20 (t, $J = 7.1$ Hz, 3H, H-2'); ^{13}C NMR (151 MHz, MeOD) δ : 172.80 (C-1), 156.95 (C-4), 139.20 (C-8), 132.18 (C-5), 128.81 (C-7, 9), 125.99 (C-6, 10), 60.91 (C-1''), 60.34 (C-1'), 30.59 (C-2), 21.83 (C-3), 19.93 (C-11), 13.09 (C-2'); ESIMS: m/z 250.1442 $[\text{M} + \text{H}]^+$, 272.1261 $[\text{M} + \text{Na}]^+$, calc. for $\text{C}_{14}\text{H}_{19}\text{NO}_3$ (249.3056).

Ethyl (E)-4-(4-methylbenzoyl)-4-[(benzyloxy)imino]butanoate (3a-2). Yield 76%; ^1H NMR (600 MHz, CDCl_3) δ : 7.52 (d, $J = 8.1$ Hz, 2H, H-6, 10), 7.40 (d, $J = 7.3$ Hz, 2H, H-3'', 7''), 7.35 (t, $J = 7.5$ Hz, 2H, H-4'', 6''), 7.30 (t, $J = 7.2$ Hz, 1H, H-5''), 7.16 (d, $J = 8.0$ Hz, 2H, H-7, 9), 5.22 (s, 2H, H-1''), 4.08 (q, $J = 7.1$ Hz, 2H, H-1'), 3.06 (dd, $J = 10.5, 5.8$ Hz, 2H, H-3), 2.57–2.52 (m, 2H, H-2), 2.35 (s, 3H, H-11), 1.20 (t, $J = 7.1$ Hz, 3H, H-2'); ^{13}C NMR (151 MHz, MeOD) δ : 172.77 (C-1), 157.28 (C-4), 139.20 (C-8), 138.02 (C-2''), 132.20 (C-5), 128.80 (C-7, 9), 128.03 (C-3'', 7''), 127.84 (C-4'', 6''), 127.48 (C-5''), 126.04 (C-6, 10), 75.86 (C-1''), 60.34 (C-1'), 30.58 (C-2), 22.02 (C-3), 20.01 (C-11), 13.08 (C-2'); ESIMS: m/z 326.1766 $[\text{M} + \text{H}]^+$, 348.1579 $[\text{M} + \text{Na}]^+$, calc. for $\text{C}_{20}\text{H}_{23}\text{NO}_3$ (325.4015).

Ethyl (E)-4-(4-methoxyphenyl)-4-[(methoxy)imino]butanoate (3b-1). Yield 76%; ^1H NMR (600 MHz, CDCl_3) δ : 7.60–7.57 (m, 2H, H-6, 10), 6.91–6.87 (m, 2H, H-7, 9), 4.11 (q, $J = 7.1$ Hz, 2H, H-1'), 3.96 (s, 3H, H-1''), 3.82 (s, 3H, H-11), 3.03–2.99 (m, 2H, H-3), 2.56–2.52 (m, 2H, H-2), 1.23 (t, $J = 7.1$ Hz, 3H, H-2'); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.73 (C-1), 160.52 (C-8), 156.46 (C-4), 127.67 (C-5), 127.64 (C-6, 10), 113.94 (C-7, 9), 61.91 (C-1''), 60.59 (C-1'), 55.32 (C-11), 30.99 (C-2), 22.28 (C-3), 14.18 (C-2'); ESIMS: m/z 266.1389 $[\text{M} + \text{H}]^+$, 288.1199 $[\text{M} + \text{Na}]^+$, calc. for $\text{C}_{14}\text{H}_{19}\text{NO}_4$ (265.3050).

Ethyl (E)-4-(4-methoxyphenyl)-4-[(benzyloxy)imino]butanoate (3b-2). Yield 78%; ^1H NMR (600 MHz, CDCl_3) δ : 7.66–7.62 (m, 2H, H-6, 10), 7.45 (d, $J = 7.3$ Hz, 2H, H-3'', 7''), 7.40 (t, $J = 7.5$ Hz, 2H, H-4'', 6''), 7.34 (t, $J = 7.3$ Hz, 1H, H-5''), 6.94–6.90 (m, 2H, H-7, 9), 5.26 (s, 2H, H-1''), 4.13 (q, $J = 7.1$ Hz, 2H, H-1'), 3.84 (s, 3H, H-11), 3.14–3.07 (m, 2H, H-3), 2.63–2.57 (m, 2H, H-2), 1.25 (t, $J = 7.2$ Hz, 3H, H-2'); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.72 (C-1), 160.57 (C-8), 156.76 (C-4), 138.11 (C-2''), 128.38 (C-3'', 7''), 128.11 (C-4'', 6''), 127.76 (C-5''), 127.72 (C-6), 127.68 (C-5), 113.93 (C-7, 9), 76.22 (C-1''), 60.60 (C-1'), 55.32 (C-11), 30.99 (C-2), 22.40 (C-3), 14.19 (C-2'); ESIMS: m/z 342.1694 $[\text{M} + \text{H}]^+$, 364.1506 $[\text{M} + \text{Na}]^+$, calc. for $\text{C}_{20}\text{H}_{23}\text{NO}_4$ (341.4009).

Ethyl (E)-4-(4-chlorophenyl)-4-[(methoxy)imino]butanoate (3c-1). Yield 75%; ^1H NMR (600 MHz, CDCl_3) δ : 7.60–7.56 (m, 2H, H-6, 10), 7.35–7.31 (m, 2H, H-7, 9), 4.10 (q, $J = 7.2$ Hz, 2H, H-1'), 3.98 (s, 3H, H-1''), 3.02–2.98 (m, 2H, H-3), 2.55–2.52 (m, 2H, H-2), 1.22 (t, $J = 7.2$ Hz, 3H, H-2'); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.46 (C-1), 155.74 (C-4), 135.21 (C-8), 133.66 (C-5), 128.71 (C-7, 9), 127.55 (C-6, 10), 62.16 (C-1''), 60.65 (C-1'), 30.77 (C-2), 22.12 (C-3), 14.15 (C-2'); ESIMS: m/z 270.0901 $[\text{M} + \text{H}]^+$, 292.0720 $[\text{M} + \text{Na}]^+$, calc. for $\text{C}_{13}\text{H}_{16}\text{ClNO}_3$ (269.7240).

Ethyl (E)-4-(4-chlorophenyl)-4-[(benzyloxy)imino]butanoate (3c-2). Yield 77%; ^1H NMR (600 MHz, CDCl_3) δ : 7.59–7.56 (m, 2H, H-6, 10), 7.41–7.34 (m, 4H, H-3'', 7'', 4'', 6''), 7.34–7.29 (m, 3H, H-7, 9, 5''), 5.22 (s, 2H, H-1''), 4.08 (q, $J = 7.1$ Hz, 2H, H-1'), 3.06–3.02 (m, 2H, H-3), 2.56–2.52 (m, 2H, H-2), 1.20 (t, $J = 7.1$

Hz, 3H, H-2'); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.47 (C-1), 156.11 (C-4), 137.74 (C-2''), 135.26 (C-8), 133.65 (C-5), 128.69 (C-7, 9), 128.40 (C-3'', 7''), 128.12 (C-4'', 6''), 127.87 (C-5''), 127.62 (C-6), 76.49 (C-1''), 60.66 (C-1'), 30.75 (C-2), 22.26 (C-3), 14.14 (C-2'); ESIMS: m/z 346.1204 $[\text{M} + \text{H}]^+$, 368.1020 $[\text{M} + \text{Na}]^+$, calc. for $\text{C}_{19}\text{H}_{20}\text{ClNO}_3$ (345.8200).

Furan-2-ylmethyl (E)-4-(4-methylbenzoyl)-4-[(methoxyimino] butanoate (4a-1). Yield 76%; ^1H NMR (600 MHz, CDCl_3) δ : 7.50 (d, $J = 8.1$ Hz, 2H, H-6, 10), 7.41 (dd, $J = 1.8, 0.8$ Hz, 1H, H-5'), 7.16 (d, $J = 8.0$ Hz, 2H, H-7, 9), 6.39 (d, $J = 3.1$ Hz, 1H, H-3'), 6.35 (dd, $J = 3.2, 1.9$ Hz, 1H, H-4'), 5.04 (s, 2H, H-1'), 3.95 (s, 3H, H-1''), 3.05–3.00 (m, 2H, H-3), 2.60–2.55 (m, 2H, H-2), 2.35 (s, 3H, H-11); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.29 (C-1), 156.63 (C-4), 149.39 (C-2'), 143.27 (C-5'), 139.27 (C-8), 132.25 (C-5), 129.25 (C-7, 9), 126.16 (C-6, 10), 110.68 (C-4'), 110.57 (C-3'), 61.96 (C-1''), 58.18 (C-1'), 30.72 (C-2), 22.22 (C-3), 21.25 (C-11); ESIMS: m/z 302 $[\text{M} + \text{H}]^+$, calc. for $\text{C}_{17}\text{H}_{19}\text{NO}_4$ (301.3371).

Furan-2-ylmethyl (E)-4-(4-methylbenzoyl)-4-[(benzyloxy)imino]butanoate (4a-2). Yield 77%; ^1H NMR (600 MHz, CDCl_3) δ : 7.50 (d, $J = 8.2$ Hz, 2H, H-6, 10), 7.41–7.40 (m, 1H, H-5'), 7.38 (d, $J = 7.3$ Hz, 2H, H-3'', 7''), 7.34 (t, $J = 7.4$ Hz, 2H, H-4'', 6''), 7.31–7.27 (m, 1H, H-5''), 7.15 (d, $J = 8.0$ Hz, 2H, H-7, 9), 6.36 (d, $J = 3.2$ Hz, 1H, H-3'), 6.35 (dd, $J = 3.2, 1.9$ Hz, 1H, H-4'), 5.20 (s, 2H, H-1''), 5.01 (s, 2H, H-1'), 3.09–3.04 (m, 2H, H-3), 2.60–2.55 (m, 2H, H-2), 2.34 (s, 3H, H-11); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.28 (C-1), 156.94 (C-4), 149.38 (C-2'), 143.25 (C-5'), 139.31 (C-8), 137.97 (C-2''), 132.24 (C-5), 129.22 (C-7, 9), 128.35 (C-3'', 7''), 128.10 (C-4'', 6''), 127.74 (C-5''), 126.22 (C-6, 10), 110.66 (C-4'), 110.55 (C-3'), 76.25 (C-1''), 58.17 (C-1'), 30.69 (C-2), 22.32 (C-3), 21.25 (C-11); ESIMS: m/z 378 $[\text{M} + \text{H}]^+$, 400 $[\text{M} + \text{Na}]^+$, calc. for $\text{C}_{23}\text{H}_{23}\text{NO}_4$ (377.4330).

Furan-2-ylmethyl (E)-4-(4-methoxyphenyl)-4-[(methoxy)imino]butanoate (4b-1). Yield 77%; ^1H NMR (600 MHz, CDCl_3) δ : 7.60–7.54 (m, 2H, H-6, 10), 7.41 (dd, $J = 1.8, 0.8$ Hz, 1H, H-5'), 6.90–6.85 (m, 2H, H-7, 9), 6.39 (d, $J = 3.1$ Hz, 1H, H-3'), 6.36 (dd, $J = 3.2, 1.9$ Hz, 1H, H-4'), 5.04 (s, 2H, H-1'), 3.94 (s, 3H, H-1''), 3.81 (s, 3H, H-11), 3.04–2.98 (m, 2H, H-3), 2.60–2.56 (m, 2H, H-2); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.33 (C-1), 160.51 (C-8), 156.29 (C-4), 149.39 (C-2'), 143.28 (C-5'), 127.63 (C-6, 10), 127.58 (C-5), 113.94 (C-7, 9), 110.70 (C-4'), 110.57 (C-3'), 61.91 (C-1''), 58.20 (C-1'), 55.31 (C-11), 30.76 (C-2), 22.19 (C-3); ESIMS: m/z 318 $[\text{M} + \text{H}]^+$, calc. for $\text{C}_{17}\text{H}_{19}\text{NO}_5$ (317.3365).

Furan-2-ylmethyl (E)-4-(4-methoxyphenyl)-4-[(benzyloxy)imino]butanoate (4b-2). Yield 78%; ^1H NMR (600 MHz, CDCl_3) δ : 7.61–7.53 (m, 2H, H-6, 10), 7.40 (s, 1H, H-5'), 7.38 (d, $J = 7.7$ Hz, 2H, H-3'', 7''), 7.34 (dd, $J = 10.9, 4.0$ Hz, 2H, H-4'', 6''), 7.29 (t, $J = 7.1$ Hz, 1H, H-5''), 6.91–6.83 (m, 2H, H-7, 9), 6.37 (d, $J = 2.8$ Hz, 1H, H-3'), 6.36–6.31 (m, 1H, H-4'), 5.19 (s, 2H, H-1''), 5.01 (s, 2H, H-1'), 3.80 (s, 3H, H-11), 3.11–3.00 (m, 2H, H-3), 2.63–2.53 (m, 2H, H-2); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.30 (C-1), 160.53 (C-8), 156.56 (C-4), 149.37 (C-2'), 143.26 (C-5'), 138.03 (C-2''), 128.34 (C-3'', 7''), 128.08 (C-4'', 6''), 127.72 (C-5''), 127.69 (C-6, 10), 127.57 (C-5), 113.90 (C-7, 9), 110.67 (C-4'), 110.56 (C-3'), 76.19 (C-1''), 58.18 (C-1'), 55.30 (C-11), 30.73 (C-2), 22.27 (C-3); ESIMS: m/z 394 $[\text{M} + \text{H}]^+$, calc. for $\text{C}_{23}\text{H}_{23}\text{NO}_5$ (393.4324).

Furan-2-ylmethyl (E)-4-(4-chlorophenyl)-4-[(methoxy)imino]butanoate (4c-1). Yield 75%; ^1H NMR (600 MHz, CDCl_3) δ : 7.58–7.54 (m, 2H, H-6, 10), 7.41 (dd, $J = 1.7, 0.6$ Hz, 1H, H-5'), 7.34–7.30 (m, 2H, H-7, 9), 6.38 (d, $J = 3.2$ Hz, 1H, H-3'), 6.36 (dd, $J = 3.2, 1.9$ Hz, 1H, H-4'), 5.04 (s, 2H, H-1'), 3.96 (s, 3H, H-1''), 3.03–2.99 (m, 2H, H-3), 2.60–2.56 (m, 2H, H-2); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.11 (C-1), 155.61 (C-4), 149.28 (C-2'), 143.32 (C-5'), 135.24 (C-8), 133.58 (C-5), 128.73 (C-7, 9), 127.55 (C-6, 10), 110.77 (C-4'), 110.58 (C-3'), 62.18 (C-1''), 58.24 (C-1'), 30.59 (C-2), 22.07 (C-3); ESIMS: m/z 322 $[\text{M} + \text{H}]^+$, calc. for $\text{C}_{16}\text{H}_{16}\text{ClNO}_4$ (321.7555).

Furan-2-ylmethyl (E)-4-(4-chlorophenyl)-4-[(benzyloxy)imino]butanoate (4c-2). Yield 76%; ^1H NMR (600 MHz, CDCl_3) δ : 7.55 (dt, $J = 4.3, 1.8$ Hz, 2H, H-6, 10), 7.41 (s, 1H, H-5'), 7.40–7.37 (m, 2H, H-3'', 7''), 7.37–7.33 (m, 2H, H-4'', 6''), 7.33–7.28 (m, 3H, H-7, 9, 5''), 6.36 (s, 1H, H-3'), 6.35 (dd, $J = 3.2, 1.7$ Hz, 1H, H-4'), 5.21 (s, 2H, H-1''), 5.01 (s, 2H, H-1'), 3.04 (dd, $J = 11.7, 4.2$ Hz, 2H, H-3), 2.61–2.56 (m, 2H, H-2); ^{13}C NMR (151 MHz, CDCl_3) δ : 172.09 (C-1), 155.95 (C-4), 149.26 (C-2'), 143.30 (C-5'), 137.69 (C-2''), 135.27 (C-8), 133.57 (C-5), 128.70 (C-7, 9), 128.41 (C-3'', 7''), 128.14 (C-4'', 6''), 127.88 (C-5''),

127.61 (C-6, 10), 110.75 (C-4'), 110.57 (C-3'), 76.51 (C-1''), 58.23 (C-1'), 30.57 (C-2), 22.17 (C-3); ESIMS: m/z 398 [M + H]⁺, calc. for C₂₂H₂₀ClNO₄ (397.8515).

(*E*)-4-(4-Methylbenzoyl)-4-[(methoxyimino)-*N*-phenylbutanamide (5a-1). Yield 78%, m.p. 94.4~95.3 °C; ¹H NMR (600 MHz, DMSO) δ: 9.91 (s, 1H, H-NH), 7.58 (t, *J* = 8.4 Hz, 4H, H-6, 10, 2', 6'), 7.31–7.26 (m, 2H, H-3', 5'), 7.22 (d, *J* = 8.0 Hz, 2H, H-7, 9), 7.02 (t, *J* = 7.4 Hz, 1H, H-4'), 3.91 (s, 3H, H-1''), 3.01–2.96 (m, 2H, H-3), 2.52–2.48 (m, 2H, H-2), 2.32 (s, 3H, H-11); ¹³C NMR (151 MHz, DMSO) δ: 170.26 (C-1), 157.46 (C-4), 139.62 (C-1'), 139.30 (C-8), 132.45 (C-5), 129.58 (C-7, 9), 129.12 (C-3', 5'), 126.56 (C-6, 10), 123.53 (C-4'), 119.56 (C-2', 6'), 62.12 (C-1''), 33.40 (C-2), 22.43 (C-3), 21.27 (C-11); ESIMS: m/z 297.1607 [M + H]⁺, 319.1411 [M + Na]⁺, calc. for C₁₈H₂₀N₂O₂ (296.3636).

(*E*)-4-(4-Methylbenzoyl)-4-[(benzyloxyimino)-*N*-phenylbutanamide (5a-2). Yield 79%, m.p. 119.5~120.5 °C; ¹H NMR (600 MHz, DMSO) δ: 9.95 (s, 1H, H-NH), 7.53 (dd, *J* = 7.5, 5.1 Hz, 4H, H-6, 10, 2', 6'), 7.38 (d, *J* = 7.4 Hz, 2H, H-3'', 7''), 7.34 (t, *J* = 7.4 Hz, 2H, H-4'', 6''), 7.30–7.25 (m, 3H, H-3', 5', 5''), 7.19 (d, *J* = 7.9 Hz, 2H, H-7, 9), 7.03 (t, *J* = 7.4 Hz, 1H, H-4'), 5.17 (s, 2H, H-1''), 3.06–2.98 (m, 2H, H-3), 2.51 (dd, *J* = 8.1, 6.6 Hz, 2H, H-2), 2.28 (s, 3H, H-11); ¹³C NMR (151 MHz, DMSO) δ: 170.46 (C-1), 157.93 (C-4), 139.50 (C-8), 139.36 (C-1'), 138.47 (C-2''), 132.34 (C-5), 129.60 (C-7, 9), 129.15 (C-3', 5'), 128.79 (C-3'', 7''), 128.27 (C-4'', 6''), 128.14 (C-5''), 126.56 (C-6, 10), 123.77 (C-4'), 119.72 (C-2', 6'), 75.80 (C-1''), 33.26 (C-2), 22.52 (C-3), 21.21 (C-11); ESIMS: m/z 373.2 [M + H]⁺, 395.1 [M + Na]⁺, calc. for C₂₄H₂₄N₂O₂ (372.4596).

(*E*)-4-(4-Methoxyphenyl)-4-[(methoxyimino)-*N*-phenylbutanamide (5b-1). Yield 78%, m.p. 125.6~126.5 °C; ¹H NMR (600 MHz, DMSO) δ: 9.95 (s, 1H, H-NH), 7.68 (d, *J* = 8.6 Hz, 2H, H-6, 10), 7.61 (d, *J* = 8.0 Hz, 2H, H-2', 6'), 7.32 (t, *J* = 7.7 Hz, 2H, H-3', 5'), 7.06 (t, *J* = 7.3 Hz, 1H, H-4'), 7.00 (d, *J* = 8.6 Hz, 2H, H-7, 9), 3.94 (s, 3H, H-1''), 3.82 (s, 3H, H-11), 3.04–2.99 (m, 2H, H-3), 2.54 (t, *J* = 8.0 Hz, 2H, H-2); ¹³C NMR (151 MHz, DMSO) δ: 170.32 (C-1), 160.62 (C-8), 157.12 (C-4), 139.61 (C-1'), 129.12 (C-3', 5'), 128.05 (C-6, 10), 127.57 (C-5), 123.54 (C-4'), 119.58 (C-2', 6'), 114.39 (C-7, 9), 62.04 (C-1''), 55.67 (C-11), 33.45 (C-2), 22.38 (C-3); ESIMS: m/z 313.1551 [M + H]⁺, 355.1367 [M + Na]⁺, calc. for C₁₈H₂₀N₂O₃ (312.3630).

(*E*)-4-(4-Methoxyphenyl)-4-[(benzyloxyimino)-*N*-phenylbutanamide (5b-2). Yield 79%, m.p. 119.0~119.6 °C; ¹H NMR (600 MHz, DMSO) δ: 9.92 (s, 1H, H-NH), 7.66–7.60 (m, 2H, H-6, 10), 7.57 (d, *J* = 7.6 Hz, 2H, H-2', 6'), 7.41 (d, *J* = 7.2 Hz, 2H, H-3'', 7''), 7.39–7.33 (m, 2H, H-4'', 6''), 7.32–7.26 (m, 3H, H-3', 5', 5''), 7.03 (t, *J* = 7.4 Hz, 1H, H-4'), 6.98–6.94 (m, 2H, H-7, 9), 5.19 (s, 2H, H-1''), 3.77 (s, 3H, H-11), 3.06–3.00 (m, 2H, H-3), 2.55–2.51 (m, 2H, H-2); ¹³C NMR (151 MHz, DMSO) δ: 170.30 (C-1), 160.66 (C-8), 157.55 (C-4), 139.59 (C-1'), 138.64 (C-2''), 129.13 (C-3', 5'), 128.78 (C-3'', 7''), 128.31 (C-4'', 6''), 128.11 (C-6, 10), 128.10 (C-5''), 127.57 (C-5), 123.56 (C-4'), 119.58 (C-2', 6'), 114.41 (C-7, 9), 75.74 (C-1''), 55.69 (C-11), 33.37 (C-2), 22.46 (C-3); ESIMS: m/z 389.1870 [M + H]⁺, 411.1676 [M + Na]⁺, calc. for C₂₄H₂₄N₂O₃ (388.4590).

(*E*)-4-(4-Chlorophenyl)-4-[(methoxyimino)-*N*-phenylbutanamide (5c-1). Yield 76%, m.p. 151.3~152.4 °C; ¹H NMR (600 MHz, DMSO) δ: 9.91 (s, 1H, H-NH), 7.71 (d, *J* = 8.5 Hz, 2H, H-6, 10), 7.55 (d, *J* = 7.6 Hz, 2H, H-2', 6'), 7.48 (dd, *J* = 8.4, 1.4 Hz, 2H, H-7, 9), 7.28 (t, *J* = 7.8 Hz, 2H, H-3', 5'), 7.03 (t, *J* = 7.3 Hz, 1H, H-4'), 3.93 (s, 3H, H-1''), 2.99 (t, *J* = 8.0 Hz, 2H, H-3), 2.54–2.47 (m, 2H, H-2); ¹³C NMR (151 MHz, DMSO) δ: 170.17 (C-1), 156.75 (C-4), 139.54 (C-1'), 134.46 (C-8), 134.13 (C-5), 129.13 (C-3', 5'), 129.03 (C-7, 9), 128.47 (C-6, 10), 123.57 (C-4'), 119.57 (C-2', 6'), 62.36 (C-1''), 33.21 (C-2), 22.35 (C-3); ESIMS: m/z 317.1048 [M + H]⁺, 339.0866 [M + Na]⁺, calc. for C₁₇H₁₇ClN₂O₂ (316.7821).

(*E*)-4-(4-Chlorophenyl)-4-[(benzyloxyimino)-*N*-phenylbutanamide (5c-1). Yield 78%, m.p. 116.0~117.9 °C; ¹H NMR (600 MHz, DMSO) δ: 9.95 (s, 1H, H-NH), 7.70 (t, *J* = 8.3 Hz, 2H, H-6, 10), 7.56 (dd, *J* = 22.3, 8.0 Hz, 2H, H-2', 6'), 7.47 (d, *J* = 7.2 Hz, 2H, H-7, 9), 7.42 (t, *J* = 6.9 Hz, 2H, H-3'', 7''), 7.36 (dd, *J* = 6.4, 5.3 Hz, 2H, H-4'', 6''), 7.33–7.25 (m, 3H, H-3', 5', 5''), 7.03 (dd, *J* = 12.2, 6.3 Hz, 1H, H-4'), 5.22 (s, 2H, H-1''), 3.13–2.98 (m, 2H, H-3), 2.55 (dt, *J* = 16.4, 8.2 Hz, 2H, H-2); ¹³C NMR (151 MHz, DMSO) δ: 170.16 (C-1), 157.17 (C-4), 139.58 (C-1'), 138.36 (C-2''), 134.54 (C-8), 134.14 (C-5), 129.12 (C-3', 5'), 129.04 (C-7, 9), 128.81 (C-3'', 7''), 128.50 (C-6, 10), 128.34 (C-4'', 6''), 128.19 (C-5''), 123.57 (C-4'), 119.59 (C-2',

6'), 76.08 (C-1''), 33.18 (C-2), 22.46 (C-3); ESIMS: m/z 393.1370 [M + H]⁺, 415.1164 [M + Na]⁺, calc. for C₂₃H₂₁ClN₂O₂ (392.8780).

3.3. Bio-Evaluation Methods

3.3.1. Cells and Cell Culture

HepG2.2.15 (clonal cells derived from human hepatoma cell line G2) cells were provided by the Chinese Academy of Medical Sciences (P.R. China) and maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum and 380 µg/mL of G418, 50 µg/mL of kanamycin, and 0.03% L-glutamine at 37 °C in a 5% CO₂ atmosphere with 100% humidity [26,33].

3.3.2. Drug Treatment

HepG2.2.15 cells were seeded at a density of 1×10^5 cells/ml (200 mL/well) in 96-well plates and maintained at 37 °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 treated with drug-containing fresh medium every 3 d for up to 9 d in a time-dependent experiment. Medium was taken at the 9th day of the treatment and stored at −20 °C until analysis. The IC₅₀ and selected index (SI) of each compound were calculated, respectively.

3.3.3. Cell Toxicity

Logarithmically growing cells were seeded in 96-well culture plates at a density of 1×10^5 cells/mL (200 mL/well). They were cultured for 24 h and then treated with various concentrations of drugs. Optical density (OD) values were read at 450 nm after 9 days and the percentage of cell death was calculated; the cells were treated with drug-containing fresh medium every 3 d for up to 9 d. After drug treatment, the cytotoxicity was measured using the MTT assay [45,46].

3.3.4. Method for HBsAg and HBeAg Inhibition Assays

The levels of HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) in the supernatant of the HepG2.2.15 cell were simultaneously detected using enzyme-linked immunosorbent assay (ELISA) kits (Rongsheng Biotechnology Co. Ltd., Shanghai, China) according to the manufacturer's instructions. The synthesized derivatives were expressed as the concentration of compound that achieved 50% inhibition (IC₅₀) to the secretion of HBsAg and HBeAg. The selectivity index (SI) was determined as the ratio of CC₅₀ to IC₅₀, which is a major pharmaceutical parameter of estimates possible for future clinical application.

4. Conclusions

In a summary, most of the compounds in the series of oxime ethers with a C₆-C₄ fragment, which were designed and virtually bioactively screened by docking with a target protein, showed anti-HBV activities in an in vitro assay. Among them, compound 5c-1 showed the most potent activity inhibiting HBsAg secretion (IC₅₀ = 39.93 µM, SI = 28.51). The results of bioactive screening showed that the stronger the compounds bound to a target protein (human leukocyte antigen A protein) in docking, the more active they were for anti-HBV in vitro.

Acknowledgments: This work was financially supported by the National Natural Science Foundation of China (No. 81760635), the Foundation of High-Level Innovation Team and Outstanding Scholar program in Guangxi college, the Department of Education of Guangxi Zhuang autonomous region (2015), and the Science and Technology Research Foundation for Traditional Chinese Medicine, Health Department of Guangxi province (gzbz14-19). We thank Jun Xu from the School of Chemical and Chemical Engineering, Sun Yat Sen University, for providing generous help in MOE docking.

Author Contributions: Wanxing Wei conceived experiments; Jie Tan and Min Zhou designed and performed the docking study, synthesis and analysis of bioactive assay data; Xinhua Cui and Zhuocai Wei performed assay experiments; Jie Tan and Min Zhou wrote the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lok, A.S. Chronic hepatitis B. *N. Engl. J. Med.* **2002**, *346*, 1682–1683. [[CrossRef](#)] [[PubMed](#)]
2. World Health Organization (WHO). *Hepatitis B Fact Sheet N°204*; WHO: Geneva, Switzerland, 2017.
3. Zhang, E.; Kosinska, A.; Lu, M.; Yan, H.; Roggendorf, M. Current status of immunomodulatory therapy in chronic hepatitis B, fifty years after discovery of the virus: Search for the “magic bullet” to kill cccDNA. *Antivir. Res.* **2015**, *123*, 193–203. [[CrossRef](#)] [[PubMed](#)]
4. Block, T.M.; Rawat, S.; Brosgart, C.L. Chronic hepatitis B: A wave of new therapies on the horizon. *Antivir. Res.* **2015**, *121*, 69–81. [[CrossRef](#)] [[PubMed](#)]
5. Scaglione, S.J.; Lok, A.S.F. Effectiveness of hepatitis B treatment in clinical practice. *Gastroenterology* **2012**, *142*, 1360–1368. [[CrossRef](#)] [[PubMed](#)]
6. Clercq, E.D.; Férir, G.; Kaptein, S.; Neyts, J. Antiviral treatment of chronic hepatitis B virus (HBV) infections. *Viruses* **2010**, *2*, 1279–1305. [[CrossRef](#)] [[PubMed](#)]
7. Pradere, U.; Garnier-Amblard, E.C.; Coats, S.J.; Amblard, F.; Schinazi, R.F. Synthesis of nucleoside phosphate and phosphonate prodrugs. *Chem. Rev.* **2014**, *114*, 9154–9218. [[CrossRef](#)] [[PubMed](#)]
8. Chotiayaputta, W.; Lok, A.S. Hepatitis B virus variants. *Nat. Rev. Gastroenterol. Hepatol.* **2009**, *6*, 453–462. [[CrossRef](#)] [[PubMed](#)]
9. Cento, V.; Hemert, F.V.; Neumann-Fraune, M.; Mirabelli, C.; Di Maio, V.C.; Salpini, R.; Bertoli, A.; Micheli, V.; Gubertini, G.; Romano, S.; et al. Anti-HBV treatment induces novel reverse transcriptase mutations with reflective effect on HBV S antigen. *J. Infect.* **2013**, *67*, 303–312. [[CrossRef](#)] [[PubMed](#)]
10. Wong, D.K.; Cheung, A.M.; O'Rourke, K.; Naylor, C.D.; Detsky, A.S.; Heathcote, J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B: A meta-analysis. *Ann. Intern. Med.* **1993**, *119*, 312–323. [[CrossRef](#)] [[PubMed](#)]
11. Perrillo, R.P.; Schiff, E.R.; Davis, G.L.; Bodenheimer, H.C., Jr.; Lindsay, K.; Payne, J.; Dienstag, J.L.; O'Brien, C.; Tamburro, C.; van Lie, R.A.W.; et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. *N. Engl. J. Med.* **1990**, *323*, 295–301. [[CrossRef](#)] [[PubMed](#)]
12. Dusheiko, G.; Bertolotti, A. Resistance to lamivudine therapy: Is there more than meets the eye. *Gut* **2011**, *54*, 4–6.
13. Naveen, C.S.; Shakya, N.; Mak, M.; Agrawal, B.; Kumar, R. Antiviral Activity of Various 1-(2'-Deoxy-β-D-lyxofuranosyl), 1-(2'-Fluoro-β-D-xylofuranosyl)1-(3'-Fluoro-β-D-arabino-furanosyl), and 2'-Fluoro-2',3'-didehydro-2',3'-dideoxyribose Pyrimidine Nucleoside Analogues against Duck Hepatitis B Virus (DHBV) and Human Hepatitis B Virus (HBV) Replication. *J. Med. Chem.* **2010**, *53*, 7156–7166.
14. Lv, Z.L.; Sheng, C.Q.; Wang, T.T.; Zhang, Y.K.; Liu, J.; Feng, J.L.; Sun, H.L.; Zhong, H.Y.; Niu, C.J.; Li, K. Design, Synthesis, and Antihepatitis B Virus Activities of Novel 2-Pyridone Derivatives. *J. Med. Chem.* **2010**, *53*, 660–668. [[CrossRef](#)] [[PubMed](#)]
15. Matthias, D.; Karen, M.; Rob, D.V.; Tim, H.M.J.; Géry, D.; Norbert de, K. Design, Synthesis, and Antiviral Evaluation of Purine-β-lactam and Purine-aminopropanol Hybrids. *J. Med. Chem.* **2012**, *55*, 5637–5641.
16. Lv, Z.G.; He, W.; Tian, X.H.; Kang, J.F.; Liu, Y.X.; Yu, W.Q.; Peng, Y.M.; Zheng, L.Y.; Wang, Q.D.; Yu, W.Q.; et al. Design, synthesis, and biological evaluation of new N4-Substituted 20-deoxy-20-fluoro-40-azido cytidine derivatives as potent anti-HBV Agents. *Eur. J. Med. Chem.* **2015**, *101*, 103–110. [[CrossRef](#)] [[PubMed](#)]
17. Imoto, S.; Kohgo, S.; Tokuda, R.; Kumamoto, H.; Aoki, M.; Amano, M.; Kuwata-Higashi, N.; Mitsuya, H.; Haraguchi, K. Design, Synthesis, and Evaluation of Anti-HBV Activity of Hybrid Molecules of Entecavir and Adefovir: Exomethylene Acycloguanine Nucleosides and Their Monophosphate Derivatives. *Nucleoside Nucleotides Nucleic Acid* **2015**, *34*, 590–602. [[CrossRef](#)] [[PubMed](#)]
18. Yang, X.Y.; Xu, X.Q.; Guan, H.; Wang, L.L.; Wu, Q.; Zhao, G.M.; Li, S. A new series of HAPs as anti-HBV agents targeting at capsid assembly. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4247–4249. [[CrossRef](#)] [[PubMed](#)]
19. Gao, L.M.; Han, Y.X.; Wang, Y.P.; Li, Y.H.; Shan, Y.Q.; Li, X.; Peng, Z.G.; Bi, C.W.; Zhang, T.; Du, N.N.; et al. Design and Synthesis of Oxymatrine Analogues Overcoming Drug Resistance in Hepatitis B Virus through Targeting Host Heat Stress Cognate 70. *J. Med. Chem.* **2011**, *54*, 869–876. [[CrossRef](#)] [[PubMed](#)]

20. Huang, T.J.; Chuang, H.; Liang, Y.C.; Lin, H.H.; Horng, J.C.; Kuo, Y.C.; Chen, C.W.; Tsai, F.Y.; Yen, S.C.; Chou, S.C.; et al. Design, Synthesis, and Bioevaluation of Paeonol Derivatives as Potential Anti-HBV Agents. *Eur. J. Med. Chem.* **2015**, *90*, 428–435. [[CrossRef](#)] [[PubMed](#)]
21. Wang, L.J.; Chen, H.; Ma, Y.B.; Huang, X.Y.; Chen, J.J.; Geng, C.A.; Zhang, X.M.; Chen, J.J. Design, Synthesis and Biological Evaluation of Caudatin Analogs as Potent Hepatitis B Virus Inhibitors. *Med. Chem.* **2015**, *11*, 165–179. [[CrossRef](#)] [[PubMed](#)]
22. Lv, J.J.; Yu, S.; Wang, Y.F.; Wang, D.; Xu, M.; Zhang, Y.J.; van Lie, R.A.W. Anti-Hepatitis B Virus Norbisabolane Sesquiterpenoids from *Phyllanthus acidus* and the Establishment of Their Absolute Configurations Using Theoretical Calculations. *J. Org. Chem.* **2014**, *79*, 5432–5447. [[CrossRef](#)] [[PubMed](#)]
23. Yan, M.H.; Cheng, P.; Jiang, Z.Y.; Ma, Y.B.; Zhang, X.M.; Zhang, F.X.; Yang, L.M.; Zheng, Y.T.; Chen, J.J. Periglaucines A–D, Anti-HBV and –HIV–1 Alkaloids from *Pericampylus glaucus*. *J. Nat. Prod.* **2008**, *71*, 760–763. [[CrossRef](#)] [[PubMed](#)]
24. Kortagere, S.; Xu, J.P.; Mankowski, M.K.; Ptak, R.G.; Cocklin, S. Structure–Activity Relationships of a Novel Capsid Targeted Inhibitor of HIV-1 Replication. *J. Chem. Inf. Model.* **2014**, *54*, 3080–3090. [[CrossRef](#)] [[PubMed](#)]
25. Niet, D.A.; Stelma, F.; Jansen, L.; Sinnige, M.J.; Remmerswaal, E.B.M.; Takkenberg, R.B.; Kootstra, N.A.; Reedink, H.W. Restoration of T cell function in chronic hepatitis B patients upon treatment with interferon based combination therapy. *J. Hepatology* **2015**, *64*, 539–546. [[CrossRef](#)] [[PubMed](#)]
26. Sun, L.; Zhang, Y.; Zhao, B.; Deng, M.M.; Liu, J.; Li, X.; Hou, J.W.; Gui, M.M.; Zhang, S.J.; Li, X.D.; et al. A new unconventional HLA-A2-restricted epitope from HBV core protein elicits antiviral cytotoxic T lymphocytes. *Protein Cell* **2014**, *5*, 317–327. [[CrossRef](#)] [[PubMed](#)]
27. Xu, W.; Chu, Y.W.; Zhang, R.H.; Xu, H.B.; Wang, Y.; Xiong, S.D. Endoplasmic reticulum targeting sequence enhances HBV-specific cytotoxic T lymphocytes induced by a CTL epitope-based DNA vaccine. *Virology* **2005**, *334*, 255–263. [[CrossRef](#)] [[PubMed](#)]
28. Yu, D.B.; Liu, H.; Shi, S.; Zou, L.B.; Zou, Z.H. A novel dendritic-cell-targeting DNA vaccine for hepatitis B induces T cell and humoral immune responses and potentiates the antiviral activity in HBV transgenic mice. *Immunol. Lett.* **2015**, *168*, 293–299. [[CrossRef](#)] [[PubMed](#)]
29. Li, J.Q.; Ge, J.; Ren, S.L.; Zhou, T.; Sun, Y.; Sun, H.L. Hepatitis B surface antigen (HBsAg) and core antigen (HBcAg) combine CpG oligodeoxynucleotides as a novel therapeutic vaccine for chronic hepatitis B infection. *Vaccine* **2015**, *33*, 4247–4254. [[CrossRef](#)] [[PubMed](#)]
30. Liu, S.; Wei, W.X.; Shi, K.C.; Cao, X.; Zhou, M.; Liu, Z.P. In vitro and in vivo anti-hepatitis B virus activities of the lignan niranthin isolated from *Phyllanthus niruri* L. *J. Ethnopharmacol.* **2014**, *155*, 1061–1067. [[CrossRef](#)] [[PubMed](#)]
31. Liu, S.; Wei, W.X.; Li, Y.B.; Lin, X.; Shi, K.C.; Cao, X.; Zhou, M. In vitro and in vivo anti-hepatitis B virus activities of the lignan nirtetralin B isolated from *Phyllanthus niruri* L. *J. Ethnopharmacol.* **2014**, *157*, 62–68. [[CrossRef](#)] [[PubMed](#)]
32. Wei, W.X.; Li, X.R.; Wang, K.W.; Zheng, Z.W.; Zhou, M. Lignans with anti-hepatitis B virus activities from *Phyllanthus niruri* L. *Phytother. Res.* **2012**, *26*, 964–968. [[CrossRef](#)] [[PubMed](#)]
33. Liu, S.; Wei, W.X.; Li, Y.B.; Liu, X.; Cao, X.J.; Lei, K.C.; Zhou, M. Design, synthesis, biological evaluation and molecular docking studies of phenylpropanoid derivatives as potent anti-hepatitis B virus agents. *Eur. J. Med. Chem.* **2015**, *95*, 473–482. [[CrossRef](#)] [[PubMed](#)]
34. Liu, S.; Li, Y.B.; Wei, W.X.; Wei, J.C. Synthesis and biological evaluation of phenylpropanoid derivatives. *Med. Chem. Res.* **2016**, *25*, 1074–1086. [[CrossRef](#)]
35. Liu, S.; Li, Y.B.; Wei, W.X.; Wang, K.W.; Wang, L.S.; Wang, J.Y. Design, Synthesis, Molecular Docking Studies and Anti-HBV Activity of Phenylpropanoid Derivatives. *Chem. Biol. Interact.* **2016**, *251*, 1–9. [[CrossRef](#)] [[PubMed](#)]
36. Imoto, H.; Sugiyama, Y.; Kimura, H.; Momose, Y. Studies on Non-Thiazolidinedion Antidiabetic Agents. Novel Oxyiminoalkanoic Acid derivatives as Potent Glucose and Lipid Lowering Agents. *Chem. Pharm. Bull.* **2003**, *51*, 138–151. [[CrossRef](#)] [[PubMed](#)]
37. Wei, X.W.; Tan, J.; Zhou, M. Anti-Hepatitis B Virus Activitive N-Phenyl-4-phenyl Butylamidoxime and Its Derivatives. CN106518727A, 2 November 2016.
38. Howe, N.J.; Blades, K.; Lamont, G.M. Synthesis of the Novel Tetrahydropyrazolo[3,4-c]pyridin-5-one Scaffold. *Synlett* **2015**, *26*, 228–232. [[CrossRef](#)]

39. Alfonsi, M.; Arcadi, A.; Chiarini, M. Sequential Rhodium-Catalyzed Stereo- and Regioselective Addition of Organoboron Derivatives to the Alkyl 4-Hydroxy-2-Alkynoates/Lactonization Reaction. *J. Med. Chem.* **2007**, *72*, 9510–9517. [[CrossRef](#)] [[PubMed](#)]
40. Mahmoodi, N.O.; Malekroudi, R.Y. Synthesis of Chiral 5-Aryltetrahydrofuran-2-ones via Yeast Bioreduction of γ -Keto Acids and Their Esters. *Rus. J. Med. Chem.* **2006**, *42*, 365–368. [[CrossRef](#)]
41. Starodubtseva, E.V.; Turova, O.V.; Vinogradov, M.G.; Gorshkova, L.S.; Struchkova, M.I. A convenient route to chiral γ -lactones via asymmetric hydrogenation of γ -ketoesters using the RuCl_3 -BINAP-HCl catalytic system. *Tetrahedron* **2008**, *64*, 11713–11717. [[CrossRef](#)]
42. Li, W.H.; Zhang, L.F.; Liu, W. Synthesis and antifungal activity of 2-carboxamide-4-aryl-1,5-benzothiazepines. *Chin. J. Org. Chem.* **2011**, *31*, 1252–1257.
43. Candeloro, V.; Bowie, J.H. 1H-1-benzazepines. The reactions of levulinic acid and β -benzoylpropionic acid with aniline and methoxyanilines. *Aust. J. Chem.* **1978**, *31*, 2031–2037. [[CrossRef](#)]
44. Liu, J.X.; Chen, K.Y.; Ren, E.C. Structural insights into the binding of hepatitis B virus core peptide to HLA-A2 alleles: Towards designing better vaccines. *Eur. J. Immunol.* **2011**, *41*, 2097–2106. [[CrossRef](#)] [[PubMed](#)]
45. Han, Y.Q.; Huang, Z.M.; Yang, X.B.; Liu, H.Z.; Wu, G.X. In vivo and in vitro antihepatitis B virus activity of total phenolics from *Oenanthe javanica*. *J. Ethnopharmacol.* **2008**, *118*, 148–153. [[CrossRef](#)] [[PubMed](#)]
46. Sells, M.A.; Chen, M.L.; Acs, G. Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA. *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 1005–1009. [[CrossRef](#)] [[PubMed](#)]

Sample Availability: Samples of the all compounds are available from the authors.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).