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Synthesis and in vitro anti-hepatitis B virus activities of some ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates

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Abstract—A series of ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates, **8a**–**11v**, were synthesized and evaluated for their anti-hepatitis B virus (HBV) activities in 2.2.15 cells. The selective indexes of inhibition on replication of HBV DNA of compounds **11s** (>8.7) and **11t** (10.8), which were introduced halogen on the phenyl ring at position 2, were greater than those of the other evaluated compounds including lamivudine (7.0). Compounds **9e**, **9h**, **9l**, and **11v** exhibited significant anti-HBV activities, and the IC_{50} values on replication of HBV DNA of these compounds were 3.6, 6.37, 5.2, and 5.4 μg/ml, respectively, which were far more potent than the positive control lamivudine 228 μg/ml.

1. Introduction

Hepatitis B virus (HBV)-infected hepatitis is one of the common most infectious diseases in the world. More than 400 million people worldwide are chronically infected by the hepatitis B virus.[1] HBV infection causes liver diseases such as cirrhosis and maybe eventually hepatocellular carcinoma. Current clinical therapies for HBV infections with interferon-α, lamivudine, and ribavirin have limited efficacy in a significant proportion of patients and often result in severe side effects.[2–4] Thus, it is urgently needed to develop more effective and reliable therapeutics for the treatment of HBV infection.

Ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates displayed a variety of biological effects, such as antiviral effects, immunostimulative, and interferon induced activity.[5,6] Their representive agent is arbidol (Fig. 1), which was launched in Russia for the prophylaxis and treatment of acute respiratory viral infections, acting as an inhibitor of virus entry and membrane fusion.[7,8] The biological properties of ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates raised our interest to focus on their derivatives as potential anti-HBV agents.

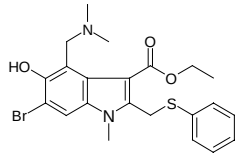

Figure 1. Structure of arbidol.

In this paper, we report the design and synthesis of several new ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylate derivatives, which were mainly modified at position 1, 4 on indole cycle and different substitutions on the phenyl ring on position 2, and we evaluate their in vitro anti-HBV activities.

2. Results and discussion

2.1. Synthetic approach

The title compounds, ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylate derivatives, were obtained as described in Scheme 1. The structures of compounds **8a**–**11v** are listed in Table 1.

The key intermediate 1-alkyl-5-hydroxy-2-methyl-1*H*-indole-3-carboxylate **3** was synthesized from commercially available **1** and an appropriate alkyl substituted amine,[9] such as methylamine and cyclopropylamine, followed by

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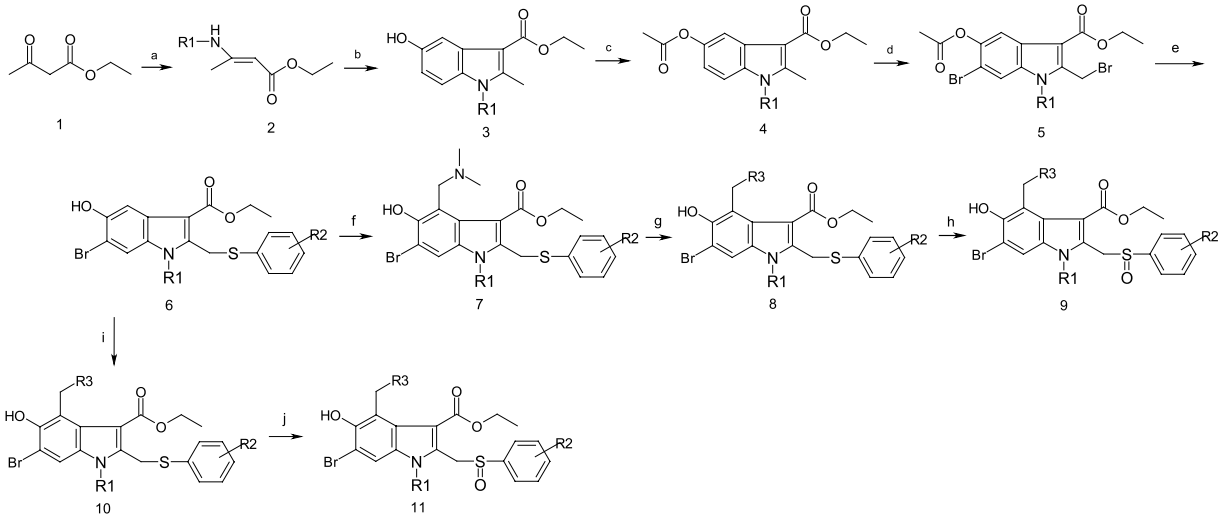
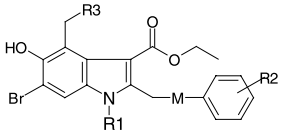


Scheme 1. Synthesis of target compounds. Reagents and conditions: (a) R_1NH_2, rt; (b) 1,4-benzoquinone/$ClCH_2CH_2Cl$, reflux; (c) CH_3COCl/pyridine/CH_3COCH_3, 40–45 °C; (d) Br_2/CCl_4, reflux; (e) R_2-substituted thiophenol/KOH/CH_3OH, rt; (f) 37% HCHO/dimethyl amine/CH_3COOH, 60 °C-reflux; (g) appropriate amines/CH_3CH_2OH, 78 °C-reflux; (h) hydrogen peroxide/acetic acid, 0 °C; (i) 37% HCHO/appropriate amines/CH_3COOH, 60 °C-reflux; (j) hydrogen peroxide/acetic acid, 0 °C.

Table 1. Structures of compounds **8a**–**11v**



Compound	R1	R2	R3	M
8a	Methyl	*m*-Methoxy	2-Methylimidazolyl	Sulfur
8b	Methyl	2-Pyridinyl	2-Aminoethanethio	Sulfur
9c	Cyclopropyl	Hydrogen	Imidazolyl	Sulfinyl
9d	Cyclopropyl	*p*-Fluoro	Imidazolyl	Sulfinyl
9e	Cyclopropyl	3′,4′-Difluoro	Imidazolyl	Sulfinyl
9f	Methyl	*p*-Nitryl	Imidazolyl	Sulfinyl
9g	Methyl	Hydrogen	2-Methylimidazolyl	Sulfinyl
9h	Methyl	3′,4′-Difluoro	2-Methylimidazolyl	Sulfinyl
9i	Methyl	*m*-Methoxy	2-Methylimidazolyl	Sulfinyl
9j	Cyclopropyl	Hydrogen	Guanidinyl	Sulfinyl
9k	Methyl	*p*-Fluoro	Guanidinyl	Sulfinyl
9l	Cyclopropyl	*p*-Fluoro	Guanidinyl	Sulfinyl
9m	Methyl	2-Pyridinyl	2-Aminoethanethio	Sulfinyl
9n	Cyclopropyl	*m*-Methoxy	Triazolyl	Sulfinyl
9o	Methyl	*m*-Methoxy	4′,5′-Dicyanoimidazolyl	Sulfinyl
11p	Methyl	Hydrogen	Dimethylamino	Sulfinyl
11q	Methyl	*p*-Fluoro	Dimethylamino	Sulfinyl
11r	Cyclopropyl	2-Pyridinyl	Dimethylamino	Sulfinyl
11s	Methyl	*p*-Fluoro	Morpholino	Sulfinyl
11t	Methyl	*m*-Chloro	Morpholino	Sulfinyl
11u	Methyl	*p*-Fluoro	Pyrrolidinyl	Sulfinyl
11v	Cyclopropyl	3′,4′-Difluoro	4-Methyl piperazinyl	Sulfinyl

Nentizescu condensation of **2** and 1,4-benzoquinone.[10] To protect the hydroxy group on position 5, acetyl chloride was added into the solution of **3** and pyridine, and gave **4** in a yield of 85%. By the two steps of bromination with bromine and reaction with electron-withdrawing group or electron-donating group substituted thiophenol, **4** was transformed into **6**.

It was of interest to examine whether the diversity of the Mannich basic group on position 4 could have an effect on potential anti-HBV activities. So we introduced heteroaromatic amines or other bases, for example, imidazole, methyl- or cyano-substituted imidazole, triazole, 2-aminosulfanyl, and so on, at position 4. But the above-mentioned Mannich bases could not be prepared directly by the standard Mannich reaction of **6** with those amines. We obtained **7** first by the Mannich reaction of **6** with dimethylamine, according to the procedure of Grinev and Pershin.[11] Then the mixture of **7** and appropriate base in anhydrous ethanol was refluxed

for 4–6 h, ultimately resulting in the formation of compounds **8a**–**b**. To relieve the cellular toxicity and increase the antiviral activity, the sulfides on some analogues of **8** were further oxidized into sulfinyl by hydrogen peroxide in glacial acetic acid and gave compounds **9c**–**o**.

Various Mannich bases were introduced at position 4 directly by the Mannich reaction of **6** with secondary amines such as dimethylamine, morpholine, *N*-methylpiperazine and pyrrolidine, and gave **10**.[11] The oxidation of **10** was undertaken to yield target compounds **11p**–**v** ultimately.

2.2. Biological activity test

All target compounds **8a**–**11v** 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, half of the evaluated compounds exhibited inhibitory effects on HBV and were superior to lamivudine.

Of these compounds, **9e** showed the most potent in vitro anti-HBV activity (IC_{50}: 3.6 μg/ml). Its analogues **9h**, **9l**, and **11v** also exhibited significant efficacy on HBV, their IC_{50} on the replication of HBV DNA being 6.37, 5.2, and 5.4 μg/ml, respectively, which were far more potent than the positive control lamivudine 228 μg/ml. The selective indexes of **9e**, **9h**, and **11v** (5.8, 6.28, and 6.35) were comparable to that of lamivudine (7.0). Although **11t** and **11s** (IC_{50}: 26.64 μg/ml, 57.6 μg/ml) were less effective than **9e**, **9h**, **9l**, and **11v**, their potencies were still 8 or 4 times of the control. SIs of **11t** and **11s** (>8.7, 10.8) were the highest in all tested compounds. All of these compounds possessed fluoro or chloro on the phenyl ring at position 2. By contrast, compounds **8a**, **9i**, and **9n** only displayed suppressant properties on the production of HBsAg and HBeAg. However, **9c**, **9j**, and **11p** were ineffective in HBV-infected 2.2.15 cells. These results suggested that the compounds substituted on the phenyl ring of position 2 possessed more anti-HBV potency than those non-substituted compounds. Particularly, the electron-withdrawing groups such as halogen were more in favor of enhancing activities than the electron-donating groups, except for nitro (**9t**).

The introduction of sulfinyl, instead of sulfide, did reduce the cytotoxicities and kept up or increased the anti-HBV activities in addition (compounds **9m** vs **8b**; **9i** vs **8a**).

Different Mannich basic functionalities introduced at position 4 seemed to have few influence on antiviral activities. But **11u** (4-pyrrolidinomethyl derivative) and **9o** (4′,5′-dicyanoimidazolylmethyl derivative) did not have inhibitory effects at the relevant maximum intoxic concentration.

Compared with the 1-methyl compound (**9k**, IC_{50}: 29.30 μg/ml), cyclopropyl at position 1 (**9l**, IC_{50}: 5.2 μg/ml) could increase the antiviral effects, whereas the cytotoxicity was enhanced correspondingly. TC_{50} values of **9k** and **9l** were 125 and 18.52 μg/ml, respectively.

Table 2. Anti-HBV activities of compounds **8a**–**11v**

Compound	TC_{50} (μg/ml)[a]	HbsAg		HBeAg		DNA replication	
		IC_{50} (μg/ml)[b]	SI[c]	IC_{50} (μg/ml)[b]	SI[c]	IC_{50} (μg/ml)[b]	SI[c]
8a	6.35	2.66	2.39	—	—	—	—
8b	8.0	—	—	—	—	—	—
9c	192.45	—	—	—	—	—	—
9d	55.56	—	—	—	—	11.4	4.87
9e	21.11	—	—	—	—	3.6	5.8
9f	40.0	—	—	—	—	—	—
9g	99.21	49.06	2.02	—	—	22.49	4.4
9h	40.0	—	—	—	—	6.37	6.28
9i	8.0	2.54	3.15	—	—	—	—
9j	111.11	—	—	—	—	—	—
9k	125	62.16	2.01	—	—	29.30	4.27
9l	18.52	—	—	—	—	5.2	3.63
9m	125	—	—	—	—	26.84	4.66
9n	111.1	61.48	1.81	33.77	3.29	—	—
9o	28.74	—	—	—	—	—	—
11p	96.23	—	—	—	—	—	—
11q	96.23	—	—	19.80	4.86	—	—
11r	346.68	61.3	5.6	—	—	—	—
11s	>500	—	—	—	—	57.6	>8.7
11t	288.68	—	—	—	—	26.64	10.8
11u	346.68	—	—	—	—	—	—
11v	34.35	—	—	—	—	5.4	6.35
Lamivudine	1600	—	—	—	—	228	7.0

[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}).

3. Conclusion

In summary, we synthesized a series of new ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylate derivatives and examined their anti-HBV activities and cytotoxicities. Most of them exhibited better bioactivities against HBV in 2.2.15 cells. According to the above results, the following conclusions can be made:

1. Substitutions of the phenyl ring at position 2 can improve anti-HBV activities. Particularly the electron-withdrawing groups such as fluoro and chloro contributed more anti-HBV effects than the electron-donating methoxy group.
2. The oxidation of sulfide into sulfinyl reduced the cellular toxicities and kept up or increased the anti-HBV activities.
3. Derivatives having different Mannich basic functionalities at the 4 position did not show significant distinctness of anti-HBV effects, though the electron-withdrawing group on the basic group could decrease it.
4. The steric changes of the alkyl group ($C \leqslant 3$) at position 1 have a slight influence on the antiviral potency of compounds.

Moreover, being the first report on ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates serving as anti-HBV agents, these results propose a lead compound in the research and development of anti-HBV and similar virus-infected hepatitis.

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. Ethyl 5-acetoxy-1-alkyl-2-methy-1*H*-lindole-3-carboxylate (4). To 500 ml of acetone were added 0.35 mol of **3** and 0.7 mol pyridine, and then 0.7 mol of acetyl chloride was added dropwise with good stirring under 40–45 °C. The resulting solution was stirred at this temperature for 4–5 h and then cooled. The solution was poured into 500 ml water, and the precipitate was collected by filtration and washed with water.

4.1.2. General procedure for the synthesis of compounds 8a–8b. To a solution of imidazole or 2-methyl-1-imidazole or aminoethanethiol (0.03 mol) in anhydrous ethanol (50 ml) was added **7** (0.01 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 and dried to give the desired compounds **8a**–**8b**.

4.1.2.1. Ethyl 6-bromo-4-[(2-methyl-1-imidazolyl)-methyl]-5-hydroxy-1-methyl-2-[(3-methoxy-phenyl)sulfanylmethyl-1*H*-indole-3-carboxylate (8a). To a solution of 2-methyl-1-imidazole (0.03 mol) in anhydrous ethanol (50 ml) was added ethyl 6-bromo-4-[(dimethylamino)methyl]-5-hydroxy-1-methyl-2-[(3-methoxyphenyl)sulfanylmethyl]-1*H*-indole-3-carboxylate (0.01 mol). The reaction mixture was further stirred with refluxing until a mass of solid appeared in the solution. The precipitate was filtered, washed with acetone (15 ml), and dried in vacuo. Then a white powder **8a** (4.9 g) was ultimately obtained. mp: 177–179 °C; ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H, J = 7.2 Hz, –OCH$_2$C***H***$_3$), 2.35 (s, 3H, 2-C***H***$_3$ of imidazolyl), 3.65 (s, 3H, –OC***H***$_3$), 3.72 (s, 3H, –NC***H***$_3$), 4.16 (q, 2H, J = 7.2Hz, –OC***H***$_2$CH$_3$), 4.67 (s, 2H, –SC***H***$_2$–), 4.81 (s, 2H, –C***H***$_2$N<), 6.33–6.57 (m, 2H, 4,5-2***H*** of imidazolyl), 6.92–7.56 (m, 4H, –Ph***H***), 7.75 (s, 1H, –**Φ*H***), 9.85 (br s, 1H, –O***H***); MS: *m*/*z* 544.0, 546.0 [MH$^+$].

4.1.2.2. Ethyl 6-bromo-4-(2-aminoethylsulfanylmethyl)-5-hydroxy-1-methyl-2-[(2-pyridinyl)sulfanylmethyl]-1*H*-indole-3-carboxylate (8b). mp: 183–185 °C; ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H, J = 6.9 Hz, –OCH$_2$C***H***$_3$), 2.94–3.10 (m, 4H, –SC***H***$_2$C***H***$_2$–), 3.77 (s, 3H, –NC***H***$_3$), 4.27 (q, 2H, J = 6.9 Hz, –OC***H***$_2$CH$_3$), 4.45 (s, 2H, –SC***H***$_2$–), 4.91 (d, 2H, J = 3 Hz, –C***H***$_2$N<), 7.14–7.69 (s, 4H, –pyridinyl ***H***), 7.74 (s, 1H, –**Φ*H***), 7.96 (br s, 2H, –N***H***$_2$), 8.71 (br s, 1H, –O***H***); MS: *m*/*z* 510.0, 512.0 [MH$^+$].

4.1.3. General procedure for the synthesis of compounds 9c–11v. Appropriate analogues of **8** or **10** (0.01 mol) were first dissolved in glacial acetic acid and water (25 ml, 10:1), and hydrogen peroxide (0.011 mol) was successively dropped into the solution at 0 °C. After stirring at 0 °C for 6–8 h, the solvent was evaporated in vacuo, and 50 ml of water was added in one portion. The resultant mixture was adjusted to pH 12 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 in most circumstances. Then, 50 ml of ether was poured into the yellow oil. The solution was cooled at 0 °C and the precipitate was filtered and dried to give **9c**–**11v**.

4.1.3.1. Ethyl 6-bromo-1-cyclopropyl-5-hydroxy-4-(1-imidazolylmethyl)-2-(phenylsulfinylmethyl)-1*H*-indole-3-carboxylate (9c). Ethyl 6-bromo-1-cyclopropyl-5-hydroxy-4-(1-imidazolyl methyl)-2-(phenylsulfanylmethyl)-1*H*-indole-3-carboxylate (0.01 mol) was dissolved in glacial acetic acid and water (25 ml, 10:1), and then hydrogen peroxide (0.011 mol) was dropped into the solution at 0 °C. After stirring at 0 °C for 6–8 h, the solvent was evaporated in vacuo, and 50 ml of water was added in one portion. The solution was adjusted to pH 12 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. Then 50 ml of ether was poured into the yellow oil. The solution was cooled at 0 °C and the precipitate was filtered and dried to give white powder **9c** (5.3 g). mp: 197–199 °C; ^{1}H NMR ($CDCl_3$): δ 0.80 (m, 2H, –CH$_2$–N–CH(C***H***$_2$)$_2$), 1.25 (m, 1H, –CH$_2$–N–C***H***(CH$_2$)$_2$),

1.35 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 1.62 (m, 2H, –CH_2–N–$CH(CH_2)_2$), 4.24 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 5.09 (s, 2H, –CH_2N<), 5.38 (s, 2H, –sulfinyl-CH_2–), 7.18–7.23 (t, 2H, 4,5-2***H*** of imidazolyl), 7.29–7.43 (m, 5H, –Ph***H***), 7.56 (s, 1H, 2-***H*** of imidazolyl), 7.66 (s, 1H, –**Φ*H***), 10.20 (br s, 1H, –O***H***); MS: *m*/*z* 542.0, 544.0 [MH^+].

4.1.3.2. Ethyl 6-bromo-1-cyclopropyl-5-hydroxy-4-(1-imidazolylmethyl)-2-[(4- fluorophenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (9d). mp: 200–202 °C; ^{1}H NMR ($CDCl_3$): δ 0.90–1.00 (m, 2H, –CH_2–N–$CH(CH_2)_2$), 1.20 (m, 2H, –CH_2–N–$CH(CH_2)_2$), 1.36 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.47 (m, 1H, –CH_2–N–$CH(CH_2)_2$), 4.32 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.74 (s, 2H, –CH_2N<), 5.91 (s, 2H, –sulfinyl-CH_2–), 6.95–7.15 (m, 4H, –Ph***H***), 7.38–7.55 (m, 3H, 3***H*** of imidazolyl), 7.71 (s, 1H, –**Φ*H***); MS: *m*/*z* 560.0, 562.0 [MH^+].

4.1.3.3. Ethyl 6-bromo-1-cyclopropyl-2-[(3,4-difluorophenyl)sulfinylmethyl]-5-hydroxy-4-(1-imidazolylmethyl)-1*H*-indole-3-carboxylate (9e). mp: 193–195 °C; ^{1}H NMR (DMSO-d_6): δ 0.95–1.04 (m, 2H, –CH_2–N–$CH(CH_2)_2$), 1.21 (m, 2H, –CH_2–N–$CH(CH_2)_2$), 1.35 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.77 (m, 1H, –CH_2–N–$CH(CH_2)_2$), 4.15 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.83 (s, 2H, –CH_2N<), 5.71 (s, 2H, –sulfinyl-CH_2–), 6.67–7.39 (m, 3H, –Ph***H***), 7.54–7.69 (m, 3H, 3***H*** of imidazolyl), 7.72 (s, 1H, –**Φ*H***); MS: *m*/*z* 578.1, 580.1 [MH^+].

4.1.3.4. Ethyl 6-bromo-5-hydroxy-4-(1-imidazolylmethyl)-1-methyl-2-[(4-cyanophenyl) sulfinylmethyl]-1*H*-indole-3-carboxylate (9f). mp: 189–191 °C; ^{1}H NMR (DMSO-d_6): δ 1.17 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 3.68 (s, 3H, –NCH_3), 4.07 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.95 (s, 2H, –CH_2N<), 5.68 (s, 2H, –sulfinyl-CH_2–), 6.72–7.36 (m, 4H, –Ph***H***), 7.69–7.72 (m, 3H, 3***H*** of imidazolyl), 7.84 (s, 1H, –**Φ*H***); MS: *m*/*z* 561.0, 563.0 [MH^+].

4.1.3.5. Ethyl 6-bromo-5-hydroxy-4-[(2-methyl-1-imidazolyl)methyl]-1-methyl-2-(phenyl-sulfinylmethyl)-1*H*-indole-3-carboxylate (9g). mp: 186–188 °C; ^{1}H NMR (DMSO-d_6): δ 1.39 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.33 (s, 3H, 2-CH_3 of imidazolyl), 3.54 (s, 3H, –NCH_3), 4.06 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.80 (s, 2H, –CH_2N<), 5.54 (s, 2H, –sulfinyl-CH_2–), 6.29–6.54 (m, 2H, 4,5-2***H*** of imidazolyl), 7.44–7.64 (m, 5H, –Ph***H***), 7.87 (s, 1H, –**Φ*H***); MS: *m*/*z* 530.1, 532.0 [MH^+].

4.1.3.6. Ethyl 6-bromo-5-hydroxy-2-[(3,4-difluorophenyl)sulfinylmethyl]-4-[(2-methyl-1- imidazolyl)methyl]-1-methyl-1*H*-indole-3-carboxylate (9h). mp: 182–184 °C; ^{1}H NMR (DMSO-d_6): δ 1.07 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.31 (s, 3H, 2-CH_3 of imidazolyl), 3.69 (s, 3H, –NCH_3), 3.98 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.89 (s, 2H, –CH_2N<), 5.48 (s, 2H, –sulfinyl-CH_2–), 6.24–6.52 (m, 2H, 4,5-2***H*** of imidazolyl), 7.26–7.61 (m, 3H, –Ph***H***), 7.90 (s, 1H, –**Φ*H***); MS: *m*/*z* 566.0, 568.0 [MH^+].

4.1.3.7. Ethyl 6-bromo-5-hydroxy-4-[(2-methyl-1-imidazolyl)methyl]-1-methyl-2-[(3-methoxyphenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (9i). mp: 179–181 °C; ^{1}H NMR (DMSO-d_6): δ 1.17 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.34 (s, 3H, 2-CH_3 of imidazolyl), 3.53 (s, 3H, –OCH_3), 3.67 (s, 3H, –NCH_3), 4.07 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.83 (s, 2H, –CH_2N<), 5.56 (s, 2H, –sulfinyl-CH_2–), 6.31–6.54 (m, 2H, 4,5-2***H*** of imidazolyl), 6.92–7.76 (m, 4H, –Ph***H***), 7.88 (s, 1H, –**Φ*H***); MS: *m*/*z* 560.1, 562.1 [MH^+].

4.1.3.8. Ethyl 6-bromo-1-cyclopropyl-4-(guanidinylmethyl)-5-hydroxy-2-(phenylsulfinylmethyl)-1*H*-indole-3-carboxylate (9j). mp: 192–194 °C; ^{1}H NMR (DMSO-d_6): δ 0.98 (m, 2H, –CH_2–N–$CH(CH_2)_2$), 1.13 (m, 2H, –CH_2–N–$CH(CH_2)_2$), 1.23 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.92 (m, 1H, –CH_2–N–$CH(CH_2)_2$), 4.19 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.51 (s, 2H, –sulfinyl-CH_2–), 5.38 (s, 2H, –CH_2N<), 6.60 (2H, –NH_2), 6.94 (1H, –C═N***H***),7.19 (m, 5H, –Ph***H***), 7.54 (s, 1H, –**Φ*H***); MS: *m*/*z* 533.0, 535.1 [MH^+].

4.1.3.9. Ethyl 6-bromo-4-(guanidinomethyl)-5-hydroxy-1-methyl-2-[(4-fluorophenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (9k). mp: 188–190 °C; ^{1}H NMR (DMSO-d_6): δ 1.36 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 3.62 (s, 3H, –NCH_3), 4.28 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.65 (s, 2H, –CH_2N<), 4.78 (s, 2H, –sulfinyl-CH_2–), 6.68 (2H, –NH_2), 6.92 (1H, –C═N***H***), 7.00–7.44 (m, 4H, –Ph***H***), 7.47 (s, 1H, –**Φ*H***); MS: *m*/*z* 525.0, 527.0 [MH^+].

4.1.3.10. Ethyl 6-bromo-1-cyclopropyl-4-(guanidinomethyl)-5-hydroxy-2-[(4-fluorophenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (9l). mp: 194–196 °C; ^{1}H NMR ($CDCl_3$): δ 0.90 (m, 2H, –N–$CH(CH_2)_2$), 1.24 (m, 2H, –N–$CH(CH_2)_2$), 1.32 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.69 (m, 1H, –N–$CH(CH_2)_2$), 4.23 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.75 (s, 2H, –CH_2N<), 4.85 (s, 2H, –sulfinyl-CH_2–), 6.80 (2H, –NH_2), 7.32 (1H, –C═N***H***), 7.39–7.60 (m, 4H, –Ph***H***), 7.83 (s, 1H, –**Φ*H***), 9.28 (br s, 1H, –O***H***); MS: *m*/*z* 551.0, 553.0 [MH^+].

4.1.3.11. Ethyl 6-bromo-4-[(2-aminoethyl)sulfanylmethyl]-5-hydroxy-1-methyl-2-[(2-pyridinyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (9m). mp: 190–192 °C; ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 3H, J = 6.9 Hz, –OCH_2CH_3), 2.97–3.16 (m, 4H, –SCH_2CH_2–), 3.74 (s, 3H, –NCH_3), 4.24 (q, 2H, J = 6.9 Hz, –OCH_2CH_3), 4.48 (d, 2H, J = 3 Hz, –CH_2N<), 5.11 (s, 2H, –sulfinyl-CH_2–), 7.18–7.64 (s, 4H, –pyridinyl ***H***), 7.79 (s, 1H, –**Φ*H***), 7.99 (br s, 2H, –NH_2), 8.91 (br s, 1H, –O***H***); MS: *m*/*z* 526.1, 528.1 [MH^+].

4.1.3.12. Ethyl 6-bromo-1-cyclopropyl-5-hydroxy-4-(1-triazolylmethyl)-2-[(3-methoxyphenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (9n). mp: 201–203 °C; ^{1}H NMR ($CDCl_3$): δ 0.89 (m, 2H, –N–$CH(CH_2)_2$), 1.16 (m, 2H, –N–$CH(CH_2)_2$), 1.47 (t, 3H, J = 7.2 Hz, –OCH_2CH_3), 2.26 (m, 1H, –N–$CH(CH_2)_2$), 3.50 (s, 3H, –OCH_3), 4.40 (q, 2H, J = 7.2 Hz, –OCH_2CH_3), 4.90 (s, 2H, –CH_2N<), 6.10 (s, 2H, –sulfinyl-CH_2–), 6.84 (2H, 2***H***

of triazolyl), 6.99–7.96 (m, 4H, –Ph*H*), 8.62 (s, 1H, –Φ*H*), 9.60 (br s, 1H, –O*H*); MS: *m*/*z* 573.0, 575.0 [MH^+].

4.1.3.13. Ethyl 6-bromo-4-(4,5-dicyano-1-imidazolylmethyl)-5-hydroxy-1-methyl-2-[(3-methoxyphenyl)sulfinylmethyl]-1*H*-indole-3-carboxylate (9o). mp: 198–200 °C; ^{1}H NMR (DMSO-d_6): δ 1.19 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 3.52 (s, 3H, –OC*H*$_3$), 3.64 (s, 3H, –NC*H*$_3$), 4.09 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.84 (s, 2H, –C*H*$_2$N<), 5.87 (s, 2H, –sulfinyl-C*H*$_2$–), 6.94 (m, 1H, 1*H* of 4,5-dicyano-1-imidazolyl), 7.00–7.90 (m, 4H, –Ph*H*), 7.97 (s, 1H, –Φ*H*), 9.34 (br s, 1H, –O*H*); MS: *m*/*z* 596.0, 598.0 [MH^+].

4.1.3.14. Ethyl 6-bromo-4-(dimethylaminomethyl)-5-hydroxy-1-methyl-2-(phenylsulfinylmethyl)-1*H*-indole-3-carboxylate (11p). mp: 178–180 °C; ^{1}H NMR ($CDCl_3$): δ 1.49 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 2.88 (m, 6H, –N(C*H*$_3$)$_2$), 3.34 (s, 3H, –NC*H*$_3$), 4.41 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.82 (s, 2H, –C*H*$_2$N<), 5.33 (s, 2H, –sulfinyl-C*H*$_2$–), 7.44–7.54 (m, 5H, –Ph*H*), 7.56 (s, 1H, –Φ*H*), 10.00 (br s, 1H, –O*H*); MS: *m*/*z* 493.0, 495.0 [MH^+].

4.1.3.15. Ethyl 6-bromo-4-(dimethylaminomethyl)-2-[(4-fluorophenyl)sulfinylmethyl]-5-hydroxy-1-methyl-1*H*-indole-3-carboxylate (11q). mp: 181–183 °C; ^{1}H NMR ($CDCl_3$): δ 1.43 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 2.40 (m, 6H, –N(C*H*$_3$)$_2$), 3.50 (s, 3H, –NC*H*$_3$), 4.22 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.35 (s, 2H, –C*H*$_2$N<), 4.60 (s, 2H, –sulfinyl-C*H*$_2$–), 7.13–7.50 (m, 4H, –Ph*H*), 7.51 (s, 1H, –Φ*H*); MS: *m*/*z* 511.0, 513.0 [MH^+].

4.1.3.16. Ethyl 6-bromo-4-(dimethylaminomethyl)-5-hydroxy-1-cyclopropyl-2-[(2-pyridinyl)- sulfinylmethyl]-1*H*-indole-3-carboxylate (11r). mp: 176–178 °C; ^{1}H NMR (DMSO-d_6): δ 0.91 (m, 2H, –N–CH(C*H*$_2$)$_2$), 1.18 (m, 2H, –N–CH(C*H*$_2$)$_2$), 1.42 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 2.29 (m, 1H, –N–C*H*(CH$_2$)$_2$), 2.38 (m, 6H, –N(C*H*$_3$)$_2$), 3.87 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.62 (s, 2H, –C*H*$_2$N<), 5.54 (s, 2H, –sulfinyl-C*H*$_2$–), 7.15–7.60 (m, 4H, –pyridinyl ***H***), 7.78 (s, 1H, –Φ*H*), 8.85 (br s, 1H, –O*H*); MS: *m*/*z* 520.1, 522.1 [MH^+].

4.1.3.17. Ethyl 6-bromo-2-[(4-fluorophenyl)sulfinylmethyl]-5-hydroxy-4- (morphorinomethyl) -1-methyl-1*H*-indole-3-carboxylate (11s). mp: 195–197 °C; ^{1}H NMR (DMSO-d_6): δ 1.31 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 2.44 (s, 4H, 4*H* of morpholino), 3.57 (s, 3H, –NC*H*$_3$), 3.62 (s, 4H, 4*H* of morpholino), 4.07 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.17 (d, 2H, –NC*H*$_2$–), 4.78 (s, 2H, –sulfinyl-C*H*$_2$–), 7.36–7.74 (m, 4H, –Ph*H*), 7.81 (s, 1H, –Φ*H*); MS: *m*/*z* 552.9, 554.9 [MH^+].

4.1.3.18. Ethyl 6-bromo-2-[(3-chlorophenyl)sulfinylmethyl]-5-hydroxy-4-(morphorinomethyl)-1-methyl-1*H*-indole-3-carboxylate (11t). mp: 188–190 °C; ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 2.49 (s, 4H, 4*H* of morpholino), 3.56 (s, 3H, –NC*H*$_3$), 3.60 (s, 4H, 4*H* of morpholino), 4.08 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.16 (d, 2H, –NC*H*$_2$–), 4.81 (s, 2H, –sulfinyl-C*H*$_2$–), 7.38–7.65 (m, 4H, –Ph*H*), 7.74 (s, 1H, –Φ*H*); MS: *m*/*z* 568.9, 570.9 [MH^+].

4.1.3.19. Ethyl 6-bromo-2-[(4-fluorophenyl)sulfinylmethyl]-5-hydroxy-1-methyl -4-(pyrrolidinomethyl)-1*H*-indole-3-carboxylate (11u). mp: 196–198 °C; ^{1}H NMR ($CDCl_3$): δ 1.42 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 1.90 (s, 4H, 4*H* of pyrrolidinyl), 2.75 (s, 4H, 4*H* of pyrrolidinyl),3.49 (s, 3H, –NC*H*$_3$), 4.28 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.31 (d, 2H, –NC*H*$_2$–), 4.60 (s, 2H, –sulfinyl-C*H*$_2$–), 7.13–7.49 (m, 4H, –Ph*H*), 7.51 (s, 1H, –Φ*H*); MS: *m*/*z* 536.9, 538.9 [MH^+].

4.1.3.20. Ethyl 6-bromo-1-cyclopropyl-2-[(3,4-difluorophenyl)sulfinylmethyl]-5-hydroxy-4-(4-methylpiperazinomethyl)-1*H*-indole-3-carboxylate (11v). mp: 191–193 °C; ^{1}H NMR ($CDCl_3$): δ 0.93–1.07 (m, 2H, –CH$_2$–N–CH(C*H*$_2$)$_2$), 1.22 (m, 2H, –CH$_2$–N–CH(C*H*$_2$)$_2$), 1.32 (s, 4H, 4*H* of piperazinyl), 1.42 (t, 3H, J = 7.2 Hz, –OCH$_2$C*H*$_3$), 2.32 (s, 4H, 4*H* of piperazinyl), 2.83 (m, 1H, –CH$_2$–N–C*H*(CH$_2$)$_2$), 4.19 (s, 3H, 4-C*H*$_3$ of piperazinyl), 4.32 (q, 2H, J = 7.2 Hz, –OC*H*$_2$CH$_3$), 4.68 (s, 2H, –C*H*$_2$N<), 4.80 (s, 2H, –sulfinyl-C*H*$_2$–), 6.99–7.28 (m, 3H, –Ph*H*), 7.71 (s, 1H, –Φ*H*); MS: *m*/*z* 610.0, 612.0 [MH^+].

4.2. Biological assay

4.2.1. In vitro anti-HBV assays. The antiviral activities of compounds **8a**–**11v** 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.[12,13] 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.[14] Intracellular HBV DNA levels were measured by quantitative Southern blot hybridization.[13] The IC_{50} and selected index of the evaluated compounds and lamivudine were calculated, respectively.

4.2.2. Cytotoxicity assay. Cytotoxicity 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.

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