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Synthesis and in vitro anti-hepatitis B virus activity of 6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ols

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ABSTRACT

A series of novel 6*H*-[1]benzothiopyrano[4,3-*b*]quinoline derivatives were prepared and evaluated for their anti-hepatitis B virus (HBV) activity and cytotoxicity in human hepatoblastoma-derived liver Hep-G2 cells. Compounds **10g**, **10h**, **10j**, **10l** and **10o** were found to be potent anti-HBV compounds with IC_{50} values less than 50 μM. The most promising compound was **10l**, with an IC_{50} value of 14.7 μM and a SI value of 12.4. This is the first report of the anti-HBV effects of 6*H*-[1]benzothiopyrano[4,3-*b*] quinolin-9-ols.

1. Introduction

HBV infection is a worldwide health problem and may lead to life-long infection, cirrhosis of the liver, liver cancer, liver failure, and death.[1,2] It is most prevalent in Asia, Africa, Southern Europe and Latin America, where the prevalence of hepatitis B surface antigen (HBsAg) carriers in the general population ranges from 2% to 20%.[3] Interferon has been shown to be effective against HBV, but it requires inconvenient injection therapy. The approved chemotherapeutic anti-HBV agents in clinical application are nucleoside analogues, such as lamivudine, adeforiv dipivoxil, entecavir, telbivudine and tenofovir. Long-term nucleoside analogues treatment will result in drug-resistance, which has made most nucleoside analogues ineffective for a huge number of patients.[4–6] Therefore, it is of great interest to synthesize non-nucleoside compounds to evaluate their anti-HBV activity.

We have previously synthesized a series of ethyl 7-hydroxy-6-methoxy-2-(substituted phenylthiomethyl)quinoline-3- carboxylates.[7] Several compounds were found to inhibit the expression of the viral antigens HBsAg or HBeAg and the replication of HBV DNA. To develop new class of anti-HBV agents, the possibility of replacing the quinoline ring with the 6*H*-[1]benzothiopyrano[4,3-*b*]quinoline ring was investigated. Some of structure features of previous quinoline derivates were also included in target compounds. In order to examine the effect of substituents on the D ring, the methyl group and the F atom were introduced at different positions of the D ring. Resulted compounds were evaluated for their inhibition of HBV in human hepatoblastoma-derived liver Hep-G2 cells (HepG2 2.2.15 cells) (Fig. 1).

2. Chemistry

General synthesis for target compounds is depicted in Scheme 1 starting from 3-hydroxy-4-methoxy-benzaldehyd **1**. Protection of the phenolic hydroxyl group of **1** with benzyl chloride in DMF at 80 °C, followed by selective nitration with HNO_3 gave the intermediate 5-(benzyloxy)-4-methoxy-2-nitrobenzaldehyde **3**[8] in good yield, which was then treated with zinc powder in alcohol to provide the known intermediate **4**. Alkylation of **5** with 3-chloropropanoic acid gave **6**, which was ring-closed in concentrated sulfuric acid to yield the known[9–12] benzothiopyranone derivatives **7**. Condensation of **7** with intermediate **4** gave **8**, which subsequently converted to **9** in the mixture of concentrated hydrochloric acid and acetic acid (1:1, V/V) at 80 °C. Here, steps f and g were accomplished in one pot. The final step involved a Mannich reaction of **9**

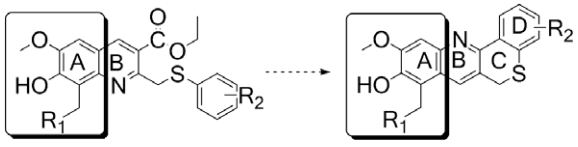

Figure 1. Structure of ethyl 7-hydroxyquinoline-3-carboxylates and 6*H*-[1]benzothiopyrano[4,3-*b*]quinolines.

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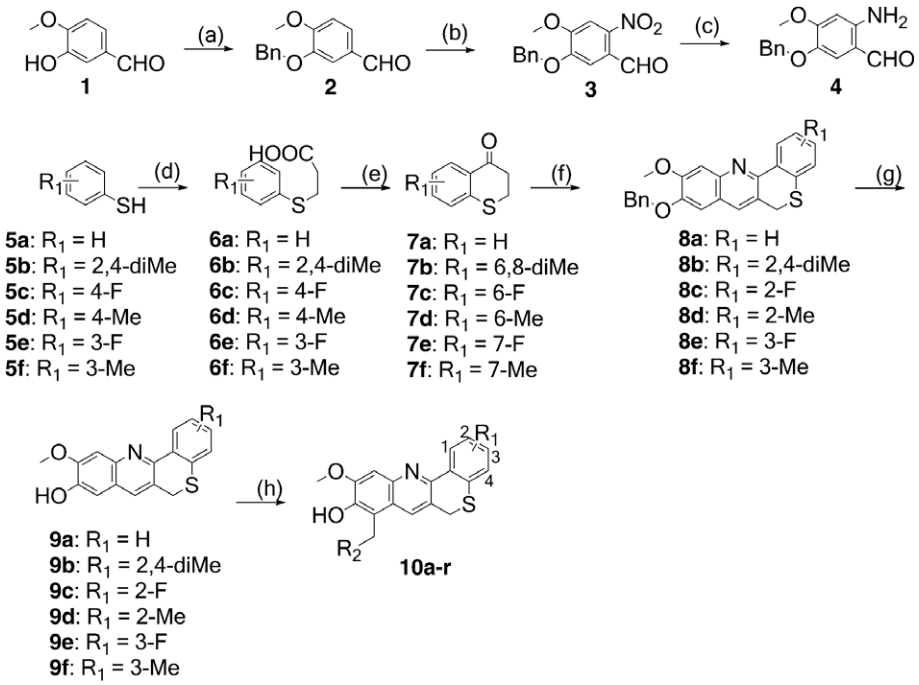

Scheme 1. Synthesis of target compounds. Reagents and conditions: (a) benzyl chloride, K_2CO_3, DMF, 80 °C, 2 h; (b) HNO_3, rt–30 °C, 1 h; (c) Zn, C_2H_5OH, reflux, 4 h; (d) 3-chloropropanoic acid, KOH, K_2CO_3, C_2H_5OH, reflux, 5 h; (e) H_2SO_4, rt, 16 h; (f) **4**,C_2H_5ONa, C_2H_5OH, reflux, 15 h; (g) HCl, AcOH, 80 °C, 15 h; (h) alkyl secondary amine, HCHO, AcOH, 45 °C, 2 h.

Table 1
The substituents, anti-HBV activity and cytotoxicity of target compounds **10a**–**r** in vitro

Compound	R_1	R_2	CC_{50}[a] (μM)	HBsAg		DNA replication	
				IC_{50}[b] (μM)	SI[c]	IC_{50} (μM)	SI
10a	H	4-Methylpiperazin-1-yl	25.2	—[d]	—	—	—
10b	H	Diethylamino	39.0	—	—	—	—
10c	H	Dimethylamino	36.5	9.3	3.9	—	—
10d	H	Morpholino	42.1	—	—	—	—
10e	2,4-diMe	Dimethylamino	32.4	13.8	2.4	—	—
10f	2,4-diMe	Morpholino	50.6	—	—	—	—
10g	2,4-diMe	Piperidin-1-yl	21.9	—	—	4.2	5.3
10h	2-F	Pyrrolidin-1-yl	434.9	—	—	47.7	9.1
10i	2-Me	4-Methylpiperazin-1-yl	527.2	—	—	106.7	4.9
10j	2-Me	Diethylamino	12.0	—	—	2.0	6.0
10k	2-Me	Dimethylamino	67.3	22.1	3.0	—	—
10l	2-Me	Morpholino	181.3	—	—	14.7	12.4
10m	2-Me	Pyrrolidin-1-yl	146.4	—	—	56.3	2.6
10n	3-F	4-Methylpiperazin-1-yl	13.5	—	—	—	—
10o	3-F	Diethylamino	20.7	—	—	2.4	8.7
10p	3-F	Morpholino	28.8	—	—	—	—
10q	3-Me	Morpholino	261.5	—	—	—	—
10r	3-Me	Pyrrolidin-1-yl	566.1	—	—	173.3	3.3
lamivudine			2183.1	—	—	240.0	9.1

[a] CC_{50} is 50% cytotoxic concentration in HepG2.2.15 cells.
[b] IC_{50} is 50% inhibitory concentration.
[c] Selectivity index (SI: TC_{50}/IC_{50}).
[d] Means no antiviral activity at the concentration lower than its TC_{50}.

to provide the desired products (**10a**–**r**, Table 1) in yields ranging from 54% to 83%.

3. Anti-HBV analysis

Stably HBV-transfected HepG2 2.2.15 cell line was derived from hepatoblastoma HepG-2 cells and had been a useful in vitro model for evaluation of anti-HBV agents.[13,14] Compounds **10a**–**r**, along with the reference antiviral drug lamivudine, were evaluated in vitro against the HepG2 2.2.15 cells. The levels of HBV DNA and the Hepatitis B surface antigen (HBsAg) in the culture supernatants were detected to determine the inhibitory effect of each compound. Toxic effects of test compounds were quantified using CC_{50} values and the results were given in Table 1.

The present study investigated several substitutions on the D ring. When R_1 was H, only compound **10c** with a dimethylamino group at R_2 position was found to inhibit HBsAg with an IC_{50} value of 9.3 μM. Introduction of a methyl group or a F atom at the 2-po-

sition of the 6*H*-[1]benzothiopyrano[4,3-*b*]quinoline ring led to compounds **10h–m** with potent inhibition against HBsAg secretion or HBV NDA production. Comparing compound **10l** with **10q**, compound **10l** with a methyl group at the 2-position showed potent activity against HBV DNA with an IC_{50} value of 14.7 μM and a SI value of 12.4, while compound 10q with a methyl group at the 3-position lost activity. This difference was also found in comparing the activity of compounds **10m** and **10r**. This result revealed that the methyl group was more preferred at the 2-position than at the 3-position. Besides, further substitution in the D ring might have a negative effect on the activity. For example, introducing another methyl group at the 4-position of compound **10l** resulted in compound **10f** without anti-HBV activity. In general, the methyl group and the F atom were tolerated at the 2-, 3- and 4-positions of the 6*H*-[1]benzothiopyrano[4,3-*b*]quinoline ring, and the methyl group at R_1 position was preferred for potent anti-HBV activity as demonstrated by compounds **10g**, **10i–m** and **10r**.

We could observe that anti-HBsAg activity was significantly affected by Mannich basic functionalities. Compounds (**10l**, **10k** and **10c**) with a dimethylamino group at the R_2 position showed selective inhibition of HBsAg secretion. In general, different Mannich basic functionalities seemed to have few influence on anti-HBV DNA activity. Although, compounds (**10i**, **10j**, **10l** and **10m**) with different Mannich basic functionalities showed different activities against HBV DNA, there was no obvious trend of influence on the anti-HBV activity by R_2 groups in all test compounds. Besides, morpholino and pyrrolidine derivatives showed less toxic than diethylamine and dimethylamine derivatives.

Compounds **10a–r** were also investigated for inhibition of HBeAg secretion. None of these compounds possessed activity up to the 50% cytotoxic concentration.

4. Conclusion

In conclusion, we presented the synthesis and in vitro anti-HBV activity evaluation of a novel series of 6*H*-[1] benzothiopyrano[4,3-*b*]quinolines. It was found that the dimethylamino group was more preferred at R_2 position for HBsAg inhibitory activity. Introduction of the methyl group at the 2-position of the 6*H*-[1]benzothiopyrano[4,3-*b*] quinoline ring would improve anti-HBV activity. One member of this series, compound **10l**, was found to be more potent than lamivudine, with an IC_{50} value of 14.7 μM and a SI value of 12.4 in vitro assays.

5. Experimental

5.1. Chemistry: general

All chemicals were obtained from commercial suppliers and used without purification. Melting points were determined by capillary tube method and are uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). ^{1}H NMR spectra were performed using Bruker 300 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). Compound **4** was synthesized according to the literature.[8]

5.2. 3-(Phenylthio)propanoic acid (6a)

Thiophenol **5a** (0.5 mol) in 250 mL of 10% KOH and 300 mL EtOH was refluxed for 5 h with 3-chloropropanoic acid (0.5 mol) in saturated solution of K_2CO_3 (0.5 mol). Then, the reaction mixture was concentrated and neutralized with 18% HCl solution. The resulting precipitate was collected and washed with H_2O and petrol ether to give **6a** as white solid. Yield: 63%; mp 58–59 °C (lit.[9], 59). MS (ES+) *m/z* 205.0 $(M+Na)^+$ 221.0 $(M+K)^+$.

5.3. 3-(2,4-Dimethylphenylthio)propanoic acid (6b)

According to the procedure used to prepare **6a**, starting from 2,4-dimethylbenzenethiol, **6b** was obtained as white solid. Yield: 45%; mp 84–85 °C (lit.[10], 84–85). MS (ES+) *m/z* 233.1 $(M+Na)^+$ 249.0 $(M+K)^+$.

5.4. 3-(4-Fluorophenylthio)propanoic acid (6c)

According to the procedure used to prepare **6a**, starting from 4-fluorobenzenethiol, **6c** was obtained as white solid. Yield: 90%; mp 75–76 °C (lit.[9], 76). MS (ES+) *m/z* 222.9 $(M+Na)^+$ 239.0 $(M+K)^+$.

5.5. 3-(4-Methylphenylthio)propanoic acid (6d)

According to the procedure used to prepare **6a**, starting from 4-methylbenzenethiol, **6d** was obtained as white solid. Yield: 77%; mp 71–72 °C (lit.[9], 71). MS (ES+) *m/z* 219.1 $(M+Na)^+$ 235.1 $(M+K)^+$.

5.6. 3-(3-Fluorophenylthio)propanoic acid (6e)

According to the procedure used to prepare **6a**, starting from 3-fluorobenzenethiol, **6e** was obtained as white solid. Yield: 90%; mp 81–82 °C (lit.[11], 82–83). MS (ES+) *m/z* 222.9 $(M+Na)^+$ 239.0 $(M+K)^+$.

5.7. 3-(3-Methylphenylthio)propanoic acid (6f)

According to the procedure used to prepare **6a**, starting from 3-methylbenzenethiol, **6f** was obtained as white solid. Yield: 84%; mp 67 °C (lit.[12], 65–66). MS (ES+) *m/z* 219.1 $(M+Na)^+$ 235.1 $(M+K)^+$.

5.8. 3,4-Dihydro-2*H*-1-benzothiopyran-4-one (7a)

According to the procedure reported[10], 3-(phenylthio)propanoic acid (**6a**) (0.2 mol) was dissolved in concentrated H_2SO_4 (200 mL) at 5 °C. The solution was removed from the cooling-bath and set aside over night. It was then poured on ice, and cooled for 1 h. The solid was filtered and recrystallised from petrol ether gave compound **7a** as solid. Yield: 54%; mp 29–30 °C (lit.[9], 29). MS (ES+) *m/z* 187.0 $(M+Na)^+$.

5.9. 6,8-Dimethyl-3,4-dihydro-2*H*-1-benzothiopyran-4-one (7b)

According to the procedure used to prepare **7a**, starting from 3-(2,4-dimethylphenylthio)propanoic acid (**6b**), **7b** was obtained as white solid. Yield: 90%; mp 38–39 °C (lit.[10], 38–39). MS (ES+) *m/z* 215.1 $(M+Na)^+$.

5.10. 6-Fluoro-3,4-dihydro-2*H*-1-benzothiopyran-4-one (7c)

According to the procedure used to prepare **7a**, starting from 3-(4-fluorophenylthio)propanoic acid (**6c**), **7c** was obtained as white solid. Yield: 86%; mp 96–97 °C (lit.[9], 96). MS (ES+) *m/z* 205.0 $(M+Na)^+$.

5.11. 6-Methyl-3,4-dihydro-2*H*-1-benzothiopyran-4-one (7d)

According to the procedure used to prepare **7a**, starting from 3-(4-methylphenylthio)propanoic acid (**6d**), **7d** was obtained as white solid. Yield: 70%; mp 40–41 °C (lit.[9], 41–42). MS (ES+) *m/z* 179.1 $(M+H)^+$.

5.12. 7-Fluoro-3,4-dihydro-2*H*-1-benzothiopyran-4-one (7e)

According to the procedure used to prepare **7a**, starting from 3-(3-fluorophenylthio)propanoic acid (**6e**), **7e** was obtained as white solid. Yield: 45%; mp 69 °C (lit.[11], 69–70). MS (ES+) *m/z* 183.0 $(M+H)^+$ 205.0 $(M+Na)^+$.

5.13. 7-Methyl-3,4-dihydro-2*H*-1-benzothiopyran-4-one (7f)

According to the procedure used to prepare **7a**, starting from 3-(3-methylphenylthio)propanoic acid (**6f**), **7f** was obtained as white solid. Yield: 30%; mp 50–51 °C (lit.[12], 51–52). MS (ES+) *m/z* 179.2 $(M+H)^+$ 201.1 $(M+Na)^+$.

5.14. 10-Methoxy-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (9a)

Na (0.32 mol) was dissolved in 200 mL EtOH, followed by addition of compounds **4** (0.02 mol) and **7a** (0.02 mol). The reaction mixture was refluxed for 15 h. Then the EtOH was removed by distillation and the residue was cooled to room temperature. AcOH (150 mL) and concentrated HCl (75 mL) were added, after which the mixture was refluxed for additional 15 h. The mixture was allowed to cool to room temperature and the solid product **9a** was collected by filtration, washed with a mixture of AcOH (10 mL) and concentrated HCl (5 mL), and dried. Yield: 14%; mp >290 °C; MS (ES+) *m/z* 296.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.98 (s, 3H), 4.20 (s, 2H), 7.34–7.61 (m, 5H), 8.20 (s, 1H), 8.40 (m, 1H).

5.15. 10-Methoxy-2,4-dimethyl-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (9b)

According to the procedure used to prepare **9a**, starting from compounds **4** and **7b**, **9b** was obtained as yellow solid. Yield: 30%; mp >290 °C; MS (ES+) *m/z* 324.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.34 (s, 3H), 2.38 (s, 3H), 3.96 (s, 3H), 4.20 (s, 2H), 7.22 (s, 1H), 7.61 (s, 1H), 7.88 (s, 1H), 8.21 (s, 1H), 8.80 (s, 1H).

5.16. 2-Fluoro-10-methoxy-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (9c)

According to the procedure used to prepare **9a**, starting from compounds **4** and **7c**, **9c** was obtained as yellow solid. Yield: 26%; mp >290 °C; MS (ES+) *m/z* 314.0 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.00 (s, 3H), 4.21 (s, 2H), 7.25–7.31 (m, 1H), 7.40–7.80 (m, 2H), 7.95 (s, 1H), 8.02 (s, 1H), 8.54 (s, 1H).

5.17. 10-methoxy-2-methyl-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (9d)

According to the procedure used to prepare **9a**, starting from compounds **4** and **7d**, **9d** was obtained as yellow solid. Yield: 41%; mp >290 °C; MS (ES+) *m/z* 310.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.41 (s, 3H), 4.04 (s, 3H), 4.27 (s, 2H), 7.50 (d, J = 7.7 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.95 (s, 1H), 8.02 (s, 1H), 8.41 (s, 1H), 8.62 (s, 1H).

5.18. 3-Fluoro-10-methoxy-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (9e)

According to the procedure used to prepare **9a**, starting from compounds **4** and **7e**, **9e** was obtained as yellow solid. Yield: 36%; mp >290 °C; MS (ES+) *m/z* 313.9 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.02 (s, 3H), 4.25 (s, 2H), 7.40–7.51 (m, 2H), 7.94 (s, 1H), 8.00 (s, 1H), 8.46–8.51 (m, 2H).

5.19. 10-Methoxy-3-methyl-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (9f)

According to the procedure used to prepare **9a**, starting from compounds **4** and **7f**, **9f** was obtained as yellow solid. Yield: 27%; mp >290 °C; MS (ES+) *m/z* 310.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.47 (s, 3H), 4.02 (s, 3H), 4.20 (s, 2H), 7.56 (d, J = 7.7 Hz, 1H), 7.69 (s, 1H), 7.95 (s, 1H), 8.03 (s, 1H), 8.43 (d, J = 7.7 Hz, 1H), 8.48 (s, 1H).

5.20. 10-Methoxy-8-((4-methylpiperazin-1-yl)methyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10a)

A solution of 1-methylpiperazine (0.025 mol), 37% HCHO (0.012 mol) and compound **9a** (0.010 mol) in AcOH was heated to 45 °C for 2–5 h. Upon completion of the reaction, as monitored by TLC, the AcOH was removed under vacuum, and the residue was poured into cool water (100 mL). The resulting mixture was adjusted to pH 8 with ammonium hydroxide at 10 °C, and the precipitate was collected by filtration, and purified by silica gel column chromatography to give the title compound **10a**. Yield: 76%; mp: 190 °C (dec); MS (ES+) *m/z* 408.2 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.17 (s, 3H), 2.39 (m, 4H), 2.59 (m, 4H), 3.96 (s, 3H), 4.07 (s, 2H), 4.21 (s, 2H), 7.34–7.41 (m, 4H), 8.23 (s, 1H), 8.47 (m, 1H); IR (KBr): 1239.5, 1384.1, 1461.3, 1506.7, 1617.8, 2806.9, 2937.8, 3428.1 cm^{-1}; Anal. Calcd for $C_{23}H_{25}N_3O_2S$: C, 67.79; H, 6.18; N, 10.31. Found: C, 67.80; H, 5.99; N, 10.46.

5.21. 8-((Diethylamino)methyl)-10-methoxy-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10b)

According to the procedure used to prepare **10a**, starting from compound **9a** and diethylamine, **10b** was obtained as yellow solid. Yield: 58%; mp: 187 °C (dec); MS (ES+) *m/z* 381.2 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.36 (t, J = 7.2 Hz, 6H), 3.20 (q, J = 7.2 Hz, 4H), 4.06 (s, 3H), 4.4.31 (s, 2H), 4.68 (s, 2H), 7.37–7.50 (m, 4H), 8.16 (s, 1H), 8.50 (m, 1H); IR (KBr): 1239.5, 1384.1, 1461.3, 1506.7, 1617.8, 2806.9, 2937.8, 3428.1 cm^{-1}; Anal. Calcd for $C_{22}H_{24}N_2O_2S$: C, 69.44; H, 6.36; N, 7.36. Found: C, 69.25; H, 6.09; N, 7.51.

5.22. 8-((Dimethylamino)methyl)-10-methoxy-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10c)

According to the procedure used to prepare **10a**, starting from compound **9a** and dimethylamine, **10c** was obtained as yellow solid. Yield: 74%; mp: 208 °C (dec); MS (ES+) *m/z* 353.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.86 (s, 6H), 4.03 (s, 3H), 4.25 (s, 2H), 4.86 (s, 2H), 7.38–7.46 (m, 3H), 7.50 (s, 1H), 8.00 (s, 1H), 8.51 (m, 1H); IR (KBr): 1254.8, 1384.5, 1592.4, 2835.5, 3426.9 cm^{-1}; Anal. Calcd for $C_{20}H_{20}N_2O_2S$: C, 68.16; H, 5.72; N, 7.95. Found: C, 68.42; H, 5.39; N, 7.80.

5.23. 10-Methoxy-8-(morpholinomethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10d)

According to the procedure used to prepare **10a**, starting from compound **9a** and morpholine, **10d** was obtained as yellow solid. Yield: 80%; mp: 194 °C (dec); MS (ES+) *m/z* 395.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.72 (m, 4H), 3.84 (m, 4H), 4.06 (s, 3H), 4.09 (s, 2H), 4.15 (s, 2H), 7.26–7.42 (m, 3H), 7.45 (s, 1H), 7.87 (s, 1H), 8.52–8.55 (m, 1H); IR (KBr): 1266.6, 1382.7, 1476.6, 1570.1, 2966.4, 3422.1 cm^{-1}; Anal. Calcd for $C_{22}H_{22}N_2O_3S$: C, 66.98; H, 5.62; N, 7.10. Found: C, 66.89; H, 5.58; N, 7.12.

5.24. 8-((Dimethylamino)methyl)-10-methoxy-2,4-dimethyl-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10e)

According to the procedure used to prepare **10a**, starting from compound **9b** and dimethylamine, **10e** was obtained as yellow solid. Yield: 61%; mp: 180 °C (dec); MS (ES+) *m/z* 381.1 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.34 (s, 3H), 2.38 (s, 3H), 2.83 (s, 6H), 4.07 (s, 3H), 4.23 (s, 2H), 4.74 (s, 2H), 7.22 (s, 1H), 7.91 (s, 1H), 8.24 (s, 1H), 8.85 (s, 1H); IR (KBr): 1274.9, 1383.9, 1495.5, 1631.5, 2944.8, 3393.1 cm^{-1}; Anal. Calcd for $C_{22}H_{24}N_2O_2S$: C, 69.44; H, 6.36; N, 7.36. Found: C, 69.43; H, 6.15; N, 7.08.

5.25. 10-Methoxy-2,4-dimethyl-8-(morpholinomethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10f)

According to the procedure used to prepare **10a**, starting from compound **9b** and morpholine, **10f** was obtained as yellow solid. Yield: 73%; mp: 246 °C (dec); MS (ES+) *m/z* 423.2 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.30 (s, 3H), 2.35 (s, 3H), 2.54 (m, 4H), 3.58(m, 4H), 3.96 (s, 3H), 4.00 (s, 2H), 4.15 (s, 2H), 7.10 (s, 1H), 7.38 (s, 1H), 8.14 (s, 1H), 8.24 (s, 1H); IR (KBr): 1252.0, 1383.7, 1466.3, 1597.8, 1673.2, 2851.3, 2918.5, 3427.8 cm^{-1}; Anal. Calcd for $C_{24}H_{26}N_2O_3S$: C, 68.22; H, 6.20; N, 6.63. Found: C, 68.43; H, 6.15; N, 6.49.

5.26. 10-Methoxy-2,4-dimethyl-8-(piperidin-1-ylmethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10g)

According to the procedure used to prepare **10a**, starting from compound **9b** and piperidine, **10g** was obtained as yellow solid. Yield: 82%; mp: 151 °C (dec); MS (ES+) *m/z* 421.1 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 1.64–1.79 (m, 6H), 2.34 (s, 3H), 2.38 (s, 3H), 3.10–3.51 (m, 4H), 4.06 (s, 3H), 4.24 (s, 2H), 4.69 (s, 2H), 7.21 (s, 1H), 7.88 (s, 1H), 8.24 (s, 1H), 8.85 (s, 1H); IR (KBr): 1280.0, 1383.3, 1476.4, 1498.6, 1629.2, 2722.0, 3013.6 cm^{-1}; Anal. Calcd for $C_{25}H_{28}N_2O_2S$: C, 71.40; H, 6.71; N, 6.66. Found: C, 71.52; H, 6.48; N, 6.70.

5.27. 2-Fluoro-10-methoxy-8-(pyrrolidin-1-ylmethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10h)

According to the procedure used to prepare **10a**, starting from compound **9c** and pyrrolidine, **10h** was obtained as yellow solid. Yield: 78%; mp: 197 °C (dec); MS (ES+) *m/z* 397.0 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 1.88–2.08 (m, 4H), 3.24–3.48 (m, 4H), 4.07 (s, 3H), 4.28 (s, 2H), 4.78 (s, 2H), 7.25–7.31 (m, 1H), 7.49–7.35 (m, 1H), 7.68 (s, 1H), 8.22–8.26 (m, 1H), 8.72 (s, 1H); IR (KBr): 1240.2, 1384.3, 1464.4, 1480.7, 1601.9, 2838.7, 2965.0, 3419.9 cm^{-1}; Anal. Calcd for $C_{22}H_{21}FN_2O_2S$: C, 66.65; H, 5.34; N, 7.07. Found: C, 66.49; H, 5.49; N, 7.10.

5.28. 10-Methoxy-2-methyl-8-((4-methylpiperazin-1-yl)methyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10i)

According to the procedure used to prepare **10a**, starting from compound **9d** and 1-methylpiperazine, **10i** was obtained as yellow solid. Yield: 57%; mp: 215 °C (dec); MS (ES+) *m/z* 422.2 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.39 (m, 4H), 2.41 (s, 3H) 2.59 (m, 4H), 3.17 (s, 3H), 3.96 (s, 3H), 4.07 (s, 2H), 4.21 (s, 2H), 7.17 (d, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.41 (s, 1H), 8.23 (s, 1H), 8.28 (s, 1H); IR (KBr): 1232.1, 1384.2, 1591.9, 2936.5, 3428.2 cm^{-1}; Anal. Calcd for $C_{24}H_{27}N_3O_2S$: C, 68.38; H, 6.46; N, 9.97. Found: C, 68.50; H, 6.49; N, 9.84.

5.29. 8-((Diethylamino)methyl)-10-methoxy-2-methyl-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10j)

According to the procedure used to prepare **10a**, starting from compound **9d** and diethylamine, **10j** was obtained as yellow solid. Yield: 67%; mp: 240 °C (dec); MS (ES+) *m/z* 395.1 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 1.34 (t, *J* = 7.2 Hz, 6H), 2.14 (s, 3H), 3.20 (m, 4H), 4.07 (s, 3H), 4.27 (s, 2H), 4.71 (s, 2H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.96 (s, 1H), 8.41 (s, 1H), 8.79 (s, 1H); IR (KBr): 1280.8, 1383.9, 1492.1, 1543.5, 1632.1, 2592.8, 2925.2, 3419.2 cm^{-1}; Anal. Calcd for $C_{23}H_{26}N_2O_2S$: C, 70.02; H, 6.64; N, 7.10. Found: C, 68.88; H, 6.59; N, 6.94.

5.30. 8-((Dimethylamino)methyl)-10-methoxy-2-methyl-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10k)

According to the procedure used to prepare **10a**, starting from compound **9d** and dimethylamine, **10k** was obtained as yellow solid. Yield: 81%; mp: 263 °C (dec); MS (ES+) *m/z* 367.1 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.43 (s, 3H), 2.88 (s, 6H), 4.03 (s, 3H), 4.21 (s, 2H), 4.87 (s, 2H), 7.23 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.49 (s, 1H), 8.15 (s, 1H), 8.39 (s, 1H); IR (KBr): 1270.9, 1384.1, 1486.7, 1630.4, 3016.8, 3377.8 cm^{-1}; Anal. Calcd for $C_{21}H_{22}N_2O_2S$: C, 68.82; H, 6.05; N, 7.64. Found: C, 68.53; H, 5.94; N, 7.45.

5.31. 10-Methoxy-2-methyl-8-(morpholinomethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10l)

According to the procedure used to prepare **10a**, starting from compound **9d** and morpholine, **10l** was obtained as yellow solid. Yield: 62%; mp: 226 °C (dec); MS (ES+) *m/z* 409.1 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.39 (s, 3H), 2.54 (m, 4H), 3.59 (m, 4H), 3.97 (s, 3H), 4.01 (s, 2H), 4.18 (s, 2H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.39 (s, 1H), 8.24 (s, 1H), 8.29 (s, 1H); IR (KBr): 1284.9, 1384.2, 1497.5, 1544.4, 1630.2, 2942.4, 3385.4 cm^{-1}; Anal. Calcd for $C_{23}H_{24}N_2O_3S$: C, 67.62; H, 5.92; N, 6.86:. Found: C, 67.78; H, 5.88; N, 6.78.

5.32. 10-Methoxy-2-methyl-8-(pyrrolidin-1-ylmethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10m)

According to the procedure used to prepare **10a**, starting from compound **9d** and pyrrolidine, **10m** was obtained as yellow solid. Yield: 79%; mp: 190 °C (dec); MS (ES+) *m/z* 393.2 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.00 (m, 4H), 2.40 (s, 3H), 3.24–3.48 (m, 4H), 4.06 (s, 3H), 4.28 (s, 2H), 4.77 (s, 2H), 7.15 (d, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.42 (s, 1H), 8.20 (s, 1H), 8.29 (s, 1H); IR (KBr): 1258.5, 1384.2, 1472.0, 1621.4, 3422.1 cm^{-1}; Anal. Calcd for $C_{23}H_{24}N_2O_2S$: C, 70.38; H, 6.16; N, 7.14. Found: C, 70.20; H, 6.00; N, 7.26.

5.33. 3-Fluoro-10-methoxy-8-((4-methylpiperazin-1-yl)methyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10n)

According to the procedure used to prepare **10a**, starting from compound **9e** and 1-methylpiperazine, **10n** was obtained as yellow solid. Yield: 54%; mp: 235 °C (dec); MS (ES+) *m/z* 426.1 $(M+H)^+$; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.17 (s, 3H), 2.38 (m, 4H), 2.57 (m, 4H), 3.95 (s, 3H), 4.09 (s, 2H), 4.25 (s, 2H), 7.08–7.22 (m, 2H), 7.34 (s, 1H), 8.23 (s, 1H), 8.41–8.51 (m, 1H); IR (KBr): 1266.5, 1382.7, 1476.6, 1621.4, 22966.4, 3422.1 cm^{-1}; Anal. Calcd for $C_{23}H_{24}FN_3O_2S$: C, 64.92; H, 5.68; N, 9.88. Found: C, 64.88; H, 5.49; N, 9.80.

5.34. 8-((Diethylamino)methyl)-3-fluoro-10-methoxy-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10o)

According to the procedure used to prepare **10a**, starting from compound **9e** and diethylamine, **10o** was obtained as yellow solid. Yield: 83%; mp: 251 °C (dec); MS (ES+) *m/z* 399.2 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.34 (t, J = 7.2 Hz, 6H), 3.19 (m, 4H), 4.05 (s, 3H), 4.30 (s, 2H), 4.68 (s, 2H), 7.20–7.26 (m, 1H), 7.33–7.37 (m, 1H), 7.58 (s, 1H), 8.51 (m, 2H); IR (KBr): 1290.9, 1386.6, 1477.0, 1600.6, 2595.2, 2943.4, 3349.3 cm^{-1}; Anal. Calcd for $C_{22}H_{23}FN_2O_2S$: C, 66.31; H, 5.82; F, 4.77; N, 7.03. Found: C, 66.02; H, 5.90; N, 6.84.

5.35. 3-Fluoro-10-methoxy-8-(morpholinomethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10p)

According to the procedure used to prepare **10a**, starting from compound **9e** and morpholine, **10p** was obtained as yellow solid. Yield: 76%; mp: 212 °C (dec); MS (ES+) *m/z* 413.0 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.54 (m, 4H), 3.59 (m, 4H), 3.97 (s, 3H), 4.02 (s, 2H), 4.26 (s, 2H), 7.14–7.23 (m, 1H), 7.30–7.34 (m, 1H), 7.36 (s, 1H), 8.27 (s, 1H), 8.46–8.51 (m, 1H); IR (KBr): 1239.7, 1384.9, 1466.7, 1505.8, 1602.4, 2861.5, 2891.1, 2938.6, 2966.5, 3427.4 cm^{-1}; Anal. Calcd for $C_{22}H_{21}FN_2O_3S$: C, 64.06; H, 5.13; N, 6.79. Found: C, 63.76; H, 5.34; N, 6.48.

5.36. 10-Methoxy-3-methyl-8-(morpholinomethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10q)

According to the procedure used to prepare **10a**, starting from compound **9f** and morpholine, **10q** was obtained as yellow solid. Yield: 69%; mp: 184 °C (dec); MS (ES+) *m/z* 409.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.33 (m, 4H), 2.54 (s, 3H), 3.59 (m, 4H), 3.96 (s, 3H), 4.02 (s, 2H), 4.20 (s, 2H), 7.18 (d, J = 7.8 Hz, 1H), 7.23 (s, 1H), 7.36 (s, 1H), 8.24 (s, 1H), 8.36 (d, J = 7.8 Hz, 1H); IR (KBr): 1249.3, 1384.2, 1464.7, 1598.7, 1688.9, 2850.6, 2918.3, 3424.2 cm^{-1}; Anal. Calcd for $C_{23}H_{24}N_2O_3S$: C, 67.62; H, 5.92; N, 6.86. Found: C, 67.48; H, 6.12; N, 6.69.

5.37. 10-Methoxy-3-methyl-8-(pyrrolidin-1-ylmethyl)-6*H*-[1]benzothiopyrano[4,3-*b*]quinolin-9-ol (10r)

According to the procedure used to prepare **10a**, starting from compound **9f** and pyrrolidine, **10r** was obtained as yellow solid. Yield: 70%; mp: 240 °C (dec); MS (ES+) *m/z* 393.1 $(M+H)^+$; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.78 (m, 4H), 2.33 (s, 3H), 2.65 (m, 4H), 3.95 (s, 3H), 4.19 (s, 2H), 4.20 (s, 2H), 7.18 (d, J = 7.8 Hz, 1H), 7.22 (s, 1H), 7.33 (s, 1H), 8.19 (s, 1H), 8.33 (d, J = 7.8 Hz, 1H); IR (KBr): 1256.3, 1385.3, 1461.5, 1484.6, 1603.5, 2883.2, 2959.3, 3446.5 cm^{-1}; Anal. Calcd for $C_{23}H_{24}N_2O_2S$: C, 70.38; H, 6.16; N, 7.14. Found: C, 70.29; H, 6.20; N, 6.89.

5.38. In vitro anti-HBV activity assay

The analysis of the anti-HBV activity was determined using previously described procedures.[13,14] Confluent cultures in 96-well flat-bottomed tissue culture plates were treated with eight consecutive daily doses of test compounds and lamivudine (purchased from Glaxo & Welcome Co.) in RPMI1640 medium with 2% fetal bovine serum. HBV nucleic acids and proteins were analyzed at the end of the treatment period (day 8). HBsAg and HBeAg were analyzed in culture medium by semi-quantitative EIA. Intracellular HBV DNA forms were extracted from culture media and analyzed by a slot blot hybridization technique.

5.39. Cytotoxicity assay

Cultures for cytotoxicity analyses were maintained on 96-well flat-bottomed plates and treated with compounds (in 0.2 mL culture medium/well) as described above. Twenty-four hours following the final addition of compounds, cytotoxicity in HepG2 2.2.15 cells was determined by uptake of Neutral red dye using methods reported elsewhere.[14] The IC_{50} and selected index of each compound were calculated, respectively.

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