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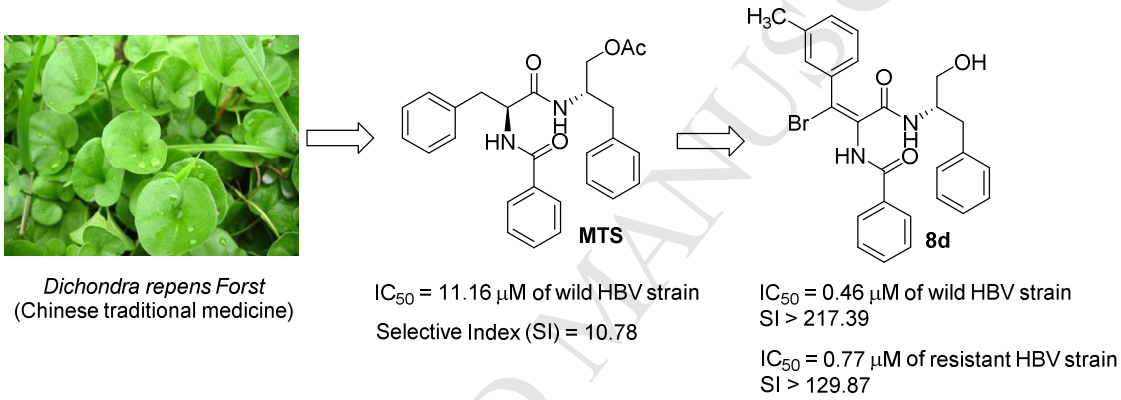
Graphical Abstract



Compound **8d** exhibited significantly anti-HBV activity against wild-type strain as well as lamivudine and entecavir resistant HBV strain.

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Abstract

As an ongoing search for potent non-nucleoside anti-HBV agents with novel structures, we described a series of phenyl propionamide derivatives (**3a-b**, **4a-e**, **7a-g**, **8a-h** and **9a-b**) by pharmacophore fusion strategy in the present work. All the compounds exhibited an anti-HBV activity to some extent. Among them, compounds **8d** and **9b** displayed most potent anti-HBV activity with IC_{50} values on HBV DNA replication of 0.46 and 0.14 μM, respectively. And the selective index values of **8d** and **9b** were more than 217.39 and 153.14, suggesting that **8d** and **9b** exhibited favorable safety profiles. Interestingly, **8d** and **9b** possessed significantly antiviral activities against lamivudine and entecavir resistant HBV mutants with IC_{50} values of 0.77 and 0.32 μM. Notably, preliminary anti-HBV action mechanism studies showed that **8d** could inhibit intracellular HBV pgRNA and RT activity of the HBV polymerase. Molecular docking studies suggested that compound **8d** could fit into the dimer-dimer interface of HBV core protein by hydrophobic interaction. In addition, in silico prediction of physicochemical properties showed that **8d** conformed well to the Lipinski's rule of five, suggesting its potential for use as a drug like molecule. Taken together, **8d** possessed significantly anti-HBV activity, low toxicity, diverse anti-HBV mechanism and favorable physicochemical properties, and warranted further investigation as a promising non-nucleoside anti-HBV candidate.

Keywords: Anti-HBV agents; Phenyl propionamide derivatives; Non-nucleoside; Synthesis.

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1. Introduction

Hepatitis B virus (HBV) infection remains a major threat to public health despite the fact that HBV vaccination programs have been extensively implemented in the past. There are about 350 million chronic HBV carriers in the world and each year about one million chronic hepatitis patients develop into more life-threatening diseases such as liver failure, cirrhosis or hepatocellular carcinoma (HCC) [1]. More depressingly, current treatment options, such as interferons (IFNs) and nucleos(t)ides-based reverse transcriptase inhibitors, do not offer a satisfactory clinical cure rate for chronic HBV infection [2]. Remarkably, nucleos(t)ide therapies typically require lifetime treatment to prevent viral rebound[3, 4], and the HBV can easily develop resistance to this kind of drugs [5, 6], while IFNs need parenteral administration and usually cause adverse effects such as “flu-like” symptoms [7]. Therefore, there is a tremendous unmet medical requirement to develop novel and safety anti-HBV agents with diverse action mechanisms [8].

In an ongoing search for potent non-nucleoside HBV inhibitors started a few years ago, we described a series of Matijing-Su (MTS) derivatives as anti-HBV agents [9-11], which possessed a phenylalanine dipeptide scaffold. Among them, Y101 was the most potent one (Figure 1) [12]. Notably, these phenylalanine dipeptide derivatives displayed a different anti-HBV action mechanism from those of conventional nucleotide drugs, and exhibited little inhibitory effect on HBV DNA polymerase. More interestingly, Y101 possessed potent antiviral activities against wild-type as well as polymerase drug resistant HBV strains. Several years ago, a series of phenyl acrylamide derivatives (AT-130) was reported as novel anti-HBV agents by interfering the assembly of HBV core particles instead of inhibiting HBV DNA polymerase [13, 14]. Excitingly, like Y101, AT-130 also displayed antiviral activities against polymerase drug resistant HBV strains. With these in mind, and considering that both Y101 and AT-130 possessed novel anti-HBV action mechanism, a series novel phenyl propionamide derivatives was designed by fusing the pharmacophore of Y101 and AT-130 (Figure 2), and their anti-HBV activities, including preliminary anti-HBV action mechanisms were evaluated in the present work.

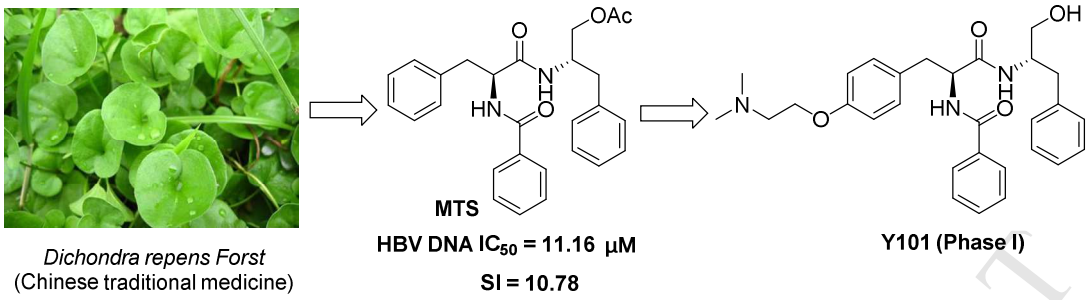


Figure 1. Chinese traditional medicine and the structures of the anti-HBV natural product compound MTS and its derivative Y101.

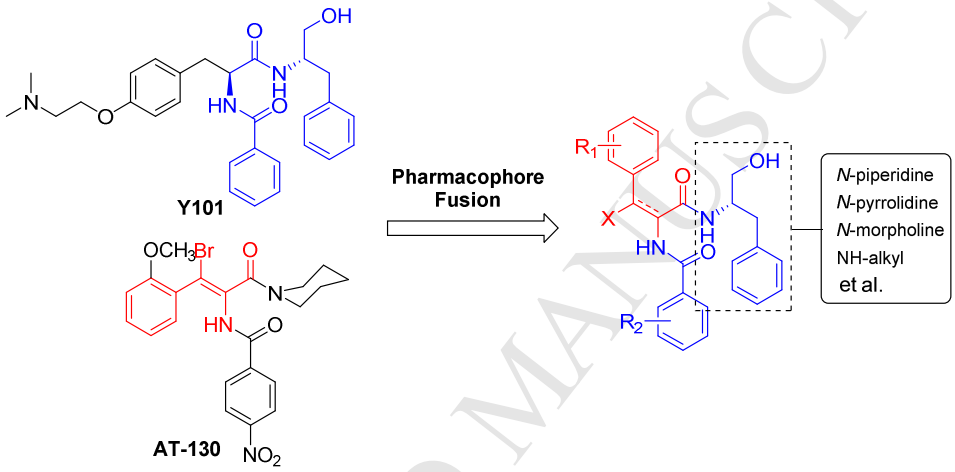
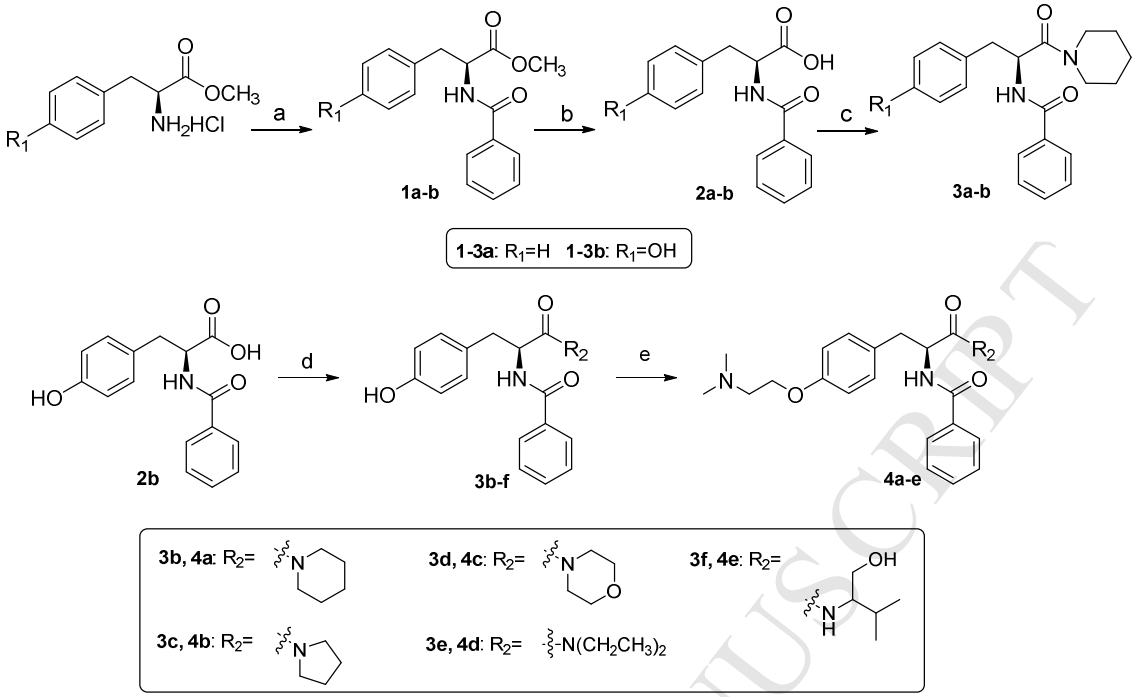


Figure 2. Structures of lead compounds and the newly designed phenyl propionamide derivatives via fused pharmacophore strategy.

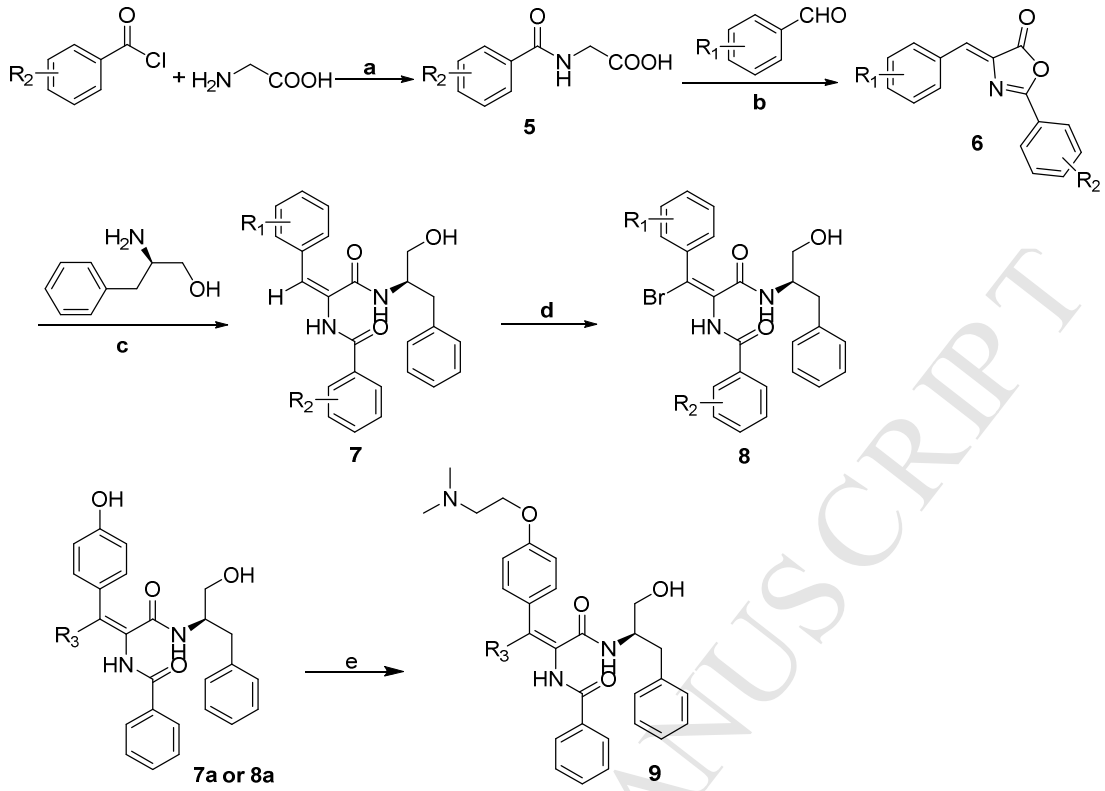
2. Chemistry

The synthetic procedure of the target compounds **3a-b** and **4a-e** is illustrated in Scheme 1. Briefly, L-Phenylalanine methyl ester hydrochloride or L-tyrosine methyl ester hydrochloride was condensed with benzoic acid to give intermediate **1a-b**, which was then hydrolyzed to yield intermediate **2a-b** as described previously. Compound **3a-b** was obtained by the condensation of **2a-b** with piperidine. With compound **2b** as the starting material, and using the procedure described for the preparation of **3a-b**, **3b-f** were prepared by changing piperidine into various amine, which was then alkylated with 2-dimethylaminoethyl chloride hydrochloride to provide alkoxyl substituted derivatives **4a-e**, respectively.



Scheme 1. **Reagents and conditions:** (a) benzoic acid, CH_2Cl_2, IBCF, NMM, 0 °C; (b) NaOH, DMF, rt.; (c) piperidine, CH_2Cl_2, IBCF, NMM, 0 °C; (d) amine, CH_2Cl_2, IBCF, NMM, 0 °C; (e) 2-dimethylaminoethyl chloride hydrochloride, 1,4-dioxane, K_2CO_3, 90 °C.

The synthetic routes of the target compounds **7a-g**, **8a-h** and **9a-b** are depicted in Scheme 2. Compound **5** was obtained by the acylation reaction with glycine and benzoyl chloride or benzoyl chloride derivatives as the raw materials, which was then reacted with benzaldehyde or benzaldehyde derivatives in the presence of acetic anhydride (Ac_2O) and sodium acetate (AcONa) to obtain compound **6**. Next, the intermediate mentioned above was reacted with L-phenylalaninol to give the target compounds **7a-g**. Finally, the crude reaction product was directly brominated with bromine to give the target compounds **8a-h**, respectively. Compound **7b** or **8b** was alkylated with 2-dimethylaminoethyl chloride hydrochloride to provide alkoxyl substituted derivative **9a** or **9b**.



Scheme 2. **Reagents and conditions:** a) NaOH (aq), HCl; b) AcONa/Ac_2O, 100 °C; c) CH_3Cl, 0 °C; d) Br_2, $CaCO_3/CHCl_3$, 0 °C; e) 2-dimethylaminoethyl chloride hydrochloride, 1,4-dioxane, K_2CO_3, 90 °C.

3. Results and discussion

3.1. Biological evaluation

All the synthesized compounds were evaluated for the in vitro anti-HBV activity and cytotoxicity in HepG2 2.2.15 cells using real-time quantitative PCR and MTT methods, respectively. The concentration of compound required for 50% inhibition of DNA replication was defined as IC_{50} and the concentration of compound that induced the death of the HepG2 2.2.15 cells cultures by 50% was defined as CC_{50}. Selectivity index (SI) was determined as the CC_{50}/IC_{50} value. Lamivudine was used as the positive control.

3.1.1. Cytotoxicity assays

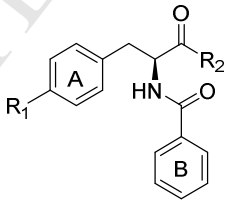
To evaluate the in vitro cytotoxic effects of the target compounds, HepG2 2.2.15 cells were treated with various doses of **3a-b**, **4a-e**, **7a-g**, **8a-h** and **9a-b** for 72 h, and then the viability of the cells was measured by the MTT assay. All measurements were performed in three replicates, and the results were presented as a relative percentage of the results of the control group. As shown in Table 1 and Table 2, most of the

compounds exhibited little (CC_{50} > 100 μM) or slight (CC_{50} = 21.44 ~ 66.71 μM) intrinsic cytotoxicity in vitro, indicating that this kind of compounds possessed a relatively safety profile.

3.1.2. Inhibitory effect on HBV DNA replication

Next, the anti-HBV activity of compounds **3a-b**, **4a-e**, **7a-g**, **8a-h** and **9a-b** was screened in the HepG2 2.2.15 cell line by determining their inhibitory effect on HBV DNA replication. Lead compound Y101 and lamivudine (3TC) were selected as the positive controls. After the treatment of the cells with the test compounds at the set concentration of 20 μM or 4 μM for 6 days, the extra cellar HBV DNA levels were quantified by real time PCR. The preliminary one dose anti-HBV screening showed that, compounds **3a-b**, **4a-e**, **7a-g** and **9a** displayed relative poor inhibitory effect on HBV DNA replication (Table 1 and Table 2), the inhibition rate of most compounds against HBV DNA replication was less than 50%, except **4a (**51.64%**)** and **7g** (51.36%). Interestingly, analogues **8a-h** and **9b** exhibited significant anti-HBV activity, the inhibition rate on HBV DNA replication of all these compounds was higher than that of the positive control Y101 (with an inhibition rate of 69.17%, Table 2). Clearly, compound **8d** and **9b** showed the most potent in vitro anti-HBV DNA activity with an inhibition rate of 87.61% and 99.45%, respectively, which was comparable with the positive control 3TC.

Table 1. Inhibitory effect of the target compounds **3a-b** and **4a-e** on HBV DNA level[a]

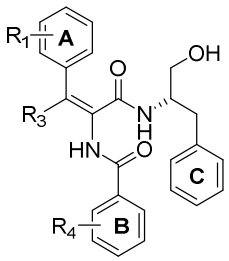


Compounds	R_1	R_2	CC_{50} ± SD (μM)	Inhibition rate (%)
3a	H		>100	42.30 ± 5.44
3b	4-OH		>100	19.48 ± 3.71
4a	4-$OCH_2CH_2N(CH_3)_2$		>100	51.64 ± 3.36
4b	4-$OCH_2CH_2N(CH_3)_2$		>100	47.62 ± 2.12
4c	4-$OCH_2CH_2N(CH_3)_2$		>100	24.90 ± 4.18
4d	4-$OCH_2CH_2N(CH_3)_2$	$N(CH_2CH_3)_2$	>100	17.47 ± 3.41

4e	4-$OCH_2CH_2N(CH_3)_2$		89.10 ± 0.117	47.32 ± 4.83
MTS			>100	60.61 ± 5.37
Y101			>100	69.17 ± 3.68
3TC			>100	92.66 ± 2.24

[a]Shown is the mean ± SD of at least three independent experiments with duplicate measurements.

Table 2. Inhibitory effect of the target compounds **7a-g**, **8a-h and 9a-b** on HBV DNA level[a]



Compounds	R_1	R_3	R_4	CC_{50} ± SD (μM)	Inhibition rate ± SD (%)
7a	4-OH	H	H	>100	24.95 ± 4.52
9a	4-$OCH_2CH_2N(CH_3)_2$	H	H	>100	37.55 ± 3.81
7b	4-Br	H	H	72.66 ± 0.091	39.05 ± 2.47 (4 μM)
7c	4-CN	H	H	>100	10.18 ± 4.66
7d	3-CH_3	H	H	>100	15.80 ± 3.40
7e	2-Cl	H	H	>100	29.75 ± 4.59
7f	2-Br	H	H	>100	26.24 ± 2.11
7g	H	H	2-F	>100	51.36 ± 2.93
8a	4-OH	Br	H	38.95 ± 0.032	63.19 ± 4.65 (4 μM)
9b	4-$OCH_2CH_2N(CH_3)_2$	Br	H	21.44 ± 0.059	99.45 ± 0.34 (4 μM)
8b	4-Br	Br	H	>100	73.06 ± 2.45
8c	4-CN	Br	H	>100	75.06 ± 3.68
8d	3-CH_3	Br	H	>100	87.61 ± 2.13
8e	2-Cl	Br	H	66.71 ± 0.013	80.15 ± 2.63
8f	2-Br	Br	H	>100	76.64 ± 5.62
8g	H	Br	2-F	>100	76.39 ± 5.91
8h	H	Br	H	>100	77.64 ± 2.69

Y101	>100	69.17 ± 3.68
3TC	>100	92.66 ± 2.24

[a]Shown is the mean ± SD of at least three independent experiments with duplicate measurements.

Subsequently, the active compounds **8a-h** and **9b** were further evaluated in HepG2 2.2.15 cells in a dose-dependent manner. The anti-HBV activity of each compound was evaluated by the combination of the IC_{50} value for inhibition of HBV DNA replication and the selectivity index (SI). As shown in Table 3, all the tested compounds displayed significant inhibitory effect on HBV DNA replication with IC_{50} values in range of 0.14-7.26 μM. Obviously, **8d** and **9b** were the top two compounds that possessed potent anti-HBV activity with IC_{50} values of 0.46 and 0.14 μM, respectively. More importantly, the SI values of **8d** and **9b** were more than 217 and 153, suggesting that **8d** and **9b** exhibited favorable safety profiles.

Table 3. Anti-HBV activity and SI values of target compounds **8a-h** and **9b**[a]

Compounds	CC_{50} ± SD (μM)	DNA replication	
		IC_{50} ± SD (μM)	SI
8a	38.95 ± 0.032	2.00 ± 0.027	19.48
9b	21.44 ± 0.059	0.14 ± 0.025	153.14
8b	>100	4.45 ± 0.009	>22.47
8c	>100	5.50 ± 0.017	18.18
8d	>100	0.46 ± 0.033	>217.39
8e	66.71 ± 0.013	1.88 ± 0.070	35.48
8f	>100	7.26 ± 0.086	>13.77
8g	>100	2.44 ± 0.051	40.98
8h	>100	3.86 ± 0.043	>25.91
3TC	>100	<0.1	>1000

[a]Shown is the mean ± SD of at least three independent experiments with duplicate measurements.

From data of Table 1-3, a preliminary structure activity relationship (SAR) conclusions can be drawn as follows: when the phenylalanine fragment in MTS derivative Y101 was replaced by piperidine (a key fragment in AT-130) or other amino substituents, such as pyrrolidine, diethylamine, morpholine, the anti-HBV activity of Y101 was obviously decreased (**3a** *vs.* MTS; **4a-e** *vs*. Y101, Table 1). Changing the phenylalanine scaffold of MTS into phenyl acrylamide, the anti-HBV activity was also significantly reduced. Notably, introducing a bromine atom into phenyl acrylamide fragment was benefit for the

anti-HBV activity of phenyl propionamide derivatives (**7a-g** *vs.* **8a-g**; **9a** vs **9b**; Y101 *vs*. **9b**, Table 2). In addition, para substituent in A ring also significantly affect the anti-HBV activity. Generally speaking, compounds with a dimethylamino ethoxy substituent exhibited higher anti-HBV activity (**4a** *vs.* **3a**, **9b** *vs.* **8h**). When dimethylamino ethoxy substituent was replaced with CN, Br or OH, the anti-HBV activity was significantly decreased (**9b** *vs.* **8a**, **8b**, **8c**). Interestingly, compound **8d**, with a meta methyl substituent in A ring, exhibited significant anti-HBV activity with a inhibition rate of 87.61%, indicating that the introduction of a meta substituent might be benefit for the anti-HBV activity. However, the precise SAR remains further investigation when more phenyl propionamide derivatives are available in the near future.

3.1.3. Assessment of activity of compounds **8a**, **8d** and **9b** *against polymerase drug resistant HBV strain*

Considering that the emergence of drug resistant strains is one of the key limitations associated with the current nucleotide analogs approved for HBV treatment, it is important to evaluate the activity of new compound against drug resistant HBV strains. Therefore, the anti-HBV effect on the lamivudine and entecavir dually resistant mutant (rtL180M + rtM204V + rtT184L) of the active compounds **8a**, **8d** and **9b** were subsequently evaluated. Lamivudine and entecavir were selected as reference drugs. Briefly, HepG2 cells transiently transfected with lamivudine and entecavir resistant strain (LERS) or wild-type strain were treated with lamivudine (100 μM), entecavir (10 μM), **8a** (1 μM), **8d** (1 μM) or **9b** (1 μM) respectively for 72 h, followed by examination of the level of HBV DNA replicative intermediate using real time PCR. The single concentration anti-HBV screening showed that lamivudine and entecavir could significantly suppress the wild-type HBV replication by 94.28% and 95.67% (Figure 3). As expected, both lamivudine and entecavir displayed poor anti-HBV activity against lamivudine and entecavir resistant strain, and the inhibition rate was only 19.37% and 17.90%, respectively. In sharp contrast, compounds **8a**, **8d** and **9b** at relatively low concentration of 1 μM can significantly inhibit the drug resistant HBV replication with a potency similar to that observed on wild-type HBV (43.17% vs 34.84%, 65.91% vs 61.36%, 80.12% vs 79.57%), indicating the effectiveness of **8a**, **8d** and **9b** on both strains.

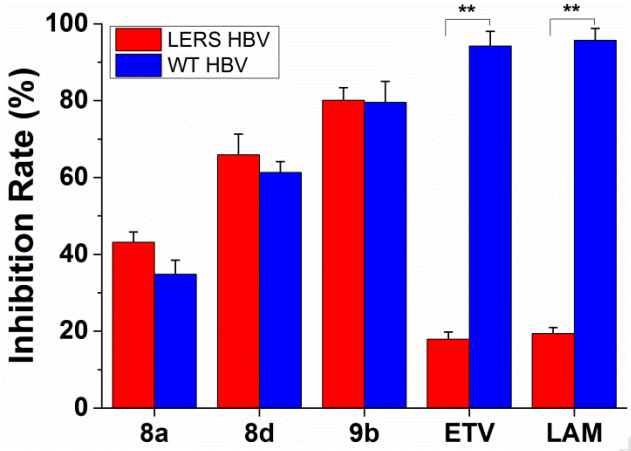


Figure 3. Antiviral effect of **8a**, **8d** and **9b** in lamivudine and entecavir resistant HBV. HepG2 cells were transiently transfected with the full genome of wide-type or lamivudine and entecavir resistant HBV, followed by 72 h treatment with lamivudine (100 μM), entecavir (10 μM), **8a** (1 μM), **8d** (1 μM) or **9b** (1 μM), respectively. HBV DNA replicative intermediate level was measured using real time PCR. $^{**}P < 0.01$ represents significant difference from the WT HBV group.

And also, compounds **8a**, **8d** and **9b** were further evaluated in a dose-dependent manner and the activity of each compound were evaluated by the combination of the IC_{50} value for inhibition of HBV DNA replication and the selectivity index (SI) (Table 4). As shown in Table, the IC_{50} values of **8d** and **9b** were 0.77 and 0.32 μM, indicating that both of them displayed potent inhibitory effect on HBV DNA replication against drug resistant strain. Notably, SI values of compounds **8d** and **9b** were more than 129.87 and 89.72, suggesting their relatively safety profiles.

Table 4. Inhibitory activity of compounds **8a**, **8d** and **9b** against lamivudine and entecavir resistant HBV strain[a]

Compounds	CC_{50} (μM)	DNA replication	
		IC_{50} (μM)	SI
8a	34.31 ± 0.016	2.97 ± 0.010	11.55
8d	>100	0.77 ± 0.013	>129.87
9b	28.71 ± 0.029	0.32 ± 0.038	89.72
3TC	>100	>100	<1

[a]Shown is the mean ± SD of at least three independent experiments with duplicate measurements.

3.2. In silico prediction of physicochemical properties

Furthermore, some key physicochemical parameters of compounds **8a**, **8d** and **9b** were predicted for their compliance with the Lipinski's rule of five using free online software (http://www.molinspiration.com/) [8]. As shown in Table 5, **8a** and **8d** are well accordance with the Lipinski's rule of five, indicating their

potential for use as drug like molecule. Considering that compound **8d** exhibited potent anti-HBV activity with low toxicity and reasonable physicochemical properties, we selected **8d** to explore the anti-HBV mechanism of the target compounds.

Table 5. Prediction of physicochemical properties[a] of **8a, 8d** and **9b**

Compounds	*nViol*	MW	natoms	miLogP	nON	nOHNH	Nrotb	TPSA	MV
Range		＜500		＜5	＜10	＜5	＜=10	＜140	
8a	0	495.37	32	3.60	6	4	8	98.65	401.40
8d	0	493.4	32	4.51	5	3	8	78.42	409.94
9b	1	566.50	37	4.17	7	3	12	90.90	481.88

[a] nViol: number of violations; natoms: number of atoms; miLogP: molinspiration predicted LogP; MW: molecular weight; nON: number of hydrogen bond acceptors; nOHNH: number of hydrogen bond donors; nrotb: number of rotatable bonds; TPSA: topological polar surface area; MV: molar volume.

*3.3. Anti-HBV mechanism studies of compound **8d***

*3.3.1. Effect of compound **8d** on HBV viral gene expression*

It is well known that pregenomic RNA (pgRNA) plays a significant role in HBV lifecycle and is associated with HBV DNA replication. Briefly, HepG2 2.2.15 cells were treated with compound **8d** at the concentration of 1 μM for 6 days. After the treatment, the total cellular RNA was extracted, and intracellular HBV RNA was detected by real time PCR. Notably, unlike 3TC, compound **8d** could inhibit 3.5 kb pgRNA expression (Table 6), suggesting that compound **8d** possessed different anti-HBV action mechanism than polymerase inhibitor 3TC.

Table 6. Effect of compound **8d** on intracellular HBV pgRNA

Compounds	Concentration (μM)	pgRNA (copies/mL)	Inhibition rate (%)
8d	1	4.13E+06	49.42
3TC	1	8.10E+06	0.81
Control	-	8.17E+06	-

*3.3.2. Evaluation of the inhibitory activity of compound **8d** on the DHBV RT activity*

Considering that compound **8d** possessed novel chemical structure different from that of nucleoside analogues, we finally evaluated whether **8d** exhibits the inhibitory effect on RT activity of DHBV polymerase in a cell-free assay, using lamivudine (3TC) as a positive control, which inhibits virus replication by acting as a chain terminator of both the RT and DNA polymerase activities of the enzyme. Interestingly, study on elongation of viral minus strand DNA showed that **8d** could inhibit the incorporation of radiolabelled dNTPs in a dose-dependent manner (Figure 4), suggesting that **8d** exhibited inhibitory effect on RT activity of DHBV polymerase. This result, together with the result on HBV viral gene expression demonstrated that compound **8d**, as a novel anti-HBV agent, possessed multiple action mechanisms.

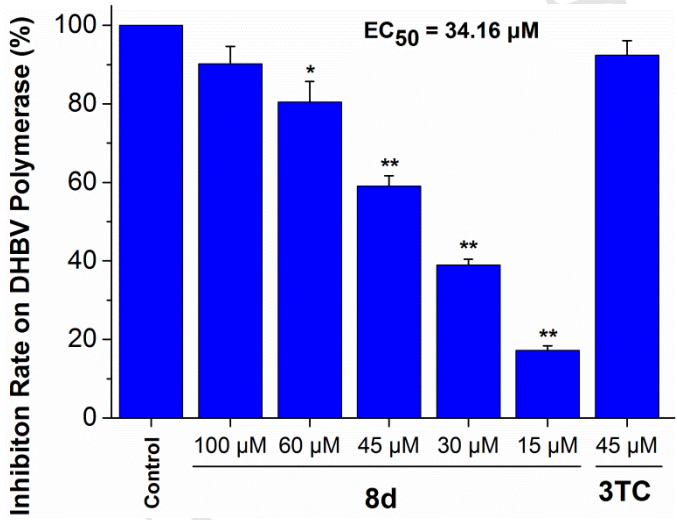


Figure 4. In vitro inhibitory activity of **8d** on the RT activity of the DHBV polymerase. The DHBV polymerase was expressed *in vitro* in a reticulocyte system. The enzymatic activity was studied in the absence or presence of increasing concentrations of nucleoside analog triphosphates. The inhibition rate of compound **8d** correspond to the concentration required to inhibit the incorporation of [α^{32}P]-dNTP in viral minus strand DNA. *P<0.05, **P<0.01 *vs*. control group.

3.3.3 Molecular docking

HBV core protein performs multiple essential roles at different stages of HBV virus life cycle, which is regarded as an excellent target for developing novel, efficacious and safe anti-HBV agents. Recently, phenyl acrylamide derivatives, such as AT-130, were reported as a HBV core protein assembly effectors thus exerting anti-HBV activity. With these in mind and considering that **8d** shared a similar phenyl

acrylamide skeleton, we preliminarily examined the interaction of **8d** with the interface of HBV core protein by Molecular docking. Interestingly, compound **8d** could be successfully docked into the dimer-dimer interface of HBV core protein, with the docking score value was of 6.00. As shown in Figure 5A, compound **8d** was fitted into a well-defined, largely hydrophobic groove composed of Pro25, Asp29, Leu30, Thr33, Leu37, Trp102, Ile105, Ser106, Thr109, Phe110, Tyr118, Phe122, Ile139, Leu140, and Ser141 in one protein subunit. Meanwhile, compound **8d** was covered by a "lid" formed by the C-terminal helix and loop (including residues Val124, Trp125, Arg127, Thr128, Pro129, Ala132, Arg133, and Pro134) of the other protein subunit (Figure 5B). It is likely that the binding affinity between HBV core protein and compound **8d** was nearly dominated by the hydrophobic interactions.

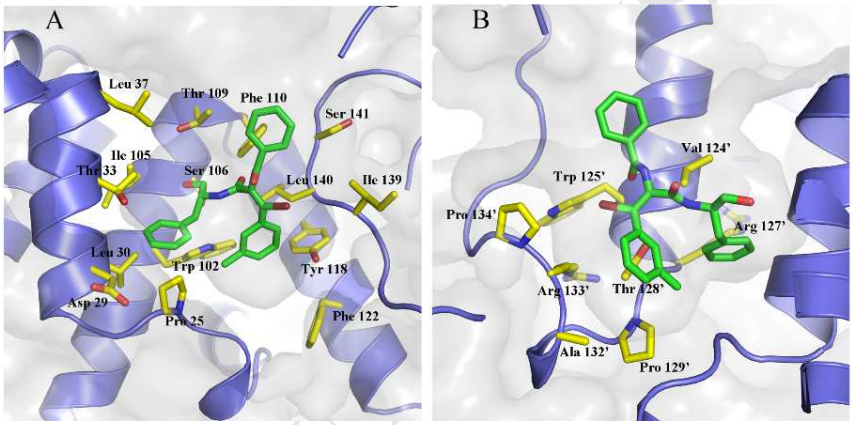


Figure 5. Binding mode of compound **8d** in the dimer-dimer interface of HBV core protein. (A) The ligand **8d** accommodates in the hydrophobic groove formed by the dimer-dimer interface, with the lid removed for clarity. (B) The ligand **8d** binds to the lid, with the groove removed for clarity. The protein and ligand **8d** are shown in cartoon and stick mode, respectively. The key resides and ligand **8d** are colored in yellow and green, respectively.

4. Conclusions

Overall, a series of phenyl propionamide derivatives (**3a-b**, **4a-e**, **7a-g**, **8a-h** and **9a-b**) was designed and synthesized as novel non-nucleoside anti-HBV agents. Among them, compounds **8d** and **9b** displayed most potent anti-HBV activity with IC_{50} values on HBV DNA replication of 0.46 and 0.14 μM, respectively. And the selective index values of **8d** and **9b** were more than 217.39 and 153.14, suggesting that **8d and 9b** exhibited favorable safety profiles. Interestingly, **8d** and **9b** possessed significantly antiviral activities

against lamivudine and entecavir resistant HBV mutants with IC_{50} values of 0.77 and 0.32 μM. Notably, preliminary anti-HBV action mechanism studies showed that **8d** could inhibit intracellular HBV pgRNA and RT activity of the HBV polymerase. Molecular docking studies suggested that compound **8d** could fit into the dimer-dimer interface of HBV core protein by hydrophobic interaction. In addition, in silico prediction of physicochemical properties showed that compounds **8d** conformed well to the Lipinski's rule of five. Considering the significantly anti-HBV activity, low toxicity, diverse anti-HBV mechanism and favorable physicochemical properties, **8d** might be novel non-nucleoside anti-HBV candidates to warrant further investigation.

5. Experimental protocols

5.1. Chemical analysis

All of the synthesized compounds were purified by column chromatography on silica gel 60 (200–300 mesh) or thin layer chromatography (TLC) on silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). Melting points were measured on a model YRT-3 apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL (400 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 with Me_4Si (TMS) as an internal standard. MS detection was performed using an Agilent G6460C triple quadrupole mass spectrometer. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. The purity of all investigated compounds in biological testing was determined as ≥ 95% unless otherwise stated. Analytical HPLC was run on the Agilent 1260 HPLC instrument, equipped with Agilent SB-C18 column and UV detection at 254 nm. Eluent system was: 80% MeOH in H_2O; flow rate = 0.2 mL/min.

*5.1.1. General procedure for the synthesis of compounds **3a-b***

Isobutyl chloroformate (IBCF) (1.1 mmol) was added dropwise to the mixture of benzoic acid (1.0 mmol), L-phenylalanine methyl ester hydrochloride (1.1 mmol) and N-methylmorpholine (NMM) (2.3 mmol) in CH_2Cl_2 (50 mL) at 0 °C within 30 min. The mixture was stirred for 30 min and the bulk of CH_2Cl_2 was removed *in vacuo*. The residue was dissolved in ethyl acetate and washed sequentially with water, 5% HCl, saturated $NaHCO_3$ solution and brine, dried with anhydrous Na_2SO_4. Removal of the solvent gave a residue which was recrystallized from ethyl acetate to afford target compound **1**. 2 M NaOH (1 mL) was added to the solution of compound **1** (1.0 mmol) in DMF (5 mL). After stirring at room

temperature for 2 h, the mixture was acidified to pH 6-7 with concentrated hydrochloric acid, and partitioned between ethyl acetate and water. The organic phase was separated and washed with brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give compound **2**. Compound **3a-b** was prepared with a similar procedure to that described for the synthesis of compound **1**, and the crude product was purified by column chromatography to yield the title compound, respectively.

*5.1.1.1. (S)-N-(1-oxo-3-phenyl-1-(piperidin-1-yl)propan-2-yl)benzamide (**3a**)*

As a yellow oil, yield: 41.2%. Analytical data for **3a**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.78-7.80 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.47-7.51 (t, 1H, Ar-H), 7.40-7.43 (t, 2H, Ar-H), 7.21-7.30 (m, 5H, Ar-H), 5.36-5.41 (m, 1H, CH), 3.46-3.57 (m, 2H, CH_2), 3.04-3.30 (m, 4H, 2×CH_2), 0.83-1.60 (m, 6H, 3×CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 169.39, 166.54, 136.32, 134.12, 131.66, 129.76 (2C), 128.58 (2C), 128.55 (2C), 127.15 (2C), 127.09, 50.24, 46.71, 43.24, 39.86, 25.98, 25.47, 24.33; ESI-MS: m/z 359.1 $[M+Na]^+$, HRESIMS: calcd for $C_{21}H_{25}N_2O_2$ $[M+H]^+$ 337.1910, found 337.1900. Purity: 99.8%.

*5.1.1.2. (S)-N-(3-(4-hydroxyphenyl)-1-oxo-1-(piperidin-1-yl)propan-2-yl)benzamide (**3b**)*

As a yellow oil, yield: 43.7%. Analytical data for **3b**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.78-7.80 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.48-7.52 (t, 1H, Ar-H), 7.41-7.43 (t, 2H, Ar-H), 7.00 (d, *J* = 8.0 Hz, 2H, H-5, 9), 6.62 (d, *J* = 8.0Hz, 2H, H-6, 8), 5.34-5.39 (m, 1H, CH), 3.46-3.57 (m, 2H, CH_2), 3.03-3.29 (m, 4H, 2×CH_2), 0.81-1.60 (m, 6H, 3×CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 169.39, 166.54, 157.09, 134.21, 131.64, 130.66 (2C), 128.61, 128.57 (2C), 127.14 (2C), 114.64 (2C), 51.45, 46.70, 46.65, 38.91, 26.05, 26.03, 24.34; ESI-MS: m/z 353.2 $[M+H]^+$, HRESIMS: calcd for $C_{21}H_{25}N_2O_3$ $[M+H]^+$ 353.1860, found 353.1852. Purity: 92.9%.

*5.1.2. General procedure for the synthesis of compounds **4a-e***

Compound **3b** (0.5 mmol) was followed by the treatment with 2-dimethylaminoethyl chloride hydrochloride (0.6 mmol) and K_2CO_3 (3.5 mmol) in dioxane (15 mL) at 90 °C for 3 h. The crude product was purified by column chromatography to yield the title compound, respectively.

*5.1.2.1. (S)-N-(3-(4-(2-(dimethylamino)ethoxy)phenyl)-1-oxo-1-(piperidin-1-yl)propan-2-yl)benzamide (**4a**)*

As a yellow oil, yield: 36.4%. Analytical data for **4a**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.77-7.80 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.47-7.51 (t, 1H, Ar-H), 7.40-7.44 (t, 2H, Ar-H), 7.10-7.12 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.82-6.84 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.31-5.36 (m, 1H, CH), 4.03-4.06 (t, 2H, OCH_2), 3.22-3.34 (m, 4H,

2×CH_2), 2.92-3.13 (m, 2H, CH_2), 2.72-2.75 (t, 2H, NCH_2), 2.35 (s. 6H, 2×CH_3), 0.90-1.92 (m, 6H, 3×CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 169.44, 166.50, 157.93, 134.21, 131.64, 130.66 (2C), 128.61, 128.57 (2C), 127.14 (2C), 114.64 (2C), 65.97, 58.25, 51.45, 46.71, 46.65, 45.82 (2C), 38.92, 26.06, 26.04, 24.35; ESI-MS: m/z 424.2 $[M+H]^+$, HRESIMS: calcd for $C_{21}H_{25}N_2O_2$ $[M+H]^+$ 424.2595, found 424.2593. Purity: 90.6%.

*5.1.2.2. (S)-N-(3-(4-(2-(dimethylamino)ethoxy)phenyl)-1-oxo-1-(pyrrolidin-1-yl)propan-2-yl)benzamide (**4b**)*

As a yellow oil, yield: 38.1%. Analytical data for **4b**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.78-7.80 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.47-7.51 (t, 1H, Ar-H), 7.40-7.44 (t, 2H, Ar-H), 7.09-7.12 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.80-6.85 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.05-5.11 (m, 1H, CH), 4.03-4.06 (t, 2H, OCH_2), 3.27-3.49 (m, 4H, 2×CH_2), 2.91-3.13 (m, 2H, CH_2), 2.72-2.75 (t, 2H, NCH_2), 2.35 (s. 6H, 2×CH_3), 1.58-1.92 (m, 4H, 2×CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 169.79, 166.61, 157.95, 134.12, 131.67, 130.56 (2C), 128.60, 128.58 (2C), 127.16 (2C), 114.62 (2C), 66.09, 58.33, 52.96, 46.50, 46.39, 45.79 (2C), 38.92, 25.91, 25.88; ESI-MS: m/z 410.2 $[M+H]^+$, HRESIMS: calcd for $C_{24}H_{32}N_3O_3$ $[M+H]^+$ 410.2438, found 410.2434. Purity: 92.2%.

*5.1.2.3. (S)-N-(3-(4-(2-(dimethylamino)ethoxy)phenyl)-1-morpholino-1-oxopropan-2-yl)benzamide (**4c**)*

As a yellow oil, yield: 37.2%. Analytical data for **4c**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.79-7.81 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.49-7.53 (t, 1H, Ar-H), 7.41-7.45 (t, 2H, Ar-H), 7.13-7.15 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.85-6.87 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.27-5.33 (m, 1H, CH), 4.03-4.06 (t, 2H, OCH_2), 2.86-3.63 (m, 5H, 4×CH_2, CH), 2.73-2.76 (t, 2H, NCH_2), 2.35 (s. 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 170.14, 166.62, 158.15, 133.95, 131.83, 130.67 (2C), 130.58, 128.66 (2C), 127.14 (2C), 114.79 (2C), 66.57, 66.23, 65.99, 58.26, 50.20, 45.88 (2C), 39.25; ESI-MS: m/z 426.2 $[M+H]^+$, HRESIMS: calcd for $C_{24}H_{32}N_3O_4$ $[M+H]^+$ 426.2387, found 426.2373. Purity: 94.0%.

*5.1.2.4. (S)-N-(1-(diethylamino)-3-(4-(2-(dimethylamino)ethoxy)phenyl)-1-oxopropan-2-yl)benzamide (**4d**)*

As a yellow oil, yield: 40.9%. Analytical data for **4d**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.78-7.80 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.47-7.51 (t, 1H, Ar-H), 7.40-7.43 (t, 2H, Ar-H), 7.12-7.14 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.81-6.83 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.20-5.26 (m, 1H, CH), 4.02-4.05 (t, 2H, OCH_2), 3.49-3.86 (m, 2H, CH_2), 2.92-3.13 (m, 4H, 4×CH_2), 2.71-2.74 (t, 2H, NCH_2), 2.34 (s. 6H, 2×CH_3), 0.97-1.09 (m, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 170.63, 166.42, 157.95, 143.13, 131.66, 130.68 (2C), 130.59, 128.67

(2C), 127.15 (2C), 114.60 (2C), 65.98, 58.36, 50.76, 45.94 (2C), 41.76, 40.58, 39.25, 14.29, 12.91; ESI-MS: m/z 412.2 $[M+H]^+$, HRESIMS: calcd for $C_{24}H_{33}N_3O_3Na$ $[M+Na]^+$ 434.2414, found 434.2419. Purity: 93.8%.

5.1.2.5.

*N-((S)-3-(4-(2-(dimethylamino)ethoxy)phenyl)-1-(((S)-1-hydroxy-3-methylbutan-2-yl)amino)-1-oxopropan-2-yl)benzamide (**4e**)*

As a yellow oil, yield: 36.9%. Analytical data for **4e**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.74-7.76 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.48-7.51 (t, 1H, Ar-H), 7.38-7.42 (t, 2H, Ar-H), 7.19-7.21 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.83-6.85 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.85-4.90 (m, 1H, CH), 4.06-4.07 (t, 2H, OCH_2), 3.42-3.53 (m, 2H, CH_2), 3.05-3.23 (m, 3H, CH_2, CH), 2.79-2.81 (t, 2H, NCH_2), 2.39 (s. 6H, 2×CH_3), 0.78-0.85 (m, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 171.59, 167.47, 157.74, 133.79, 131.91, 130.52 (2C), 129.17, 128.67 (2C), 127.22 (2C), 114.87 (2C), 65.55, 63.06, 58.02, 57.23, 55.65, 45.58 (2C), 37.94, 28.87, 19.45, 18.78; ESI-MS: m/z 442.2 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{35}N_3O_4Na$ $[M+Na]^+$ 464.2510, found 464.2527. Purity: 98.4%.

*5.1.3. General procedure for the synthesis of compounds **7a-g*** [15]

Compounds **5** was obtained by the acylation reaction with glycine (0.01 mol) and benzoyl chloride or benzoyl chloride derivatives (0.02 mol) as the raw materials in sodium hydroxide solution, which was then reacted with benzaldehyde or benzaldehyde derivatives (0.01 mol) in the presence of acetic anhydride (Ac_2O) (10 mL) and sodium acetate (AcONa) (0.01 mol) to obtain compounds **6**. Next, the intermediate mentioned above was reacted with L-phenylalaninol (0.01 mol). The crude product was purified by column chromatography to yield the title compound **7a-g**, respectively.

5.1.3.1.

*(S,E)-N-(3-((1-hydroxy-3-phenylpropan-2-yl)amino)-1-(4-hydroxyphenyl)-3-oxoprop-1-en-2-yl)benzamide (**7a**)*

As a white powder, yield: 39.3%; mp: 82.5-83.6 °C. Analytical data for **7a**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.92 (s, 1H, OH), 7.98-8.00 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.83-7.85 (d, *J* = 8.0 Hz, 1H, NH), 7.52-7.58 (m, 5H, Ar-H), 7.17-7.25 (m, 5H, Ar-H), 7.11-7.13 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.06 (s, 1H, CH=), 4.75-4.77 (t, 1H, OH), 4.02-4.07 (m, 1H, CH), 3.30-3.44 (m, 2H, CH_2), 2.75-2.90 (m, 2H, CH_2); ^{13}C NMR

(DMSO, 100 MHz, δ ppm): 166.42, 165.41, 150.87, 139.75, 134.15, 132.49, 132.29, 131.01, 130.91 (2C), 129.75 (2C), 128.90 (2C), 128.66 (2C), 128.42 (2C), 127.97, 126.43, 122.48 (2C), 62.82, 53.66, 36.84; ESI-MS: m/z 417.2 $[M+H]^+$, HRESIMS: calcd for $C_{22}H_{25}N_2O_4$ $[M+H]^+$ 417.1809, found 417.1821. Purity: 98.9%.

5.1.3.2.

(S,E)-N-(1-(4-bromophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide

(7b)

As a white powder, yield: 48.1%; mp: 85.5-86.4°C. Analytical data for **7b**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.90 (s, 1H, NH), 7.98-8.00 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.89-7.91 (d, *J* = 8.8 Hz, 1H, NH), 7.46-7.62 (m, 7H, Ar-H), 7.16-7.27 (m, 5H, Ar-H), 6.99 (s, 1H, CH=), 4.74-4.77 (t, 1H, OH), 3.99-4.08 (m, 1H, CH), 3.32-3.49 (m, 2H, CH_2), 2.76-2.92 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.36, 165.38, 139.68, 134.19, 134.03, 132.36, 131.99 (2C), 131.73, 131.62 (2C), 129.73 (2C), 128.94 (2C), 128.68 (2C), 128.39 (2C), 127.33, 126.49, 122.12, 62.84, 53.67, 36.83; ESI-MS: m/z 479.01 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{24}BrN_2O_3$ $[M+H]^+$ 479.0965, found 479.0952. Purity: 99.9%.

5.1.3.3.

(S,E)-N-(1-(4-cyanophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide

(7c)

As a white powder, yield: 50.6%; mp: 94.5-95.3°C. Analytical data for **7c**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 10.01 (s, 1H, NH), 8.02-8.04 (d, *J* = 8.0 Hz, 1H, NH), 7.97-7.99 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.81-7.83 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.66-7.69 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.59-7.63 (t, 1H, Ar-H), 7.51-7.55 (t, 2H, Ar-H), 7.17-7.28 (m, 5H, Ar-H), 7.00 (s, 1H, CH=), 4.75-4.77 (t, 1H, OH), 4.01-4.07 (m, 1H, CH), 3.32-3.50 (m, 2H, CH_2), 2.76-2.93 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.35, 165.18, 139.95, 139.69, 133.90, 133.79, 132.85 (2C), 132.46, 130.25 (2C), 129.73 (2C), 128.95 (2C), 128.69 (2C), 128.43 (2C), 126.50, 126.07, 119.26, 110.82, 62.86, 53.78, 36.83; ESI-MS: m/z 426.2 $[M+H]^+$, HRESIMS: calcd for $C_{26}H_{24}N_3O_3$ $[M+H]^+$ 426.1812, found 426.1798. Purity: 99.6%.

5.1.3.4. (S,E)-N-(3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-(m-tolyl)prop-1-en-2-yl)benzamide

(7d)

As a white powder, yield: 47.2%; mp: 70.9-71.6°C. Analytical data for **7d**: ^{1}H NMR (DMSO, 400 MHz,

δ ppm): 9.87 (s, 1H, NH), 7.98-8.80 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.79-7.81 (d, *J* = 8.0 Hz, 1H, NH), 7.58-7.61 (t, 1H, Ar-H), 7.51-7.55 (t, 2H, Ar-H), 7.10-7.35 (m, 9H, Ar-H), 7.06 (s, 1H, CH=), 4.73-4.76 (t, 1H, OH), 3.99-4.07 (m, 1H, CH), 3.31-3.47 (m, 2H, CH_2), 2.76-2.91 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.49, 165.44, 139.70, 137.99, 134.71, 134.26, 132.26. 130.79, 130.53, 129.76 (2C), 129.25, 128.92, 128.90 (2C), 128.67 (2C), 128.32 (2C), 126.88, 126.46, 62.81, 53.58, 36.82, 21.50; ESI-MS: m/z 415.2 $[M+H]^+$, HRESIMS: calcd for $C_{26}H_{27}N_2O_3$ $[M+H]^+$ 415.2016, found 415.2007. Purity: 100.0%.

5.1.3.5.

(S,E)-N-(1-(2-chlorophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide

(7e)

As a white powder, yield: 43.9%; mp: 107.7-108.5°C. Analytical data for **7e**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.83 (s, 1H, NH), 8.00-8.02 (d, *J* = 8.0 Hz, 1H, NH), 7.93-7.94 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.49-7.61 (m, 5H, Ar-H), 7.18-7.34 (m, 7H, Ar-H), 7.11 (s, 1H, CH=), 4.76-4.79 (t, 1H, OH), 4.00-4.08 (m, 1H, CH), 3.33-3.52 (m, 2H, CH_2), 2.77-2.95 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.49, 165.18, 139.73, 133.79, 133.67, 133.35, 133.22, 132.23, 130.37, 130.10, 130.01, 129.73 (2C), 128.86 (2C), 128.68 (2C), 128.38 (2C), 127.57, 126.46, 123.88, 62.94, 53.77, 36.80, ESI-MS: m/z 435.2 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{24}ClN_2O_3$ $[M+H]^+$435.1470, found 435.1461. Purity: 99.8%.

5.1.3.6.

(S,E)-N-(1-(2-bromophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide

(7f)

As a white powder, yield: 41.5%; mp: 99.7-100.4°C. Analytical data for **7f**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.83 (s, 1H, NH), 7.98-8.00 (d, *J* = 8.0 Hz, 1H, NH), 7.90-7.92 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.68-7.70 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.47-7.59 (m, 4H, Ar-H), 7.17-7.29 (m, 7H, Ar-H), 7.05 (s, 1H, CH=), 4.74-4.77 (t, 1H, OH), 3.98-4.07 (m, 1H, CH), 3.31-3.50 (m, 2H, CH_2), 2.75-2.93 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.54, 165.16, 139.71, 135.11, 133.97, 133.21, 132.99, 132.33, 130.56, 130.25, 129.73 (2C), 128.84 (2C), 128.72 (2C), 128.38 (2C), 128.09, 126.46, 126.34, 124.53, 62.91, 53.77, 36.78; ESI-MS: m/z 479.1 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{24}BrN_2O_3$ $[M+H]^+$ 479.0965, found 479.0952. Purity: 97.5%.

5.1.3.7.

*(S,E)-2-fluoro-N-(3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)benzamide (**7g**)*

As a white powder, yield: 45.7%; mp: 105.7-106.2°C. Analytical data for **7g**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.80 (s, 1H, NH), 7.78-7.80 (d, *J* = 8.0 Hz, 1H, NH), 7.67-7.71 (t, 1H, Ar-H), 7.58-7.62 (m, 3H, Ar-H), 7.17-7.41 (m, 10H, Ar-H), 7.05 (s, 1H, CH=), 4.79-4.81 (t, 1H, OH), 4.00-4.08 (m, 1H, CH), 3.38-3.48 (m, 2H, CH_2), 2.75-2.93 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.15, 163.86, 158.68, 139.63, 134.58, 130.82, 130.79. 130.11, 129.83 (2C), 129.73 (2C), 129.20, 129.01 (2C), 128.96, 128.69 (2C), 126.51, 125.08, 124.00, 116.87, 62.76, 53.58, 36.92; ESI-MS: m/z 419.2 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{24}FN_2O_3$ $[M+H]^+$ 419.1766, found 419.1757. Purity: 99.3%.

*5.1.4. General procedure for the synthesis of compounds **8a-h*** [15]

Finally, the crude reaction product (**7a-h**) (0.01 mol) was directly brominated with bromine (0.015 mol) and anhydrous calcium carbonate (0.01 mol) in $CHCl_3$. The crude product was purified by column chromatography to yield the title compound **8a-h**, respectively.

5.1.4.1.

*(S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-1-(4-hydroxyphenyl)-3-oxoprop-1-en-2-yl)benzamide (**8a**)*

As a yellow powder, yield: 18.0%, m.p.223.7-224.6 °C. Analytical data for **8a**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.87 (s, 1H, OH), 9.80 (br, 1H, NH), 7.99-8.01 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.64-7.66 (d, *J* = 8.0 Hz, 1H, NH), 7.59-7.63 (t, 1H, Ar-H), 7.51-7.55 (t, 2H, Ar-H), 7.08-7.23 (m, 7H, Ar-H), 6.68-6.70 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.19-4.27 (m, 1H, OH), 3.73-3.81 (m, 1H, CH), 3.01-3.21 (m, 2H, CH_2), 2.44-2.62 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.65, 163.47, 158.80, 139.47, 133.65, 132.42, 131.21, 129.58 (4C), 128.97 (2C), 128.87, 128.61 (2C), 128.40 (2C), 126.35, 122.69, 115.44 (2C), 62.16, 53.36, 36.25; ESI-MS: m/z 495.1 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{24}BrN_2O_4$ $[M+H]^+$ 495.0914, found 495.1089. Purity: 98.5%.

5.1.4.2.

*(S,Z)-N-(1-bromo-1-(4-bromophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide (**8b**)*

As a white powder, yield: 15.4%, m.p.99.7-101.1 °C. Analytical data for **8b**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.90 (s, 1H, NH), 7.96-7.98 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.84-7.87 (d, *J* = 8.0 Hz, 1H, NH), 7.57-7.61 (t, 1H, Ar-H), 7.49-7.52 (t, 2H, Ar-H), 7.43-7.45 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.13-7.28 (m, 5H, Ar-H), 7.03-7.05 (d, *J* = 8.0 Hz, 2H, Ar-H), 3.70-3.77 (m, 1H, CH), 3.01-3.21 (m, 2H, CH_2), 2.39-2.62 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.58, 162.98, 139.47, 137.68, 134.43, 133.47, 132.31, 131.64 (2C), 129.50 (2C), 129.09 (2C), 128.99 (2C), 128.61 (2C), 128.46 (2C), 126.34, 122.85, 119.54, 62.23, 53.46, 36.17; ESI-MS: m/z 558 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{23}Br_2N_2O_3$ $[M+H]^+$ 557.0070, found 557.0065. Purity: 94.2%.

5.1.4.3.

*(S,Z)-N-(1-bromo-1-(4-cyanophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide (**8c**)*

As a white powder, yield: 15.9%, m.p.98.8-99.4 °C. Analytical data for **8c**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.96 (s, 1H, NH), 7.97-7.99 (m, 2H, Ar-H), 7.66-7.68 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.57-7.61 (t, 1H, Ar-H), 7.49-7.53 (t, 2H, Ar-H), 7.38-7.40 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.14-7.19 (m, 3H, Ar-H), 7.02-7.04 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.20 (br, 1H, OH), 3.71-3.79 (m, 1H, CH), 3.00-3.21 (m, 2H, CH_2), 2.38-2.63 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.73, 162.82, 143.04, 139.41, 135.58, 133.36, 132.79, 132.54 (2C), 130.41 (2C), 129.43 (2C), 129.04 (2C), 128.62 (2C), 128.52 (2C), 126.35, 119.01, 118.00, 62.21, 53.46, 36.10; ESI-MS: m/z 504 $[M+H]^+$, HRESIMS: calcd for $C_{26}H_{23}BrN_3O_3$ $[M+H]^+$ 504.0917, found 504.0906. Purity: 97.1%.

5.1.4.4.

*(S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-(m-tolyl)prop-1-en-2-yl)benzamide (**8d**)*

As a white powder, yield: 14.3%, m.p.98.9-100.6 °C. Analytical data for **8d**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.91 (s, 1H, NH), 8.01-8.03 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.67-7.69 (d, *J* = 8.0 Hz, 1H, NH), 7.60-7.62 (t, 1H, Ar-H), 7.52-7.56 (t, 2H, Ar-H), 7.06-7.21 (m, 9H, Ar-H), 3.73-3.75 (m, 1H, CH), 2.99-3.17 (m, 2H, CH_2), 2.41-2.58 (m, 2H, CH_2), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.62, 163.18, 139.38, 138.29, 137.78, 133.82, 133.54, 132.63, 130.05, 129.54 (2C), 129.00 (2C), 128.99, 128.61 (2C), 128.45 (2C), 127.98, 126.80, 126.36, 121.52, 61.87, 53.34, 36.08, 31.43; ESI-MS: m/z 493

$[M+H]^+$, HRESIMS: calcd for $C_{26}H_{25}BrN_2O_3Na$ $[M+Na]^+$ 515.0941, found 515.0942. Purity: 97.4%.

5.1.4.5.

*(S,Z)-N-(1-bromo-1-(2-chlorophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benz amide (**8e**)*

As a white powder, yield: 19.7%, m.p. 79.3-82 °C. Analytical data for **8e**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.64 (d, *J* = 8.0 Hz, 1H, NH), 7.73-8.00 (m, 3H, NH, Ar-H), 7.57-7.60 (t, 1H, Ar-H), 7.46-7.53 (m, 3H, Ar-H), 7.02-7.41 (m, 8H, Ar-H), 4.54-4.61 (t, 1H, OH), 3.65-3.70 (m, 1H, CH), 2.97-3.16 (m, 2H, CH_2), 2.35-2.63 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.09, 162.11, 139.20, 137.48, 135.77, 133.54, 132.97, 132.79, 131.63, 130.13, 129.79, 129.52 (2C), 128.97 (2C), 128.63 (2C), 128.58 (2C), 127.66, 126.41, 62.10, 51.98, 36.19; ESI-MS: m/z 513.1 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{23}BrClN_2O_3$ $[M+H]^+$ 513.0575, found 513.0569. Purity: 95.5%.

5.1.4.6.

*(S,Z)-N-(1-bromo-1-(2-bromophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benz amide (**8f**)*

As a white powder, yield: 17.1%, m.p.58.8-59.9 °C. Analytical data for **8f**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.94 (s, 1H, NH), 7.99-8.01 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.63-7.65 (d, *J* = 8.0 Hz, 1H, NH),7.57-7.61 (t, 1H, Ar-H), 7.49-7.53 (t, 2H, Ar-H), 7.35-7.36 (d, *J* = 4.0 Hz, 1H, Ar-H), 7.10-7.30 (m, 6H, Ar-H), 7.02-7.04 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.40-4.60 (t, 1H, OH), 3.63-3.70 (m, 1H, CH), 2.94-3.13 (m, 2H, CH_2), 2.33-2.63 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.03, 162.01, 139.42, 139.13, 135.52, 133.56, 133.34, 131.64, 131.39, 131.13, 129.54 (2C), 128.96 (2C), 128.64 (2C), 128.59 (2C), 128.23, 126.42, 123.29, 121.09, 61.98, 51.92, 36.17; ESI-MS: m/z 559 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{23}Br_2N_2O_3$ $[M+H]^+$ 557.0070, found 557.0064. Purity: 96.8%.

5.1.4.7.

*(S,E)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-2-fluorobenz amide (**8g**)*

As a white powder, yield: 16.3%, m.p.124.6-125.4°C. Analytical data for **8g**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.91 (s, 1H, NH), 8.01-8.03 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.67-7.69 (d, *J* = 8.0 Hz, 1H, NH), 7.60-7.64 (t, 1H, Ar-H), 7.52-7.56 (t, 2H, Ar-H), 7.06-7.27 (m, 9H, Ar-H), 3.73-3.75 (m, 1H, CH),

2.98-3.18 (m, 2H, CH_2), 2.41-2.58 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.38, 166.02, 162.54, 139.37, 138.13, 137.82, 134.05, 133.00, 129.88 (2C), 128.55 (2C), 129.16, 129.11, 128.98 (2C), 128.86, 128.63 (2C), 127.19, 126.37, 116.99, 62.38, 53.26, 36.12; ESI-MS: m/z 497 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{22}BrFN_2O_3Na$ $[M+Na]^+$ 519.0690, found 519.0686. Purity: 95.1%.

5.1.4.8.

*(S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)benzamide (**8h**)*

As a yellow powder, yield: 29.4%, m.p. 89.7-90.5°C. Analytical data for **8h**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.95 (s, 1H, NH), 8.01-8.03 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.76-7.78 (d, *J* = 8.0 Hz, 1H, NH), 7.60-7.65 (t, 1H, Ar-H), 7.52-7.57 (t, 2H, Ar-H), 7.07-7.32 (m, 10H, Ar-H), 3.70-3.80 (m, 1H, CH), 2.97-3.17 (m, 2H, CH_2), 2.39-2.59 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.6, 163.11, 139.40, 138.39, 133.93, 133.50, 129.91, 129.59 (2C), 129.56 (2C), 129.01 (2C), 128.99, 128.67 (2C), 128.63 (2C), 128.45 (2C), 126.27, 121.21, 62.05, 53.31, 36.10; ESI-MS: m/z 479 $[M+H]^+$, HRESIMS: calcd for $C_{25}H_{23}BrN_2O_3Na$ $[M+Na]^+$ 501.0784, found 501.0781. Purity: 98.6%.

*5.1.5. General procedure for the synthesis of compounds **9a-b***

Compound **7a** or **8a** (0.01 mol) was alkylated with 2-dimethylaminoethyl chloride hydrochloride (0.012 mol) to provide alkoxyl substituted derivatives **9a** or **9b**. The crude product was purified by column chromatography to yield the title compound, respectively.

5.1.5.1.

*(S,E)-N-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide (**9a**)*

The title compound was obtained starting from **7a** and 2-dimethylaminoethyl chloride hydrochloride. As a white powder, yield: 24.9%, m.p. 114.1-114.9 °C. Analytical data for **9a**: 1H NMR (DMSO, 400 MHz, δ ppm): 9.77 (s, 1H, NH), 7.97-7.99 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.90-7.93 (d, *J* = 8.0 Hz, 1H, NH),7.45-7.58 (m, 5H, Ar-H), 7.16-7.25 (m, 5H, Ar-H), 7.00 (s, 1H, CH=), 6.88-6.90 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.24-4.31 (m, 1H, CH_2), 3.97-4.02 (m, 4H, 2×CH_2), 2.76-2.85 (8, 2H, CH_2), 2.54-2.57 (t, 2H, CH_2), 2.15 (s, 6H, 2×CH_3); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.26, 165.84, 159.32, 138.83, 134.26, 132.20, 131.58 (2C), 129.70 (2C), 129.26, 128.87 (2C), 128.76 (2C), 128.73, 128.40 (2C), 127.12, 126.71, 115.04 (2C), 66.36,

65.25, 58.12, 50.15, 46.03 (2C), 37.14; ESI-MS: m/z 488.2 $[M+H]^+$, HRESIMS: calcd for $C_{29}H_{34}N_3O_4$ $[M+H]^+$ 488.2544, found 488.2561. Purity: 99.0%.

5.1.5.2.

*(S,Z)-N-(1-bromo-1-(4-(2-(dimethylamino)ethoxy)phenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide (**9b**)*

The title compound was obtained starting from **8a** and 2-dimethylaminoethyl chloride hydrochloride. As a white powder, yield: 13.6%, m.p.183.0-183.8 °C. Analytical data for **9b**: 1H NMR (DMSO, 400 MHz, δ ppm): 8.27-8.30 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.12-7.15 (dd, 2H, *J* = 8.0 Hz, 8.0 Hz, Ar-H), 8.04-8.02 (d, *J* = 8.0 Hz, 1H, NH), 7.59-7.61 (m, 3H, Ar-H), 7.16-7.31 (m, 5H, Ar-H), 7.06-7.08 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.98-5.01 (t, 1H, OH), 4.20-4.26 (m, 1H, CH), 4.12-4.15 (t, 2H, CH_2), 3.49-3.55 (m, 2H, CH_2), 2.91-2.96 (m, 2H, CH_2), 2.64-2.67 (t, 2H, CH_2), 2.23 (s, 6H, $2\times CH_3$); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 161.01, 160.25, 157.84, 139.54, 131.67, 130.14, 129.75, 129.73, 129.68 (4C), 128.75 (4C), 126.88, 126.57, 126.51, 119.87, 114.90 (2C), 66.48, 62.57, 58.13, 52.85, 46.08 (2C), 37.16; ESI-MS: m/z 566.2 $[M+H]^+$, HRESIMS: calcd for $C_{29}H_{33}BrN_3O_4$ $[M+H]^+$ 566.1649, found 566.1654. Purity: 95.0%.

5.2. Biological assays

HepG2 cell lines were obtained from 302 military hospital of china. The cell lines were cultivated in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.38 mg/mL of G418. Cytotoxicity, anti-HBV activities of compounds and effect on HBV viral gene expression were evaluated on the HepG2 2.2.15 cell line (stably transfected cell lines containing HBV genome on a plasmid). Determination of antiviral efficacy against nucleoside analogue-resistant HBV was evaluated on the HepG2 cell line which were transiently transfected respectively with plasmids containing drug-resistant mutant (rtL180M + rtM204V + rtT184L).

5.2.1. Cytotoxicity assays

Cytotoxicity induced by the target compounds in cultures of HepG2 2.2.15 cells was also determined. Briefly, HepG2 2.2.15 cells were grown to confluence in 96-well culture plates, and then treated with test compounds (in 0.2 mL culture medium/well), respectively. Untreated control cultures were maintained on each 96-well plate. The cytotoxicity was determined by measuring MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye uptake, as determined from the

absorbance at 490 nm relative to untreated cells, at 24 hours following 72 h of treatment.

5.2.2. Inhibitory effect on HBV DNA replication

For detection of extracellular replication intermediates, HepG2 2.2.15 cells were seeded in 24 well culture plates at a density of 2×10^5 cells per well and allowed to recover for 24 h. Culture medium was replaced by assay medium containing the compound that is being tested or 3TC. Every 2 days, medium was changed. After 6 days, intracellular HBV DNA was extracted using method reported before[16], and the levels were quantified by real time PCR.

5.2.3. Determination of antiviral efficacy against nucleoside analogue-resistant HBV [17]

HepG2 cells per well (2×10^5) were plated into 24-well plates. After overnight incubation, the cells were transiently transfected respectively with plasmids containing drug-resistant mutant (rtL180M + rtM204V + rtT184L) for 24 h. The supernatant was discarded, followed by PBS wash. Then, the cells were cultivated with fresh medium containing lamivudine (100 μM), Entecavir (10 μM), **8a** (1 μM), **8d** (1 μM) or **9b** (10 μM). After 72 h treatment, HBV DNA replicative intermediate was extracted using the method mentioned above [16], and the level was quantified using real time PCR.

5.3. *Anti-HBV action mechanism of compound **8d***

*5.3.1. Effect of compound **8d** on HBV viral gene expression* [18, 19]

HepG2 2.2.15 cells were cultured in 6-well plates at a density of 6×10^5 cells, and were treated with compounds for 6 days. Total RNA was extracted from HepG2 2.2.15 cells by the use of TRIzol reagent (Invitrogen), and then detected by quantitative real time PCR.

*5.3.2. Evaluation of the Inhibitory Activity of Compounds **8d** on the DHBV RT Activity*

The DHBV polymerases were obtained from Beijing Weijian Jiye Institute of Biotechnology (302 military hospital of china). The expression of the DHBV polymerases and the analysis of elongation of viral minus strand DNA were performed as described in detail [20]. The reaction mixture included [α^{32}P]-dNTP (0.165 μmol/L; 3,000 Ci/mmol) of which the inhibitor is the analog, whereas the 3 other cold dNTPs were used at 100 μmol/L each.

5.3.3. Molecular docking

For the purpose of investigating the possible binding interactions between HBV core protein and compound **8d**, Surflex docking module in Sybyl X-2.1 was used for molecular docking studies [21]. The

crystal structure of HBV core protein complex with NVR-010-001-E2 (PDB ID: 5EOI) was obtained from the RCSB Protein Data Bank [22]. The space where ligand NVR-010-001-E2 placed was selected as the active pocket, and all water molecules were removed. The missing hydrogen atoms were added by biopolymer module during protein preparation, the docking parameters were kept as default values.

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References and notes

[1] M.Y. Zhu, W. Li, Y. Lu, X. Dong, B. Lin, Y. Chen, X.E. Zhang, J.L. Guo, M.S. Li, HBx drives alpha fetoprotein expression to promote initiation of liver cancer stem cells through activating PI3K/AKT signal pathway, Int. J. Cancer, 140 (2017) 1346-1355.

[2] B.Y. Winer, T. Huang, B.E. Low, C. Avery, M.A. Pais, G. Hrebikova, E. Siu, L. Chiriboga, M.V. Wiles, A. Ploss, Recapitulation of treatment response patterns in a novel humanized mouse model for chronic hepatitis B virus infection, Virology, 502 (2017) 63-72.

[3] J. Wang, T. Shen, X.B. Huang, G.R. Kumar, X.M. Chen, Z.Z. Zeng, R.Y. Zhang, R. Chen, T. Li, T.Y. Zhang, Q. Yuan, P.C. Li, Q. Huang, R. Colonno, J.D. Jia, J.L. Hou, M.A. McCrae, Z.L. Gao, H. Ren, N.S. Xia, H. Zhuang, F.M. Lu, Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound, J. Hepatol., 65 (2016) 700-710.

[4] Y.J. Wang, L. Yang, J.P. Zuo, Recent developments in antivirals against hepatitis B virus, Virus Res., 213 (2016) 205-213.

[5] G.Q. Yin, B. Zhong, Efficacy of interferon for chronic hepatitis B in patients with nucleoside and nucleotide combination therapy failure, J. Gastroen. Hepatol., 31 (2016) 248-255.

[6] S. Manzoor, M. Saalim, M. Imran, S. Resham, J. Ashraf, Hepatitis B virus therapy: What's the future holding for us?, World J. Gastroentero, 21 (2015) 12558-12575.

[7] J. Liu, A. Kosinska, M. Lu, M. Roggendorf, New therapeutic vaccination strategies for the treatment of chronic hepatitis B, Virol Sin, 29 (2014) 10-16.

[8] H. Jia, F. Bai, N. Liu, X. Liang, P. Zhan, C. Ma, X. Jiang, X. Liu, Design, synthesis and evaluation of pyrazole derivatives as non-nucleoside hepatitis B virus inhibitors, Eur. J. Med. Chem., 123 (2016) 202-210.

[9] B.X. Xu, Z.M. Huang, C.X. Liu, Z.G. Cai, W.D. Pan, P.X. Cao, X.J. Hao, G.Y. Liang, Synthesis and anti-hepatitis B virus activities of Matijing-Su derivatives, Bioorg. Med. Chem., 17 (2009) 3118-3125.

[10] J.Y. Qiu, B.X. Xu, Z.M. Huang, W.D. Pan, P.X. Cao, C.X. Liu, X.J. Hao, B.A. Song, G.Y. Liang, Synthesis and biological evaluation of Matijing-Su derivatives as potent anti-HBV agents, Bioorg. Med. Chem., 19 (2011) 5352-5360.

[11] J.Y. Qiu, B.X. Xu, Q.N. Gong, W.D. Pan, C.X. Liu, Z.M. Huang, X.K. Gu, G.Y. Liang, Synthesis and Biological Evaluation of Matijin-Su Derivatives as Potential Antihepatitis B Virus and Anticancer Agents, Chem. Biodivers., 13 (2016) 1584-1592.

[12] Z.X. Hu, Y.G. Zhang, Q. An, B.X. Xu, W.D. Pan, P.X. Cao, C.X. Liu, Z.M. Huang, W. Xia, J.Y. Qiu, G.Y. Liang, Development of a practical and scalable synthesis of anti-HBV drug Y101, Tetrahedron, 70 (2014) 9592-9600.

[13] S.P. Katen, Z.N. Tan, S.R. Chirapu, M.G. Finn, A. Zlotnick, Assembly-Directed Antivirals Differentially Bind Quasiequivalent Pockets to Modify Hepatitis B Virus Capsid Tertiary and Quaternary Structure, Structure, 21 (2013) 1406-1416.

[14] L.C. Li, S.R. Chirapu, M.G. Finn, A. Zlotnick, Phase Diagrams Map the Properties of Antiviral Agents Directed against Hepatitis B Virus Core Assembly, Antimicrob. Agents. Ch, 57 (2013) 1505-1508.

[15] P.Y. Wang, D. Naduthambi, R.T. Mosley, C.R. Niu, P.A. Furman, M.J. Otto, M.J. Sofia, Phenylpropenamide derivatives: Anti-hepatitis B virus activity of the Z isomer, SAR and the search for novel analogs, Bioorg. Med. Chem. Lett, 21 (2011) 4642-4647.

[16] W.E. DELANEY, H.C. ISOM. Hepatitis B Virus Replication in Human HepG2 Cells Mediated by Hepatitis B Virus Recombinant Baculovirus, Hepatology, (28) 1998 1134-1146.

[17] L.M. Gao, Y.X. Han, Y.P. Wang, Y.H. Li, Y.Q. Shan, X. Li, Z.G. Peng, C.W. Bi, T.A. Zhang, N.N. Du, J.D. Jiang, D.Q. Song, Design and Synthesis of Oxymatrine Analogues Overcoming Drug Resistance in Hepatitis B Virus through Targeting Host Heat Stress Cognate 70, J. Med. Chem., 54 (2011) 869-876.

[18] Y.B. Xu, L. Yang, G.F. Wang, X.K. Tong, Y.J. Wang, Y. Yu, J.F. Jing, C.L. Feng, P.L. He, W. Lu, W.

Tang, J.P. Zuo, Benzimidazole derivative, BM601, a novel inhibitor of hepatitis B virus and HBsAg secretion, Antivir. Res., 107 (2014) 6-15.

[19] Y.J. Wang, D. Lu, Y.B. Xu, W.Q. Xing, X.K. Tong, G.F. Wang, C.L. Feng, P.L. He, L. Yang, W. Tang, Y.H. Hu, J.P. Zuo, A Novel Pyridazinone Derivative Inhibits Hepatitis B Virus Replication by Inducing Genome-Free Capsid Formation, Antimicrob. Agents. Ch, 59 (2015) 7061-7072.

[20] B. Seigneres, C. Pichoud, P. Martin, P. Furman, C. Trepo, F. Zoulim, Inhibitory activity of dioxolane purine analogs on wild-type and lamivudine-resistant mutants of hepadnaviruses, Hepatology., 36 (2002) 710-722.

[21] SYBYL_X2.1 is availabe from Tripos Associates Inc., S. H. R., St. Louis, MO 631444, USA.

[22] K. Klumpp, A.M. Lam, C. Lukacs, R. Vogel, S.P. Ren, C. Espiritu, R. Baydo, K. Atkins, J. Abendroth, G.C. Liao, A. Efimov, G. Hartman, O.A. Flores, High-resolution crystal structure of a hepatitis B virus replication inhibitor bound to the viral core protein, P. Natl. Acad. Sci. USA., 112 (2015) 15196-15201.

Highlights

1. Novel phenyl propionamide derivatives were synthesized as anti-HBV agents.
2. **8d** exhibited potent anti-HBV activity against wild and drug resistant HBV strains.
3. **8d** could inhibit HBV pgRNA expression and RT activity of DHBV polymerase.