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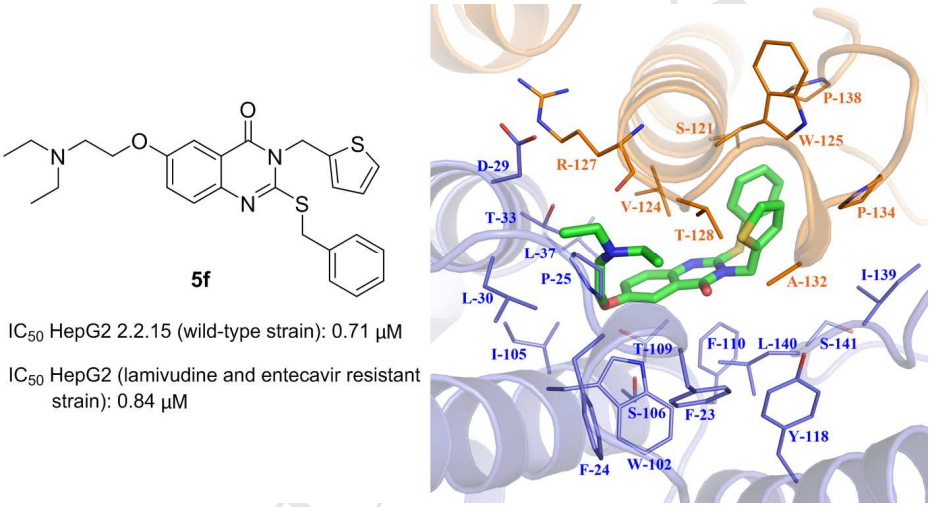
Graphical Abstract



Compound **5f** exhibited significantly anti-HBV activity against wild and drug (lamivudine and entecavir) resistant HBV strains, and could well fit into the dimer-dimer interface of HBV core protein dominated by hydrophobic interactions.

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Abstract

Hepatitis B virus (HBV) infection is a worldwide public health issue. Search for novel non-nucleoside anti-HBV agents is of great importance. In the present study, a series of quinazolinones derivatives (**4a-t** and **5a-f**) were synthesized and evaluated as novel anti-HBV agents. Among them, compounds **5e** and **5f** could significantly inhibit HBV DNA replication with IC_{50} values of 1.54 μM and 0.71 μM, respectively. Interestingly, the selective index values of **5f** was higher than that of lead compound **K284-1405**, suggesting **5f** possessed relatively safety profile than **K284-1405**. Notably, **5e** and **5f** exhibited remarkably anti-HBV activities against lamivudine and entecavir resistant HBV strain with IC_{50} values of 1.90 and 0.84 μM, confirming their effectiveness against resistant HBV strain. In addition, molecular docking studies indicated that compounds **5e** and **5f** could well fit into the dimer-dimer interface of HBV core protein dominated by hydrophobic interactions. Notably, their binding modes were different from the lead compound **K284-1405**, which may be attributed to the additional substituent groups in the quinazolinone scaffold. Taken together, **5e** and **5f** possessed novel chemical structure and potent anti-HBV activity against both drug sensitive and resistant HBV strains, thus warranting further research as potential non-nucleoside anti-HBV candidates.

Keywords: Anti-HBV agents; Synthesis; Quinazolinone derivatives; HBV core protein

1. Introduction

Hepatitis B virus (HBV) infection, a worldwide health threat, may cause acute, chronic hepatitis,

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liver cirrhosis, liver fibrosis or hepatocelullar carcinoma (HCC) [1-3]. Statistically, there are about 350 million people worldwide are chronically infected by HBV [4, 5]. Despite the availability of the effective prophylactic vaccine, HBV infection and complications still result in 686000 deaths per year [6-8]. Currently, interferons (IFNs) and nucleos(t)ides-based reverse transcriptase inhibitors have been approved for the treatment of HBV infection. Unfortunately, due to the limitations and side effects of the current treatment, the clinical cure rate for patients with chronic HBV infection is not satisfactory [9-11]. For IFNs therapy, parenteral administration is mandatory, and IFNs usually results in adverse effect, such as “flu-like” symptoms [12]. For nucleos(t)ide therapy, patients are required to take drugs for life to prevent HBV viral rebound [13]. What's more frustrating is that HBV can develop resistance to nucleos(t)ide drugs [14, 15]. Therefore, there is a clear and urgent need to develop anti-HBV agents with novel structure and mode of action.

It is well-known that HBV core protein performs crucial roles at HBV virus life cycle via affecting the encapsidation of pregenomic RNA, the maturation and maintenance of the stable HBV virion and blocking virion secretion [16]. Consequently, HBV core protein is considered as a promising target for the development of novel, safe and effective anti-HBV agents [17, 18]. The latest research confirmed that several compounds can prevent encapsidation of pgRNA by modulate the kinetics/thermodynamics of HBV core protein aggregation, thus inhibiting HBV DNA synthesis and infectious virion production [7, 19-22]. Recently, the crystal structure of the HBV core protein in complex with a heteroaryldihydropyrimidine (HAP) derivate NVR-010-001-E2 was reported. Meanwhile, the mode of action of this compound with HBV core protein was described in detail [23]. Based on these research findings, our group obtained a compound with quinazolinone scaffold (compound **K284-1405**, Figure 1) as a new HBV core protein inhibitor by molecular docking based virtual screening. Preliminary anti-HBV activity studies showed that compound **K284-1405** displayed potential anti-HBV activity with an IC_{50} value on HBV DNA replication of 4.07 μM, and the selective index (SI) values was more than 17.57. Herein, to obtain novel anti-HBV agents with more potent anti-HBV activities, we firstly synthesized a series novel quinazolinones derivatives (Figure 1), and then evaluated their anti-HBV activities against both drug sensitive and resistant HBV strains in the present work. In addition, the mode of binding between the active compounds and HBV core protein were also

proposed by docking studies in this context.

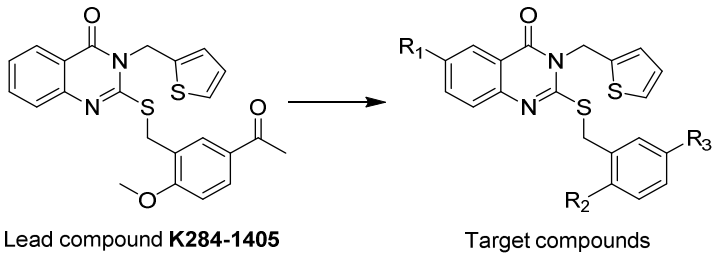
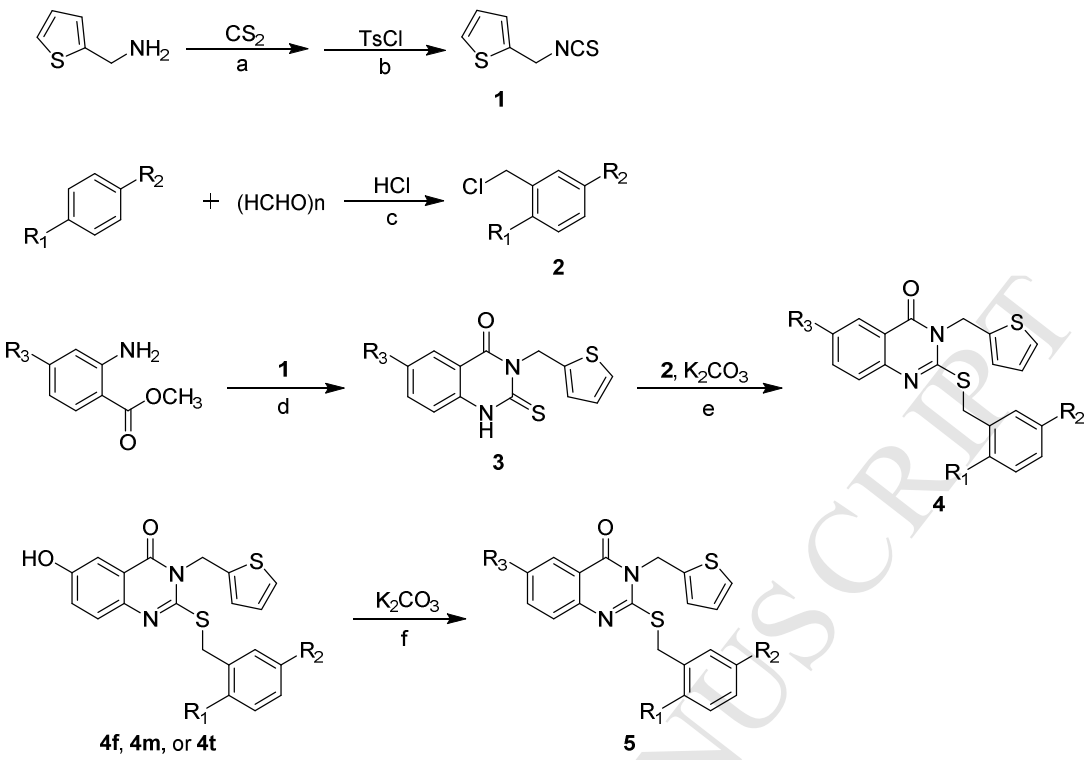


Figure 1. Chemical structures of lead compound **K284-1405** and the quinazolinone derivatives.

2. Chemistry

Synthetic procedures of the novel quinazolinone derivatives **4a-t** and **5a-f** were illustrated in Scheme 1. Briefly, 2-thiophenemethylamine was reacted with CS_2 and toluene sulfonyl chloride in THF to obtain compound **1**. Compound **2** was obtained by the chloromethylation reaction with benzene or benzene derivatives and paraformaldehyde was selected as the raw materials. Methyl anthranilate or methyl anthranilate derivatives was condensed with 2-(isothiocyanatomethyl) thiophene to give intermediate **3**, which was then reacted with compound **2** mentioned above in the presence of K_2CO_3 and 1,4-dioxane to obtain target compounds **4a-t**, respectively. Compound **4f**, **4m**, or **4t** was alkylated with 2-dimethylaminoethyl chloride hydrochloride or 2-diethylaminoethyl chloride hydrochloride to provide alkoxyl substituted derivative **5a-f**, respectively.



4a-f	R_1: OCH_3;	R_2: $COCH_3$;	R_3: F, Cl, Br, I, CH_3, OH
5a-b	R_1: OCH_3;	R_2: $COCH_3$;	R_3: $OCH_2CH_2N(CH_3)_2$, $OCH_2CH_2N(CH_2CH_3)_2$
4g-m	R_1: OCH_3;	R_2: H;	R_3: H, F, Cl, Br, I, CH_3, OH
5c-d	R_1: OCH_3;	R_2: H;	R_3: $OCH_2CH_2N(CH_3)_2$, $OCH_2CH_2N(CH_2CH_3)_2$
4n-t	R_1: H;	R_2: H;	R_3: H, F, Cl, Br, I, CH_3, OH
5e-f	R_1: H;	R_2: H;	R_3: $OCH_2CH_2N(CH_3)_2$, $OCH_2CH_2N(CH_2CH_3)_2$

Scheme 1. Synthetic route of target compounds **4a-t** and **5a-f**. Reagents and conditions: a) TEA, THF, 0 °C; b) TsCl, 0 °C; c) HCl, 80 °C; d) TEA, EtOH, reflux; e) 1,4-dioxane, K_2CO_3, 90 °C; f) 2-dimethylaminoethyl chloride hydrochloride or 2-diethylaminoethyl chloride hydrochloride, 1,4-dioxane, K_2CO_3, 90 °C.

3. Results and discussion

3.1. Biological evaluation

The intrinsic cytotoxicity of the target compounds against HepG2 2.2.15 cells was determined by MTT. The dose of the test compound that results in 50% death of HepG2 2.2.15 cells was defined as CC_{50}. The anti-HBV activities of the target compounds were assessed in HepG2 2.2.15 cells using real time quantitative PCR (RT-PCR) assay. The concentration of the test compound that inhibits HBV DNA replication by 50% was defined as IC_{50}. Selectivity index (SI) was calculated as the CC_{50}/IC_{50} value. Classic anti-HBV agent lamivudine (3TC) was selected as the positive control.

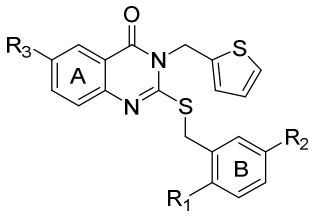
3.1.1. Cytotoxicity of the target compounds

Firstly, the intrinsic cytotoxicity of the target compounds against HepG2 2.2.15 cells was measured by MTT assay. Briefly, HepG2 2.2.15 cells were incubated with various concentrations of target compounds **4a-t** and **5a-f** for 72 h, respectively. Then the cell viability was determined by MTT assay. As seen from Table 1, most of the test compounds display little (CC_{50} > 100 μM) or slight (CC_{50} = 8.76-89.04 μM) intrinsic cytotoxicity in vitro, suggesting that the target compounds possessed a relatively safety profile.

3.1.2. Effect of the target compounds on HBV DNA replication

Subsequently, the inhibitory effect of the target compounds **4a-t** and **5a-f** on HBV DNA replication was screened in the HepG2 2.2.15 cell line by RT-PCR assay. Compound **K284-1405** and lamivudine (3TC) were used as the lead compound and positive control, respectively. Specifically, HepG2 2.2.15 cells was treated with the test compounds at the non-toxic dose (4 μM) for 6 days, then the extracellular HBV DNA levels were quantified by RT-PCR. As shown in Table 1, at non-toxic dose (4 μM) most test compounds can inhibit HBV DNA replication to some extent. Among them, some of the target compounds displayed more potent anti-HBV activity than lead compound **K284-1405**, for their inhibition rate on HBV DNA replication was much higher than that of **K284-1405** (47.08%). Obviously, compounds **5f** was the most potent ones with a HBV DNA inhibition rate of 98.42%, respectively, which was comparable with the positive control 3TC.

Table 1. Inhibitory effect of the target compounds **4a-t** and **5a-f** on HBV DNA replication



Compounds	R_1	R_2	R_3	CC_{50} (μM)	Inhibition rate (%) (4 μM)
4a	OCH_3	$COCH_3$	6-F	89.04	30.67
4b	OCH_3	$COCH_3$	6-Cl	74.62	40.74
4c	OCH_3	$COCH_3$	6-Br	>100	45.96

4d	OCH_3	$COCH_3$	6-I	81.13	45.41
4e	OCH_3	$COCH_3$	6-CH_3	>100	56.15
4f	OCH_3	$COCH_3$	6-OH	>100	3.26
5a	OCH_3	$COCH_3$	6-$OCH_2CH_2N(CH_3)_2$	17.99	33.88
5b	OCH_3	$COCH_3$	6-$OCH_2CH_2N(CH_2CH_3)_2$	8.76	41.96
4g	OCH_3	H	H	>100	76.27
4h	OCH_3	H	6-F	>100	79.61
4i	OCH_3	H	6-Cl	>100	63.27
4j	OCH_3	H	6-Br	>100	64.15
4k	OCH_3	H	6-I	>100	65.87
4l	OCH_3	H	6-CH_3	>100	69.43
4m	OCH_3	H	6-OH	>100	0.59
5c	OCH_3	H	6-$OCH_2CH_2N(CH_3)_2$	14.70	53.89
5d	OCH_3	H	6-$OCH_2CH_2N(CH_2CH_3)_2$	10.14	75.97
4n	H	H	H	>100	11.03
4o	H	H	6-F	>100	9.53
4p	H	H	6-Cl	>100	16.06
4q	H	H	6-Br	>100	16.72
4r	H	H	6-I	>100	1.87
4s	H	H	6-CH_3	>100	13.79
4t	H	H	6-OH	>100	5.62
5e	H	H	6-$OCH_2CH_2N(CH_3)_2$	21.13	83.28
5f	H	H	6-$OCH_2CH_2N(CH_2CH_3)_2$	15.79	98.42
K284-1405	OCH_3	$COCH_3$	H	71.51	47.08
3TC				>100	94.31

To further confirm the anti-HBV activity, the inhibitory effect on HBV DNA replication of active compounds **4g**, **4h** and **5d-f** at different concentrations was subsequently determined by RT-PCR assay. As shown in Table 2, all the tested compounds could significantly inhibit HBV DNA replication with IC_{50} values in range of 0.71-2.81 μM. Clearly, **5e** and **5f** exhibited most potent anti-HBV activity with IC_{50} values of 1.54 and 0.71 μM, respectively. In addition, the SI value of compound **5f** was 22.24, which was higher than that of lead compound **K284-1405** (SI: 17.57), indicating that **5f** possessed relatively safety profile than **K284-1405**.

Table 2. Anti-HBV activity and SI values of target compounds **4g**, **4h** and **5d-f**

Compounds	CC_{50} (μM)	DNA replication	
		IC_{50} (μM)	SI
4g	>100	2.81	>35.59
4h	>100	2.48	>40.48
5d	10.14	1.93	5.25
5e	21.13	1.54	13.72
5f	15.79	0.71	22.24
K284-1405	71.51	4.07	17.57
3TC	>100	<0.1	>1000

Based on the data shown in Tables 1-2, the structure activity relationship (SAR) can be drawn as follows: Acetyl substituent in ring B was not necessary for the anti-HBV activity. When the acetyl substituent was removed, the anti-HBV activity was all increased (**4g** *vs.* **K284-1405**; **4a-f** *vs*. **4h-m**, Table 1). In sharp contrary, the methoxy group in ring B was crucial for the anti-HBV activity of the target compounds. After removing the methoxy group, the anti-HBV activity was remarkably decreased (**4n-t** *vs*. **4g-m**, Table 1). Among them, compounds **4n-t** were inactive at a test concentration of 4 μM ,confirming that the methoxy substituent played a significant role in the anti-HBV activity. In addition, the anti-HBV activity was significantly weakened when introducing a hydroxy substituent in ring A (**4f**, **4m**, **4t** *vs*. **K284-1405**), suggesting that the introduction of a meta substituent might be not conducive to the anti-HBV activity. Interestingly, when the hydroxy substituent was replaced with dimethylamino ethoxy substituent or diethylamine ethoxy, the anti-HBV activity was significantly increased (**4f** *vs.* **5a-b**; **4m** *vs.* **5c-d**; **4t** *vs*. **5e-f**). One plausible explanation is that introduction of dimethylamino ethoxy substituent or diethylamine ethoxy group might form an additional interactions with HBV core protein, thus increasing the affinity of the target compound with HBV core protein. However, the precise SAR remains further investigation when more quinazolinone derivatives are available in the near future.

3.1.3. Anti-HBV activity of compounds **5e** and **5f** *against polymerase drug resistant HBV strain*

As mentioned above, one of the main limitations of the nucleos(t)ide therapy is the emergence

of drug resistance. Therefore, it is of great interest to investigate whether the active compounds **5e** and **5f** exhibit anti-HBV activities against drug resistant HBV strains. HepG2 cells, transiently transfected with lamivudine and entecavir resistant strain mutant (rtL180M + rtM204V + rtT184L) (LERS) was served as an evaluation model. Lamivudine and entecavir were used as reference drugs. After the treatment of HepG2 cells (LERS HBV) or wild-type strain with target compounds **5e** (4 μM), **5f** (4 μM), lamivudine (100 μM) or entecavir (10 μM) respectively for 72 h, the level of HBV DAN replicative intermediate was examined by RT-PCR. As shown in Figure 2, lamivudine and entecavir displayed potent anti-HBV activity against wild-type strain with an inhibition rate on HBV DNA replication of 90.67% and 98.07%. As was expected, lamivudine and entecavir resistant strain was indeed resistant to both lamivudine and entecavir, and their inhibition rate of HBV DNA replication was only 17.73% and 22.58%, respectively. Interestingly, both compound **5e** and **5f** exhibited potent anti-HBV activity against resistant HBV cell strain. Notably, their inhibition rates on HBV DNA replication were respectively 80.91% and 93.99%, which was comparable with that on wild-type HBV cell strain (81.95% and 96.09%, respectively).

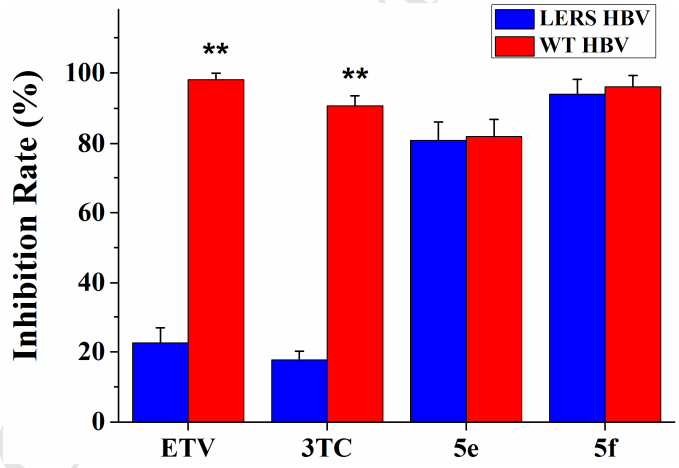


Figure 2. Anti-HBV activities of **5e** and **5f** against lamivudine and entecavir resistant HBV strain. Briefly, the full genome of wide-type or lamivudine and entecavir resistant HBV were transiently transfected into HepG2 cells. Then the cells were treated with entecavir (10 μM), lamivudine (100 μM), **5e** (4 μM) or **5f** (4 μM) for 72 h, respectively. Finally, HBV DNA replicative intermediate level was determined by RT-PCR.

In order to confirm the anti-HBV activity against resistant HBV strain, we further determined the IC_{50} values for inhibition of HBV DNA replication and the SI values of compounds **5e** and **5f** in lamivudine and entecavir resistant HBV strain. Data in Table 3 showed that the IC_{50} values of

5e and **5f** were 1.90 and 0.84 μM, respectively, confirming their potent inhibitory effect on HBV DNA replication against drug resistant strain. Meanwhile, SI values of compound **5f** was 21.40, indicating **5f** possessed relatively safety profile.

Table 3. Anti-HBV activities of compounds **5e** and **5f** against lamivudine and entecavir resistant HBV strain

Compounds	CC_{50} (μM)	DNA replication	
		IC_{50} (μM)	SI
5e	26.72	1.90	14.06
5f	17.98	0.84	21.40
3TC	>100	>100	<1

3.2. Molecular docking

It is well-known that 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 safety anti-HBV agents. Therefore, we preliminarily investigated the binding interactions of the lead compound **K284-1405** and its two analogs **5e** and **5f** with HBV core protein by molecular docking, and compound **NVR-010-001-E2** was also studied for comparison (Figure 3). It can be seen that all three compounds fitted well into the dimer-dimer interface of HBV core protein, and the bindings were almost dominated by the hydrophobic interactions in light of the fact that the binding interface composed of a large number of hydrophobic residues (Figure 3). In detail, each compound was accommodated into a well-defined hydrophobic groove which consisted of residues Phe23, Phe24, Pro25, Asp29, Leu30, Thr33, Leu37, Trp102, Ile105, Ser106, Thr109, Phe110, Tyr118, Ile139, Leu140 and Ser141 in one protein subunit (Figure 3A-D). Meanwhile, each compound was also covered by a "lid" formed by the C-terminal helix of the other protein subunit (Figure 3E-H). Notably, compared with compounds **K284-1405** and **NVR-010-001-E2**, compounds **5e** and **5f** had additional substituent groups in quinazolinone scaffold, which inserted into a new hydrophobic pocket composed of the residues (Ser121, Pro138 and Phe156) in the lid. It is likely that the main difference of binding patterns between compounds **5e** (or **5f**) and **NVR-010-001-E2** was in the lid rather than the hydrophobic groove. Moreover, the binding affinities of compounds **5e** and **5f** were stronger than those of **K284-1405** and **NVR-010-001-E2**, with the corresponding docking scores of 7.03, 8.42, 6.58 and 6.36, respectively.

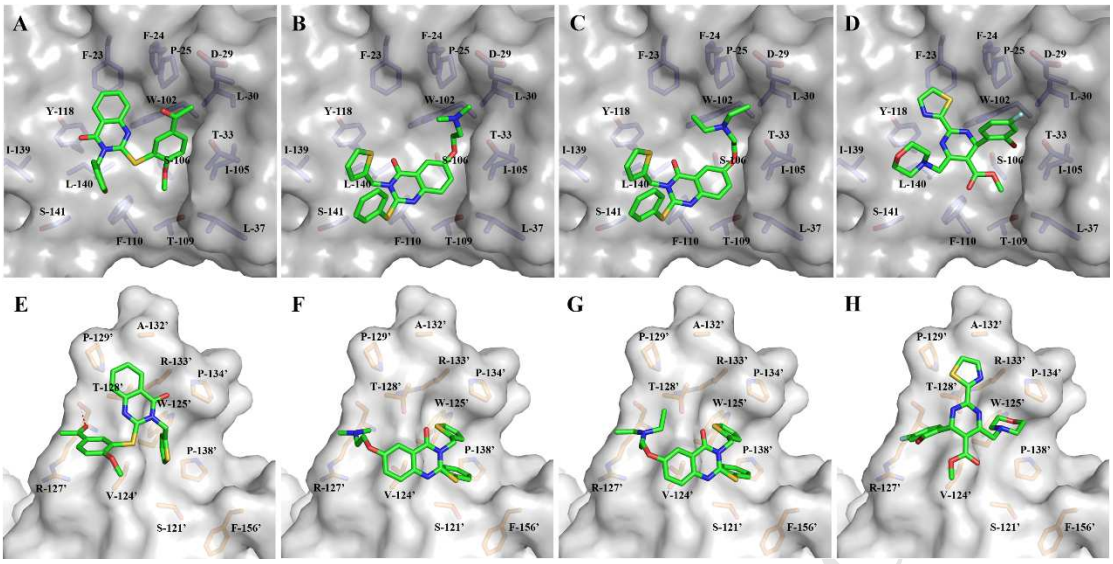


Figure 3. Molecular docking predicted binding modes of compounds **K284-1405**, **5e** and **5f**. The crystal structure of **NVR-010-001-E2** interacted with HBV core protein is used for comparison. (A-D) Compounds **K284-1405**, **5e**, **5f** and **NVR-010-001-E2** sit in the hydrophobic groove of the contact domain (gray surface) on one dimer and the lid is removed for clarity. (E-H) Binding surface to the lid, with the contact domain removed for clarity.

4. Conclusions

In summary, twenty-six quinazolinone derivatives (**4a-t** and **5a-f**) were synthesized and evaluated as novel non-nucleoside anti-HBV agents. Among them, compounds **5e** and **5f** could significantly inhibit HBV DNA replication with IC_{50} values of 1.54 and 0.71 μM, respectively, thus displaying potent anti-HBV activities. Notably, **5e** and **5f** also possessed remarkably anti-HBV activities against lamivudine and entecavir resistant HBV strain with IC_{50} values of 1.90 and 0.84 μM, confirming their effectiveness against resistant HBV strain. In addition, molecular docking studies showed that compounds **5e** and **5f** could well fit into the dimer-dimer interface of HBV core protein by hydrophobic interactions. Considering the novel chemical structure and potent anti-HBV activity against both wild and resistant HBV strains, **5e** and **5f** might be warranted for the further investigation as novel non-nucleoside anti-HBV candidates.

5. Experimental protocols

5.1. Chemical analysis

All of the synthesized compounds were purified by column chromatography on silica gel 60

(Qingdao Ocean Chemical Company, China). Melting points of individual compounds were determined on a model YRT-3 apparatus and uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were performed on a JEOL (400 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 (Representative NMR spectra were shown in supplementary material). MS detection was recorded using an Agilent G6125BA single quadrupole mass spectrometer. All solvents were reagent grade and, when necessary, were purified and dried by standards methods.

*5.1.1. General procedure for the synthesis of compounds **1-4***

CS_2 (2.3 mL, 30 mmol) was added dropwise to the mixture of 2-thiophenemethylamine (30 mmol) and triethylamine (12 mL) in THF (25 mL) at 0 °C within 30 min. After stirring at room temperature for 1 h, the mixture was cooled down to 0 °C and then add the toluene sulfonyl chloride (6 g, 31.5 mmol). After stirring at room temperature for 1 h, the mixture was dispersed in 5% HCl (100 mL) and petroleum ether, washed sequentially with water, saturated $NaHCO_3$ solution and brine, dried with anhydrous Na_2SO_4 and evaporated *in vacuo* to give compound **1**. HCl (10.5 mL) was added to the mixture of benzene or benzene derivatives (6.65 mmol) and paraformaldehyde (11.97 mmol). After stirring at 60 °C for 10 h, the mixture was cooled down to room temperature and then the mixture was dispersed in water and ethyl acetate, 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 to afford compound **2**. A solution of methyl anthranilate or methyl anthranilate derivatives (0.01 mol) and 2-(isothiocyanatomethyl)thiophene (1.55 g, 0.01 mol) in the mixture of triethylamine and ethanol was heated at reflux temperature for 4 h. The reaction mixture was cooled down to room temperature and then filtered to give intermediate **3**, which was then reacted with compound **2** (0.01 mmol) mentioned above in the presence of K_2CO_3 and 1,4-dioxane. After reflux for 10 h, the mixture was cooled down to room temperature and dissolved in ethyl acetate, 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 ethanol to obtain target compound **4**, respectively.

*5.1.1.1. 2-((5-acetyl-2-methoxybenzyl)thio)-6-fluoro-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one **(4a)***

As a yellow powder, yield: 17%; m.p.158.3-162.8 °C. Analytical data for **4a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.24 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 7.89 (dd, *J* = 8.4 Hz, 2.4

Hz, 1H, Ar-H), 7.84 (dd, *J* = 8.4, 3.2 Hz, 1H, Ar-H), 7.68 (dd, *J* = 8.8, 4.8 Hz, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 6.91 (m, 2H, Ar-H), 5.42 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 2.50 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 196.65, 162.64, 161.58, 155.27, 144.18, 136.98, 132.01, 130.48, 129.93, 128.87, 128.40, 126.47, 126.31, 125.07, 123.35, 123.11, 112.06, 111.83, 110.11, 56.01, 42.52, 31.56, 26.39; ESI-MS: m/z 454.9 $[M+H]^+$.

5.1.1.2.

*2-((5-acetyl-2-methoxybenzyl)thio)-6-chloro-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4b**)*

As a white powder, yield: 16%; m.p.170.3-173.6 °C. Analytical data for **4b**: ^{1}H NMR (400 MHz, DMSO, δ ppm): 8.21 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 7.93 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.88 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.73 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.44 (d, *J* = 5.2 Hz, 1H, Ar-H), 7.13-7.18 (m, 2H, Ar-H), 6.97 (t, 1H, Ar-H), 5.36 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 3.95 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO, δ ppm): 201.44, 167.58, 166.48, 164.82, 162.05, 150.70, 142.23, 140.39, 136.69, 135.74, 136.48, 134.62, 133.76, 133.47, 131.85, 130.76, 129.45, 125.22, 116.12, 61.51, 41.07, 36.06, 31.68; ESI-MS: m/z 470.9 $[M+H]^+$.

5.1.1.3.

*2-((5-acetyl-2-methoxybenzyl)thio)-6-bromo-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4c**)*

As a yellow powder, yield: 15%; m.p.143.3-150.6 °C. Analytical data for **4c**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.33 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.23 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 7.88 (dd, *J* = 8.8 Hz, 2.0 Hz, 1H, Ar-H), 7.78 (dd, *J* = 8.4, 2.0 Hz, 1H, Ar-H), 7.55 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 6.91 (m, 2H, Ar-H), 5.41 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 2.50 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.64, 162.65, 161.57, 160.53, 156.63, 146.23, 137.80, 136.87, 132.02, 130.51, 129.93, 129.68, 128.91, 127.96, 126.41, 124.94, 120.84, 119.04, 110.12, 56.01, 42.55, 31.61, 26.40; ESI-MS: m/z 514.8 $[M+H]^+$.

*5.1.1.4. 2-((5-acetyl-2-methoxybenzyl)thio)-6-iodo-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4d**)*

As a white powder, yield: 16%; m.p.170.3-177.6 °C. Analytical data for **4d**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.54 (s, 1H, Ar-H), 8.23 (br, 1H, Ar-H), 7.96 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.89 (d, *J* = 6.8 Hz, 1H, Ar-H), 7.42 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.20 (br, 2H, Ar-H), 6.90-6.93 (m, 2H, Ar-H), 5.41 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 2.51 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.67, 161.57, 160.29, 156.83, 146.69, 143.37, 136.88, 135.98, 132.04,

130.51, 129.93, 128.90, 128.00, 126.48, 126.35, 124.95, 121.16, 116.31, 110.12, 56.02, 42.56, 31.61, 26.41; ESI-MS: m/z 563.2 $[M+H]^+$.

5.1.1.5.

*2-((5-acetyl-2-methoxybenzyl)thio)-6-methyl-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4e**)*

As a white powder, yield: 6%; m.p.189.5-191.0 °C. Analytical data for **4e**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.25 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.98 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.90 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, Ar-H), 7.61 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.54 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H, Ar-H), 7.17-7.20 (m, 2H, Ar-H), 6.88-6.92 (m, 2H, Ar-H), 5.41 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 3.95 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.43 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.76, 161.70, 161.56, 154.87, 145.36, 137.36, 136.16, 136.04, 132.13, 130.31, 129.87, 128.69, 126.56, 126.41, 126.19, 125.84, 125.22, 119.16, 110.13, 56.00, 42.40, 31.45, 26.47, 21.33; ESI-MS: m/z 450.9 $[M+H]^+$.

5.1.1.6.

2-((5-acetyl-2-methoxybenzyl)thio)-6-hydroxy-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one ***(4f)***

As a yellow powder, yield: 12%; m.p.218.5-220.0 °C. Analytical data for **4f**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.20 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.91 (dd, *J* =8.8, 2.4 Hz, 1H, Ar-H), 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.38-7.40 (m, 2H, Ar-H), 7.28 (dd, *J* = 8.8 Hz, 3.2 Hz, 1H, Ar-H), 7.15 (dd, *J* = 7.6 Hz, 1.2 Hz, 1H, Ar-H), 7.12 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.94 (dd, *J* = 4.8 Hz, 3.2 Hz, 1H, Ar-H), 5.35 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 3.95 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.52, 161.67, 160.87, 156.18, 152.37, 140.79, 138.01, 131.85, 130.77, 129.78, 128.67, 128.09, 126.93, 126.89, 125.05, 124.82, 120.21, 111.18, 109.77, 55.31, 49.15, 31.23, 26.86; ESI-MS: m/z 452.9 $[M+H]^+$.

*5.1.1.7. 2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4g**)*

As a white powder, yield: 6%; m.p.134.4-136.6 °C. Analytical data for **4g**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.22 (br, 1H, Ar-H), 7.68 (br, 1H, Ar-H), 7.62 (br, 1H, Ar-H), 7.51 (br, 1H, Ar-H), 7.37 (br, 1H, Ar-H), 7.23-7.26 (m, 3H, Ar-H), 6.90 (br, 3H, Ar-H), 5.44 (s, 2H, CH_2), 4.60 (s, 2H, CH_2), 3.89 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.76, 157.81, 156.44, 147.46, 137.34, 134.55, 131.21, 129.28, 128.78, 127.18, 126.42, 126.20, 126.12, 125.77, 124.66, 120.53, 119.47, 110.65, 55.63, 42.45, 31.84; ESI-MS: m/z 395.1 $[M+H]^+$.

*5.1.1.8. 6-fluoro-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4h**)*

As a white powder, yield: 23%; m.p.116.1-122.8 °C. Analytical data for **4h**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.86 (dd, *J* = 8.4 Hz, 2.8 Hz, 1H, Ar-H), 7.61 (dd, *J* = 8.8 Hz, 4.8 Hz, 1H, Ar-H), 7.49 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.39-7.44 (m, 1H, Ar-H), 7.18-7.29 (m, 3H, Ar-H), 6.87-6.92 (m, 3H, Ar-H), 5.43 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 3.89 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 157.82, 155.78, 144.28, 137.06, 131.16, 129.35, 128.93, 128.49, 128.40, 126.46, 126.32, 124.48, 123.19, 122.95, 120.53, 112.05, 111.81, 110.68, 55.63, 42.53, 31.89; ESI-MS: m/z 413.2 $[M+H]^+$.

*5.1.1.9. 6-chloro-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4i**)*

As a white powder, yield: 25%; m.p.114.8-122.9 °C. Analytical data for **4i**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.18 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.61 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, Ar-H), 7.54 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.28 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.19-7.22 (m, 2H, Ar-H), 6.87-6.91 (m, 3H, Ar-H), 5.42 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 3.89 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 156.01, 153.05, 152.20, 141.24, 132.20, 130.17, 126.48, 126.43, 124.64, 124.22, 123.06, 121.71, 121.62, 119.60, 115.78, 115.68, 105.91, 105.52, 50.88, 37.79, 27.19; ESI-MS: m/z 428.8 $[M+H]^+$.

*5.1.1.10. 6-bromo-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4j**)*

As a white powder, yield: 25%; m.p.134.2-139.3 °C. Analytical data for **4j**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.35 (dd, *J* = 6.8 Hz, 2.8 Hz, 1H, Ar-H), 7.75 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H, Ar-H), 7.47-7.49 (m, 2H, Ar-H), 7.20-7.30 (m, 3H, Ar-H), 6.87-6.93 (m, 3H, Ar-H), 5.42 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 3.89 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 159.91, 157.94, 157.66, 146.20, 138.25, 137.47, 131.52, 129.91, 129.03, 128.91 (2C), 127.19, 127.08, 123.96, 120.79, 120.77, 118.68, 111.58, 56.13, 42.94, 31.90; ESI-MS: m/z 472.8 $[M+H]^+$.

*5.1.1.11. 6-iodo-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4k**)*

As a gray powder, yield: 7%; m.p.126.6-131.7 °C. Analytical data for **4k**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.55 (s, 1H, Ar-H), 7.93 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.48 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.35 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.28 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.20-7.22 (m, 2H, Ar-H), 6.87-6.92 (m, 3H, Ar-H), 5.42 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 3.89 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 160.31, 157.82, 157.40, 146.73, 143.19, 136.95, 135.95, 131.19, 129.40, 128.98, 128.03, 126.46, 126.38, 124.35, 121.14, 120.55, 110.68, 89.46, 55.64, 42.56, 31.99; ESI-MS: m/z 520.8 $[M+H]^+$.

*5.1.1.12. 2-((2-methoxybenzyl)thio)-6-methyl-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4l**)*

As a white powder, yield: 32%; m.p. 108.1-110.2 °C. Analytical data for **4l**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.02 (s, 1H, Ar-H), 7.50-7.56 (m, 3H, Ar-H), 7.27 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.18-7.22 (m, 2H, Ar-H), 6.86-6.90 (m, 3H, Ar-H), 5.44 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 3.88 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.76, 157.82, 155.42, 145.47, 137.48, 136.00, 135.86, 131.22, 129.26, 128.72, 126.67, 126.39, 126.18, 125.91, 124.74, 120.54, 119.17, 110.65, 55.63, 42.43, 31.85, 21.34; ESI-MS: m/z 408.9 $[M+H]^+$.

*5.1.1.13. 6-hydroxy-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4m**)*

As a yellow powder, yield: 28%; m.p. 113.0-115.0 °C. Analytical data for **4m**: ^{1}H NMR (400 MHz, DMSO, δ ppm): 7.50 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.50 (dd, *J* = 7.2 Hz, 1.6 Hz, 1H, Ar-H), 7.37-7.38 (m, 2H, Ar-H), 7.21-7.27 (m, 2H, Ar-H), 7.10 (d, *J* = 1.6 Hz, 1H, Ar-H), 6.99 (d, *J* = 8.1 Hz, 1H, Ar-H), 6.82-6.91 (m, 3H, Ar-H), 5.30 (s, 2H, CH_2), 4.44 (s, 2H, CH_2), 3.79 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO, δ ppm): 160.90, 157.93, 156.27, 152.70, 140.81, 138.14, 131.40, 129.76, 128.68, 128.19, 127.03, 126.95, 124.94, 124.41, 120.78, 120.17, 111.59, 109.79, 55.45, 42.65, 31.56; ESI-MS: m/z 410.9 $[M+H]^+$.

*5.1.1.14. 2-(benzylthio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4n**)*

As a white powder, yield: 17%; m.p.99.1-101.3 °C. Analytical data for **4n**: ^{1}H NMR (400 MHz, DMSO, δ ppm): 8.25 (dd, *J* = 8.8 Hz, 1.2 Hz, 1H, Ar-H), 7.71 (t, 1H, Ar-H), 7.59 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.46-7.48 (m, 2H, Ar-H), 7.28-7.40 (m, 4H, Ar-H), 7.20-7.23 (m, 2H, Ar-H), 6.93 (t, 1H, Ar-H), 5.46 (s, 2H, CH_2), 4.56 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO, δ ppm): 162.56, 157.14, 146.94, 146.68, 141.15, 134.38, 133.83, 131.09, 130.44, 128.55, 128.26 (2C), 128.00 (3C), 127.76 (2C), 126.77, 43,83, 25.42; ESI-MS: m/z 364.9 $[M+H]^+$.

*5.1.1.15. 2-(benzylthio)-6-fluoro-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4o**)*

As a white powder, yield: 8%; m.p.157.2-167.1 °C. Analytical data for **4o**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.87 (dd, *J* = 8.4 Hz, 2.8 Hz, 1H, Ar-H), 7.58 (dd, *J* = 8.8 Hz, 4.8 Hz, 1H, Ar-H), 7.47 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.28-7.47 (m, 4H, Ar-H), 7.21-7.25 (m, 2H, Ar-H), 6.92 (t, 1H, Ar-H), 5.45 (s, 2H, CH_2), 4.55 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 160.46, 155.81, 144.21, 137.59, 137.06, 129.96 (2C), 129.44, 129.36, 128.99 (3C), 127.99, 127.19, 127.13, 124.17, 123.93, 111.80, 43.00, 36.30; ESI-MS: m/z 382.9 $[M+H]^+$.

*5.1.1.16. 2-(benzylthio)-6-chloro-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4p**)*

As a yellow powder, yield: 18%; m.p.139.6-145.5 °C. Analytical data for **4p**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.20 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.62 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.46 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.28-7.35 (m, 3H, Ar-H), 7.22 (d, *J* = 4.4 Hz, 2H, Ar-H), 6.92 (t, 1H, Ar-H), 5.45 (s, 2H, CH_2), 4.55 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO, δ ppm): 160.09, 157.10, 145.93, 137.49, 136.96, 135.64, 130.74, 129.97 (2C), 129.00 (3C), 128.81, 128.02, 127.22, 127.14, 125.99, 120.48, 43.06, 36.36; ESI-MS: m/z 437.0 $[M+K]^+$.

*5.1.1.17. 2-(benzylthio)-6-bromo-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4q**)*

As a white powder, yield: 16%; m.p.145.3-147.6 °C. Analytical data for **4q**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.35 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.76 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H, Ar-H), 7.45 (d, *J* = 6.8 Hz, 3H, Ar-H), 7.21-7.34 (m, 5H, Ar-H), 6.92 (t, 1H, Ar-H), 5.44 (s, 2H, CH_2), 4.54 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO, δ ppm): 159.95, 157.22, 146.18, 138.34, 137.48, 136.94, 129.98 (2C), 129.08, 129.00 (3C), 128.95, 128.02, 127.22, 127.14, 120.85, 118.80, 43.07, 36.37; ESI-MS: m/z 465.1 $[M+Na]^+$.

*5.1.1.18. 2-(benzylthio)-6-iodo-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4r**)*

As a white powder, yield: 13%; m.p.144.6-145.2 °C. Analytical data for **4r**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.56 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.93 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H, Ar-H), 7.45 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.27-7.34 (m, 4H, Ar-H), 7.20-7.22 (m, 2H, Ar-H), 6.91 (t, 1H, Ar-H), 5.43 (s, 2H, CH_2), 4.54 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO, δ ppm): 159.73, 157.17, 146.44, 143.80, 137.51, 136.93, 135.20, 129.98 (2C), 129.00 (3C), 128.71, 128.02, 127.22, 127.13, 121.06, 91.27, 43.03, 36.37; ESI-MS: m/z 490.8 $[M+H]^+$.

*5.1.1.19. 2-(benzylthio)-6-methyl-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4s**)*

As a white powder, yield: 18%; m.p.151.9-152.7 °C. Analytical data for **4s**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.04 (s, 1H, Ar-H), 7.46-7.50 (m, 4H, Ar-H), 7.21-7.33 (m, 5H, Ar-H), 6.91 (br, 1H, Ar-H), 5.46 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 2.45 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.73, 154.54, 145.53, 137.44, 136.43, 136.03 (2C), 129.53 (2C), 128.74 (3C), 127.75, 126.61, 126.48, 126.27, 126.03, 119.31, 42.52, 36.97, 21.37; ESI-MS: m/z 378.9 $[M+H]^+$.

*5.1.1.20. 2-(benzylthio)-6-hydroxy-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**4t**)*

As a white powder, yield: 15%; m.p.213.8-215.0 °C. Analytical data for **4t**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.01 (s, 1H, OH), 7.45-7.47 (m, 3H, Ar-H), 7.39 (dd, *J* = 5.2 Hz, 1.2 Hz, 1H, Ar-H), 7.35 (d, *J* = 3.6 Hz, 1H, Ar-H),7.19-7.30 (m, 4H, Ar-H), 7.12 (d, *J* = 3.6 Hz, 1H, Ar-H),

6.92 (dd, *J* = 5.2 Hz, 3.6 Hz, 1H, Ar-H), 5.33 (s, 2H, CH_2), 4.48 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 160.88, 156.22, 152.29, 140.79, 138.10, 137.29, 129.92 (2C), 128.96 (2C), 128.72, 128.26, 127.91, 127.08, 127.04, 124.92, 120.23, 109.75, 42.76, 36.16; ESI-MS: m/z 381.2 $[M+H]^+$.

*5.1.2. General procedure for the synthesis of compounds **5a-f***

Finally, Compound **4f**, **4m** or **4t** (0.01 mol) was alkylated with 2-dimethylaminoethyl chloride hydrochloride or 2-diethylaminoethyl chloride hydrochloride (0.012 mol) in the presence of K_2CO_3 and 1,4-dioxane to provide alkoxyl substituted derivative **5a-f**. The crude product was further purified via column chromatography to produce the title compound, respectively.

5.1.2.1.

*2-((5-acetyl-2-methoxybenzyl)thio)-6-(2-(dimethylamino)ethoxy)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**5a**)*

As a white powder, yield: 10%; m.p.70.2-72.8 °C. Analytical data for **5a**: ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 8.23 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.89 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, Ar-H), 7.61 (d, *J* =9.2 Hz, 1H, Ar-H), 7.57 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.35 (dd, *J* = 8.8 Hz, 2.8 Hz, 1H, Ar-H), 7.18-7.25 (m, 2H, Ar-H), 6.88-6.92 (m, 2H, Ar-H), 5.41 (s, 2H, CH_2), 4.54 (s, 2H, CH_2), 4.32 (t, 2H, OCH_2), 3.95 (s, 3H, CH_3), 3.09 (t, 2H, NCH_2), 2.59 (s, 6H, CH_3), 2.49 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.77, 161.58, 161.53, 155.96, 153.68, 142.72, 137.23, 132.06, 130.36, 129.87, 128.77, 127.97, 126.45, 126.25, 125.20, 125.00, 120.06, 110.12, 107.58, 64.71, 57.13, 56.01, 44.67 (2C), 42.52, 31.41, 26.46; ESI-MS: m/z 524.3 $[M+H]^+$.

5.1.2.2.

*2-((5-acetyl-2-methoxybenzyl)thio)-6-(2-(diethylamino)ethoxy)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**5b**)*

As a yellow oil, yield: 9%. Analytical data for **5b**: ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 8.24 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.91 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H, Ar-H), 7.61 (m, 2H, Ar-H), 7.34 (dd, *J* = 8.4 Hz, 3.2 Hz, 1H, Ar-H), 7.18-7.22 (m, 2H, Ar-H), 6.89-6.93 (m, 2H, Ar-H), 5.42 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 4.16 (t, 2H, OCH_2), 3.96 (s, 3H, CH_3), 2.94 (t, 2H, NCH_2), 2.65-2.70 (m, 4H, $N(CH_2CH_3)_2$), 2.50 (s, 3H, CH_3), 1.09 (t, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.76, 162.64, 161.66, 161.57, 156.86, 153.18, 142.34, 137.35, 132.09, 130.30, 129.89, 128.73,

127.69, 126.43, 126.21, 125.28, 120.07, 110.13, 107.25, 66.70, 56.00, 51.45, 47.83 (2C), 42.50, 31.37, 26.46, 11.72 (2C); ESI-MS: m/z 552.3 $[M+H]^+$.

5.1.2.3.

*6-(2-(dimethylamino)ethoxy)-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**5c**)*

As a yellow powder, yield: 25%; m.p.82.5-83.2 °C. Analytical data for **5c**: ^{1}H NMR (400 MHz, DMSO, δ ppm): 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.45-7.47 (m, 2H, Ar-H), 7.38-7.41 (m, 2H, Ar-H), 7.22-7.26 (m, 1H, Ar-H), 7.11 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.99 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.91 (dd, *J* = 5.2 Hz, 3.6 Hz, 1H, Ar-H), 6.84 (t, 1H, Ar-H), 5.33 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 4.15 (t, 2H, OCH_2), 3.80 (s, 3H, CH_3), 2.69 (t, 2H, NCH_2), 2.24 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO, δ ppm): 160.87, 157.94, 156.90, 153.97, 142.04, 137.94, 131.46, 129.82, 128.82, 128.32, 127.05 (2C), 125.34, 124.30, 120.79, 119.92, 111.60, 107.65, 66.59, 57.90, 56.13, 45.85 (2C), 42.77, 31.64; ESI-MS: m/z 482.2 $[M+H]^+$.

5.1.2.4.

*6-(2-(diethylamino)ethoxy)-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**5d**)*

As a yellow oil, yield: 25%. Analytical data for **5d**: ^{1}H NMR (400 MHz, DMSO, δ ppm): 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.45-7.47 (m, 2H, Ar-H), 7.37-7.39 (m, 2H, Ar-H), 7.24 (t, 1H, Ar-H), 7.11 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.99 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.91 (dd, *J* = 5.2 Hz, 3.6 Hz, 1H, Ar-H), 6.85 (t, 1H, Ar-H), 5.33 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 4.10 (t, 2H, OCH_2), 3.80 (s, 3H, CH_3), 2.80 (t, 2H, NCH_2), 2.55 (m, 4H, $N(CH_2CH_3)_2$), 0.95 (t, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, DMSO, δ ppm): 162.85, 160.87, 157.93, 156.97, 153.94, 141.99, 137.93, 131.46, 129.83, 128.82, 128.32, 127.06, 125.32, 124.28, 120.79, 119.91, 111.59, 107.66, 56.13, 55.45, 51.61, 47.52 (2C), 42.77, 31.64, 12.25 (2C); ESI-MS: m/z 510.3 $[M+H]^+$.

*5.1.2.5. 2-(benzylthio)-6-(2-(dimethylamino)ethoxy)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**5e**)*

As a white powder, yield: 13%; m.p.75.8-77.2 °C. Analytical data for **5e**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.55 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.45-7.47 (m, 3H, Ar-H), 7.37-7.40 (m, 2H, Ar-H), 7.25-7.30 (m, 2H, Ar-H), 7.19-7.23 (m, 1H, Ar-H), 7.13 (dd, *J* = 3.6 Hz, 1.2 Hz, 1H, Ar-H), 6.92 (dd, *J* = 5.2 Hz, 3.2 Hz, 1H, Ar-H), 5.35 (s, 2H, CH_2), 4.50 (s, 2H, CH_2), 4.14 (t, 2H, OCH_2), 2.67

(t, 2H, NCH_2), 2.20 (s, 6H, CH_3); ^{13}C NMR (100 MHz, DMSO, δ ppm): 160.87, 156.98, 153.48, 141.96, 137.91, 137.22, 129.95 (2C), 128.97(2C), 128.83, 128.32, 127.94, 127.10 (2C), 125.38, 119.97, 107.63, 66.65, 57.90, 45.89 (2C), 42.87, 36.20; ESI-MS: m/z 452.3 $[M+H]^+$.

*5.1.2.6. 2-(benzylthio)-6-(2-(diethylamino)ethoxy)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**5f**)*

As a yellow powder, yield: 13%; m.p.108.8-110.2 °C. Analytical data for **5f**: ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.55 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.45-7.48 (m, 3H, Ar-H), 7.36-7.40 (m, 2H, Ar-H), 7.26-7.30 (m, 2H, Ar-H), 7.20-7.24 (m, 1H, Ar-H), 7.13 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.92 (dd, *J* = 5.2 Hz, 3.6 Hz, 1H, Ar-H), 5.35 (s, 2H, CH_2), 4.50 (s, 2H, CH_2), 4.08 (t, 2H, OCH_2), 2.76 (t, 2H, NCH_2), 2.49-2.54 (m, 4H, $N(CH_2CH_3)_2$), 0.94 (t, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 165.63, 161.84, 158.19, 146.67, 142.68, 141.97, 134.70 (2C), 133.73 (2C), 133.59, 133.06, 132.70, 131.85(2C), 130.09, 124.74, 112.40, 72.20, 56.42, 52.27 (2C), 47.62, 40.97, 17.17 (2C); ESI-MS: m/z 480.3 $[M+H]^+$.

5.2. Biological assays

HepG2 cell lines, provided by 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 and anti-HBV activities of the target compounds were evaluated on HepG2 2.2.15 cell line, which was a stably transfected cell line containing HBV genome on a plasmid. Antiviral efficacy against nucleoside analogue-resistant HBV was conducted on HepG2 cell line transiently transfected with plasmids containing drug-resistant mutant (rtL180M + rtM204V + rtT184L).

5.2.1. Cytotoxicity assay

The cytotoxicity of the target compounds against HepG2 2.2.15 cells was determined by MTT assay. Briefly, HepG2 2.2.15 cells were seeded in 96-well culture plates and then cultured for 24 h. The exponentially growing cells were incubated with test compounds (in 0.2 mL culture medium/well) for 72 h, respectively. The cytotoxicity was determined by analyzing MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) absorbance at 490 nm, as determined from the absorbance relative to untreated cells.

5.2.2. Determination the effect on HBV DNA replication

HepG2 2.2.15 cells (2×10^5 cells/well) were seeded in 24 well culture plates and cultured for 24 h. Then, culture medium was replaced by assay medium containing the test compound or positive control 3TC at the indicated concentrations. The medium was changed every 2 days. After 6 days incubation, the intracellular HBV DNA was extracted according to the method described previously [24], and the HBV DNA levels were quantified by RT-PCR.

5.2.3. Antiviral efficacy against nucleoside analogue-resistant HBV strain [25]

HepG2 cells, transiently transfected respectively with plasmids containing drug-resistant mutant (rtL180M + rtM204V + rtT184L) were selected as an evaluation model [26]. Briefly, HepG2 cells (2×10^5) were plated into 24-well plates. After 24 incubation, the supernatant was discarded, followed by PBS wash. The cells were then incubated with fresh medium containing **5e** (4 μM), **5f** (4 μM), lamivudine (100 μM) or Entecavir (10 μM), respectively. After 72 h incubation, HBV DNA replicative intermediate was extracted according to the method mentioned above [24], and the level was quantified by RT- PCR.

5.3. *Molecular docking*

Surflex molecular docking module in Sybyl X-2.1 (SYBYL_X2.1 is availabe from Tripos Associates Inc., S Hanley Rd., St. Louis, MO 631444, USA) was used for conducting molecular docking based virtual screening and investigating the possible binding interactions between HBV core protein and its inhibitors. The crystal structure of HBV core protein complex with NVR-010-001-E2 (PDB ID: 5E0I) was obtained from the RCSB Protein Data Bank [23]. The ChemDiv database, a commercially available small molecule database from TopScience Co. (Shanghai, China), was consulted as a screening library. Because only 2D structural information was available, the compounds in the ChemDiv database were preprocessed by the dbtranslate module in Sybyl-X2.1. The space where ligand NVR-010-001-E2 placed was selected as the binding site of inhibitor, 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. The binding affinity of inhibitors were evaluated by docking score.

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Highlights

1. Novel quinazolinone derivatives were synthesized and evaluated as anti-HBV agents.
2. **5e** and **5f** exhibited potent anti-HBV activity against wild and resistant HBV strains.
3. **5e** and **5f** could well fit into the dimer-dimer interface of HBV core protein.