Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 2552–2558

Synthesis and in vitro anti-hepatitis B virus activities of some ethyl 5-hydroxy-1*H*-indole-3-carboxylates

Chunshen Zhao, Yanfang Zhao, Huifang Chai and Ping Gong*

School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China

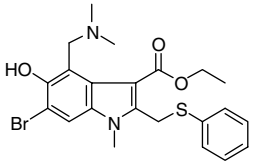
Received 16 October 2005; revised 15 November 2005; accepted 16 November 2005
Available online 1 December 2005

Abstract—A series of ethyl 5-hydroxy-1*H*-indole-3-carboxylates $\mathbf{6_A}$–$\mathbf{10_T}$ were synthesized and evaluated for their anti-hepatitis B virus (HBV) activities in 2.2.15 cells. The IC_{50} and selective index of inhibition on replication of HBV DNA of compounds $\mathbf{10_L}$ (1.52 μg/ml, 9.38) and $\mathbf{10_P}$ (2.00 μg/ml, 8.85) were higher than those of the other evaluated compounds including lamivudine (7.02). Compounds $\mathbf{7_E}$ and $\mathbf{10_J}$ exhibited significant anti-HBV activities, and the IC_{50} values on replication of HBV DNA of these compounds were 24.90 and 15.41 μg/ml, respectively, which were far more potent than the positive control lamivudine 228.00 μg/ml.

1. Introduction

Hepatitis B virus (HBV)-infected hepatitis is the common infectious disease in the world. Conservative estimates place the number of persons chronically infected with HBV at more than 300 million.[1,2] HBV infection can cause severe liver diseases such as cirrhosis and liver cancer. Currently approved agents of chronic HBV treatment include interferon-α and the nucleoside analogues lamivudine and adefovir dipivoxil.[3] However, the side effects of interferon and the viral resistance of nucleoside analogues make the current treatment regimens far from satisfactory.[4,5] New anti-HBV drugs with novel mechanisms of action are desperately needed.

Arbidol (Fig. 1) and its derivatives displayed a variety of biological effects, such as antiviral effects, immunostimulative and interferon-induced activities.[6–8] With arbidol as the lead compound, Chai et al. synthesized some new ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates and disclosed their favorable anti-HBV activities first.[9] They were shown to be potent inhibitors of HBV replication in vitro. The results encouraged us greatly to perform further research on these to find more potent anti-HBV remedies.



Arbidol

Figure 1. Structure of arbidol.

We are interested in exploring the modification in replacing bromo with hydrogen on the 6-position of the indole cycle to assess whether hydrophobic and electronic changes could affect efficacy. In this paper, we synthesized a new series of ethyl 5-hydroxy-1*H*-indole-3-carboxylate derivatives and evaluated their anti-HBV activities. The other modifications were mainly focused on position 1, 4 on the indole cycle, and different arylsulfinylmethyl or arylsulfonylmethyl substitutions on position 2 were incorporated to investigate their influences on antiviral activities.

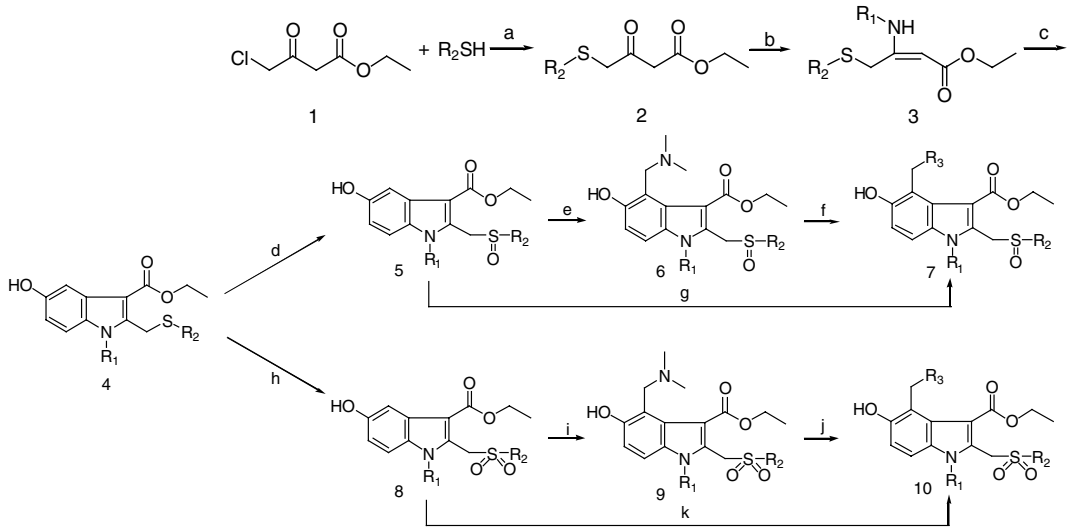
2. Results and discussion

2.1. Synthetic approach

The title compounds ethyl 5-hydroxy-1*H*-indole-3-carboxylate derivatives were obtained as described in Scheme 1. The structures of compounds $\mathbf{6_A}$–$\mathbf{10_T}$ are listed in Table 1.

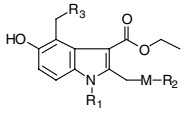
Keywords: Anti-hepatitis B virus activity; Ethyl 5-hydroxy-1*H*-indole-3-carboxylates; Synthesis.
* Corresponding author. Tel./fax: +86 24 23986426; e-mail: saieho@126.com

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.11.033



Scheme 1. Synthesis of target compounds. Reagents and conditions: (a) $R_2SH/KOH/CH_3OH$, rt; (b) R_1NH_2, 45 °C; (c) 1,4-benzoquinone/$ClCH_2CH_2Cl$, reflux; (d) sodium perborate tetrahydrate/CH_3COOH, 45–50 °C; (e) 37% HCHO/dimethyl amine/CH_3COOH, 55 °C reflux; (f) appropriate amines/CH_3CH_2OH, 78 °C reflux; (g) 37% HCHO/appropriate amines/CH_3COOH, 55 °C reflux; (h) sodium wolframate dihydrate/hydrogen peroxide/sodium bisulfite/$CH_3OH/CHCl_3$, 20 °C; (i) 37% HCHO/dimethyl amine/CH_3COOH, 55 °C reflux; (j) appropriate amines/CH_3CH_2OH, 78 °C reflux; (k) 37% HCHO/appropriate amines/CH_3COOH, 55 °C reflux.

Table 1. Structures of compounds $\mathbf{6_A}$–$\mathbf{10_T}$



Compound	R_1	R_2	R_3	M
$\mathbf{6_A}$	Cyclopropyl	Phenyl	Dimethylamino	Sulfinyl
$\mathbf{7_B}$	Methyl	*p*-Methylphenyl	Pyrrolidinyl	Sulfinyl
$\mathbf{7_C}$	Methyl	*m*-Methoxyphenyl	Pyrrolidinyl	Sulfinyl
$\mathbf{7_D}$	Cyclopropyl	*m*,*p*-Dimethoxyphenyl	Pyrrolidinyl	Sulfinyl
$\mathbf{7_E}$	Cyclopropyl	Phenyl	2-Methylimidazolyl	Sulfinyl
$\mathbf{7_F}$	Cyclopropyl	2-Pyridinyl	2-Methylimidazolyl	Sulfinyl
$\mathbf{7_G}$	Methyl	*m*-Methoxyphenyl	Triazolyl	Sulfinyl
$\mathbf{9_H}$	Methyl	*m*,*p*-Dimethoxyphenyl	Dimethylamino	Sulfonyl
$\mathbf{10_I}$	Methyl	*p*-Methylphenyl	Pyrrolidinyl	Sulfonyl
$\mathbf{10_J}$	Methyl	*m*-Methoxyphenyl	Pyrrolidinyl	Sulfonyl
$\mathbf{10_K}$	Methyl	*m*,*p*-Dimethoxyphenyl	Pyrrolidinyl	Sulfonyl
$\mathbf{10_L}$	Cyclopropyl	2-Furanylmethyl	Pyrrolidinyl	Sulfonyl
$\mathbf{10_M}$	Methyl	*m*,*p*-Dimethoxyphenyl	Morpholino	Sulfonyl
$\mathbf{10_N}$	Cyclopropyl	2-Furanylmethyl	Morpholino	Sulfonyl
$\mathbf{10_O}$	Cyclopropyl	2-Furanylmethyl	Piperidinyl	Sulfonyl
$\mathbf{10_P}$	Cyclopropyl	*p*-Methylphenyl	Imidazolyl	Sulfonyl
$\mathbf{10_Q}$	Cyclopropyl	*p*-Methoxyphenyl	Imidazolyl	Sulfonyl
$\mathbf{10_R}$	Cyclopropyl	*p*-Methoxyphenyl	2-Methylimidazolyl	Sulfonyl
$\mathbf{10_S}$	Cyclopropyl	*m*,*p*-Difluorophenyl	2-Methylimidazolyl	Sulfonyl
$\mathbf{10_T}$	Cyclopropyl	*p*-Fluorophenyl	Triazolyl	Sulfonyl

We introduced methyl or cyclopropyl at position 1 to investigate the influence of the steric changes of alkyl group at position 1 on the antiviral potency. The sulfur of arbidol was transformed into sulfoxide or sulfone to study the influence on antiviral activity and cytotoxicity. According to the structure–activity relationship study (SAR) of our previous work, the Mannich basic functionalities at position 4 showed important influence on antiviral activity. Mannich bases possessing different electronic effects and aqueous solubilities such as dimethylamino, piperidinyl, pyrrolidinyl, morpholino, 1-imidazolyl, 2-methyl-1-imidazolyl, and triazolyl were introduced to position 4. To get more SAR information, other structural changes were made, mainly the change of electron-withdrawing groups and electron-donating group substitutions on the aryl ring on position 2.

The synthesis of target ethyl 5-hydroxy-1*H*-indole-3-carboxylates started from commercially available **1** and various arylthiols. Alkylamination of **2**, then Nenitsescu condensation of **3** with 1,4-benzoquinone, gave the key intermediate 1-alkyl-5-hydroxy-2-(arylsulfanylmethyl)-1*H*-indole-3-carboxylate **4**.[10] Being reacted with sodium perborate or sodium wolframate and hydrogen peroxide, **4** was oxidized into **5** and **8**, respectively. Mannich reaction of **5** and **8** with different secondary alkylamines led to $\mathbf{6_A}$, $\mathbf{7_{B–D}}$, $\mathbf{9_H}$, and $\mathbf{10_{I–O}}$. For the preferred 4-arylaminomethylene series, amino exchanging reaction proceeded on the 4-dimethylaminomethylene ones of **6** and **9**, allowing appropriate heteroaromatic amines to afford $\mathbf{7_{E–G}}$ and $\mathbf{10_{P–T}}$.

In our previous work, Mannich reaction of **4** was first approached, then the oxidation with hydrogen peroxide was carried out to oxidize sulfide into sulfoxide and sulfone. Unfortunately, it was difficult to control the process of oxidation here with the above method, because the nitrogen at position 4 could be also oxidized and yield side products. By using two types of oxidants, sodium perborate or sodium wolframate, the oxidation of **4** was completed at different phases perfectly as expected, followed by two steps of Mannich reaction and amino exchanging reaction. With this modified synthetic route, the good yield and pure compounds **5** and **8** were obtained and used in the next step without isolation and purification. Furthermore, the purity of target compounds was raised and the total yield was improved correspondingly.

2.2. Biological activity test

All target compounds $\mathbf{6_A}$–$\mathbf{10_T}$ were evaluated for their cytotoxicities and anti-HBV activities, namely the ability to inhibit the replication of HBV DNA and the production of HBsAg and HBeAg in HBV-infected 2.2.15 cells. The results are summarized in Table 2.

As shown in Table 2, $\mathbf{10_L}$ showed the most potent in vitro anti-HBV activity in all the tested compounds. Its IC_{50} is 1.52 μg/ml, which was 150 times that of the control lamivudine 228.00 μg/ml. Compounds $\mathbf{10_J}$ and $\mathbf{10_P}$ also exhibited significant efficacy on HBV (IC_{50}: 15.41, 2.00 μg/ml) on replication of HBV DNA (structures were listed in Fig. 2). The selective indexes of $\mathbf{10_L}$, $\mathbf{10_P}$, and $\mathbf{10_J}$ (9.38, 8.85, and 6.93) were higher than or comparable to that of lamivudine (7.02). Although SI of $\mathbf{7_E}$ (2.57) were lower than the control, its anti-HBV potency (24.90 μg/ml) was still 9 times that of lamivudine. However, compounds $\mathbf{10_O}$, $\mathbf{10_M}$, and $\mathbf{10_N}$ only displayed suppressant properties on the production of HBsAg or even no anti-HBV activity at a concentration lower than TC_{50}. This suggested that the Mannich basic functionalities introduced to position 4 had substantial influence on antiviral activities. Particularly, the heterocyclic groups such as pyrrolidinyl and imidazolyl were more in favor of enhancing activities than the other groups.

$\mathbf{10_J}$ and $\mathbf{10_I}$ exhibited good or slight anti-HBV effects, but $\mathbf{7_B}$ and $\mathbf{7_C}$ were inactive at noncytotoxic concentrations. This revealed that introduction of sulfonyl instead

Table 2. Anti-HBV activities of compounds $\mathbf{6_A}$–$\mathbf{10_T}$

Compound	TC_{50} (μg/ml)[a]	HBsAg[d]		HBeAg[d]		DNA replication[d]	
		IC_{50} (μg/ml)[b]	SI[c]	IC_{50} (μg/ml)[b]	SI[c]	IC_{50} (μg/ml)[b]	SI[c]
$\mathbf{6_A}$	53.40	—	—	—	—	—	—
$\mathbf{7_B}$	42.77	—	—	—	—	—	—
$\mathbf{7_C}$	106.83	—	—	—	—	—	—
$\mathbf{7_D}$	74.07	—	—	—	—	—	—
$\mathbf{7_E}$	64.10	—	—	—	—	24.90	2.57
$\mathbf{7_F}$	1154.70	—	—	533.43	2.16	—	—
$\mathbf{7_G}$	666.67	—	—	—	—	—	—
$\mathbf{9_H}$	128.30	—	—	—	—	—	—
$\mathbf{10_I}$	35.61	—	—	15.93	2.24	—	—
$\mathbf{10_J}$	106.83	—	—	15.41	6.93	15.41	6.93
$\mathbf{10_K}$	106.83	—	—	—	—	—	—
$\mathbf{10_L}$	14.26	—	—	—	—	1.52	9.38
$\mathbf{10_M}$	74.07	—	—	—	—	—	—
$\mathbf{10_N}$	154.08	—	—	—	—	—	—
$\mathbf{10_O}$	4.75	1.96	2.42	—	—	—	—
$\mathbf{10_P}$	17.70	—	—	—	—	2.00	8.85
$\mathbf{10_Q}$	74.07	—	—	—	—	—	—
$\mathbf{10_R}$	74.74	—	—	—	—	—	—
$\mathbf{10_S}$	154.08	—	—	—	—	—	—
$\mathbf{10_T}$	192.30	—	—	—	—	—	—
Arbidol	74.49	47.45	1.57	—	—	22.85	3.26
Lamivudine	1600.00	—	—	—	—	228.00	7.02

[a] TC_{50} is 50% cytotoxic concentration in 2.2.15 cells.
[b] IC_{50} is 50% inhibitory concentration.
[c] Selective Index (SI: TC_{50}/IC_{50}).
[d] — means no antiviral activity at the concentration lower than its TC_{50}.

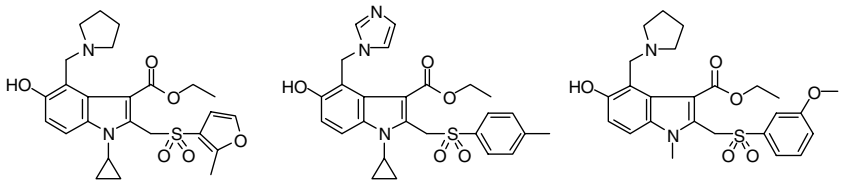


Figure 2. Chemical structures of typical compounds that showed potent anti-HBV activity.

of sulfinyl increased antiviral activities and showed less influence on the cytotoxicities in addition.

Compared with our previous results, the removal of the 6-bromo group from the indole cycle shows little distinctness at anti-HBV efficacy and cytotoxicities. This implies that 6-bromo group might not be the antiviral pharmacophore. Different aryl substitutions on position 2 did not exhibit significant influence on antiviral activities. It seemed that one electron-donating group substitution or non-substitution was good to keep up antiviral activity. This trend seemed to be not consistent with our previous observation on ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates.

3. Conclusion

In summary, we synthesized a series of new ethyl 5-hydroxy-1*H*-indole-3-carboxylate derivatives and examined their anti-HBV activities and cytotoxicities in 2.2.15 cells. According to the above results, the following conclusions could be made:

1. Mannich basic functionalities introduced to position 4 had substantial influence on antiviral activities. Particularly, the heterocyclic groups such as pyrrolidinyl and imidazolyl were more in favor of enhancing activities than the other groups.
2. The introduction of sulfonyl instead of sulfinyl increased anti-HBV activities and showed less influence on the cytotoxicities in addition.
3. Different aryl substitutions on position 2 seemed to have less influence on antiviral activities. It seemed that one electron-donating group substitution or non-substitution was good to keep up antiviral activity.
4. 6-Bromo group might not be the pharmacophore of anti-HBV effect.

4. Experimental

4.1. Chemistry

All melting points were obtained with a Veego melting point apparatus and are uncorrected. Proton (^{1}H) nuclear magnetic resonance spectroscopy was performed using Bruker ARX-300, 300 MHz spectrometers with Me_4Si as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS.

4.1.1. Synthesis of arylsulfanylacetoxyacetate ester (2). The appropriate arylthiol **1** (0.11 mol) was dropped into a solution (100 ml) of kalium hydroxide (0.12 mol) in methanol at rt and stirred for 0.5 h, then chloroacetoxyacetate ester (0.1 mol) was added and the mixture was stirred at rt for 6 h. The mixture was filtered and the solvent was evaporated in vacuo. After being extracted by methylene chloride, washed by water, and dried, the solution was evaporated in vacuo to give **2** (70–75%).

4.1.2. Synthesis of 3-alkylamino-4-arylsulfanyl-2-crotonate (3). The appropriate alkyl-substituted amine (0.14 mol) was added into **2** (0.07 mol) at rt, then the mixture was heated to 45 °C for 18 h with continuous stirring. After being extracted by methylene chloride, washed by water, and dried, the solution was evaporated in vacuo to give **3** (73–80%).

4.1.3. Synthesis of 1-alkyl-2-(arylsulfanylmethyl)-5-hydroxy-1*H*-indole-3-carboxylate (4). Compound **3** (0.1 mol) was added dropwise to a solution of 1,4-benzoquinone in 1,2-dichloroethane (100 ml) by keeping the mixture boiling and heated for 8 h. The solution was cooled and the precipitate was collected by filtration and dried to give **4** (66–68%).

4.1.4. Synthesis of 1-alkyl-2-(arylsulfinylmethyl)-5-hydroxy-1*H*-indole-3-carboxylate (5). Compound **4** (0.01 mol) was first dissolved in glacial acetic acid (40 ml), sodium perborate tetrahydrate (0.01 mol) successively added into the solution. After stirring at 45–50 °C for 3–4 h, most of the acetic acid was evaporated in vacuo. Then the solution was poured into water and the precipitate was filtered, washed by acetone, and gave **5** (81–89%).

4.1.5. General procedure for the synthesis of compounds 6_A–7_D. To a 300 ml of acetic acid were successively added appropriate alkylamine (0.25 mol), 37.7% solution of formalin (0.11 mol), and **5** (0.1 mol). The reaction mixture was stirred at 50–55 °C for 8 h. The solvent was evaporated in vacuo, and 200 ml of water was added in one portion. The resultant mixture was adjusted to pH 10 with sodium hydroxide and extracted with methylene chloride. The organic phase was dried over sodium sulfate and evaporated in vacuo to yield a yellow oil. The oil was dissolved in acetone (50 ml) and cooled at 0 °C overnight. The solid was filtered and dried to obtain 6_A–7_D (72–77%).

4.1.5.1. Ethyl 4-(dimethylaminomethyl)-5-hydroxy-1-cyclopropyl-2-(phenylsulfinylmethyl)-1*H*-indole-3-carboxylate (6_A). Mp: 176–178 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 1H, –N–CH(CH_2)$_2$), 1.04 (m, 1H, –N–CH(CH_2)$_2$), 1.15 (m, 2H, –N–CH(CH_2)$_2$), 1.27 (t, 3H, J = 7.2 Hz,

–OCH_2CH_3), 2.51 (m, 1H, –N–C*H*$(CH_2)_2$), 2.88 (m, 6H, –$N(CH_3)_2$), 4.24 (q, 2H, *J* = 7.2Hz, –OCH_2CH_3), 4.82 (s, 2H, –CH_2N<), 5.71 (s, 2H, –CH_2–sulfinyl–), 7.38–7.55 (m, 5H, –Ph*H*), 7.56 (d, 1H, –Φ*H*), 7.58 (d, 1H, –Φ*H*),10.00 (br s, 1H, –O*H*); MS: *m*/*z* 441.0 [MH^+].

4.1.5.2. Ethyl 4-(pyrrolidinylmethyl)-5-hydroxy-1-methyl-2-[(4-methylphenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (7_B). Mp: 180–182 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 1.87 (m, 4H, –pyrrolidinyl *H*), 2.46 (s, 3H, *p*-CH_3 of phenyl), 2.73 (m, 4H, –pyrrolidinyl *H*), 3.77 (s, 3H, –NCH_3), 4.02 (q, 2H, *J* = 7.2 Hz, –OCH_2CH_3), 4.26 (s, 2H, –CH_2N<), 4.67 (s, 2H, –CH_2–sulfinyl–), 6.94–7.26 (m, 4H, –Ph*H*), 7.57 (d, 2H, –Φ*H*); MS: *m*/*z* 455.0 [MH^+].

4.1.5.3. Ethyl 4-(pyrrolidinylmethyl)-5-hydroxy-1-methyl-2-[(3-methoxyphenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (7_C). Mp: 191–193 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 1.87 (m, 4H, –pyrrolidinyl *H*), 2.73 (m, 4H, –pyrrolidinyl *H*), 3.66 (s, 3H, –NCH_3), 3.69 (s, 3H, *m*-OCH_3 of phenyl), 4.12 (q, 2H, *J* = 7.2 Hz, –OCH_2CH_3), 4.36 (s, 2H, –CH_2N<), 4.80 (s, 2H, –CH_2–sulfinyl–), 7.08–7.37 (m, 4H, –Ph*H*), 7.55 (d, 2H, –Φ*H*); MS: *m*/*z* 471.1 [MH^+].

4.1.5.4. Ethyl 4-(pyrrolidinylmethyl)-5-hydroxy-1-cyclopropyl-2-[(3,4-dimethoxyphenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (7_D). Mp: 183–185 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 2H, –N–CH(CH_2)$_2$), 1.12 (m, 2H, –N–CH(CH_2)$_2$), 1.50 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 2.04 (m, 4H, –pyrrolidinyl *H*), 3.24 (m, 1H, –N–C*H*$(CH_2)_2$), 3.59 (m, 4H, –pyrrolidinyl *H*), 3.59 (s, 3H, *m*-OCH_3 of phenyl), 3.89 (s, 3H, *p*-OCH_3 of phenyl), 4.42 (q, 2H, *J* = 7.2 Hz, –OCH_2CH_3), 4.75 (d, 2H, –NCH_2–), 5.15 (s, 2H, –CH_2–sulfinyl–), 6.70 (s, 1H, 2-H of phenyl), 6.83 (d, 2H, –Ph*H*), 7.43 (d, 2H, –Φ*H*); MS: *m*/*z* 527.0 [MH^+].

4.1.6. General procedure for the synthesis of compounds $7_{E–G}$. To a solution of imidazole or 2-methyl-1-imidazole (0.1 mol) in anhydrous ethanol (50 ml) was added **6** (0.02 mol) at 78 °C with constant stirring. The solid was dissolved about 1 h later. The reaction mixture was further stirred with refluxing until a mass of solid appeared in the solution. The precipitate was filtered, dried, and gave the desired compounds $7_{E–G}$ (75–78%).

4.1.6.1. Ethyl 4-(2-methyl-1-imidazolylmethyl)-5-hydroxy-1-cyclopropyl-2-(phenylsulfinylmethyl)-1*H*-indole-3-carboxylate (7_E). Mp: 188–190 °C; ^{1}H NMR ($CDCl_3$) δ 0.99 (m, 2H, –CH_2–N–CH(CH_2)$_2$), 1.26 (m, 2H, –CH_2–N–CH(CH_2)$_2$), 1.35 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 2.48 (s, 3H, 2-CH_3-1-imidazolyl), 3.09 (m, 2H, –CH_2–N–C*H*$(CH_2)_2$), 4.27 (q, 2H, *J* = 7.2 Hz, –OCH_2CH_3), 5.11 (s, 2H, –CH_2N<), 5.69 (s, 2H, –CH_2–sulfinyl–), 6.78 (d, 2H, –imidazolyl *H*), 7.14–7.93 (m, 5H, –Ph*H*), 7.86–7.90 (d, 2H, –Φ*H*); MS: *m*/*z* 478.0 [MH^+].

4.1.6.2. Ethyl 4-(2-methyl-1-imidazolylmethyl)-5-hydroxy-1-cyclopropyl-2-[(2-pyridinyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (7_F). Mp: 177–179 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (m, 2H, –CH_2–N–CH(CH_2)$_2$), 1.25(m, 2H, –CH_2–N–CH(CH_2)$_2$), 1.34 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 2.46 (s, 3H, 2-CH_3-1-imidazolyl), 3.06 (m, 2H, –CH_2–N–C*H*$(CH_2)_2$), 4.28 (q, 2H, *J* = 7.2 Hz, –OCH_2CH_3), 5.10 (s, 2H, –CH_2N<), 5.67 (s, 2H, –CH_2–sulfinyl–), 6.77 (d, 2H, imidazolyl *H*), 7.15–7.46 (m, 4H, –pyridinyl *H*), 7.88–7.91 (d, 2H, –Φ*H*); MS: *m*/*z* 479.0 [MH^+].

4.1.6.3. Ethyl 4-(1-triazolylmethyl)-5-hydroxy-1-methyl-2-[(3-methoxyphenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (7_G). Mp: 174–176 °C; ^{1}H NMR (DMSO-d_6) δ 1.43 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 3.50 (s, 3H, –NCH_3), 3.66 (s, 3H, *m*-OCH_3-phenyl), 4.15 (q, 2H, *J* = 7.2Hz, –OCH_2CH_3), 4.83 (s, 2H, –CH_2N<), 5.71 (s, 2H, –CH_2–sulfinyl–), 7.03–7.14 (m, 2H, triazolyl *H*), 7.52–7.64 (m, 4H, –Ph*H*), 7.97 (d, 2H, –Φ*H*); MS: *m*/*z* 469.1 [MH^+].

4.1.7. Synthesis of 1-alkyl-2-(arylsulfonylmethyl)-5-hydroxy-1*H*-indole-3-carboxylate (8). Compound **4** (0.01 mol) was first dissolved in methanol and chloroform (40 ml, 1:1), sodium wolframate dihydrate (catalyst) and hydrogen peroxide (0.05 mol) were successively dropped into the solution and stirred at 20 °C for 1.5 h. A solution of sodium bisulfite (0.04 mol) in water was added into the mixture, then methanol and chloroform were evaporated in vacuo, and 50 ml of water was added in one portion. The precipitate was filtered, washed by acetone, dried, and gave **8** (83–86%).

4.1.8. General procedure for the synthesis of compounds 9_H–10_O. To a 300 ml of acetic acid were successively added dimethylamine or *N*-methyl-piperazine (0.25 mol), 37.7% solution of formalin (0.11 mol), and **8** (0.1 mol). The reaction mixture was stirred at 50–55 °C for 8 h. The solvent was evaporated in vacuo, and 200 ml of water was added in one portion. The resultant mixture was adjusted to pH 10 with sodium hydroxide and extracted with methylene chloride. The organic phase was dried over sodium sulfate and evaporated in vacuo to yield a yellow oil. The oil was dissolved in acetone (50 ml) and cooled at 0 °C overnight. The solid was filtered and dried to obtain 9_H–10_O (76–79%).

4.1.8.1. Ethyl 4-(dimethylaminomethyl)-5-hydroxy-1-methyl-2-[(3,4-dimethoxyphenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (9_H). Mp: 194–196 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 2.86 (m, 6H, –$N(CH_3)_2$), 3.57 (s, 3H, –NCH_3), 3.74 (s, 3H, *m*-OCH_3-phenyl), 3.92 (s, 3H, *p*-OCH_3-phenyl), 4.02 (q, 2H, *J* = 7.2 Hz, –OCH_2CH_3), 4.08 (s, 2H, –CH_2N<), 5.11 (s, 2H, –CH_2–sulfonyl–), 6.75–6.91 (m, 3H, –Ph*H*), 7.19–7.34 (d, 2H, –Φ*H*); MS: *m*/*z* 491.0 [MH^+].

4.1.8.2. Ethyl 4-(pyrrolidinylmethyl)-5-hydroxy-1-methyl-2-[(4-methylphenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_I). Mp: 182–184 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, *J* = 7.2 Hz, –OCH_2CH_3), 1.88 (m, 4H, –pyrrolidinyl *H*), 2.44 (s, 3H, *p*-CH_3 of phenyl), 2.71 (m, 4H, –pyrrolidinyl *H*), 3.78 (s, 3H, –NCH_3), 4.00 (q, 2H,

J = 7.2 Hz, –OCH_2CH$_3$), 4.27 (s, 2H, –CH_2N<), 5.09 (s, 2H, –CH_2–sulfonyl–), 6.96–7.25 (m, 4H, –PhH), 7.54 (d, 2H, –ΦH); MS: m/z 471.1 [MH$^+$].

4.1.8.3. Ethyl 4-(pyrrolidinylmethyl)-5-hydroxy-1-methyl-2-[(3-methoxyphenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_J). Mp: 179–181 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 1.90 (m, 4H, –pyrrolidinyl H), 3.35 (m, 4H, –pyrrolidinyl H), 3.68 (s, 3H, –NCH_3), 3.70 (s, 3H, m-OCH_3 of phenyl), 4.15 (q, 2H, J = 7.2 Hz, –OCH_2CH$_3$), 4.80 (s, 2H, –CH_2N<), 5.39 (s, 2H, –CH_2–sulfonyl–), 7.09–7.36 (m, 4H, –PhH), 7.56 (d, 2H, –ΦH); MS: m/z 487.0 [MH$^+$].

4.1.8.4. Ethyl 4-(pyrrolidinylmethyl)-5-hydroxy-1-methyl-2-[(3,4-dimethoxyphenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_K). Mp: 184–186 °C; ^{1}H NMR (CDCl$_3$) δ 1.32 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 1.90 (m, 4H, –pyrrolidinyl H), 2.73 (m, 4H, –pyrrolidinyl H), 3.75 (s, 3H, –NCH_3), 3.79 (s, 3H, m-OCH_3-phenyl), 3.91 (s, 3H, p-OCH_3-phenyl), 4.04 (q, 2H, J = 7.2 Hz, –OCH_2CH$_3$), 4.28 (s, 2H, –CH_2N<), 5.12 (s, 2H, –CH_2–sulfonyl–), 6.94–7.28 (m, 3H, –PhH), 7.56 (d, 2H, –ΦH); MS: m/z 517.1 [MH$^+$].

4.1.8.5. Ethyl 4-(pyrrolidinylmethyl)-5-hydroxy-1-cyclopropyl-2-[(2-furanylmethyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_L). Mp: 195–197 °C; ^{1}H NMR (CDCl$_3$) δ 0.96 (m, 2H, –N–CH(CH_2)$_2$), 1.22 (m, 2H, –N–CH(CH_2)$_2$), 1.37 (t, 3H, J = 7.2Hz, –OCH$_2$CH_3), 1.93 (m, 4H, –pyrrolidinyl H), 2.70 (m, 4H, –pyrrolidinyl H), 2.78 (m, 1H, –N–CH(CH$_2$)$_2$), 4.09 (d, 2H, –CH_2–furanyl–), 4.40 (q, 2H, J = 6.9 Hz, –OCH_2CH$_3$), 4.52 (d, 2H, J = 3 Hz, –CH_2N<), 5.15 (s, 2H, –CH_2–sulfonyl–), 6.41–6.88 (m, 3H, –furanyl H), 7.41–7.45 (d, 2H, –ΦH); MS: m/z 487.0 [MH$^+$].

4.1.8.6. Ethyl 4-(morpholinomethyl)-5-hydroxy-1-methdimethoxyphenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_M). Mp: 192–194 °C; ^{1}H NMR (CDCl$_3$) δ 1.31 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 2.58 (m, 4H, –morpholino H), 3.55 (s, 3H, –NCH_3), 3.73 (m, 4H, –morpholino H), 3.75 (s, 3H, m-OCH_3-phenyl), 3.93 (s, 3H, p-OCH_3-phenyl), 4.03 (q, 2H, J = 7.2 Hz, –OCH_2CH$_3$), 4.09 (s, 2H, –CH_2N<), 5.07 (s, 2H, –CH_2–sulfonyl–), 6.78–6.93 (m, 3H, –PhH), 7.18–7.36 (d, 2H, –ΦH); MS: m/z 533.0 [MH$^+$].

4.1.8.7. Ethyl 4-(morpholinomethyl)-5-hydroxy-1-cyclopropyl-2-[(2-furanylmethyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_N). Mp: 196–198 °C; ^{1}H NMR (CDCl$_3$) δ 0.95 (m, 2H, –N–CH(CH_2)$_2$), 1.22 (m, 2H, –N–CH(CH_2)$_2$), 1.38 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 2.59 (m, 4H, –morpholino H), 2.69 (m, 1H, –N–CH(CH$_2$)$_2$), 3.71 (m, 4H, –morpholino H), 4.04 (d, 2H, –CH_2–furanyl–), 4.35 (q, 2H, J = 6.9 Hz, –OCH_2CH$_3$), 4.55 (d, 2H, J = 3Hz, –CH_2N<), 5.12 (s, 2H, –CH_2–sulfonyl–), 6.45–6.93 (m, 3H, –furanyl H), 7.40–7.44 (d, 2H, –ΦH); MS: m/z 503.0 [MH$^+$].

4.1.8.8. Ethyl 4-(piperidinylmethyl)-5-hydroxy-1-cyclopropyl-2-[(2-furanylmethyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_O). Mp: 200–202 °C; ^{1}H NMR (CDCl$_3$) δ 0.98 (m, 2H, –N–CH(CH_2)$_2$), 1.21 (m, 2H, –N–CH(CH_2)$_2$), 1.41 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 1.66 (m, 6H, –piperidinyl H), 2.68 (m, 1H, –N–CH(CH$_2$)$_2$), 3.43 (m, 4H, –piperidinyl H), 4.05 (d, 2H, –CH_2–furanyl–), 4.38 (q, 2H, J = 6.9 Hz, –OCH_2CH$_3$), 4.51 (d, 2H, J = 3 Hz, –CH_2N<), 5.10 (s, 2H, –CH_2–sulfonyl–), 6.40–6.90 (m, 3H, –furanyl H), 7.43–7.46 (d, 2H, –ΦH); MS: m/z 501.1 [MH$^+$].

4.1.9. General procedure for the synthesis of compounds $10_{P–T}$. To a solution of imidazole (0.1 mol) in anhydrous ethanol (50 ml) was added **9** (0.02 mol) at 78 °C with constant stirring. The solid was dissolved about 1 h later. The reaction mixture was further stirred with refluxing until a mass of solid appeared in the solution. The precipitate was filtered, dried, and gave the desired compounds $10_{P–T}$ (73.2%).

4.1.9.1. Ethyl 4-(1-imidazolylmethyl)-5-hydroxy-1-cyclopropyl-2-[(4-methylphenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_P). Mp: 197–199 °C; ^{1}H NMR (DMSO-d_6) δ 1.01 (m, 2H, –N–CH(CH_2)$_2$), 1.14 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 1.28 (m, 2H, –N–CH(CH_2)$_2$), 2.39 (s, 3H, p-CH_3-phenyl), 3.01 (m, 1H, –N–CH(CH$_2$)$_2$), 3.96 (q, 2H, J = 7.2 Hz, –OCH_2CH$_3$), 5.27 (s, 2H, –CH_2–sulfonyl), 5.69 (s, 2H, –CH_2N<), 7.10–7.41 (m, 3H, –imidazolyl H), 7.44–7.61 (m, 4H, –PhH), 7.67 (d, 2H, –ΦH); MS: m/z 492.0 [MH$^+$].

4.1.9.2. Ethyl 4-(1-imidazolylmethyl)-5-hydroxy-1-cyclopropyl-2-[(4-methoxyphenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_Q). Mp: 177–179 °C; ^{1}H NMR (DMSO-d_6) δ 1.02 (m, 2H, –N–CH(CH_2)$_2$), 1.17 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 1.29 (m, 2H, –N–CH(CH_2)$_2$), 3.05 (m, 1H, –N–CH(CH$_2$)$_2$), 3.93 (s, 3H, p-OCH_3-phenyl), 3.99 (q, 2H, J = 7.2 Hz, –OCH_2CH$_3$), 5.28 (s, 2H, –CH_2–sulfonyl), 5.66 (s, 2H, –CH_2N<), 7.15–7.43 (m, 3H, –imidazolyl H), 7.48–7.62 (m, 4H, –PhH), 7.69 (d, 2H, –ΦH); MS: m/z 510.0 [MH$^+$].

4.1.9.3. Ethyl 4-(2-methyl-1-imidazolylmethyl)-5-hydroxy-1-cyclopropyl-2-[(4-methoxyphenyl) sulfonylmethyl]-1*H*-indole-3-carboxylate (10_R). Mp: 178–180 °C; ^{1}H NMR (DMSO-d_6) δ 1.09 (m, 2H, –N–CH(CH_2)$_2$), 1.12 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 1.28 (m, 2H, –N–CH(CH_2)$_2$), 2.33 (s, 3H, 2-CH_3-1-imidazolyl), 2.96 (m, 1H, –N–CH(CH$_2$)$_2$), 3.91 (s, 3H, p-OCH_3-phenyl), 4.01 (q, 2H, J = 7.2 Hz, –OCH_2CH$_3$), 5.29 (s, 2H, –CH_2N<), 5.36 (s, 2H, –CH_2–sulfonyl–), 6.24–6.58 (m, 2H, –imidazolyl H), 6.93–7.66 (m, 4H, –PhH), 7.72–7.79 (d, 2H, –ΦH); MS: m/z 524.0 [MH$^+$].

4.1.9.4. Ethyl 4-(2-methyl-1-imidazolylmethyl)-5-hydroxy-1-cyclopropyl-2-[(3,4-difluoro-phenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_S). Mp: 181–183 °C; ^{1}H NMR (DMSO-d_6) δ 1.06 (m, 2H, –N–CH(CH_2)$_2$), 1.10 (t, 3H, J = 7.2 Hz, –OCH$_2$CH_3), 1.27 (m, 2H, –N–CH(CH_2)$_2$), 2.30 (s, 3H, 2-CH_3-1-imidazolyl), 2.98 (m, 1H, –N–CH(CH$_2$)$_2$), 3.95 (q, 2H, J = 7.2Hz, –OCH_2CH$_3$), 5.30 (s, 2H, –CH_2N<), 5.39 (s, 2H, –CH_2–sulfonyl–), 6.25–6.51 (m, 2H, –imidazolyl H),

6.99–7.68 (m, 3H, –Ph*H*), 7.70–7.80 (d, 2H, –Φ*H*), 9.59 (br s, 1H, –O*H*); MS: *m*/*z* 530.0 [MH^+].

4.1.9.5. Ethyl 4-(1-triazolylmethyl)-5-hydroxy-1-cyclopropyl-2-[(4-fluorophenyl)sulfonylmethyl]-1*H*-indole-3-carboxylate (10_T). Mp: 192–194 °C; ^{1}H NMR ($CDCl_3$) δ 0.97 (m, 2H, –N–CH(CH_2)$_2$), 1.34 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 1.61 (m, 2H, –N–CH(CH_2)$_2$), 3.15 (m, 1H, –N–C*H*(CH_2)$_2$), 4.08 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 5.30 (d, 2H, –NCH_2–), 5.94 (s, 2H, –CH_2–sulfinyl–), 7.06–7.15 (m, 2H, –triazolyl *H*), 7.53–7.60 (m, 4H, –Ph*H*), 7.97–8.02 (d, 2H, –Φ*H*); MS: *m*/*z* 498.9 [MH^+].

4.2. Biological assay

4.2.1. In vitro anti-HBV assays. The antiviral activities of compounds $\mathbf{6_A}$–$\mathbf{10_T}$ against HBV in 2.2.15 cells were evaluated by methods reported elsewhere. The in vitro anti-HBV activities included the ability to inhibit the production of HBsAg and HBeAg, and the replication of HBV DNA in HBV-infected 2.2.15 cells. For the antiviral analyses, confluent cultures of 2.2.15 cells were maintained on 96-well flat-bottomed tissue culture plates in RPMI 1640 medium with 2% fetal bovine serum.[11,12] Cultures were treated with eight consecutive daily doses of the test compounds and lamivudine (purchased by Glaxo & Welcome Co.). The cell control was set up. Medium was changed daily with fresh test compounds and positive control. HBV nucleic acid and protein levels were measured eight days after the first treatment. Extracellular HBV surface (HBsAg) and e (HBeAg) antigen levels produced from 2.2.15 cells were evaluated by semiquantitative enzyme immunoassay (EIA) methods using commercial kits (HBsAg, Abbott Laboratories; HBeAg, Diasorin, Inc.) as previously described.[13] Intracellular HBV DNA levels were measured by quantitative Southern blot hybridization.[12] The IC_{50} and selected index of the evaluated compounds and lamivudine were calculated, respectively.

4.2.2. Cytotoxicity assay. Cytotoxity induced by the test compounds in cultures of 2.2.15 cells was also determined. Briefly, 2.2.15 cells were grown to confluence in 96-well flat-bottomed tissue culture plates and treated with test compound (in 0.2 ml culture medium/well) as described above. Untreated control cultures were maintained on each 96-well plate. Toxicity was determined by measuring neutral red dye uptake, as determined from the absorbance at 510 nm relative to untreated cells, at 24 h following day 9 of treatment.

Acknowledgment

This work was supported by a grant from the National Natural Science Foundation of China (20372047).

References and notes

1. De clercq, E. *J. Clin. Virol.* **2004**, *30*, 115–133.
2. Ching, L. L.; Vlad, R.; Man-Fung, Y.; Thierry, P. *Lancet* **2003**, *362*, 2089–2094.
3. Lee, W. M. *N. Engl. J. Med.* **1997**, *337*, 1733–1745.
4. Humphries, J. C.; Dixon, J. S. *Intervirology* **2003**, *46*, 413–420.
5. Ganem, D.; Prince, A. M. *N. Engl. J. Med.* **2004**, *350*, 1118–1129.
6. Leneva, I. A.; Fadeeva, N. I.; Fedykina, I. T. *Abstract 187*, 7th International Conference on Antiviral Research, 1994.
7. Drugs Future **1992**, *17*, 1079–1081.
8. Glushkov, R. G.; Guskova, T. A.; Krylova, L., et al. *Vestn. Ross. Akad. Med. Nauk* **1993**, *3*, 36–40.
9. Chai, H. F.; Zhao, Y. F.; Zhao, C. S.; Gong, P. *Bio. Med. Chem*., in press. Available online: 23 September 2005.
10. Monti, S. A. *J. Org. Chem.* **1966**, *31*, 2669.
11. Sells, M. A.; Zelent, A. Z.; Shvartsman, M.; Acs, G. *J. Virol.* **1988**, *62*, 2836–2844.
12. Korba, B. E.; Gerin, J. L. *Antiviral Res.* **1992**, *19*, 55–70.
13. Korba, B. E.; Gerin, J. L. *Antiviral Res.* **1995**, *28*, 225–242.