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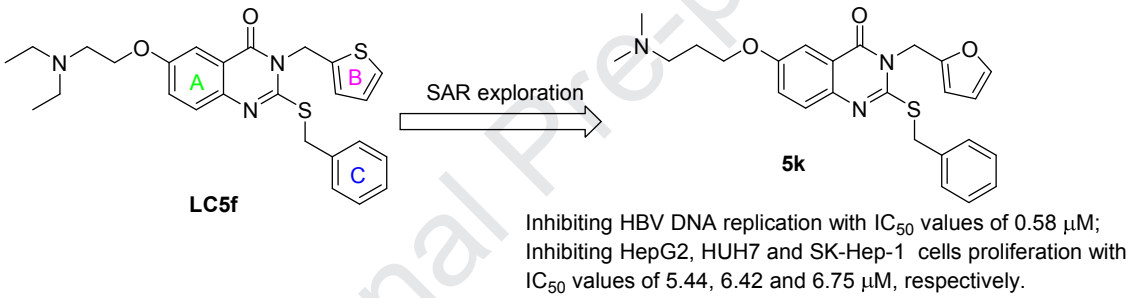
Graphical Abstract



Compound **5k** exhibited more potent in vitro anti-HBV and anti-HCC activities than lead compound **LC5f** and positive control 5-fluorouracil and sorafenib, respectively.

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Abstract

As a continuation of earlier works, a series of novel quinazolinone derivatives (**5a**-**s**) were synthesized and evaluated for their in vitro anti-HBV and anti-hepatocellular carcinoma cell (HCC) activities. Among them, compounds **5j** and **5k** exhibited most potent inhibitory effect on HBV DNA replication in both drug sensitive and resistant (lamivudine and entecavir) HBV strains. Interestingly, besides the anti-HBV effect, compound **5k** could significantly inhibit the proliferation of HepG2, HUH7 and SK- cells, with IC_{50} values of 5.44, 6.42 and 6.75 μM, respectively, indicating its potential anti-HCC activity. Notably, the in vitro anti-HCC activity of **5k** were more potent than that of positive control 5-fluorouracil and sorafenib. Further studies revealed that compound **5k** could induce HepG2 cells apoptosis by dose-dependently upregulating Bad and Bax expression and decreasing Bcl-2 and Bcl-xl protein level. Considering the potent anti-HBV and anti-HCC effect, compound **5k** might be a promising lead to develop novel therapeutic agents towards HBV infection and HBV-induced HCC.

Keywords: Anti-HBV agents; Quinazolinone derivatives; Non-nucleoside; Anti-HCC activity.

1. Introduction

Hepatitis B virus (HBV) infection is a worldwide threat to public health. According to the World Health Organization (WHO), more than 257 million people are living with HBV infection globally [1, 2]. Up to 30% HBV carriers may develop progressive chronic liver disease, including hepatitis, fibrosis, cirrhosis and

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even hepatocellular carcinoma (HCC) [3-6]. It was also reported that the majority of HCC cases was associated with HBV-infection [7, 8]. Up to now, several anti-HBV drugs are available in clinic, such as nucleos(t)ides-based reverse transcriptase inhibitors and interferons, however, the clinical cure rate for chronic HBV infection are not satisfactory, mainly because of the viral resistance and drug adverse effects [9-13]. Therefore, it is of great importance to develop novel anti-HBV agents with diverse structure and action mechanism to treat HBV infection and related diseases [14, 15].

Recently, a series of quinazolinones derivatives were prepared as potential anti-HBV agents by us [16]. Among them, compound **LC5f** could significant inhibit HBV DNA replication in wild-type and drug resistant HBV strains with IC_{50} values of 0.71 and 0.84 μM, respectively (Figure 1). Previous structure activity relationship study indicated that the introduction of diethylamine ethoxy substituents in ring A could significantly affect the anti-HBV activity. This led us to expect that the modification of compound **LC5f** would improve the anti-HBV activity. In this study, in order to further investigate the effect of substitutions on anti-HBV activity comprehensively, we prepared novel quinazolinones derivatives by introducing various alkoxy substituents (ie. dimethylamino ethoxy, diethylamine ethoxy or dimethylamino propoxy) in ring A of **LC5f**, replacing thiophenyl (ring B of **LC5f**) with furanyl or phenyl by bioisosterism principle, and/or introducing methoxy or acetyl in ring C of **LC5f**, respectively (Figure 1). Thus, nineteen compounds were synthesized, and their anti-HBV activities were subsequently evaluated in wild-type and polymerase drug resistant HBV strains. In addition, considering that HBV infection is a major global cause of HCC [17, 18], the in vitro anti-HCC activities of the target compounds were also investigated in the present work.

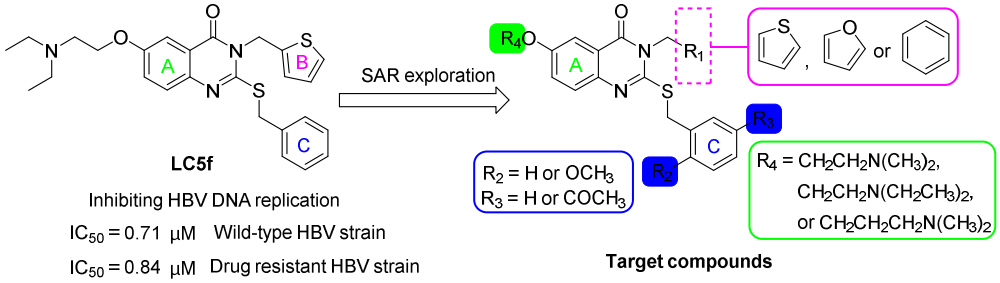
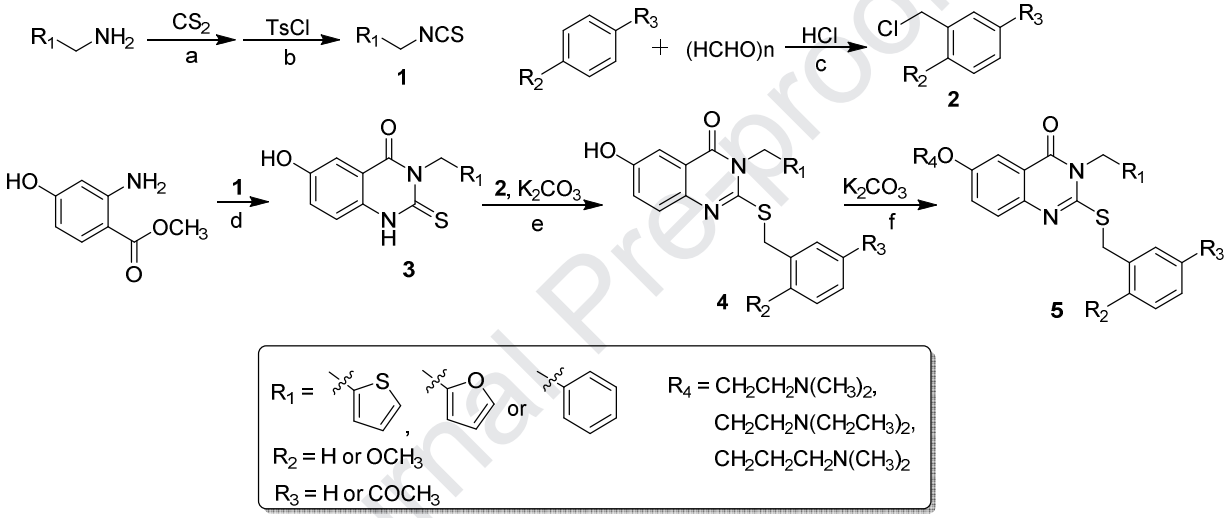


Figure 1. Chemical structures of **LC5f** and target compounds

2. Chemistry

The target compounds, quinazolinone derivatives **5a-s**, were prepared according to the following

synthetic procedures (Scheme 1). Briefly, various methylamine derivatives was firstly treated with CS_2 and toluene sulfonyl chloride to afford intermediate **1**. Chloromethylation intermediate **2** was prepared by reacting benzene or benzene derivatives with paraformaldehyde in the presence of hydrochloric acid. Methyl 2-amino-4-hydroxybenzoate was condensed with the isothiocyanate intermediate **1** to give intermediate **3**, which was subsequently reacted with intermediate **2** to prepare intermediate **4**. Finally, the target compounds **5a-s** were prepared by alkylating intermediate **4** with 2-dimethylaminoethyl chloride hydrochloride, 2-diethylaminoethyl chloride hydrochloride or 3-dimethylaminopropyl chloride hydrochloride, respectively.



Scheme 1. Synthetic route of target compounds **5a-s**. Reagents and conditions: a) TEA, THF, 0 °C; b) TsCl, 0 °C; c) HCl, 80 °C; d) TEA, EtOH, reflux; e) K_2CO_3, 1,4-dioxane, 90 °C; f) 2-dimethylaminoethyl chloride hydrochloride, 2-diethylaminoethyl chloride hydrochloride or 3-dimethylaminopropyl chloride hydrochloride, K_2CO_3, 1,4-dioxane, 90 °C.

3. Results and discussion

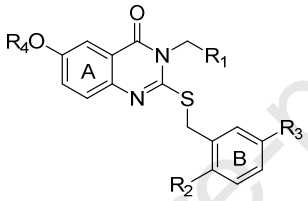
3.1 Virological assays of anti-HBV activities in vitro

3.1.1 Inhibitory effect on HBV DNA replication

The intrinsic cytotoxicity of the target compounds were firstly investigated in HBV-infected HepG2 2.2.15 cells by MTT assay. Data showed that most of the CC_{50} values of the target compounds were more than 10 μM (Table 1). Next, the in vitro anti-HBV activity of the target compounds was determined in HepG2 2.2.15 cells according to previous method [19, 20]. Briefly, HepG2 2.2.15 cells were treated with target compounds at the concentration of 0.8 μM (a non-toxic concentration far below CC_{50}) for 6 days, and

then the extra cellar HBV DNA levels were measured by real time PCR (RT-PCR) assay. **LC5f** and lamivudine (3TC) were selected as the reference and positive controls, respectively. As shown in Table 1, the intracellular HBV DNA levels in cells was decreased to some extent, especially in **5e**, **5f**, **5g**, **5j**, **5k**, **5q** and **5r** treated group, indicating their significant anti-HBV activity. Clearly, **5f**, **5j**, **5k**, **5q** and **5r** were the most potent ones, and their the inhibition rate on HBV DNA replication was 67.07%, 67.45%, 67.31%, 65.53% and 64.51%, respectively, which was much higher than that of reference control **LC5f** (with an inhibition rate of 53.59%).

Table 1. Intrinsic cytotoxicity and inhibitory effect of the target compounds **5a-s** on HBV DNA level[a]



					HepG2 2.2.15	
					[a] CC_{50}	Inhibition rate (%)
Compds	R_1	R_2	R_3	R_4	(μM)	(0.8 μM)
5a		OCH_3	$COCH_3$	$CH_2CH_2CH_2N(CH_3)_2$	9.63	0.16
5b		OCH_3	H	$CH_2CH_2CH_2N(CH_3)_2$	>10	21.65
5c		OCH_3	$COCH_3$	$CH_2CH_2N(CH_3)_2$	>10	20.68
5d		OCH_3	$COCH_3$	$CH_2CH_2N(CH_2CH_3)_2$	>10	45.19
5e		OCH_3	$COCH_3$	$CH_2CH_2CH_2N(CH_3)_2$	>10	54.24
5f		OCH_3	H	$CH_2CH_2N(CH_3)_2$	>10	67.07
5g		OCH_3	H	$CH_2CH_2N(CH_2CH_3)_2$	>10	52.63
5h		OCH_3	H	$CH_2CH_2CH_2N(CH_3)_2$	8.38	34.38
5i		H	H	$CH_2CH_2N(CH_3)_2$	>10	21.69
5j		H	H	$CH_2CH_2N(CH_2CH_3)_2$	>10	67.45

5k		H	H	$CH_2CH_2CH_2N(CH_3)_2$	>10	67.31
5l		OCH_3	$COCH_3$	$CH_2CH_2N(CH_3)_2$	>10	15.64
5m		OCH_3	$COCH_3$	$CH_2CH_2N(CH_2CH_3)_2$	>10	4.93
5n		OCH_3	H	$CH_2CH_2N(CH_3)_2$	>10	11.83
5o		OCH_3	H	$CH_2CH_2N(CH_2CH_3)_2$	>10	9.80
5p		OCH_3	H	$CH_2CH_2CH_2N(CH_3)_2$	>10	3.66
5q		H	H	$CH_2CH_2N(CH_3)_2$	>10	65.53
5r		H	H	$CH_2CH_2N(CH_2CH_3)_2$	>10	64.51
5s		H	H	$CH_2CH_2CH_2N(CH_3)_2$	>10	42.84
LC5f					>10	53.59
3TC					>100	84.42

[a] CC_{50} represents the dose of compound required to cause 50% death of the HepG2 2.2.15 cells.

To further confirm the anti-HBV activity, the dose-dependent inhibitory effect of active compounds **5e**, **5f**, **5g**, **5j**, **5k**, **5q** and **5r** on HBV DNA replication were subsequently determined in HepG2 2.2.15 cells by RT-PCR assay as mentioned above. As shown in Table 2, the IC_{50} values of the tested compounds on HBV DNA replication were in range of 0.58-0.79 μM, confirming their promising anti-HBV activity. Among them, **5j** and **5k** were the most potent ones, and their IC_{50} values on HBV DNA replication was 0.63 and 0.58 μM, respectively. Notably, **5j** and **5k** had the largest SI values, indicating their relative safety profiles.

Table 2. Anti-HBV activities and SI values of target compounds **5e**, **5f**, **5g**, **5j**, **5k**, **5q** and **5r**

Compds	DNA replication	
	[a] IC_{50} (μM)	[b] SI
5e	0.79	>12.66
5f	0.72	>13.89
5g	0.77	>12.99
5j	0.63	>15.87
5k	0.58	>17.24

5q	0.68	>14.71
5r	0.66	>15.15
LC5f	0.77	>12.99
3TC	<0.1	>1000

[a] IC_{50} represents the dose of compound required to cause 50% inhibition of DNA replication. [b] SI means selectivity index, which was calculated as the CC_{50} (shown in Table 1)/IC_{50} (shown in Table 2) value.

Subsequently, we determined the inhibitory effect of **5j** and **5k** on the production of HBsAg in HBV-infected 2.2.15 cells according to our previous method [21]. Unfortunately, compounds **5j**, **5k** and lamivudine displayed relatively poor inhibitory effect on HBsAg production with an inhibitory rate of 13.64%, 4.55% and 9.09%, respectively.

According to the bioactivity results shown in Tables 1 and 2, structure and anti-HBV activity relationship could be preliminarily drawn as follows: chemical structure of ring B in the quinazolinone derivatives significantly affect the anti-HBV activity. When the thiophenyl was replaced with a furanyl by bioisosterism principle, the anti-HBV activity was obviously increased (**5e** *vs.* **5a**; **5h** *vs.* **5b**; **5j** *vs.* **LC5f**). In sharp contrary, after changing the thiophenyl with a phenyl, the anti-HBV activity was remarkably decreased (**5p** *vs.* **5b**). In addition, methoxyl at position-2 and acetyl at position-5 on ring C of the quinazolinone derivatives might play an adverse effect on the anti-HBV activities. When the methoxy or acetyl group was removed, the anti-HBV activity was generally increased (**5j** *vs.* **5g**; **5k** *vs.* **5h**; **5q** *vs.* **5n**; **5r** *vs.* **5o**; **5s** *vs.* **5p**; **5g** *vs.* **5d**; **5f** *vs.* **5c**; **5o** *vs.* **5**m), which was consistent with our previous findings [16].

*3.1.2 Anti-HBV effect of **5j** and **5k** against drug resistant HBV strain*

Considering that drug resistance is one of the major limitations of the nucleos(t)ide therapy, we next evaluated whether **5j** and **5k** could inhibit HBV DNA replication in lamivudine and entecavir-resistant HBV strain. The inhibitory effect on HBV DNA replication in wild-type (WT) strain was served as the control group. As shown in Figure 2, both nucleoside drugs lamivudine (3TC, 100 μM) and entecavir (ETV, 10 μM) could significantly decrease the HBV DNA levels in WT strain with replication rates of 90.00% and 96.90%, respectively. In sharp contrast, such anti-HBV effect was dramatically attenuated in drug resistant strain, for the inhibition rate on HBV DNA replication were only 15.69% and 18.92%,

respectively. Interestingly, both compound **5j** and **5k** displayed significant anti-viral effect against lamivudine and entecavir-resistant mutant HBV strain, and the HBV DNA replication inhibition rates on resistant strain were comparable with that on wild-type strain (58.89% and 64.03% *vs.* 60.64% and 61.07%, respectively).

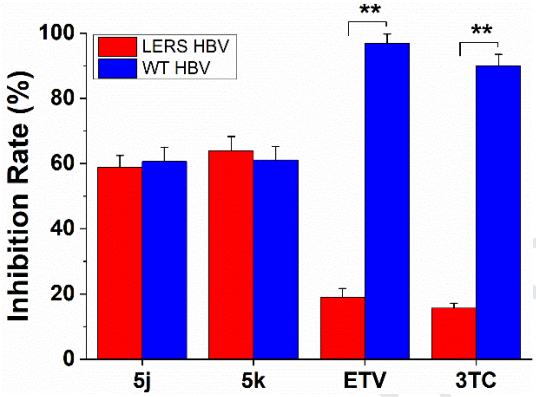


Figure 2. Inhibitory effect of **5j** and **5k** on HBV DNA replication in wild-type and drug resistant HBV strains. Briefly, the full genome of wide-type or plasmids containing lamivudine and entecavir-resistant (LERS) mutant (rtL180M + rtM204V + rtT184L) were transiently transfected into HepG2 2.2.15 cells, and the cells were then incubated in the presence or absence of lamivudine (3TC, 100 μM), entecavir (ETV, 10 μM), **5j** (0.8 μM) or **5k** (0.8 μM) for 72 h, respectively. Finally, the replicative intermediate levels of HBV DNA in both strains was determined using RT-PCR. ***P* < 0.01 represents significant difference from the WT HBV group.

3.2 In vitro anti-HCC activities of the target compounds

3.2.1 Inhibitory effect on HCC cells proliferation

As mentioned above, HBV infection is one of the leading cause for HCC, and we have confirmed that the newly synthetic quinazolinones derivatives displayed potent anti-HBV activities. Therefore, we were interested to see whether these compounds could have an anti-HCC activity. Firstly, we determined the anti-proliferation effect of the target compounds on HepG2 cells according to previous methods [22-24]. Classic anti-cancer drug 5-fluorouracil (5-Fu) and sorafenib were served as the positive control, respectively. As shown in Table 3, compounds **5a**, **5b**, **5e**, **5h** and **5k** could obviously inhibit HepG2 cells proliferation with IC_{50} values of 8.98, 8.81, 9.40, 6.76, and 5.44 μM, respectively. Notably, compounds **5h** and **5k** were the most potent ones, and their IC_{50} values were even lower than that of positive control 5-Fu

(IC_{50} = 8.69 μM) and sorafenib (IC_{50} = 7.67 μM), indicating their potent anti-HCC activity in vitro.

Table 3. In vitro anti-HCC activity of the target compounds **5a-s**

	HepG2		HepG2		HepG2
Compds	IC_{50} (μM)	**Compds**	IC_{50} (μM)	**Compds**	IC_{50} (μM)
5a	8.98	**5h**	6.76	**5o**	>20
5b	8.81	**5i**	17.54	**5p**	11.79
5c	11.33	**5j**	17.32	**5q**	>20
5d	16.99	**5k**	5.44	**5r**	>20
5e	9.40	**5l**	18.97	**5s**	>20
5f	>100	**5m**	>20	**5-Fu**	8.69
5g	18.98	**5n**	>20	**sorafenib**	7.67

To further confirm the anti-HCC activity, the in vitro anti-proliferation activity of compound **5k** against two other human HCC cell lines (HUH7 and SK-Hep-1 cell lines) was subsequently evaluated by MTT assay. As shown in Table 4, compound **5k** could also significantly inhibit HUH7 and SK-Hep-1 cells proliferation, with IC_{50} values of 6.42 and 6.75 μM, respectively. Notably, the IC_{50} values of **5k** were lower than that of positive control sorafenib (IC_{50} = 7.92 and 9.64 μM, respectively), confirming its potential anti-HCC effect.

Table 4. In vitro anti-HCC activity of the target compounds **5k**

	in vitro inhibition of HCC proliferation (IC_{50}, μM)	
Compds	HUH7	SK-Hep-1
5k	6.42	6.75
sorafenib	7.92	9.64

*3.2.2 Preliminary anti-HCC mechanism of the target compound **5k***

To understand the preliminary mechanism of anti-HCC effect, we firstly determined the morphology changes of HepG2 cells treated with **5k** by Hoechst 33342 staining under fluorescent microscopy [22]. As shown in Figure 3a, after treatment with **5k** (5, 10 or 20 μM) for 48 h, the morphology of HepG2 cells was significantly changed (ie. nucleus fragmentation and chromatin condensation), indicating the cell apoptosis.

Next, we performed the Annexin V/PI assay to further evaluate the apoptosis induction ability of **5k** by flow cytometry [25-27]. As shown in Figure 3b and 3d, after the incubation with **5k** (5, 10 or 20 μM) for 48 h, apoptotic HepG2 cells at early (Annexin-V+/PI−) and late (Annexin-V+/PI+) stages were significantly increased to 17.95%, 37.98% and 71.98%, respectively.

It is well-known that Bcl-2 family proteins, including pro-apoptotic proteins (ie. Bax and Bad) and anti-apoptotic proteins (ie. Bcl-2 and Bcl-xl), play a significant role in HCC development. In addition, the expression of Bcl-2 is a poor predictor for HCC prognosis [28, 29], and Bcl-2 family proteins are also related to chronic active hepatitis and cirrhosis [30]. Therefore, we next determined whether **5k** could affect the expression of apoptosis-related proteins by western blot assay [31]. As shown in Figure 3c and 3e, **5k** could dose-dependently upregulate Bad and Bax expression and decrease Bcl-2 and Bcl-xl protein level. These results indicated that the apoptosis induction capacity of **5k** was achieved, at least partly, by interfering with the expression of apoptosis-related proteins.

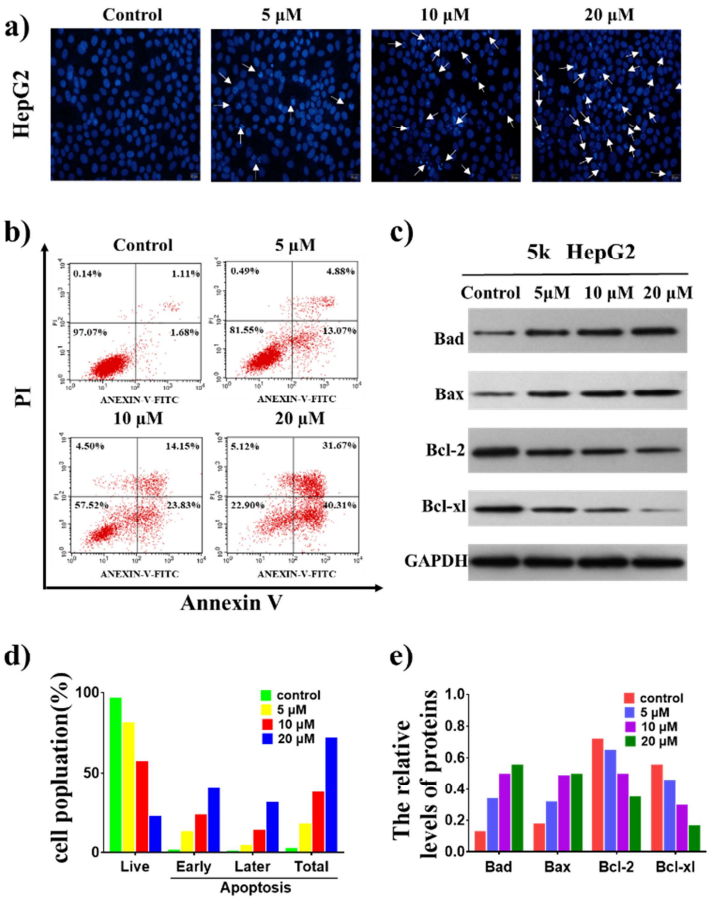


Figure 3. Apoptosis induction effect of **5k** in HepG2 cells. (a) Alterations of morphology and nuclear changes (white

arrow marked cells). HepG2 cells were incubated with **5k** (5, 10 or 20 μM) for 48 h, and then stained by Hoechst 33342 and visualized by fluorescence microscopy; (b) HepG2 cells were incubated in the absence or presence of **5k** (5, 10 or 20 μM) for 48 h, and then cells were collected and stained with Annexin V/PI to analyze early apoptotic (Annexin-V+/PI−) and late apoptotic (Annexin-V+/PI−) cell fractions by flow cytometric analysis; (c) Effect of **5k** on apoptosis-related proteins was determined by western blotting assay. HepG2 cells were treated with **5k** (5, 10 or 20 μM) for 48 h, and then harvested and lysed to detect the expression of Bad, Bax, Bcl-2 and Bcl-xl; (d) The percentage of cell distribution; (e) Density ratios of Bad, Bax, Bcl-2 and Bcl-xl to GADPH.

4. Conclusions

In summary, nineteen quinazolinone derivatives (**5a-s**) were obtained and their in vitro anti-HBV and anti-HCC activities were subsequently evaluated. Among them, compounds **5j** and **5k** exhibited potent anti-HBV activities in vitro with IC_{50} values of 0.63 and 0.58 μM, respectively. In addition, compounds **5j** and **5k** could also obviously inhibit the HBV DNA replication in lamivudine and entecavir-resistant HBV stains. Interestingly, besides the anti-HBV activities, compound **5k** possessed significant anti-HCC effect against HepG2, HUH7 and SK-Hep-1 cells, with IC_{50} values of 5.44, 6.42 and 6.75 μM, respectively. Notably, the in vitro anti-HCC activities of **5k** were more potent than that of positive control 5-fluorouracil and sorafenib. Preliminary anti-HCC mechanism studies showed that compound **5k** could induce HepG2 cells apoptosis by dose-dependently upregulating Bad and Bax expression and decreasing Bcl-2 and Bcl-xl protein level. Considering the potent anti-HBV and anti-HCC effect, compound **5k** might be a promising lead to develop novel therapeutic agents towards HBV infection and HBV-induced HCC, and warranted further research.

5. Experimental protocols

5.1. Chemical analysis

The synthesized products in the presnet work were purified by column chromatography using 200-300 mesh silica gel 60 or thin layer chromatography (TLC) using silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). Melting points of the target compounds were determined by using a model YRT-3 apparatus and are uncorrected. Subsequently, the structure of the target compounds were

analyzed by ^{1}H NMR and ^{13}C NMR (JEOL, 400 MHz), IR (Shimadzu, FTIR-8400S) and MS (Agilent 6460 and Agilent 6530 QTOF spectrometer) routinely. The purity of target compounds was determined by high-performance liquid chromatography (HPLC, see the Supplementary Material). Individual compounds with a purity of >95% were used for subsequent experiments. All solvents were reagent grade and, when necessary, were purified and dried by standards methods.

*5.1.1. General synthetic procedure of target compounds **5a-s***

CS_2 (2.3 mL, 30 mmol) was added dropwise to the mixture of methylamine derivative (30 mmol) and triethylamine (12 mL) in THF (25 mL) at 0 °C within 30 min. After stirring for 1 h at room temperature, the mixture was then cooled down to 0 °C and the toluene sulfonyl chloride (6 g, 31.5 mmol) were added in. After stirring for another 1 h at room temperature, the mixture was diluted with 5% HCl (100 mL) and petroleum ether, sequentially washed with water, saturated $NaHCO_3$ solution and brine, dried with anhydrous Na_2SO_4 and evaporated *in vacuo* to obtain compound **1**. HCl (10.5 mL) was added to the mixture of benzene or benzene derivatives (6.65 mmol) and paraformaldehyde (11.97 mmol), and then stirred for 10 h at 60 °C. After cooling down to room temperature, the mixture was then dispersed with water and ethyl acetate, and sequentially washed with water, 5% HCl, saturated $NaHCO_3$ solution and brine, dried with anhydrous Na_2SO_4. The solvent was then removed by evaporation to give compound **2**. Methyl 2-amino-4-hydroxybenzoate (0.01 mol) and isothiocyanate derivatives (0.01 mol) was heated in the presence of triethylamine and ethanol at reflux temperature for 4 h. After cooling down to room temperature, the mixture was filtered to give intermediate **3**, which was subsequently reacted with intermediate **2** (0.01 mmol) in the presence of K_2CO_3 and 1,4-dioxane under reflux for 10 h. Then, the mixture was cooled down to room temperature, dissolved with ethyl acetate, sequentially washed 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 then recrystallized in ethanol to obtain intermediate **4**. Finally, the target compounds **5a-s** were prepared by alkylating intermediate **4** (5 mmol) with 2-dimethylaminoethyl chloride hydrochloride, 2-diethylaminoethyl chloride hydrochloride or 3-dimethylaminopropyl chloride hydrochloride (6 mmol) in the presence of 1, 4-dioxane and K_2CO_3, respectively. Spectra of the target compounds were shown in the Supplementary Material.

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

The title compound was obtained starting from thiazol-2-ylmethanamine, 1-(4-methoxyphenyl)ethan-1-one, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a yellow powder, yield: 10%; m.p.97.1-100.1 °C. Analytical data for **5a**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.25 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.90 (dd, *J* = 8.4 Hz, 2.4 Hz, 1H, Ar-H), 7.58-7.61 (m, 2H, Ar-H), 7.32 (dd, *J* = 8.8 Hz, 2.8 Hz, 1H, Ar-H), 7.21 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.20 (dd, *J* = 5.2 Hz, 1.2 Hz, 1H, Ar-H), 6.89-6.93 (m, 2H, Ar-H), 5.42 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 4.09 (t, *J* = 7.2 Hz, 2H, OCH_2), 3.96 (s, 3H, OCH_3), 2.48-2.52 (m, 5H, CH_2, CH_3), 2.29 (s, 6H, $N(CH_3)_2$), 1.98-2.02 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.74, 161.64, 161.57, 156.98, 153.11, 142.28, 137.37, 132.09, 130.30, 129.88, 128.74, 127.69, 126.42, 126.22, 125.30, 125.15, 120.09, 110.12, 107.27, 66.68, 56.33, 56.00, 45.34(2C), 42.49, 31.35, 27.19, 26.47; IR (KBr, cm^{-1}): *v* 2945.62, 1674.42, 1600.92, 1577.77, 1550.77, 1492.90, 1473.62, 1269.16, 1238.30, 1222.87, 1122.57, 1022.27, 946.88, 831.07; ESI-MS: *m/z* $[M + H]^+$ 538.2; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_4S_2$, 538.1834, found 538.1844. Purity: 95.4%.

*5.1.1.2. 6-(3-(dimethylamino)propoxy)-2-((2-methoxybenzyl)thio)-3-(thiophen-2-ylmethyl)quinazolin-4(3H)-one (**5b**)*

The title compound was obtained starting from thiazol-2-ylmethanamine, anisole, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a yellow oil, yield: 27%. Analytical data for **5b**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.60 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.56 (d, *J* = 8.8 Hz, 1.6 Hz, 1H, Ar-H), 7.52 (dd, *J* = 8.0 Hz, 1.6 Hz, 1H, Ar-H), 7.19-7.31 (m, 4H, Ar-H), 6.87-6.92 (m, 3H, Ar-H), 5.44 (s, 2H, CH_2), 4.58 (s, 2H, CH_2), 4.11 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.89 (s, 3H, CH_3), 2.56 (t, *J* = 6.4 Hz, 2H, NCH_2), 2.34 (s, 6H, $N(CH_3)_2$), 2.03-2.06 (m, 2H, OCH_2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.69, 157.81, 156.81, 153.70, 142.43, 137.45, 131.19, 129.23, 128.76, 127.78, 126.40, 126.20, 124.99, 124.76, 120.52, 120.05, 110.65, 107.22, 66.57, 56.28, 55.63, 45.17(2C), 42.50, 31.73, 27.02; IR (KBr, cm^{-1}): *v* 2943.37, 1670.31, 1549.24, 1489.74, 1467.76, 1354.03, 1246.02, 1145.72, 1107.14;

ESI-MS: *m/z* $[M + H]^+$ 496.3; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{30}N_3O_3S_2$, 496.1729, found 496.1714. Purity: 95.4%.

5.1.1.3.

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

The title compound was obtained starting from furan-2-ylmethanamine, 1-(4-methoxyphenyl)ethan-1-one, methyl 2-amino-4-hydroxybenzoate and 2-dimethylaminoethyl chloride hydrochloride. As a brown powder, yield: 20%; m.p.169.4-172.9 °C. Analytical data for **5c**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.26 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.92 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, Ar-H), 7.64 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.60 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.39 (dd, *J* = 8.8 Hz, 2.8 Hz, 1H, Ar-H), 7.34 (dd, *J* = 1.6 Hz, 0.8 Hz,, 1H, Ar-H), 6.94 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.38 (dd, *J* = 3.2 Hz, 0.8 Hz, 1H, Ar-H), 6.30 (dd, *J* = 3.2 Hz, 2.0 Hz, 1H, Ar-H), 5.32 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 4.19 (t, *J* = 5.6 Hz, 2H, OCH_2), 3.96 (s, 3H, CH_3), 2.84 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.51 (s, 6H, CH_3), 2.40 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.73, 161.66, 161.56, 156.74, 153.69, 148.95, 142.44, 132.05, 130.26, 129.91, 127.73, 125.41, 125.35, 119.99, 110.51, 110.13, 109.71, 107.24, 66.07, 58.03, 55.98, 45.66 (2C), 40.65, 31.35, 26.45; IR (KBr, cm^{-1}): *v* 3330.36, 2924.07, 1673.39, 1600.92, 1577.77, 1550.77, 1427.32, 1357.89, 1270.17, 1226.73, 1157.29, 1076.28, 1014.56, 824.89; ESI-MS: m/z 508.4 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{27}H_{30}N_3O_5S$, 508.1906, found 508.1903. Purity: 96.3%.

5.1.2.4.

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

The title compound was obtained starting from furan-2-ylmethanamine, 1-(4-methoxyphenyl)ethan-1-one, methyl 2-amino-4-hydroxybenzoate and 2-diethylaminoethyl chloride hydrochloride. As a yellow oil, yield: 19%. Analytical data for **5d**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.26 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.92 (dd, *J* = 8.4 Hz, 2.4 Hz, 1H, Ar-H), 7.63 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.60 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.33-7.36 (m, 2H, Ar-H), 6.93 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.39 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.30 (dd, *J* = 3.2 Hz, 1.6 Hz, 1H, Ar-H), 5.31 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 4.14 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.96 (s, 3H, CH_3), 2.93 (t, *J* = 6.0 Hz, 2H, NCH_2), 2.63-2.69 (m, 4H, $N(CH_2CH_3)_2$), 2.51 (s,

3H, CH_3), 1.09 (t, *J* = 6.8 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.66, 161.63, 161.53, 156.95, 153,52, 148.96, 142.40, 142.24, 132.02, 130.24, 129.85, 127.64, 125.35, 125.29, 120.00, 110.50, 110.10, 109.67, 107.28, 66.98, 55.95, 51.56, 47.88 (2C), 40.61, 31.31, 26.42, 11.91 (2C); IR (KBr, cm^{-1}): *v* 2931.80, 1670.31, 1600.92, 1546.91, 1488.71, 1354.03, 1259.91, 1149.11, 1014.56, 821.68; ESI-MS: *m/z* $[M + H]^+$ 536.5; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{29}H_{34}N_3O_5S$, 536.2219, found 536.2215. Purity: 95.1%.

5.1.1.5.

*2-((5-acetyl-2-methoxybenzyl)thio)-6-(3-(dimethylamino)propoxy)-3-(furan-2-ylmethyl)quinazolin-4(3H)-one (**5e**)*

The title compound was obtained starting from furan-2-ylmethanamine, 1-(4-methoxyphenyl)ethan-1-one, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a yellow powder, yield: 10%; m.p.96.1-97.2 °C. Analytical data for **5e**: ^1H NMR (400 MHz, DMSO, δ ppm): 8.21 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.93 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, Ar-H), 7.66 (d, *J* = 9.6 Hz, 1H, Ar-H), 7.57 (d, *J* = 0.8 Hz, 1H, Ar-H), 7.43-7.45 (m, 2H, Ar-H), 7.14 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.40 (dd, *J* = 3.2 Hz, 1.6 Hz, 1H, Ar-H), 6.36 (d, *J* = 3.2 Hz, 1H, Ar-H), 5.24 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 4.10 (t, *J* = 2.4 Hz, 2H, OCH_2), 3.93 (s, 3H, OCH_3), 2.49 (s, 3H, CH_3), 2.38 (t, *J* = 3.2 Hz, 2H, NCH_2), 2.16 (s, 6H, $N(CH_3)_2$), 1.87-1.90 (m, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO, δ ppm): 196.70, 161.69, 160.77, 157.14, 153.96, 149.19, 143.21, 141.85, 131.84, 130.87, 129.81, 128.20, 125.23, 124.98, 119.93, 111.31, 111.21, 109.50, 107.55, 69.92, 56.71, 56.07, 45.67 (2C), 40.44, 31.25, 27.22, 26.92; IR (KBr, cm^{-1}): *v* 3362.17, 2723.49, 1600.92, 1554.63, 1485.63, 1337.89, 1267.09, 1153.43, 1018.41, 825.53, 786.96; ESI-MS: m/z 522.4 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_5S$, 522.2063, found 522.2058. Purity: 95.3%.

*5.1.1.6. 6-(2-(dimethylamino)ethoxy)-3-(furan-2-ylmethyl)-2-((2-methoxybenzyl)thio)quinazolin-4(3H)-one (**5f**)*

The title compound was obtained starting from furan-2-ylmethanamine, anisole, methyl 2-amino-4-hydroxybenzoate and 2-dimethylaminoethyl chloride hydrochloride. As a grey powder, yield: 26%; m.p.97.1-98.5 °C. Analytical data for **5f**: ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.60 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.56 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.51 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H, Ar-H), 7.37 (dd, *J* = 8.8 Hz, 2.8 Hz,

1H, Ar-H), 7.32 (dd, *J* = 2.0 Hz, 0.8 Hz, 1H, Ar-H), 7.22-7.26 (m, 1H, Ar-H), 6.86-6.89 (m, 2H, Ar-H), 6.38 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.28 (dd, *J* = 3.2 Hz, 1.6 Hz, 1H, Ar-H), 5.31 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 4.15 (t, *J* = 5.2 Hz, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 2.76 (t, *J* = 5.2 Hz, 2H, NCH_2), 2.34 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.76, 157.79, 156.76, 154.13, 149.05, 142.49, 142.41, 131.16, 129.18, 127.76, 125.40, 124.87, 120.51, 119.92, 111.64, 110.48, 109.70, 107.06, 66.25, 58.24, 55.62, 45.86 (2C), 40.65, 31.68; IR (KBr, cm^{-1}): *v* 2927.94, 2828.65, 1678.07, 1546.91, 1502.05, 1457.93, 1244.52, 1107.14, 1029.99, 752.24; ESI-MS: m/z 466.3 $[M+H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{28}N_3O_4S$, 466.1801, found 466.1796. Purity: 95.4%.

5.1.2.7.

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

The title compound was obtained starting from furan-2-ylmethanamine, anisole, methyl 2-amino-4-hydroxybenzoate and 2-diethylaminoethyl chloride hydrochloride. As a yellow oil, yield: 21%. Analytical data for **5g**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.60 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.57 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.52 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H, Ar-H), 7.31-7.34 (m, 2H, Ar-H), 7.24-7.29 (m, 1H, Ar-H), 6.87-6.91 (m, 2H, Ar-H), 6.39 (dd, *J* = 3.2 Hz, 0.8 Hz, 1H, Ar-H), 6.29 (dd, *J* = 3.2 Hz, 2.0 Hz, 1H, Ar-H), 5.33 (s, 2H, CH_2), 4.57 (s, 2H, CH_2), 4.16 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.88 (s, 3H, CH_3), 2.95 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.65-2.71 (m, 4H, $N(CH_2CH_3)_2$), 1.10 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.75, 157.79, 156.83, 154.06, 149.07, 142.40(2C), 131.16, 129.18, 127.72, 125.22, 124.87, 120.50, 119.97, 110.64, 110.48, 109.69, 107.22, 66.88, 55.61, 51.56, 47.88 (2C), 40.65, 31.68, 11.89 (2C); IR (KBr, cm^{-1}): *v* 2966.52, 2935.66, 1678.07, 1549.24, 1491.79, 1465.90, 1438.90, 1346.31, 1246.02, 1157.29, 1107.14, 1029.99, 752.24; ESI-MS: m/z 494.3 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{27}H_{32}N_3O_4S$, 494.2114, found 494.2109. Purity: 99.0%.

5.1.1.8.

*6-(3-(dimethylamino)propoxy)-3-(furan-2-ylmethyl)-2-((2-methoxybenzyl)thio)quinazolin-4(3H)-one (**5h**)*

The title compound was obtained starting from furan-2-ylmethanamine, anisole, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a yellow oil, yield: 54%. Analytical data for **5h**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.58 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.56 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.50 (dd, *J* = 6.8 Hz, 1.6 Hz, 1H, Ar-H), 7.23-7.31 (m, 3H, Ar-H), 6.87 (m, 2H, Ar-H),

6.37 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.28 (dd, *J* = 2.8 Hz, 0.8 Hz, 1H, Ar-H), 5.31 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 4.10 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 2.63 (t, *J* = 7.2 Hz, 2H, NCH_2), 2.37 (s, 6H, $N(CH_3)_2$), 2.05-2.09 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 160.65, 157.75, 156.91, 153.99, 149.08, 142.341, 142.31, 131.13, 129.17, 127.72, 124.97, 124.78, 120.49, 119.96, 110.63, 110.51, 109.64, 107.28, 66.76, 56.36, 55.55, 45.48(2C), 40.63, 31.68, 27.38; IR (KBr, cm^{-1}): *v* 2939.52, 1678.07, 1546.16, 1489.74, 1467.16, 1432.28, 1346.31, 1246.02, 1157.29, 1107.14, 1029.99, 833.25, 752.24; ESI-MS: m/z 480.3 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{30}N_3O_4S$, 480.1957, found 480.1952. Purity: 99.3%.

*5.1.1.9. 2-(benzylthio)-6-(2-(dimethylamino)ethoxy)-3-(furan-2-ylmethyl)quinazolin-4(3H)-one (**5i**)*

The title compound was obtained starting from furan-2-ylmethanamine, (chloromethyl)benzene, methyl 2-amino-4-hydroxybenzoate and 2-dimethylaminoethyl chloride hydrochloride. As a yellow powder, yield: 30%; m.p.91.2-92.8 °C. Analytical data for **5i**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.60 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.45 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.24-7.36 (m, 5H, Ar-H), 6.38 (d, *J* = 2.8 Hz, 1H, Ar-H), 6.29 (dd, *J* = 3.2 Hz, 2.0 Hz, 1H, Ar-H), 5.32 (s, 2H, CH_2), 4.52 (s, 2H, CH_2), 4.14 (t, *J* = 5.6 Hz, 2H, OCH_2), 2.76 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.34 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.65, 156.93, 153.33, 148.91, 142.49, 142.35, 136.52, 129.48 (2C), 128.69 (2C), 127.76, 127.68, 125.45, 120.03, 110.54, 109.79, 107.07, 66.36, 58.27, 45.92 (2C), 40.71, 36.90; IR (KBr, cm^{-1}): *v* 2939.52, 2768.12, 1676.47, 1553.35, 1492.81, 1350.17, 1226.73, 1225.03, 1157.29, 1076.26, 1028.04, 833.25, 786.96, 702.09; ESI-MS: m/z 436.3 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{24}H_{26}N_3O_3S$, 436.1695, found 436.1689. Purity: 98.8%.

*5.1.1.10. 2-(benzylthio)-6-(2-(diethylamino)ethoxy)-3-(furan-2-ylmethyl)quinazolin-4(3H)-one (**5j**)*

The title compound was obtained starting from furan-2-ylmethanamine, (chloromethyl)benzene, methyl 2-amino-4-hydroxybenzoate and 2-diethylaminoethyl chloride hydrochloride. As a white powder, yield: 20%; m.p.92.7-94.3 °C. Analytical data for **5j**: ^{1}H NMR (400 MHz, DMSO, δ ppm): 7.59 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.46-7.50 (m, 3H, Ar-H), 7.42 (dd, *J* = 8.8 Hz, 3.2 Hz, 1H, Ar-H), 7.30-7.34 (m, 2H, Ar-H), 7.27 (d, *J* = 7.2 Hz, 1H, Ar-H), 6.41 (dd, *J* = 3.2 Hz, 2.0 Hz, 1H, Ar-H), 6.37 (d, *J* = 2.8 Hz, 1H, Ar-H), 5.27 (s, 2H, CH_2), 4.52 (s, 2H, CH_2), 4.11 (t, *J* = 6.0 Hz, 2H, OCH_2), 2.80 (t, *J* = 6.0 Hz, 2H, NCH_2), 2.52-2.58 (m, 4H, $N(CH_2CH_3)_2$), 0.97 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ

ppm): 161.63, 157.01, 153.26, 148.94, 142.48, 142.25, 136.52, 129.48 (2C), 128.68 (2C), 127.72, 127.68, 125.27, 120.08, 110.55, 109.79, 107.23, 67.04, 51.61, 47.92 (2C), 40.70, 36.90, 12.00 (2C); IR (KBr, cm^{-1}): *v* 2966.52, 2927.15, 1680.57, 1550.27, 1486.66, 1350.17, 1226.73, 1157.29, 1072.42, 827.97, 700.75; ESI-MS: m/z 464.3 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{30}N_3O_3S$, 464.2008, found 464.2001. Purity: 98.0%.

*5.1.1.11. 2-(benzylthio)-6-(3-(dimethylamino)propoxy)-3-(furan-2-ylmethyl)quinazolin-4(3H)-one (**5k**)*

The title compound was obtained starting from furan-2-ylmethanamine, (chloromethyl)benzene, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a yellow powder, yield: 30%; m.p.89.2-91.3 °C. Analytical data for **5k**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.59 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.52 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.46 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.25-7.33 (m, 5H, Ar-H), 6.38 (d, *J* = 3.2 Hz, 1H, Ar-H), 6.29 (dd, *J* = 3.2 Hz, 2.0 Hz, 1H, Ar-H), 5.32 (s, 2H, CH_2), 4.52 (s, 2H, CH_2), 4.09 (t, *J* = 6.4 Hz, 2H, OCH_2), 2.46 (t, *J* = 6.8 Hz, 2H, NCH_2), 2.60 (s, 6H, $N(CH_3)_2$), 1.97-2.00 (m, 2H, OCH_2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 161.65, 157.05, 153.23, 148.93, 142.48, 142.23, 136.53, 129.48 (2C), 128.69 (2C), 127.73, 127.68, 125.07, 120.10, 110.53, 109.76, 107.35, 66.77, 56.36, 45.47 (2C), 40.70, 36.90, 27.36; IR (KBr, cm^{-1}): *v* 2943.37, 2761.96, 1677.49, 1547.19, 1489.74, 1350.17, 1226.73, 1157.29, 1103.28, 833.25, 702.09; ESI-MS: m/z 450.3 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{25}H_{28}N_3O_3S$, 450.1851, found 450.1846. Purity: 99.3%.

*5.1.1.12. 2-((5-acetyl-2-methoxybenzyl)thio)-3-benzyl-6-(2-(dimethylamino)ethoxy)quinazolin-4(3H)-one (**5l**)*

The title compound was obtained starting from phenylmethanamine, 1-(4-methoxyphenyl)ethan-1-one, methyl 2-amino-4-hydroxybenzoate and 2-dimethylaminoethyl chloride hydrochloride. As a yellow oil, yield: 7%. Analytical data for **5l**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.23 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.89 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H, Ar-H), 7.66 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.60 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.21-7.53 (m, 6H, Ar-H), 6.89 (d, *J* = 8.8 Hz, 1H, Ar-H), 5.33 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 4.16 (t, *J* = 5.6 Hz, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 2.79 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.49 (s, 3H, CH_3), 2.36 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.78, 162.05, 161.52, 156.83, 154.11, 142.47, 135.68, 132.02, 130.25, 129.79, 128.60 (2C), 127.74, 127.68, 127.60 (2C), 125.47, 125.28, 119.94, 110.12, 107.15, 66.23, 58.15, 55.94, 47.42, 45.81(2C), 31.30, 26.47; IR (KBr, cm^{-1}): *v* 2924.07, 1677.49, 1600.54, 1550.27,

1489.74, 1353.28, 1260.94, 1168.60, 1024.96, 827.97, 714.09; ESI-MS: m/z 518.3 $[M+H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{29}H_{32}N_3O_4S$, 518.2114, found 518.2102. Purity: 95.0%.

*5.1.1.13. 2-((5-acetyl-2-methoxybenzyl)thio)-3-benzyl-6-(2-(diethylamino)ethoxy)quinazolin-4(3H)-one (**5m**)*

The title compound was obtained starting from phenylmethanamine, 1-(4-methoxyphenyl)ethan-1-one, methyl 2-amino-4-hydroxybenzoate and 2-diethylaminoethyl chloride hydrochloride. As a yellow powder, yield: 21%; m.p.116.9-118.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.91 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H, Ar-H), 7.66 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.60 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.24-7.38 (m, 7H, Ar-H), 6.91 (d, *J* = 8.8 Hz, 1H, Ar-H), 5.34 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 4.15 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.92 (s, 3H, OCH_3), 2.94 (t, *J* = 6.0 Hz, 2H, NCH_2), 2.65-2.70 (m, 4H, $N(CH_2CH_3)_2$), 2.51 (s, 3H, CH_3), 1.09 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.78, 162.08, 161.53, 156.97, 154.02, 142.38, 135.71, 132.06, 130.23, 129.83, 128.60 (2C), 127.67(2C), 127.64 (2C), 125.37, 125.35, 120.01, 110.11, 107.26, 66.94, 55.94, 51.54, 47.87 (2C), 47.43, 31.30, 26.48, 11.90(2C); IR (KBr, cm^{-1}): *v* 2965.11, 1673.39, 1600.92, 1546.91, 1489.05, 1357.89, 1262.99, 1228.11, 1170.65, 1026.13, 829.39; ESI-MS: m/z 546.2 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{31}H_{36}N_3O_4S$, 546.2427, found 546.2421. Purity: 95.5%.

*5.1.1.14. 3-benzyl-6-(2-(dimethylamino)ethoxy)-2-((2-methoxybenzyl)thio)quinazolin-4(3H)-one (**5n**)*

The title compound was obtained starting from phenylmethanamine, anisole, methyl 2-amino-4-hydroxybenzoate and 2-dimethylaminoethyl chloride hydrochloride. As a yellow oil, yield: 43%. Analytical data for **5n**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.57-7.62 (m, 2H, Ar-H), 7.49 (dd, *J* = 7.6 Hz, 2.0 Hz, 1H, Ar-H), 7.22-7.39 (m, 9H, Ar-H), 5.35 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 4.17 (t, *J* = 4.8 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 2.80 (t, *J* = 4.2 Hz, 2H, NCH_2), 2.38 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 162.13, 157.75, 156.76, 154.64, 142.61, 135.78, 131.13, 129.15(2C), 128.57(2C), 127.79, 127.70, 127.63, 125.41, 124.87, 120.48, 119.93, 110.64, 107.04, 66.24, 58.23, 55.59, 47.49, 45.84(2C), 31.65; IR (KBr, cm^{-1}): *v* 2935.66, 1674.21, 1546.91, 1489.74, 1354.03, 1244.52, 1165.00, 1107.14, 1029.99, 834.13, 752.24; ESI-MS: m/z 476.2 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{27}H_{30}N_3O_3S$, 476.2008, found 476.1998. Purity: 96.3%.

*5.1.1.15. 3-benzyl-6-(2-(diethylamino)ethoxy)-2-((2-methoxybenzyl)thio)quinazolin-4(3H)-one (**5o**)*

The title compound was obtained starting from phenylmethanamine, anisole, methyl 2-amino-4-hydroxybenzoate and 2-diethylaminoethyl chloride hydrochloride. As a yellow oil, yield: 47%. Analytical data for **5o**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.60 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.59 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.49 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H, Ar-H), 7.23-7.36 (m, 7H, Ar-H), 6.86-6.90 (m, 2H, Ar-H), 5.35 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 4.16 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 2.96 (t, *J* = 5.2 Hz, 2H, NCH_2), 2.66-2.71 (m, 4H, $N(CH_2CH_3)_2$), 1.10 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 162.14, 157.75, 156.85, 154.56, 142.52, 135.80, 131.13, 129.16, 128.57(2C), 127.76, 127.71(2C), 127.63, 125.24, 124.85, 120.49, 119.97, 110.65, 107.20, 66.87, 55.59, 51.56, 45.84 (2C), 47.49, 31.66, 11.87(2C); IR (KBr, cm^{-1}): *v* 2933.31, 2831.50, 1674.21, 1546.91, 1489.74, 1462.04, 1357.89, 1244.52, 1165.00, 1107.14, 1029.99, 752.24; ESI-MS: m/z 504.2 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{29}H_{34}N_3O_3S$, 504.2321, found 504.2309. Purity: 95.1%.

*5.1.1.16. 3-benzyl-6-(3-(dimethylamino)propoxy)-2-((2-methoxybenzyl)thio)quinazolin-4(3H)-one (**5p**)*

The title compound was obtained starting from phenylmethanamine, anisole, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a yellow oil, yield: 50%. Analytical data for **5p**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.57-7.60 (m, 2H, Ar-H), 7.49 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H, Ar-H), 7.23-7.34 (m, 7H, Ar-H), 6.96-6.89 (m, 2H, Ar-H), 5.35 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 4.11 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 2.55 (t, *J* = 6.8 Hz, 2H, NCH_2), 2.33 (s, 6H, $N(CH_3)_2$), 2.02-2.06 (m, 2H, OCH_2CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 162.11, 157.75, 156.91, 154.52, 142.47, 135.82, 131.13, 129.14, 128.56(2C), 127.72(3C), 127.62, 125.04, 124.89, 120.47, 120.01, 110.64, 107.30, 66.75, 56.38, 55.59, 47.46, 45.46(2C), 31.64, 27.35; IR (KBr, cm^{-1}): *v* 2935.66, 2837.89, 1674.21, 1546.91, 1495.89, 1467.16, 1357.89, 1244.52, 1168.86, 1107.14, 1029.99, 752.24; ESI-MS: m/z 490.3 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_3S$, 490.2164, found 490.2156. Purity: 95.9%.

*5.1.1.17. 3-benzyl-2-(benzylthio)-6-(2-(dimethylamino)ethoxy)quinazolin-4(3H)-one (**5q**)*

The title compound was obtained starting from phenylmethanamine, (chloromethyl)benzene, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a white powder, yield: 16%; m.p.102.4-105.7 °C. Analytical data for **5q**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.61 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.56 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.42 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.38 (dd, *J* = 9.2 Hz, 3.2 Hz, 1H,

Ar-H), 7.24-7.33 (m, 8H, Ar-H), 5.36 (s, 2H, CH_2), 4.49 (s, 2H, CH_2), 4.20 (t, *J* = 5.2 Hz, 2H, OCH_2), 2.84 (t, *J* = 5.2 Hz, 2H, NCH_2), 2.41 (s, 6H, $N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 162.06, 156.75, 153.93, 142.55, 136.51, 135.63, 129.45(2C), 128.66(4C), 127.85, 127.74, 127.65(3C), 125.37, 120.03, 107.18, 66.00, 58.02, 47.53, 45.63(2C), 36.85; IR (KBr, cm^{-1}): *v* 2933.31, 2554.71, 2449.60, 1670.35, 1540.01, 1489.05, 1357.89, 1222.87, 1168.86, 1029.99, 840.96, 705.95; ESI-MS: m/z 446.2 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{26}H_{28}N_3O_2S$, 446.1902, found 446.1894. Purity: 95.3%.

*5.1.1.18. 3-benzyl-2-(benzylthio)-6-(2-(diethylamino)ethoxy)quinazolin-4(3H)-one (**5r**)*

The title compound was obtained starting from phenylmethanamine, (chloromethyl)benzene, methyl 2-amino-4-hydroxybenzoate and 2-diethylaminoethyl chloride hydrochloride. As a white powder, yield: 25%; m.p.94.2-97.8 °C. Analytical data for **5r**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.60 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.55 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.42 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.23-7.34 (m, 9H, Ar-H), 5.35 (s, 2H, CH_2), 4.48 (s, 2H, CH_2), 4.16 (t, *J* = 6.0 Hz, 2H, OCH_2), 2.95 (t, *J* = 6.0 Hz, 2H, NCH_2), 2.65-2.71 (m, 4H, $N(CH_2CH_3)_2$), 1.10 (t, *J* = 7.2 Hz, 6H, $N(CH_2CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 162.07, 156.94, 153.81, 142.42, 136.53, 135.67, 129.46(2C), 128.67(2C), 128.65(2C), 127.78, 127.73, 127.66(3C), 125.26, 120.07, 107.28, 66.81, 51.51, 47.87(3C), 47.52, 36.85, 11.80(2C); IR (KBr, cm^{-1}): *v* 2966.52, 2931.80, 1670.31, 1549.24, 1485.63, 1454.33, 1357.89, 1276.88, 1226.73, 1168.86, 1029.99, 834.13, 702.09; ESI-MS: m/z 474.2 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_2S$, 474.2215, found 474.2207. Purity: 96.5%.

*5.1.1.19. 3-benzyl-2-(benzylthio)-6-(3-(dimethylamino)propoxy)quinazolin-4(3H)-one (**5s**)*

The title compound was obtained starting from phenylmethanamine, (chloromethyl)benzene, methyl 2-amino-4-hydroxybenzoate and 3-dimethylaminopropyl chloride hydrochloride. As a white powder, yield: 36%; m.p.93.7-96.7 °C. Analytical data for **5s**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.61 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.54 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.38-7.40 (m, 2H, Ar-H), 7.21-7.32 (m, 9H, Ar-H), 5.33 (s, 2H, CH_2), 4.47 (s, 2H, CH_2), 4.09 (t, *J* = 6.4 Hz, 2H, OCH_2), 2.45 (t, *J* = 7.2 Hz, 2H, NCH_2), 2.25 (s, 6H, $N(CH_3)_2$), 1.96-1.99 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 162.02, 157.14, 153.71, 142.33, 136.56, 135.75, 129.47(2C), 128.67(2C), 128.65(2C), 127.78, 127.70(3C), 127.66, 125.10, 120.15, 107.39, 66.88, 56.41, 47.50, 45.64(2C), 36.86, 27.54; IR (KBr, cm^{-1}): *v* 3379.29, 2939.46, 2757.86, 1670.31, 1616.35, 1577.77, 1485.43, 1428.18, 1355.33, 1275.30, 1221.95, 1165.00, 1103.28, 1072.42, 983.70,

948.98, 833.25, 779.24, 563.21; ESI-MS: m/z 460.2 $[M + H]^+$; ESI-HRMS (TOF): *m/z* $[M + H]^+$ calcd for $C_{27}H_{30}N_3O_2S$, 460.2059, found 460.2049. Purity: 95.2%.

5.2. Biological assays

The anti-HBV activities and intrinsic cytotoxicity of the target compounds were determined in HepG2 2.2.15 cell line, a stably transfected cell line containing HBV genome on a plasmid. The cell line was provided by 302 military hospital of china, and 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 anti-HCC activities and preliminary anti-HCC action mechanism of the target compounds were evaluated in HepG2 cell line.

5.2.1. Cytotoxicity assay

The intrinsic cytotoxicity and in vitro anti-HCC activities of the target compounds were measured by MTT assay. Briefly, HepG2 2.2.15 (used to determine the intrinsic cytotoxicity) or HepG2 (used to evaluate the in vitro anti-HCC effect) cells were seeded in 96-well culture plates and cultured for 24 h to maintain the cells in exponential growth phase. Then, cells were cultured for another 72 h in the presence or absence of the test compounds (in 0.2 mL culture medium/well), respectively. Finally, the cell viability was measured by analyzing MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) absorbance at 490 nm, as calculated from the absorbance relative to untreated cells at 24 h after MTT treatment.

5.2.2. In vitro anti-HBV activity assay.

The vitro anti-HBV activity of the target compounds was evaluated by detecting their inhibitory effect on HBV DNA replication according to previous method [19, 20]. Briefly, HepG2 2.2.15 cells (2×10^5 cells/well) were seeded in 24 well culture plates and cultured for another 24 h to recover from the trypsinization. After that, the culture medium was replaced with a fresh medium containing the test compound or 3TC. Subsequently, medium was changed every 2 days. After 6 days incubation, intracellular HBV DNA was extracted, and quantified by real time PCR.

HepG2 2.2.15 cells, transiently transfected with plasmids containing lamivudine and entecavir-resistant mutant (rtL180M + rtM204V + rtT184L), were used to evaluate the antiviral efficacy of the target

compounds against nucleoside analogue-resistant HBV.

5.2.3. Hoechst 33342 Staining

HepG2 cells (5×10^4 cells/well) were seeded in six-well plates, and then incubated in the presence of **5k** (5, 10 or 20 μM) for 48 h. After the incubation, the cells were gently washed with PBS, fixed in 4% paraformaldehyde for 30 min, and subsequently stained with Hoechst 33342 (20 μg/mL, KeyGen, Nanjing, China) at room temperature for 15 min in the darkness. Finally, morphological changes of cells was observed using fluorescence microscopy (Olympus, BX41).

5.2.4. Cell Apoptosis Analysis

Annexin V staining was performed with an apoptosis assay kit (KeyGen, Nanjing, China). Briefly, HepG2 cells were incubated with or without **5k** at the indicated concentrations (5, 10 or 20 μM) for 48 h, and then gently washed twice with PBS, centrifuged, and reincubated in annexin V binding buffer (500 μL). The cells were then harvested, washed, and stained with 5 μL annexin V-FITC and 5 μL propidium iodide (PI) for 15 min in the darkness. Subsequently, apoptosis was analyzed by a FACS Calibur flow cytometer (Becton-Dickinson, San Jose, CA, USA).

5.2.5. Western blotting analysis of apoptotic proteins Bad, Bax, Bcl-2 and Bcl-xl

HepG2 cells lysates (40 μg protein/lane) from each group were separated SDS-PAGE and then transferred to polyvinyl difluoride membranes by electroblotting. After incubation in blocking solution (5% nonfat milk), the blots were incubated with primary antibodies at 4°C overnight, respectively. Primary antibodies were mouse anti-Bad, Bax, Bcl-2 and Bcl-xl (Santa Cruz Biotechnology, USA). The blots were subsequently washed with 1× Tris-Buffered Saline Tween-20 (TBST) solution, incubated with secondary antibodies for 2 h, and washed with 1× TBST solution. The blots were detected using an enhanced chemiluminescence system, and imaged using a G:BOX chemiXR5 digital imaging system. The protein bands' relative intensities were analyzed by Gel-Pro32 software.

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Highlights

1. Quinazolinone derivatives were synthesized and evaluated as anti-HBV and anti-HCC agents.
2. **5j** and **5k** exhibited potent anti-HBV activity against wild and resistant HBV strains.
3. **5k** exhibited more potent in vitro anti-HCC activity than 5-fluorouracil and sorafenib.
4. **5k** induced HepG2 cells apoptosis by regulating the expression of Bcl-2 family proteins.

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.