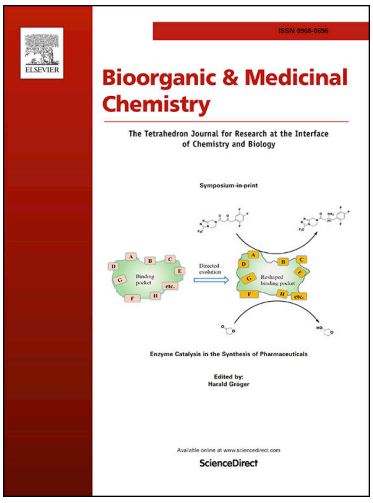
Journal Pre-proofs



Synthesis and evaluation of new phenyl acrylamide derivatives as potent non-nucleoside anti-HBV agents

Xiaoke Gu, Yinpeng Zhang, Yueting Zou, Xin Li, Mingyu Guan, Qingqing Zhou, Jingying Qiu

PII: S0968-0896(20)30722-7
DOI: https://doi.org/10.1016/j.bmc.2020.115892
Reference: BMC 115892

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 14 September 2020
Revised Date: 17 November 2020
Accepted Date: 20 November 2020

Please cite this article as: X. Gu, Y. Zhang, Y. Zou, X. Li, M. Guan, Q. Zhou, J. Qiu, Synthesis and evaluation of new phenyl acrylamide derivatives as potent non-nucleoside anti-HBV agents, *Bioorganic & Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.bmc.2020.115892

Synthesis and evaluation of new phenyl acrylamide derivatives as potent non-nucleoside anti-HBV agents

Xiaoke Gu[a, #], Yinpeng Zhang[b, #], Yueting Zou[b], Xin Li[a], Mingyu Guan[a], Qingqing Zhou[b], Jingying Qiu[a, b, *]

[a] *Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, Xuzhou 221004, People's Republic of China*

[b] *Department of Pharmaceutical Analysis, School of Pharmacy, Xuzhou Medical University, Xuzhou 221004, People's Republic of China*

Abstract

As a continuation of our previous work, a series of new phenyl acrylamide derivatives (**4Aa-g**, **4Ba-t**, **5** and **6a-c**) were designed and synthesized as non-nucleoside anti-HBV agents. Among them, compound **4Bs** could potently inhibit HBV DNA replication in wild-type and lamivudine (3TC)/entecavir resistant HBV mutant strains with IC_{50} values of 0.19 and 0.18 μM, respectively. Notably, the selective index value of **4Bs** was above 526, indicating the favorable safety profile. Interestingly, unlike nucleoside analogue 3TC, **4Bs** could significantly inhibit 3.5 kb pgRNA expression. Molecular docking study revealed that **4Bs** could fit well into the dimer-dimer interface of HBV core protein by hydrophobic, π−π and H-bond interactions. Considering the potent anti-HBV activity, low toxicity and diverse anti-HBV mechanism from that of nucleoside anti-HBV agent 3TC, compound **4Bs** might be a promising lead to develop novel non-nucleoside anti-HBV therapeutic agents, and warranted further investigation.

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

1. Introduction

Hepatitis B virus (HBV) infection is a major public health problem worldwide. Statistically, of the approximately 5% of the world's population (350 million people) that is chronic HBV carriers[1, 2], and about 20% of them will finally develop into HBV-related cirrhosis or hepatocellular carcinoma (HCC).[3-5]

* Corresponding author. Tel.: +86-516-83262138; fax: +86-516-83262630; e-mail: jingyqiu@xzhmu.edu.cn (J. Q.)
# These two authors contributed equally to this work.

Interferons (IFNs) and nucleos(t)ides-based reverse transcriptase inhibitors are the main treatment options in clinic, however, both of them do not provide satisfactory clinical outcomes for chronic HBV infection.[6-8] For instance, the long-term administration of nucleos(t)ide analogs always cause an increased viral resistance.[9, 10] Therefore, there is an urgent requirement to develop novel non-nucleoside anti-HBV agents.[11]

Y101, a dipeptide derivative of herbal ingredient, was discovered by our research group.[12] As a novel anti-HBV agent, Y101 is currently in phase I clinical trials for the treatment of HBV infection in China.[13] Our previous work on the modification of Y101 led to novel analogs **8d** and **8g**, which displayed potent in vitro anti-HBV activities.[14] The IC_{50} values of **8d** and **8g** against wild-type HBV DNA replication were 0.46 and 2.44 μM, respectively. Previous structure activity relationship (SAR) study indicated that the 3-bromo-3-phenylacrylamide moiety was significant for the anti-HBV activity of **8d**, and the introduction of a meta substituent on A ring, such as a methyl substituent, was benefit for the anti-HBV activity. To further investigate the effect of meta substituents on the anti-HBV activities, we prepared eleven phenylacrylamide derivatives by introducing various meta substituents into the A ring. In addition, we previously found that the introduction of an ortho-F substituent on B ring could improve the anti-HBV activities. In order to obtain more structurally diverse derivatives and further investigate the effect of different substituents on the anti-HBV activity, we also synthesized twenty new compounds with various substituents on B ring, thus thirty-one target compounds were obtained in total. Subsequently, their anti-HBV activities against wild and drug resistant HBV strains, and the preliminary action mechanism were investigated in the present work.

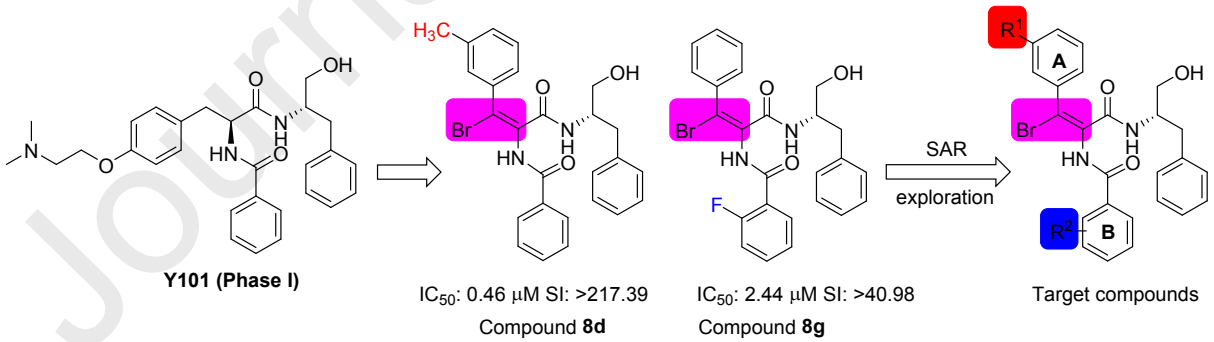


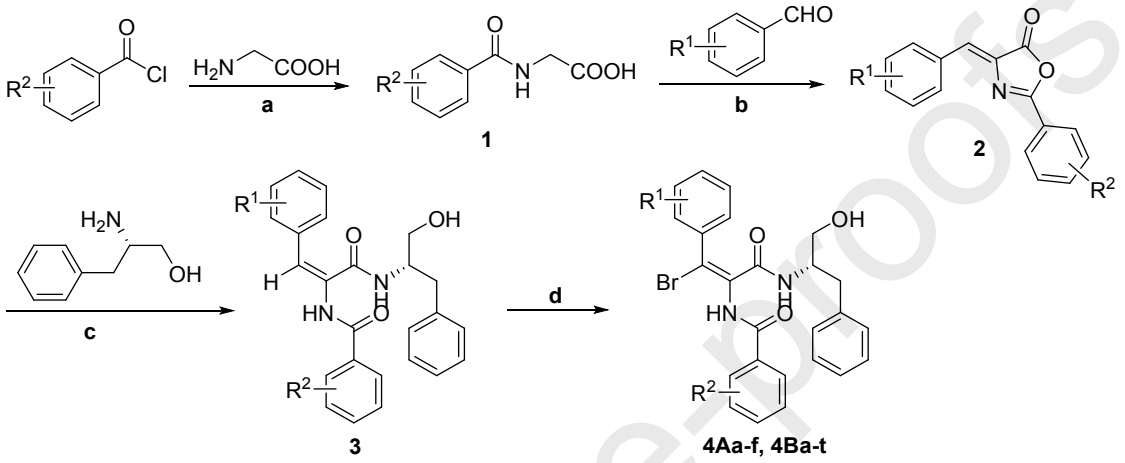
Figure 1. Chemical structures of Y101, **8d**, **8g** and the target compounds

2. Results and Discussion

2.1. Chemistry

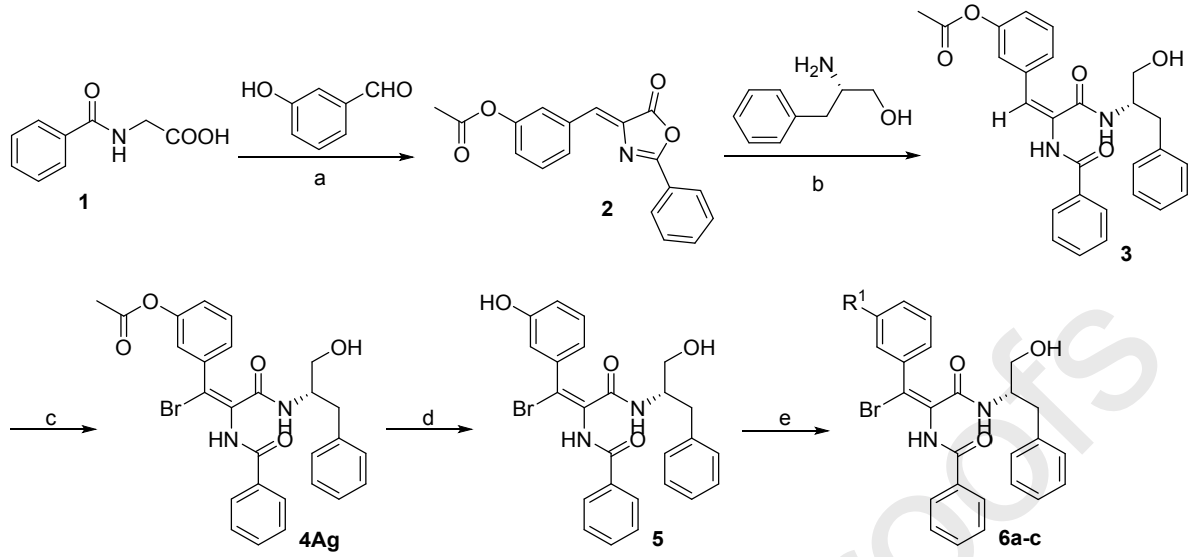
The synthetic procedures of the target compounds **4Aa-f** and **4Ba-t** were depicted in Scheme 1. Briefly,

compounds **1** were obtained by the acylation reaction with glycine and benzoyl chloride or benzoyl chloride derivatives as the raw material, which were then reacted with benzaldehyde or benzaldehyde derivatives in the presence of acetic anhydride (Ac_2O) and sodium acetate (AcONa) to obtain compounds **2**. Next, the intermediates **2** were reacted with L-phenylalaninol to give intermediates **3**, which were finally brominated to give the target compounds **4Aa-f** and **4Ba-t**, respectively.



Scheme 1. **Reagents and conditions:** a) NaOH (aq), HCl; b) AcONa/Ac_2O, 100 °C; c) CH_2Cl_2, reflux; d) Br_2, $CaCO_3$/$CHCl_3$, 0 °C.

The synthetic route of the target compounds **4Ag**, **5** and **6a-c** was depicted in Scheme 2. Briefly, compound **1** was reacted with 3-hydroxybenzaldehyde in the presence of acetic anhydride (Ac_2O) and sodium acetate (AcONa) to obtain compound **2**. Subsequently, the intermediate **2** was reacted with L-phenylalaninol to give intermediate **3**. Finally, the crude product **3** was directly brominated with bromine to give the target compound **4Ag**. After the hydrolysis of **4Ag**, the intermediate **5** was successfully obtained, which was finally alkylated with 2-dimethylaminoethyl chloride hydrochloride, 2-diethylaminoethyl chloride hydrochloride or 3-dimethylaminopropyl chloride hydrochloride to provide alkoxyl substituted derivatives **6a-c**, respectively.



Scheme 2. **Reagents and conditions:** a) AcONa/Ac_2O, 100 °C; b) CH_2Cl_2, reflux; c) Br_2, $CaCO_3$/$CHCl_3$, 0 °C; d) DMF, NaOH, rt.; e) 2-dimethylaminoethyl chloride hydrochloride, 2-diethylaminoethyl chloride hydrochloride or 3-dimethylaminopropyl chloride hydrochloride, 1,4-dioxane, K_2CO_3, 90 °C.

2.2. Biological evaluation

2.2.1. Cytotoxicity assays

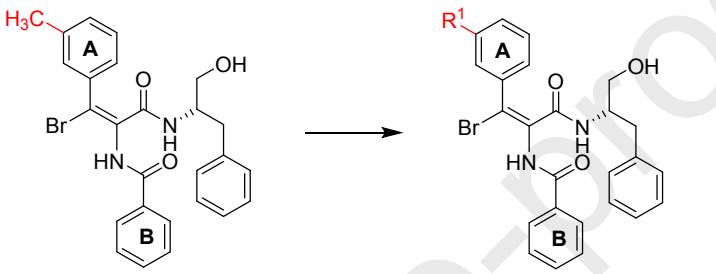
An ideal anti-HBV drug should inhibit HBV DNA replication at a non-toxic concentration. Therefore, we firstly determined the in vitro intrinsic cytotoxicity of the target compounds against HepG2 2.2.15 cells by MTT assay. As shown in Table 1, most of the target compounds displayed little (CC_{50} > 100 μM) or very low (CC_{50} = 83.57 ~ 99.51 μM) intrinsic cytotoxicity against HepG2 2.2.15 cells, indicating their relatively safety profile.

2.2.2. Inhibitory effect on replication of HBV DNA

Next, we investigated the in vitro anti-HBV activity of the target compounds with various meta substituents on A ring by determining their effect on HBV DNA levels in HepG2 2.2.15 cells using real time PCR assay.[15] Lead compound **8d** (0.8 μM) and lamivudine (3TC, 0.8 μM) were used as positive controls. As shown in Table 1, when HepG2 2.2.15 cells were treated with the target compounds at a non-toxic dose (0.8 μM) for 6 days, the extra cellar HBV DNA levels were decreased, with inhibition rates of 14.37-58.38%. Among them, compounds **6a** and **6b** displayed the most potent anti-HBV activity with inhibition rates of 58.38% and 56.46%, respectively. Notably, when the meta methyl of compound **8d** was replaced with other substituents,

such as nitryl (compound **4Aa**), trifluoromethyl (compound **4Ab**), halogen (compounds **4Ac-e**), methoxyl (compound **4Af**) and hydroxyl (compound **5**), the in vitro anti-HBV activity was decreased, confirming that a meta methyl was indeed benefit for the improvement of anti-HBV activity. Interestingly, when meta hydroxyl group was alkylated with dimethylamino ethyl (compound **6a**), diethylamine ethyl (compound **6b**) or dimethylamino propyl (compound **6c**), the anti-HBV activity was significantly increased, which was consistent with our previous findings that such substituents were beneficial for anti-HBV activity.[14]

Table 1. Inhibitory effect of the target compounds **4Aa-g**, **5** and **6a-c** (0.8 μM) on HBV DNA levels



Lead compound **8d** Target compound **4Aa-g, 5** and **6a-c**

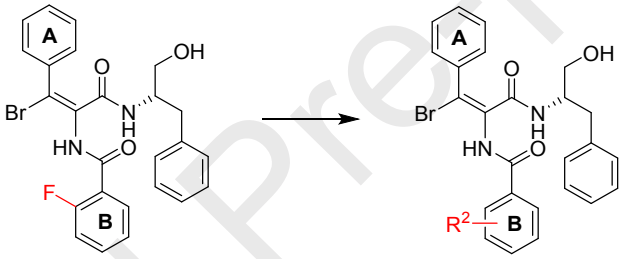
Compounds	R^1	[a]CC_{50} (μM)	Inhibition rate (%)	Compounds	R^1	CC_{50} (μM)	Inhibition rate (%)
4Aa	NO_2	93.72	2.28	**5**	OH	99.51	29.63
4Ab	CF_3	>100	23.07	**6a**	$OCH_2CH_2N(CH_3)_2$	94.66	58.38
4Ac	F	>100	42.61	**6b**	$OCH_2CH_2N(CH_2CH_3)_2$	83.57	56.46
4Ad	Cl	>100	48.16	**6c**	$OCH_2CH_2CH_2N(CH_3)_2$	85.64	37.72
4Ae	Br	>100	14.37	**8d**	/	>100	57.31
4Af	OCH_3	>100	48.90	3TC	/	>100	86.23
4Ag	$OCOCH_3$	>100	32.35				

[a] CC_{50}: defined as the concentration that induced 50% death of HepG2 2.2.15 cells.

Subsequently, we determined the in vitro anti-HBV activity of the target compounds with various substituents on B ring (Table 2), and selected lead compound **8g** (0.8 μM) and lamivudine (3TC, 0.8 μM) as positive controls. Data showed that lead compound **8g** (with an ortho-F atom on B ring) exhibited moderate in vitro anti-HBV activity with an inhibition of control of 33.62%. When the ortho-F atom was changed into ortho-Cl (compound **4Ba**) or Br atom (compound **4Bb**), the anti-HBV activity of the compound was decreased. And the introduction of an ortho-electron-donating group (ie, OCH_3 or CH_3) in B ring also resulted

in a dramatic decrease in the inhibition of control, suggesting that ortho-electron-donating group was detrimental to the in vitro anti-HBV activity. In addition, when a substituent was introduced into the meta position of B ring (Compounds **4Be-i**), the in vitro anti-HBV activity was almost lost. Notably, the introduction of a para-substituent, especially an electron withdrawing substituent, such as F, Cl or Br atom, could significantly increase the anti-HBV activity. Clearly, para-F and Cl atom were the best, for the inhibition rates of compound **4Bj** and **4Bk** were 74.94% and 70.65%, respectively, which were much higher than that of lead compound **8g**. Interestingly, introduction of a second substituent in the B ring (compounds **4Bo-t**) could significantly affect the anti-HBV activity. Among them, compound **4Bs** was the most potent one with an inhibition rate of 87.98%, which was even higher than that of classic anti-HBV drug 3TC (86.23%).

Table 2. Inhibitory effect of the target compounds **4Ba-t** (0.8 μM) on HBV DNA level



Lead compound **8g** Target compound **4Ba-t**

Compounds	R^2	CC_{50} (μM)	Inhibition of control (%)	Compounds	R^2	CC_{50} (μM)	Inhibition of control (%)
4Ba	2-Cl	>100	13.93	**4Bl**	4-Br	>100	43.00
4Bb	2-Br	>100	17.19	**4Bm**	4-OCH_3	>100	6.25
4Bc	2-OCH_3	>100	11.86	**4Bn**	4-CH_3	>100	47.36
4Bd	2-CH_3	>100	5.75	**4Bo**	2-F, 4-F	>100	58.08
4Be	3-F	>100	9.07	**4Bp**	3-F, 4-F	>100	73.65
4Bf	3-Cl	>100	5.36	**4Bq**	2-Cl, 4-F	>100	56.08
4Bg	3-Br	>100	2.48	**4Br**	2-CH_3, 4-F	>100	83.22
4Bh	3-OCH_3	>100	4.89	**4Bs**	2-Cl, 4-Cl	>100	87.98
4Bi	3-CH_3	>100	2.94	**4Bt**	3-Cl, 5-Cl	>100	83.76
4Bj	4-F	>100	74.91	**8g**	/	>100	33.62

4Bk	4-Cl	>100	70.65	3TC	/	>100	86.23

To further confirm the in vitro anti-HBV activity, we subsequently evaluated the IC_{50} values for the HBV DNA replication of the active compounds, and also determined their selectivity index (SI). As shown in Table 3, all the tested compounds could remarkably inhibit HBV DNA replication with IC_{50} values of 0.19-0.48 μM, indicating their potent in vitro anti-HBV activity. Clearly, compound **4Bs** was the most prominent one, with an IC_{50} value of 0.19 μM. Notably, **4Bs** exhibited a high SI value of above 526, indicating that **4Bs** possessed a relatively favorable safety profile.

Table 3. Anti-HBV activity and SI values of target compounds **4Bj**, **4Bk**, **4Bp** and **4Br-t**

Compounds	CC_{50} (μM)	DNA replication	
		[a]IC_{50} (μM)	[b]SI
4Bj	>100	0.38 ± 0.043	>263
4Bk	>100	0.43 ± 0.040	>232
4Bp	>100	0.48 ± 0.047	>208
4Br	>100	0.33 ± 0.044	>303
4Bs	>100	0.19 ± 0.015	>526
4Bt	>100	0.27 ± 0.05	>370
3TC	>100	0.031 ± 0.008	>1000

[a] IC_{50} : defined as the concentration of compound required for 50% inhibition of HBV DNA replication, and expressed as means ± SD of triplicate experiments. [b] SI : Selectivity index (SI), calculated by dividing the CC_{50} value by the IC_{50} value.

*2.2.3. In vitro anti-HBV activity of compounds **4Bj** and **4Bs** against polymerase drug resistant HBV strain*

As mentioned above, during the long-term administration of nucleoside drugs, HBV strains will gradually become resistant, thus resulting in the treatment failure. Therefore, we next evaluated the anti-HBV effect of the active compounds **4Bj** and **4Bs** on 3TC and entecavir dually resistant mutant (rtL180M + rtM204V + rtT184L). As shown in Figure 2, nucleoside drugs 3TC and entecavir could remarkably inhibit the wild-type HBV DNA replication with inhibition rates of 90.01% and 96.82%, respectively, while the HBV DNA replication inhibition rates of 3TC and entecavir resistant strain were significantly decreased to 18.58% and 20.71%, respectively. Notably, compounds **4Bj** and **4Bs** exhibited potent inhibitory effect on HBV DNA replication of drug resistant strain with inhibition rates of 72.90% and 87.02%, respectively, which were

comparable to that of wild type strain.

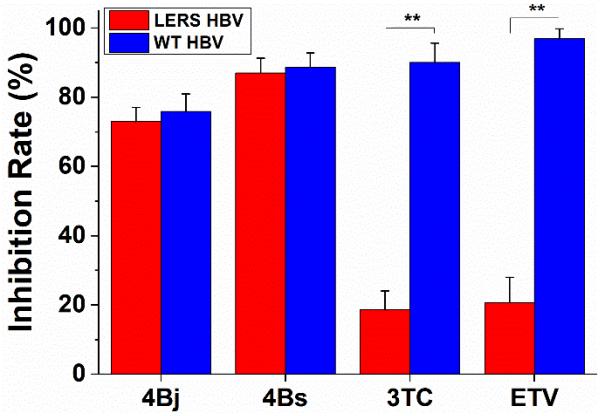


Figure 2. Antiviral effect of **4Bj** and **4Bs** in lamivudine and entecavir resistant HBV. HepG2 cells were transiently transfected with the full genome of wide-type or lamivudine and entecavir resistant HBV, followed by 72 h treatment with lamivudine (100 μM), entecavir (10 μM), **4Bj** (0.8 μM), **4Bs** (0.8 μM) respectively. HBV DNA replicative intermediate level was measured using real time PCR. $^{**}P < 0.01$ represents significant difference from the WT HBV group.

To further confirm the anti-resistant HBV activity, we investigated whether compounds **4Bj** and **4Bs** could dose-dependently inhibit the HBV DNA replication in drug resistant HBV strain. As shown in Table 4, the IC_{50} values for HBV DNA replication of **4Bj** and **4Bs** were 0.43 and 0.18 μM, respectively, validating their potent inhibitory effect on HBV DNA replication in drug resistant strain. Excitingly, the SI values of compound **4Bj** and **4Bs** were above 232 and 556, respectively, further confirming their relatively safety profiles.

Table 4. Inhibitory activity of compounds **4Bj** and **4Bs** against lamivudine and entecavir resistant HBV strain

Compounds	CC_{50} (μM)	DNA replication	
		[a] IC_{50} (μM)	[b] SI
4Bj	>100	0.43 ± 0.035	>232
4Bs	>100	0.18 ± 0.021	>556
3TC	>100	>100	/

[a] IC_{50} : defined as the concentration of compound required for 50% inhibition of HBV DNA replication, and expressed as means ± SD of triplicate experiments. [b] SI : Selectivity index (SI), calculated by dividing the CC_{50} value by the IC_{50} value.

*2.2.4. Inhibitory Effect of compound **4Bs** on HBV viral gene expression*

Considering that pregenomic RNA (pgRNA) plays a crucial role in HBV lifecycle and is related to HBV DNA replication, we subsequently determined whether compound **4Bs** could inhibit HBV viral gene

expression according to the previous methods.[16, 17] Briefly, HepG2 2.2.15 cells were firstly incubated with 1 μM compound **4Bs** for 6 days, and then the total cellular RNA was extracted and subsequently detected by real time PCR. 3TC was used as a nucleoside analog control. As shown in Table 5, 3TC displayed little inhibitory effect on pgRNA expression. In sharp contrast, compound **4Bs** could significantly inhibit 3.5 kb pgRNA expression with an inhibition of control of 63.13%, suggesting that compound **4Bs** may possess a different anti-HBV action mechanism from that of nucleoside analog 3TC.

Table 5. Effect of compound **4Bs** on intracellular HBV pgRNA

Compounds	Concentration (μM)	pgRNA (copies/mL)	Inhibition of control (%)
4Bs	1	2.54E+06	63.13
3TC	1	6.73E+06	0.26
Control	-	6.89E+06	-

2.2.5. Potential effect on HBV core protein

It is well-known that HBV core protein performs multiple roles in HBV life cycle, and is essential for HBV replication, viral assembly, and cccDNA maintenance.[18] Moreover, as a viral protein, HBV core protein is not present in human cells.[19] Therefore, HBV core protein is regarded as a promising target for developing novel, virus-selective, and efficacious anti-HBV agents.[19, 20] Considering the advantages of HBV core protein as non-nucleoside anti-HBV drug target, we analyzed the binding mode of **4B** with HBV core protein by molecular docking to preliminarily understand the potential effect of **4Bs** on HBV core protein.

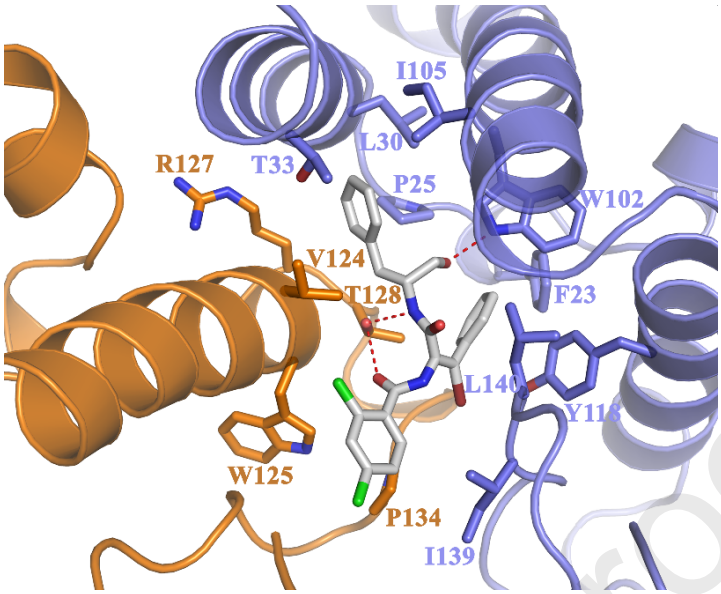


Figure 3. Binding modes of compound **4Bs** with HBV capsid protein Y132A (PDB code 5e0i). The binding pocket was at Chains B and C interface. Chains B and C were shown as cartoon model, and colored in blue and orange, respectively. Compound **4Bs** was highlighted in white stick. The key residues in the active site were shown as blue and orange line. Red dash lines indicated the hydrogen bonds between compounds and core protein (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

As shown in Figure 3, compound **4Bs**, as compared to the reference ligand, was flexibly docked into the dimer-dimer interface of HBV core protein. Generally, **4Bs** was stabilized in a well-defined hydrophobic cavity composed of Phe23, Trp102, Tyr118, Val124, Arg127 Ile139, Leu140 (Chain B) and Pro25, Leu30, Thr33, Ile105 Trp125,Thr128, Pro134 (Chain C). Meanwhile, several main interactions were predicted between **4Bs** and the HBV core protein, including that π−π interactions were indicated between the benzene ring in the phenyl acrylamide scaffold of **4Bs** with the phenyl ring of Phe23 and Tyr118 of HBV core protein; another π−π interactions were observed between the benzene ring in the phenyl acrylamide scaffold and 3,4-dichlorophenyl of **4Bs** with Trp102 and Trp 125 of HBV core protein, respectively; two hydrogen bonds (3.09 and 2.86 Å) occurred between amide carbonyl, amino of phenyl acrylamide scaffold of **4Bs** with Thr128 of HBV core protein, respectively; and another hydrogen bond (2.70 Å) was observed between hydroxyl group of phenylpropanolamine of **4Bs** with Trp102. The molecular docking studies indicated that compound **4Bs** displayed molecular interactions with HBV core protein, and might be a potential inhibitor of HBV core protein.

3. Conclusions

In summary, thirty-one compounds with phenyl acrylamide scaffold were designed and prepared as non-nucleoside anti-HBV agents. Among them, compound **4Bs** exhibited the most potent inhibitory effect on HBV DNA replication with an IC_{50} value of 0.19 μM. In addition, compound **4Bs** possessed a high selective index values (above 526), indicating that the safety profile was relatively favorable. Notably, **4Bs** also displayed a significant antiviral effect on 3TC and entecavir resistant HBV mutants with an IC_{50} value was 0.18 μM. More interestingly, unlike nucleoside analog 3TC, **4Bs** could significantly inhibit 3.5 kb pgRNA expression. Molecular docking study revealed that compound **4Bs** could fit well into the dimer-dimer interface of HBV core protein by hydrophobic, π–π and H-bond interactions. Considering the potent anti-HBV activity, low toxicity and diverse anti-HBV mechanism from that of nucleoside anti-HBV drug 3TC, compound **4Bs** might be a promising lead to develop novel non-nucleoside anti-HBV therapeutic agents, and warranted further investigation.

4. Experimental protocols

4.1. Chemistry

Compounds were purified by column chromatography using silica gel 60 (200–300 mesh) or thin layer chromatography (TLC) using silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). Melting points were determined with a model YRT-3 apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were run on a JEOL (400 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 with Me_4Si (TMS) as an internal standard. Mass detection was performed on an Agilent G6460C triple quadrupole mass spectrometer. Analytical HPLC was run on the Waters Alliance E2695 HPLC instrument, equipped with Chiralcel OD-RH column and UV detection at 254 nm.. Individual compounds with a purity of >95% were used for subsequent experiments. All solvents were reagent grade and were purified and dried by standard methods when necessary.

*4.1.1. General procedure for the synthesis of compounds **4Aa-f** and **4Ba-t***

Compounds **1** were 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 were 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 **2**. Next, the intermediates mentioned

above were reacted with L-phenylalaninol (0.01 mol) to yield the crude products **3**, respectively. Finally, the crude products **3** (0.01 mol) were directly brominated with bromine (0.015 mol) and anhydrous calcium carbonate (0.01 mol) in $CHCl_3$ to give the crude products, which were purified by column chromatography to yield the title compounds **4Aa-f** and **4Ba-t**, respectively.

*4.1.1.1. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-1-(3-nitrophenyl)-3-oxoprop-1-en- 2-yl)benzamide (**4Aa**)*

As a yellow powder, yield: 32.7%, m.p.163.4-164.7 °C. Analytical data for **4Aa**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 10.08 (s, 1H, NH), 8.15-8.16 (m, 3H, Ar-H), 8.04 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.54-7.71 (m, 5H, Ar-H), 7.04-7.18 (m, 5H, Ar-H), 4.14 (t, d = 4.0 Hz, 1H, OH), 3.75 (br, 1H, CH), 2.98-3.20 (m, 2H, CH_2), 2.40-2.62 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.67, 162.86, 147.69, 139.88, 139.30, 136.07, 135.81, 133.37, 132.79, 130.38, 129.35 (2C), 129.05 (2C), 128.55 (2C), 128.52 (2C), 126.33, 124.30, 124.17, 117.18, 61.96, 53.51, 36.08; ESI-MS: m/z 546.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}BrN_3O_5$, 524.0821, found 524.0831.

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

As a white powder, yield: 28.2%, m.p.173.1-174.3 °C. Analytical data for **4Ab**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.99 (s, 1H, NH), 7.97-8.01 (m, 3H, Ar-H, NH), 7.48-7.68 (m, 7H, Ar-H), 7.08-7.21 (m, 3H, Ar-H), 7.00-7.02 (m, 2H, Ar-H), 3.65-3.70 (m, 1H, CH), 2.91-3.10 (m, 2H, CH_2), 2.31-2.53 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.66, 162.88, 139.51, 139.27, 135.35, 133.56, 133.40, 132.75, 130.01, 129.46 (2C), 129.23 (d, $^2J_{CF}$ = 31.8 Hz), 129.03 (2C), 128.61 (2C), 128.50 (2C), 126.36, 126.19, 126.03, 125.75 (d, $^1J_{CF}$ = 270.3 Hz), 118.33, 61.78, 53.42, 36.02; ESI-MS: m/z 569.1 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{23}BrF_3N_2O_3$, 547.0844, found 547.0856.

*4.1.1.3. (S,Z)-N-(1-bromo-1-(3-fluorophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide (**4Ac**)*

As a white powder, yield: 32.7%, m.p.164.3-165.8 °C. Analytical data for **4Ac**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.94 (s, 1H, NH), 7.99 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.90 (d, *J* = 8.4 Hz, 1H, NH), 7.59 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.50 (t, *J* = 6.0 Hz, 2H, Ar-H), 7.03-7.22 (m, 9H, Ar-H), 4.37 (t, *J* = 5.6 Hz, 1H, OH), 3.67-3.75 (m, 1H, CH), 2.94-3.15 (m, 2H, CH_2), 2.37-2.57 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ

ppm): 165.63, 163.18 (d, $^{1}J_{CF}$ = 232.8 Hz), 162.96, 140.65 (d, $^{3}J_{CF}$ = 232.8 Hz), 139.37, 134.88, 133.43, 132.71, 130.74 (d, $^{3}J_{CF}$ = 7.5 Hz), 129.49 (2C), 129.01 (2C), 128.61 (2C), 128.48 (2C), 126.36, 125.75, 118.70, 116.63 (d, $^{2}J_{CF}$ = 20.8 Hz), 116.56 (d, $^{2}J_{CF}$ = 22.6 Hz), 61.96, 53.41, 36.09; ESI-MS: m/z 519.1 [M + Na]$^{+}$; ESI-HRMS (TOF): *m/z* [M + H]$^{+}$ calcd for $C_{25}H_{23}BrFN_2O_3$, 497.0876, found 497.0884.

*4.1.1.4. (S,Z)-N-(1-bromo-1-(3-chlorophenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide (**4Ad**)*

As a white powder, yield: 28.4%, m.p.162.6-163.7 °C. Analytical data for **4Ad**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.94 (s, 1H, NH), 7.98 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.93 (d, *J* = 8.4 Hz, 1H, NH), 7.59 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.50 (t, *J* = 7.2 Hz, 2H, Ar-H), 7.35-7.38 (m, 2H, Ar-H), 7.27 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.03-7.21 (m, 6H, Ar-H), 4.37 (t, *J* = 7.2 Hz, 1H, OH), 3.69-3.74 (m, 1H, CH), 2.95-3.16 (m, 2H, CH_2), 2.37-2.57 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.62, 162.94, 140.42, 139.36, 135.01, 133.43, 133.15, 132.71, 130.59, 129.50 (2C), 129.24, 129.01 (2C), 128.62 (2C), 128.50 (2C), 128.32, 118.51, 61.91, 53.45, 36.09; ESI-MS: m/z 535.0 [M + Na]$^{+}$; ESI-HRMS (TOF): *m/z* [M + H]$^{+}$ calcd for $C_{25}H_{23}BrClN_2O_3$, 513.0581, found 513.0585.

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

As a yellow powder, yield: 26.2%, m.p.185.8-186.6 °C. Analytical data for **4Ae**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.95 (s, 1H, NH), 7.98 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.95 (d, *J* = 8.4 Hz, 1H, NH), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.48-7.52 (m, 4H, Ar-H), 7.11-7.26 (m, 5H, Ar-H), 7.05 (d, *J* = 8.0 Hz, 2H, Ar-H), 4.38 (br, 1H, OH), 3.69-3.74 (m, 1H, CH), 2.97-3.16 (m, 2H, CH_2), 2.38-2.56 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.62, 162.93, 140.64, 139.35, 135.01, 133.43, 132.72, 132.33, 132.02, 130.84, 129.51 (2C), 129.02 (2C), 128.69, 128.62 (2C), 128.49 (2C), 126.38, 121.62, 118.44, 61.89, 53.45, 36.10; ESI-MS: m/z 559.0 [M + Na]$^{+}$; ESI-HRMS (TOF): *m/z* [M + H]$^{+}$ calcd for $C_{25}H_{23}Br_2N_2O_3$, 557.0075, found 557.0079.

*4.1.1.6. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-1-(3-methoxyphenyl)-3-oxoprop-1-en-2-yl)benzamide (**4Af**)*

As a yellow powder, 26.3%, m.p.86.4-87.8 °C. Analytical data for **4Af**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.92 (s, 1H, NH), 8.00 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.76 (d, *J* = 8.4 Hz, 1H, NH), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.50 (t, *J* = 6.8 Hz, 2H, Ar-H), 7.08-7.21 (m, 4H, Ar-H), 7.04 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.86-

6.92 (m, 3H, Ar-H), 4.34 (t, *J* = 6.8 Hz, 1H, OH), 3.67-3.74 (m, 4H, CH, OCH_3), 2.93-3.14 (m, 2H, CH_2), 2.36-2.53 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.59, 163.16, 159.26, 139.61, 139.36, 134.19, 133.53, 132.63, 129.79, 129.51 (2C), 129.00 (2C), 128.60 (2C), 128.43 (2C), 126.34, 121.93, 120.58, 115.40, 115.15, 61.85, 55.69, 53.35, 36.10; ESI-MS: m/z 531.1 $[M + Na]^+$; ESI-HRMS (TOF): m/z $[M + H]^+$ calcd for $C_{26}H_{26}BrN_2O_4$, 509.1076, found 509.1047.

*4.1.1.7. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-2-chlorobenzamide (**4Ba**)*

As a white powder, yield: 36.4%, m.p.167.8-168.6 °C. Analytical data for **4Ba**: ^1H NMR (DMSO, 400 MHz, δ ppm): 10.13 (s, 1H, NH), 7.81 (d, *J* = 8.4 Hz, 1H, NH), 7.40-7.56 (m, 4H, Ar-H), 7.26-7.29 (m, 5H, Ar-H), 7.04-7.21 (m, 5H, Ar-H), 4.34 (br, 1H, OH), 3.70-3.75 (m, 1H, CH), 2.93-3.12 (m, 2H, CH_2), 2.35-2.56 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.49, 162.74, 139.44, 138.35, 135.70, 133.66, 132.00, 130.79, 130.29, 130.01, 129.60, 129.53 (4C), 128.66 (4C), 127.54, 126.37, 119.86, 61.94, 53.25, 36.09; ESI-MS: m/z 535.4 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}BrClN_2O_3$, 513.0581, found 513.0578.

*4.1.1.8. (S,Z)-2-bromo-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en -2- yl)benzamide (**4Bb**)*

As a white powder, yield: 29.6%, m.p.174.4-175.8 °C. Analytical data for **4Bb**: ^1H NMR (DMSO, 400 MHz, δ ppm): 10.16 (s, 1H, NH), 7.82 (d, *J* = 8.4 Hz, 1H, NH), 7.72 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.57 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H, Ar-H), 7.50 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.43 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.15-7.35 (m, 8H, Ar-H), 7.09 (d, *J* = 6.8 Hz, 2H, Ar-H), 3.72-3.78 (m, 1H, CH), 2.97-3.17 (m, 2H, CH_2), 2.40-2.60 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.37, 162.73, 139.44, 138.37, 137.86, 133.66, 133.40, 132.06, 129.99, 129.59 (3C), 129.52 (2C), 128.66 (2C), 128.64 (2C), 127.97, 126.37, 120.02, 119.62, 61.96, 53.27, 36.12; ESI-MS: m/z 557.0 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}Br_2N_2O_3$, 557.0075, found 557.0069.

*4.1.1.9. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-2-methoxybenzamide (**4Bc**)*

As a white powder, yield: 38.5%, m.p. 127.3-128.9 °C. Analytical data for **4Bc**: ^1H NMR (DMSO, 400 MHz, δ ppm): 10.12 (s, 1H, NH), 8.00 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.95 (dd, *J* = 8.0, 2.0 Hz, 1H, NH), 7.60 (t,

J = 7.2 Hz, 1H, Ar-H), 7.04-7.28 (m, 12H, Ar-H), 4.32 (t, *J* = 6.0 Hz, 1H, OH), 4.05 (s, 3H, OCH_3), 3.69-3.74 (m, 1H, CH), 2.96-3.19 (m, 2H, CH_2), 2.35-2.59 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 163.50, 163.25, 157.89, 137.04, 136.93, 134.32, 133.64, 133.07, 129.61, 129.45 (2C), 129.25 (2C), 128.61 (2C), 128.59 (2C), 126.59, 121.63, 119.70, 113.63, 111.48, 63.03, 56.32, 52.86, 36.78; ESI-MS: m/z 531.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{25}BrN_2O_4$, 509.1076, found 509.1066.

*4.1.1.10. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-2-methylbenzamide (**4Bd**)*

As a white powder, yield: 34.6%, m.p. 137.7-138.6 °C. Analytical data for **4Bd**: ^1H NMR (DMSO, 400 MHz, δ ppm): 9.84 (s, 1H, NH), 7.77 (d, *J* = 8.0 Hz, 1H, NH), 7.49 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.36 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.23-7.30 (m, 7H, Ar-H), 7.13-7.20 (m, 3H, Ar-H), 7.05 (d, *J* = 6.8 Hz, 2H, Ar-H), 4.33 (t, *J* = 6.0 Hz, 1H, OH), 3.70-3.75 (m, 1H, CH), 2.91-3.14 (m, 2H, CH_2), 2.35-2.55 (m, 5H, CH_2, CH_3); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 168.40, 163.04, 139.46, 138.38, 136.56, 135.77, 134.02, 131.12, 130.56, 129.61 (2C), 129.52 (3C), 128.64 (4C), 128.14, 126.35, 126.00, 120.30, 62.05, 53.23, 36.11, 20.08; ESI-MS: m/z 515.1 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{26}BrN_2O_3$, 493.1127, found 493.1119.

*4.1.1.11. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-3-fluorobenzamide(**4Be**)*

As a white powder, yield: 32.4%, m.p. 157.1-158.7 °C. Analytical data for **4Be**: ^1H NMR (DMSO, 400 MHz, δ ppm): 10.07 (s, 1H, NH), 7.77-7.84 (m, 3H, Ar-H, NH), 7.54-7.59 (m, 1H, Ar-H), 7.45 (t, *J* = 8.4 Hz, 1H, Ar-H), 6.99-7.29 (m, 10H, Ar-H), 4.35 (br, 1H, OH), 3.70 (br, 1H, CH), 2.94-3.13 (m, 2H, CH_2), 2.36-2.56 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.37, 163.67 (d, $^1J_{CF}$ = 243.2 Hz), 162.91, 139.44, 138.37, 135.88 (d, $^3J_{CF}$ = 6.8 Hz), 133.70, 131.28 (d, $^3J_{CF}$ = 8.0 Hz), 129.56 (5C), 128.68 (2C), 128.60 (2C), 126.35, 124.71 (d, $^4J_{CF}$ = 2.1 Hz), 121.86, 119.66 (d, $^2J_{CF}$ = 21.3 Hz), 115.38 (d, $^2J_{CF}$ = 22.9 Hz), 62.05, 53.32, 36.11; ESI-MS: m/z 497.1 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}BrFN_2O_3$, 497.0876, found 497.0866.

*4.1.1.12. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-3-chlorobenzamide (**4Bf**)*

As a white powder, yield: 33.4%, m.p. 140.7-141.9 °C. Analytical data for **4Bf**: ^1H NMR (DMSO, 400 MHz, δ ppm): 10.12 (s, 1H, NH), 8.04 (t, *J* = 5.6 Hz, 1H, Ar-H), 7.93 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.80 (d, *J* =

8.4 Hz, 1H, NH), 7.68 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.54 (t, *J* = 8.0 Hz,1H, Ar-H), 7.03-7.30 (m, 10H, Ar-H), 3.68-3.73 (m, 1H, CH), 2.92-3.12 (m, 2H, CH_2), 2.35-2.57 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.39, 162.88, 139.54, 138.39, 135.56, 133.80, 133.69, 132.43, 131.03, 129.56 (5C), 128.67 (2C), 128.60 (2C), 128.31, 127.24, 126.34, 121.92, 62.08, 53.33, 36.12; ESI-MS: m/z 535.1 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}BrClN_2O_3$, 513.0581, found 513.0573.

*4.1.1.13. (S,Z)-3-bromo-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)benzamide (**4Bg**)*

As a white powder, yield: 32.5%, m.p. 103.9-104.4 °C. Analytical data for **4Bg**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 10.11 (s, 1H, NH), 8.18 (s, 1H, Ar-H), 7.96 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.77-7.80 (m, 2H, Ar-H, NH), 7.47 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.03-7.27 (m, 10H, Ar-H), 3.68-3.71 (br, 1H, CH), 2.92-3.13 (m, 2H, CH_2), 2.36-2.57 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.32, 162.88, 139.46, 138.40, 135.73, 135.33, 133.68, 131.27, 131.18, 129.56 (5C), 128.67 (2C), 128.60 (2C), 127.62, 126.35, 122.25, 121.93, 62.10, 53.33, 36.11; ESI-MS: m/z 579.0 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}Br_2N_2O_3$, 557.0075, found 557.0060.

*4.1.1.14. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-3-methoxybenzamide (**4Bh**)*

As a white powder, yield: 32.6%, m.p. 180.3-181.6 °C. Analytical data for **4Bh**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.92 (s, 1H, NH), 7.75 (d, *J* = 8.4 Hz, 1H, NH), 7.55-7.58 (m, 2H, Ar-H), 7.41 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.02-7.29 (m, 10H, Ar-H), 4.43 (br, 1H, OH), 3.80 (s, 3H, OCH_3), 3.70-3.75 (m, 1H, CH), 2.94-3.13 (m, 2H, CH_2), 2.35-2.54 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.39, 163.08, 159.73, 139.41, 138.41, 134.90, 133.98, 130.18, 129.60 (2C), 129.56 (3C), 128.68 (2C), 128.63 (2C), 126.36, 121.21, 120.74, 118.61, 113.42, 62.00, 55.92, 53.31, 36.09; ESI-MS: m/z 531.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{26}BrN_2O_4$, 509.1076, found 509.1065.

*4.1.1.15. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-3-methylbenzamide (**4Bi**)*

As a white powder, yield: 29.4%, m.p. 149.3-150.6 °C. Analytical data for **4Bi**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.85 (s, 1H, NH), 7.72-7.80 (m, 3H, Ar-H, NH), 7.38-7.39 (m, 2H, Ar-H), 7.28 (br, 5H, Ar-H), 7.12-7.18 (m, 3H, Ar-H), 7.02-7.04 (m, 2H, Ar-H), 3.66-3.74 (m, 1H, CH), 2.92-3.12 (m, 2H, CH_2), 2.34-

2.58 (m, 5H, CH_2, CH_3); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.45, 162.66, 139.47, 138.23, 133.70, 131.27, 131.18, 129.59 (2C), 129.49 (2C), 128.97, 128.65 (2C), 128.63 (2C), 127.34, 126.95, 126.34, 120.12, 61.93, 53.32, 36.05; ESI-MS: m/z 515.1 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{26}BrN_2O_3$, 493.1127, found 493.1116.

*4.1.1.16. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-4-fluorobenzamide (**4Bj**)*

As a white powder, yield: 31.6%, m.p. 160.3-161.9 °C. Analytical data for **4Bj**: ^1H NMR (DMSO, 400 MHz, δ ppm): 9.99 (s, 1H, NH), 8.03-8.08 (m, 2H, Ar-H), 7.77 (d, J = 8.4 Hz, 1H, NH), 7.03-7.38 (m, 12H, Ar-H), 3.68-3.73 (m, 1H, CH), 2.91-3.11 (m, 2H, CH_2), 2.34-2.55 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 166.16 (d, $^1J_{CF}$ = 208.2 Hz), 164.61, 163.04, 139.44, 138.39, 133.90, 131.32 (d, $^3J_{CF}$ = 9.2 Hz, 2C), 130.05 (d, $^4J_{CF}$ = 2.0 Hz), 129.56 (4C), 128.67 (2C), 128.61 (2C), 126.35, 121.43, 121.43, 116.11 (d, $^2J_{CF}$ = 21.5 Hz, 2C), 62.04, 53.32, 36.09; ESI-MS: m/z 519.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}BrFN_2O_3$, 497.0876, found 497.0872.

*4.1.1.17. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-4-chlorobenzamide (**4Bk**)*

As a white powder, yield: 29.7%, m.p. 166.6-167.1 °C. Analytical data for **4Bk**: ^1H NMR (DMSO, 400 MHz, δ ppm): 10.04 (s, 1H, NH), 8.00 (d, J = 8.4 Hz, 2H, Ar-H), 7.76 (d, J = 8.4 Hz, 1H, NH), 7.60 (d, J = 8.4 Hz, 2H, Ar-H), 7.02-7.27 (m, 10H, Ar-H), 3.67-3.72 (m, 1H, CH), 2.91-3.12 (m, 2H, CH_2), 2.34-2.55 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.68, 162.98, 139.43, 138.37, 137.49, 133.79, 132.32, 130.42 (2C), 129.56 (5C), 129.11 (2C), 128.67 (2C), 128.61 (2C), 126.35, 121.59, 62.03, 53.31, 36.09; ESI-MS: m/z 535.3 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{23}BrClN_2O_3$, 513.0581, found 513.0576.

*4.1.1.18. (S,Z)-4-bromo-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)benzamide (**4Bl**)*

As a white powder, yield:32.6%, m.p. 103.9-104.5°C. Analytical data for **4Bl**: ^1H NMR (DMSO, 400 MHz, δ ppm): 10.04 (s, 1H, NH), 7.92 (d, J = 8.8 Hz, 2H, Ar-H), 7.71-7.76 (m, 3H, NH, Ar-H), 7.27 (br, 5H, Ar-H), 7.02-7.16 (m, 5H, Ar-H), 3.67-3.72 (m, 1H, CH), 2.90-3.11 (m, 2H, CH_2), 2.34-2.55 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.82, 162.97, 139.43, 138.36, 133.78, 132.68, 132.05 (2C), 130.57 (2C),

129.56 (5C), 128.68 (2C), 128.61 (2C), 126.50, 126.36, 121.55, 62.03, 53.31, 36.08; ESI-MS: m/z 578.9 [M + Na]$^+$; ESI-HRMS (TOF): *m/z* [M + H]$^+$ calcd for $C_{25}H_{23}Br_2N_2O_3$, 557.0075, found 557.0066.

*4.1.1.19. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-4-methoxybenzamide (**4Bm**)*

As a white powder, yield: 30.8%, m.p. 146.9-147.3 °C. Analytical data for **4Bm**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.75 (s, 1H, NH), 7.99 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.73 (d, *J* = 8.4 Hz, 1H, NH), 7.27 (br, 5H, Ar-H), 7.02-7.19 (m, 7H, Ar-H), 4.31 (t, *J* = 5.6 Hz, 1H, OH), 3.81 (s, 3H, OCH_3), 3.67-3.76 (m, 1H, CH), 2.92-3.11 (m, 2H, CH_2), 2.33-2.52 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.05, 163.20, 162.82, 139.41, 138.46, 134.21, 130.47 (2C), 129.87, 129.61 (2C), 129.56 (2C), 129.48, 128.62 (4C), 126.35, 125.65, 120.49, 114.23 (2C), 113.90, 62.04, 56.02, 53.30, 36.12; ESI-MS: m/z 531.1 [M + Na]$^+$; ESI-HRMS (TOF): *m/z* [M + H]$^+$ calcd for $C_{26}H_{26}BrN_2O_4$, 509.1076, found 509.1074.

*4.1.1.20. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-4-methylbenzamide (**4Bn**)*

As a white powder, yield: 31.4%, m.p. 128.8-129.4 °C. Analytical data for **4Bn**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.79 (s, 1H, NH), 7.89 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.70 (d, *J* = 8.8 Hz, 1H, NH), 7.28-7.31 (m, 7H, Ar-H), 7.12-7.18 (m, 3H, Ar-H), 7.04 (d, *J* = 8.0 Hz, 2H, Ar-H), 3.68-3.73 (m, 1H, CH), 2.92-3.12 (m, 2H, CH_2), 2.34-2.53 (m, 5H, CH_2, CH_3); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.49, 163.14, 142.73, 139.40, 138.42, 134.09, 130.75, 129.61 (2C), 129.55 (2C), 129.52 (2C), 128.65 (2C), 128.62 (3C), 128.49 (2C), 126.35, 120.75, 62.02, 53.30, 36.12, 21.61; ESI-MS: m/z 515.0 [M + Na]$^+$; ESI-HRMS (TOF): *m/z* [M + H]$^+$ calcd for $C_{26}H_{26}BrN_2O_3$, 493.1127, found 493.1127.

*4.1.1.21. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-2,4-difluorobenzamide (**4Bo**)*

As a white powder, yield: 31.7%, m.p. 139.9-140.4 °C. Analytical data for **4Bo**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.76 (d, *J* = 3.6 Hz, 1H, NH), 7.78-7.83 (m, 2H, Ar-H, NH), 7.03-7.44 (m, 12H, Ar-H), 4.34 (br, 1H, OH), 3.68-73 (br, 1H, CH), 2.91-3.12 (m, 2H, CH_2), 2.34-2.55 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 164.42 (dd, $^1J_{CF}$ = 256.5 Hz, $^3J_{CF}$ = 13.1 Hz), 162.86, 162.75 (dd, $^1J_{CF}$ = 249.8 Hz, $^3J_{CF}$ = 12.5 Hz), 160.26, 136.82, 136.66, 138.16, 134.70 (dd, $^3J_{CF}$ = 20.4 Hz, $^3J_{CF}$ = 2.7 Hz), 132.64, 129.95, 129.39 (2C), 129.26 (2C), 128.74 (2C), 128.63 (2C), 126.67, 115.84 (dd, $^2J_{CF}$ = 10.6 Hz, $^4J_{CF}$ = 3.7 Hz), 113.10 (dd, $^2J_{CF}$

= 20.3 Hz, $^4J_{CF}$ = 2.7 Hz), 104.96 (dd, $^2J_{CF}$ = 26.3 Hz, $^2J_{CF}$ = 26.2 Hz), 62.05, 52.33, 36.60; ESI-MS: m/z 537.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{22}BrF_2N_2O_3$, 515.0782, found 515.0784.

*4.1.1.22. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-3,4-difluorobenzamide (**4Bp**)*

As a white powder, yield: 32.8%, m.p. 186.6-187.1 °C. Analytical data for **4Bp**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 10.08 (s, 1H, NH), 8.05 (br, 1H, Ar-H), 7.87 (br, 1H, Ar-H), 7.79 (d, *J* = 8.0 Hz, 1H, NH), 7.62 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.05-7.27 (m, 10H, Ar-H), 4.35 (br, 1H, OH), 3.70 (br, 1H, CH), 2.90-3.11 (m, 2H, CH_2), 2.35-2.57 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 163.56, 162.87, 151.73 (dd, $^1J_{CF}$ = 250.3 Hz, $^2J_{CF}$ = 12.4 Hz), 150.98 (dd, $^1J_{CF}$ = 245.4 Hz, $^2J_{CF}$ = 13.0 Hz), 139.43, 138.35, 133.66, 130.98, 129.61, 129.55 (4C), 128.67 (2C), 128.59 (2C), 126.34, 126.22 (2C), 121.95, 118.42 (dd, $^2J_{CF}$ = 37.8 Hz, $^3J_{CF}$ = 17.6 Hz), 62.04, 53.33, 36.11; ESI-MS: m/z 537.1 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{22}BrF_2N_2O_3$, 515.0782, found 515.0783.

*4.1.1.23. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-3,4-dichlorobenzamide (**4Bq**)*

As a white powder, yield: 27.4%, m.p. 195.2-196.1 °C. Analytical data for **4Bq**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 10.13 (s, 1H, NH), 7.81 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.59-7.63 (m, 1H, Ar-H), 7.56 (dd, *J* = 9.2, 2.4 Hz, 1H, Ar-H), 7.03-7.35 (m, 11H, Ar-H), 4.33 (br, 1H, OH), 3.67-3.73 (m, 1H, CH), 2.91-3.13 (m, 2H, CH_2), 2.35-2.52 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.72, 164.23 (d, $^1J_{CF}$ = 248.3 Hz), 162.66, 139.44, 138.31, 133.61, 132.41 (d, $^3J_{CF}$ = 10.1 Hz), 131.96 (d, $^3J_{CF}$ = 10.4 Hz), 129.58 (2C), 129.52 (3C), 128.65 (5C), 126.37, 119.85, 117.88 (d, $^2J_{CF}$ = 26.4 Hz), 114.94 (d, $^2J_{CF}$ = 21.5 Hz), 61.94, 53.25, 36.08; ESI-MS: m/z 553.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{22}BrClFN_2O_3$, 531.0486, found 531.0485.

*4.1.1.24. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-4-fluoro-2-methylbenzamide (**4Br**)*

As a white powder, yield: 32.0%, m.p. 180.9-182.2 °C. Analytical data for **4Br**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.92 (s, 1H, NH), 7.78 (br, 1H, NH), 7.55 (br, 1H, Ar-H), 7.05-7.26 (m, 12H, Ar-H), 4.35 (br, 1H, OH), 3.72 (br, 1H, CH), 2.94-3.10 (m, 2H, CH_2), 2.40-2.52 (m, 5H, CH_2, CH_3); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 167.50, 164.42 (d, $^1J_{CF}$ = 235.7 Hz), 162.97, 140.36 (d, $^3J_{CF}$ = 8.8 Hz), 139.48, 138.35, 133.93,

132.17, 130.64, 129.59 (2C), 129.52 (3C), 128.63 (4C), 126.35, 120.47, 117.86 (d, $^2J_{CF}$ = 21.1 Hz), 112.89 (d, $^2J_{CF}$ = 21.4 Hz), 61.06, 53.24, 36.09, 20.13; ESI-MS: m/z 533.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{25}BrFN_2O_3$, 511.1033, found 511.1016.

*4.1.1.25. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-2,4-dichlorobenzamide (**4Bs**)*

As a white powder, yield: 35.3%, m.p. 169.3-170.4 °C. Analytical data for **4Bs**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 10.20 (s, 1H, NH), 7.84 (br, 1H, NH), 7.72 (br, 1H, Ar-H), 7.55 (br, 2H, Ar-H), 7.05-7.27 (m, 10H, Ar-H), 4.36 (br, 1H, OH), 3.71 (br, 1H, CH), 2.93-3.09 (m, 2H, CH_2), 2.39-2.52 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.64, 162.62, 139.44, 138.27, 133.73, 134.59, 133.49, 132.09, 131.37, 129.82, 129.78, 129.57 (2C), 129.52 (2C), 128.65 (4C), 127.78, 126.37, 119.94, 61.93, 53.26, 36.07; ESI-MS: m/z 569.0 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{22}BrCl_2N_2O_3$, 547.0191, found 547.0169.

*4.1.1.26. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxo-1-phenylprop-1-en-2-yl)-3,5-dichlorobenzamide (**4Bt**)*

As a white powder, yield: 28.5%, m.p. 196.6-197.1 °C. Analytical data for **4Bt**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 10.20 (s, 1H, NH), 7.83 (br, 1H, NH), 7.72 (br, 1H, Ar-H), 7.55 (br, 2H, Ar-H), 7.05-7.27 (m, 10H, Ar-H), 4.35 (br, 1H, OH), 3.71 (br, 1H, CH), 2.94-3.09 (m, 2H, CH_2), 2.40-2.52 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 164.64, 162.62, 139.44, 138.28, 135.72, 134.59, 133.50, 132.09, 131.37, 129.83, 129.57 (2C), 129.52 (2C), 128.65 (5C), 127.78, 126.37, 119.92, 61.94, 53.26, 36.07; ESI-MS: m/z 569.1 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{22}BrCl_2N_2O_3$, 547.0191, found 547.0163.

*4.1.2. General procedure for the synthesis of compounds **4Ag**, **5** and **6a-c***

Compound **1** was reacted with 3-hydroxybenzaldehyde (0.01 mol) in the presence of acetic anhydride (Ac_2O) (10 mL) and sodium acetate (AcONa) (0.01 mol) to obtain compound **2**. Next, the intermediate mentioned above was reacted with L-phenylalaninol (0.01 mol) to give the crude product **3**. Finally, the product **3** (0.01 mol) was directly brominated with bromine (0.015 mol) and anhydrous calcium carbonate (0.01 mol) in $CHCl_3$ to give the target compound **4Ag**. The target compound **4Ag** (0.01 mol) was then hydrolyzed to prepare the compound **5**. Finally, compound **5** was alkylated with 2-dimethylaminoethyl chloride hydrochloride, 2-diethylaminoethyl chloride hydrochloride or 3-dimethylaminopropyl chloride

hydrochloride (0.012 mol) in the presence of 1, 4-dioxane and K_2CO_3 to provide the crude product of alkoxyl substituted derivatives, respectively, which was purified by column chromatography to yield the title compounds **6a-c**, respectively.

*4.1.2.1. (S,Z)-3-(2-benzamido-1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-1-yl)phenyl acetate (**4Ag**)*

As a yellow powder, yield: 32.1%, m.p.140.3-141.5 °C. Analytical data for **4Ag**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.94 (s, 1H, NH), 7.98 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.84 (d, *J* = 8.8 Hz, 1H, NH), 7.59 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.50 (t, *J* = 8.0 Hz, 2H, Ar-H), 7.29 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.04-7.18 (m, 9H, Ar-H), 4.34 (t, *J* = 5.6 Hz, 1H, OH), 3.68-3.73 (m, 1H, CH), 2.94-3.14 (m, 2H, CH_2), 2.37-2.56 (m, 2H, CH_2), 2.24 (s, 3H, CH_3); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 169.56, 165.63, 162.96, 150.50, 139.68, 139.40, 134.59, 133.46, 132.69, 129.74, 129.57 (2C), 129.01 (2C), 128.61 (2C), 128.48 (2C), 126.98, 126.36, 123,17, 122.92, 119.48, 61.90, 53.37, 36.08, 21.41; ESI-MS: m/z 559.5 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{27}H_{26}BrN_2O_5$, 537.1025, found 537.1025.

*4.1.2.2. (S,Z)-N-(1-bromo-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-1-(3-hydroxyphenyl)-3-oxoprop-1-en-2-yl)benzamide (**5**)*

As a white powder, yield: 29.3%, m.p.180.4-181.2 °C. Analytical data for **5**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.89 (s, 1H, NH), 9.59 (s, 1H, Ar-OH), 7.98 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.66 (d, *J* = 8.8 Hz, 1H, NH), 7.58 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.50 (t, *J* = 7.2 Hz, 2H, Ar-H), 7.02-7.18 (m, 6H, Ar-H), 6.82 (s, 1H, Ar-H), 6.69-6.73 (m, 2H, Ar-H), 4.32 (br, 1H, OH), 3.68-3.73 (m, 1H, CH), 2.92-3.14 (m, 2H, CH_2), 2.37-2.51 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.55, 163.20, 157.48, 139.42, 139.37, 133.79, 133.54, 132.61, 129.66, 129.58 (2C), 128.99 (2C), 128.63 (2C), 128.41 (2C), 126.36, 121.17, 120.38, 116.73, 116.56, 61.77, 53.30, 36.05; ESI-MS: m/z 517.5 $[M + Na]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{24}BrN_2O_4$, 495.0919, found 495.0924.

*4.1.2.3. (S,Z)-N-(1-bromo-1-(3-(2-(dimethylamino)ethoxy)phenyl)-3-((1-hydroxy-3-phenylpropan-2-yl)amino)-3-oxoprop-1-en-2-yl)benzamide (**6a**)*

As a white powder, yield: 26.5%, m.p.89.5-91.4 °C. Analytical data for **6a**: ^{1}H NMR (DMSO, 400 MHz, δ ppm): 9.91 (s, 1H, NH), 7.98 (d, J = 7.2 Hz, 2H, Ar-H), 7.75 (d, *J* = 8.8 Hz, 1H, NH), 7.58 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.50 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.09-7.20 (m, 4H, Ar-H), 7.03 (d, *J* = 8.0 Hz, 2H, Ar-H), 6.84-

6.92 (m, 3H, Ar-H), 4.32 (t, *J* = 5.6 Hz, 1H, OH), 4.00 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.65-3.73 (m, 1H, CH), 2.91-3.13 (m, 2H, CH_2), 2.59 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.33-2.51 (m, 2H, CH_2), 2.17 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.58, 163.19, 158.46, 139.59, 139.34, 134.20, 133.50, 132.65, 129.82, 129.51 (2C), 129.01 (2C), 128.61 (2C), 128.44 (2C), 126.35, 121.97, 120.42, 115.91, 116.73, 66.28, 61.80, 58.02, 53.35, 45.95 (2C), 36.09; ESI-MS: m/z 566.1 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{29}H_{33}BrN_3O_4$, 566.1654, found 566.1635.

*4.1.2.4. (S,Z)-N-(1-bromo-1-(3-(2-(diethylamino)ethoxy)phenyl)-3-((1-hydroxy-3-phenylpropan-2-yl) amino)-3-oxoprop-1-en-2-yl)benzamide (**6b**)*

As a white powder, yield: 26.7%, m.p.93.4-94.2 °C. Analytical data for **6b**: ^1H NMR (DMSO, 400 MHz, δ ppm): 9.89 (s, 1H, NH), 7.98 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.74 (d, *J* = 8.4 Hz, 1H, NH), 7.58 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.50 (t, *J*=7.6 Hz, 2H, Ar-H), 7.10-7.21 (m, 4H, Ar-H), 7.03 (d, *J* = 6.8 Hz, 2H, Ar-H), 6.86-6.93 (m, 3H, Ar-H), 4.04 (t, *J* = 5.6 Hz, 2H, OCH_2), 3.66-3.72 (m, 1H, CH), 2.94-3.13 (m, 2H, CH_2), 2.87 (br, 2H, NCH_2), 2.63 (br, 4H, N(CH_2)2), 2.35-2.51 (m, 2H, CH_2), 0.97 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.58, 163.16, 158.28, 139.63, 139.36, 134.21, 133.48, 132.66, 129.84, 129.52 (2C), 129.01 (2C), 128.61 (2C), 128.45 (2C), 126.35, 122.08, 120.44, 115.92, 115.75, 65.98, 61.78, 53.38, 51.39, 47.48 (2C), 36.07, 11.60; ESI-MS: m/z 594.1 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{31}H_{37}BrN_3O_4$, 594.1967, found 594.1979.

*4.1.2.5. (S,Z)-N-(1-bromo-1-(3-(3-(dimethylamino)propoxy)phenyl)-3-((1-hydroxy-3-phenylpropan-2-yl) amino)-3-oxoprop-1-en-2-yl)benzamide (**6c**)*

As a white powder, yield: 25.8%, m.p.88.7-89.5 °C. Analytical data for **6c**: ^1H NMR (DMSO, 400 MHz, δ ppm): 9.89 (s, 1H, NH), 7.98 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.73 (d, *J* = 8.4 Hz, 1H, NH), 7.58 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.50 (t, *J* = 6.8 Hz, 2H, Ar-H), 7.08-7.20 (m, 4H, Ar-H), 7.03 (d, *J* = 6.8 Hz, 2H, Ar-H), 6.85-6.91 (m, 3H, Ar-H), 3.95 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.65-3.73 (m, 1H, CH), 2.94-3.13 (m, 2H, CH_2), 2.35-2.52 (m, 4H, 2×CH_2), 2.20 (s, 6H, $N(CH_3)_2$), 1.82-1.89 (m, 2H, CH_2); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.58, 163.17, 158.49, 139.59, 139.37, 134.15, 133.49, 132.66, 129.82, 129.53 (2C), 129.01 (2C), 128.61 (2C), 128.45 (2C), 126.35, 121.97, 120.58, 115.86, 115.64, 66.10, 61.80, 55.70, 53.37, 44.81 (2C), 36.07, 26.47; ESI-MS: m/z 580.2 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{30}H_{35}BrN_3O_4$, 580.1811, found 580.1820.

4.2. Biological assays

HepG2 2.2.15 cells, stably transfected with HBV genome using a plasmid (302 military hospital of china), 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. The cytotoxicity, effect of the target compounds on HBV DNA replication and viral gene expression were evaluated on HepG2 2.2.15 cells. Antiviral efficacy against nucleoside analogue-resistant HBV stains was evaluated on HepG2 cell line transiently transfected with plasmids containing drug-resistant mutant (rtL180M + rtM204V + rtT184L).

4.2.1. Cytotoxicity assays

Intrinsic cytotoxicity of the target compounds against HepG2 2.2.15 cells were measured by MTT assay. Briefly, cells were seeded in 96-well culture plates and cultured for 24 h. Then, cells were continue to incubate with the target compounds (in 0.2 mL culture medium/well) for 72 h. Untreated control cultures were maintained on each 96-well plate. Cell viability was finally determined by measuring MTT absorbance at 490 nm, and calculated from the absorbance relative to untreated cells.

4.2.2. Inhibitory effect on replication of HBV DNA

HepG2 2.2.15 cells (2×10^5/well) were seeded in 24 well culture plates and recovered for 24 h. Culture medium was replaced by assay medium containing the tested compound or 3TC at the indicated dose. Medium was then changed every 2 days. After 6 days incubation, the intracellular HBV DNA was extracted and quantified by real time PCR according to the previous method.[21]

4.2.3. Antiviral activity against nucleoside analogue-resistant HBV stains

HepG2 cells (2×10^5/well) were plated into 24-well plates. After 24 h incubation, the cells were transiently transfected with plasmids containing drug-resistant mutant (rtL180M + rtM204V + rtT184L) for 24 h. Then the supernatant was removed followed by washing with PBS. Cells were subsequently treated with fresh medium containing lamivudine (100 μM), Entecavir (10 μM), **4Bj** (0.8 μM) or **4Bs** (0.8 μM). After 72 h incubation, HBV DNA replicative intermediate was extracted and quantified using real time PCR using the method mentioned above.[22]

*4.2.4. Effect of compound **4Bs** on HBV viral gene expression* [16, 17]

HepG2 2.2.15 cells (6×10^5/well) were cultured in 6-well plates, and treated with tested compound for 6

days. Total RNA of the treated HepG2 2.2.15 cells was extracted using TRIzol reagent (Invitrogen), and detected by quantitative real time PCR.

4.2.5. Molecular docking

The crystal structure of HBV core protein complex (PDB code: 5gmz) was obtained from the Protein Data Bank, which contained a single point mutation at position Y132A in the N-terminal assembly domain. Molecular docking was performed by the Sybyl X-2.1 software according to previous methods.[23, 24] Briefly, all the receptor and ligands were prepared using the Sybyl X-2.1Tools module. During the protein preparation, the missing hydrogen atoms were added by biopolymer module, and all water molecules were abandoned. The amino acids around the ligand in the crystal structure of HBV core protein were selected and defined as the active pocket. Docking parameters were kept at default settings. The pose with the highest binding score was used for further docking analysis.

Acknowledgements

This work was supported by the grants from the National Natural Science Foundation of China (NSFC No. 81703359), the Natural Science Foundation of Jiangsu province (No. BK20181151 and BK20171179), the Six Talent Peaks Project in Jiangsu Province (Grant No. 2017-YY-039) and Jiangsu Provincial 333 High-level Talents Cultivation Project (BRA201927) and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (No. KYCX19_2251). We thank to Public Experimental Research Center of Xuzhou Medical University for providing chemical structure analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

References

1. Alagarsamy V, Chitra K, Saravanan G, et al. An overview of quinazolines: Pharmacological significance and recent developments. *Eur J Med Chem*. 2018; 151: 628-685.
2. Ma CQ, Xu W, Yang QY, et al. Osteopetrosis-Associated Transmembrane Protein 1 Recruits RNA Exosome To Restrict Hepatitis B Virus Replication. *J Virol*. 2020; 94.
3. Zhu MY, Li W, Lu Y, et al. HBx drives alpha fetoprotein expression to promote initiation of liver cancer stem cells through activating PI3K/AKT signal pathway. *Int J Cancer*. 2017; 140: 1346-

1355.

4. Zhang L, Chen Y, Zhang L-J, et al. HBV induces different responses of the hepatocytes and oval cells during HBV-related hepatic cirrhosis. *Cancer Lett*. 2019; 443: 47-55.
5. Tang J, Huber AD, Pineda DL, et al. 5-Aminothiophene-2, 4-dicarboxamide analogues as hepatitis B virus capsid assembly effectors. *Eur J Med Chem*. 2019; 164: 179-192.
6. Tang L, Kottilil SWilson E. Strategies to eliminate HBV infection: an update. *Future Virology*. 2020; 15: 35-51.
7. Feng S, Gao L, Han XC, et al. Discovery of Small Molecule Therapeutics for Treatment of Chronic HBV Infection. *Acs Infectious Diseases*. 2018; 4: 257-277.
8. Yu J, Jia HY, Guo XW, et al. Design, synthesis, and evaluation of novel heteroaryldihydropyrimidine derivatives as non-nucleoside hepatitis B virus inhibitors by exploring the solvent-exposed region. *Chemical Biology & Drug Design*. 2020; 95: 567-583.
9. Do AReau NS. Chronic Viral Hepatitis: Current Management and Future Directions. *Hepatol Commun*. 2020; 4: 329-341.
10. Sari O, Boucle S, Cox BD, et al. Synthesis of sulfamoylbenzamide derivatives as HBV capsid assembly effector. *Eur J Med Chem*. 2017; 138: 407-421.
11. Pan T, Ding Y, Wu L, et al. Design and synthesis of aminothiazole based Hepatitis B Virus (HBV) capsid inhibitors. *Eur J Med Chem*. 2019; 166: 480-501.
12. Hu Z, An Q, Li K, et al. Identification, Synthesis, and Strategy for Minimization of Potential Impurities in the Preclinical Anti-HBV Drug Y101. *Org Process Res Dev*. 2013; 17: 1156-1167.
13. Hu Z, Liao H, An Q, et al. Process development of clinical anti-HBV drug Y101: identification and synthesis of novel impurities. *Res Chem Intermediat*. 2015; 42: 2577-2595.
14. Qiu J, Gong Q, Gao J, et al. Design, synthesis and evaluation of novel phenyl propionamide derivatives as non-nucleoside hepatitis B virus inhibitors. *Eur J Med Chem*. 2018; 144: 424-434.
15. Qiu J, Chen W, Zhang Y, et al. Assessment of quinazolinone derivatives as novel non-nucleoside hepatitis B virus inhibitors. *Eur J Med Chem*. 2019; 176: 41-49.
16. Xu YB, Yang L, Wang GF, et al. Benzimidazole derivative, BM601, a novel inhibitor of hepatitis B virus and HBsAg secretion. *Antivir Res*. 2014; 107: 6-15.

17. Wang YJ, Lu D, Xu YB, et al. A Novel Pyridazinone Derivative Inhibits Hepatitis B Virus Replication by Inducing Genome-Free Capsid Formation. *Antimicrob Agents Ch*. 2015; 59: 7061-7072.
18. Toyama M, Sakakibara N, Takeda M, et al. Pyrimidotriazine derivatives as selective inhibitors of HBV capsid assembly. *Virus Res*. 2019; 271: 197677.
19. Klumpp K, Lam AM, Lukacs C, et al. High-resolution crystal structure of a hepatitis B virus replication inhibitor bound to the viral core protein. *P Natl Acad Sci USA*. 2015; 112: 15196-15201.
20. Qiu Z, Lin X, Zhou M, et al. Design and synthesis of orally bioavailable 4-methyl heteroaryldihydropyrimidine based hepatitis B virus (HBV) capsid inhibitors. *J Med Chem*. 2016; 59: 7651-7666.
21. Delaney WEIsom HC. Hepatitis B virus replication in human HepG2 cells mediated by hepatitis B virus recombinant baculovirus. *Hepatology*. 1998; 28: 1134-1146.
22. Gao L-M, Han Y-X, Wang Y-P, 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.
23. Liu L, Kai X, Chen Q, et al. Identification of protein arginine methyltransferase 7 (PRMT7) inhibitor by virtual screening and biological evaluation in vitro. *Med Chem Res*. 2019; 28: 125-132.
24. An Y, Meng C, Chen Q, et al. Discovery of small molecule sirt1 activator using high-throughput virtual screening, molecular dynamics simulation, molecular mechanics generalized born/surface area (MM/GBSA) calculation, and biological evaluation. *Med Chem Res*. 2020; 29: 255-261.

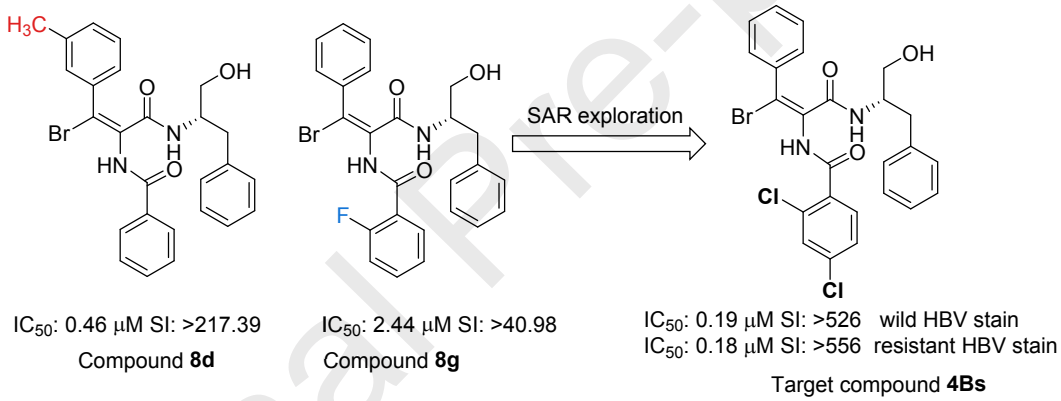
Synthesis and evaluation of new phenyl acrylamide derivatives as potent non-nucleoside anti-HBV agents

Xiaoke Gu[a, #], Yinpeng Zhang[b, #], Yueting Zou[b], Xin Li[a], Mingyu Guan[a], Qingqing Zhou[b], Jingying Qiu[a, b, *]

[a] *Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, Xuzhou 221004, People's Republic of China*

[b] *Department of Pharmaceutical Analysis, School of Pharmacy, Xuzhou Medical University, Xuzhou 221004, People's Republic of China*

Graphical Abstract



Compound **4Bs** exhibited potent anti-HBV activity against wild and drug (lamivudine and entecavir) resistant HBV strains, and possessed a favorable safety profile with a SI value of above 526.

* Corresponding author. Tel.: +86-516-83262138; fax: +86-516-83262630; e-mail: jingyqiu@xzhmu.edu.cn (J. Q.)
# These two authors contributed equally to this work.

Highlights

1. Phenyl acrylamide derivatives were synthesized and evaluated as novel anti-HBV agents.
2. **4Bs** exhibited potent anti-HBV activity against wild and resistant HBV strains.
3. **4Bs** possessed a favorable safety profile with a SI value of above 526.
4. **4Bs** inhibited 3.5kb pgRNA expression and fitted well into the interface of HBV core protein.

Declaration of Interest Statement

The authors declared that there was no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.