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Synthesis and biological evaluation of 1*H*-benzimidazol-5-ols as potent HBV inhibitors

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ABSTRACT

A new series of 1-methyl-1*H*-benzimidazol-5-ol derivatives were synthesized and evaluated for their anti-hepatitis B virus (HBV) activity and cytotoxicity in the HepG2.2.15 cell line. Some of the analogues in this series displayed inhibitory activity superior to lamivudine. Of them, compound **13b** was the most potent one, showing an IC_{50} value of 7.8 μM and a SI value of 13.0.

Hepatitis B virus (HBV) is a major cause of acute and chronic hepatitis which could lead to liver cirrhosis, liver failure and hepatocellular carcinoma.[1] An estimated 360 million people are chronically infected by HBV throughout the world with 0.5–1.2 million global deaths per year.[2] Nowadays, at least two different treatment options, including interferon and nucleoside analogues such as lamivudine, adefovir dipivoxil and entecavir are considered as antiviral therapy for chronic hepatitis B infection.[3] Unfortunately, interferon-α is effective in only one-third of patients and the treatment is greatly hampered by significant adverse effects.[4] The long-term usage of nucleoside analogues in HBV carriers may eventually lead to the development of virus drug resistance.[5,6] Therefore, the search for compounds with novel anti-HBV target and mechanism is still urgently needed.

We previously reported that ethyl 5-hydroxy-1*H*-indole-3-carboxylate analogues (A, Fig. 1) exhibited significant antiviral activity in vitro against HBV.[7–10] We have recently reported our efforts to replace the indole nucleus by quinoline[11,12] and hydroxyimidazo[1,2-*a*]pyridine[13] that produced potent HBV inhibitor. As a continuation of this work, we describe here the synthesis and structure–activity relationships (SARs) of new 1-methyl-1*H*-benzimidazol-5-ol derivatives (B, Fig. 1).

1-Methyl-1*H*-benzimidazol-5-ols **11a–11f** were achieved with an efficient synthetic route (Scheme 1). Protection of the phenolic hydroxyl group of commercially available 4-nitrophenol **1** with benzyl chloride in the presence of K_2CO_3 at 80 °C, followed by reduction of nitro group, gave 4-(benzyloxy)benzenamine **3**, which was treated with 2-chloroacetyl chloride to yield compound **4**.

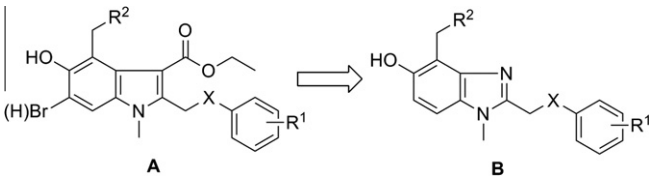

Figure 1. Modifications of ethyl 5-hydroxy-1*H*-indole-3-carboxylates **A** to 1-methyl-1*H*-benzimidazol-5-ols **B**.

Nitration of compound **4** with a mixture of nitric acid and sulfuric acid in chloroform formed compound **5**. Compound **6** was obtained by methylation of compound **5** with dimethyl sulfate in the presence of NaH in dry DMF. Treatment of compound **6** with substituted thiophenols **7** gave thioethers **8**, followed by reduction of the nitro group and cyclization in one pot to afford benzimidazoles **9**, which were subsequently deprotected in a 2:1 mixture of acetic acid and hydrochloric acid to afford **10**. The target compounds **11a–f** were then synthesized via a Mannich reaction of **10** with formaldehyde and different secondary alkylamines.

To efficiently explore the SARs around the 4-position of the benzimidazole, a modified route introducing an imidazolyl or 2-methylimidazolyl group was developed (Scheme 2). The dimethylamine derivatives **12** were obtained via a Mannich reaction. And then treatment of **12** with imidazole or 2-methylimidazole in 1,4-dioxane generated seven *N*-heteroaromatic derivatives **13a–h** in good yields. With the purpose of investigating the influence of the oxidation state of the sulfur atom on the anti-HBV activity and cytotoxicity, we prepared 2-sulfinyl analogues **14a–c** and 2-sulfonyl analogues **15a–c** (Scheme 3). The spectral data of all

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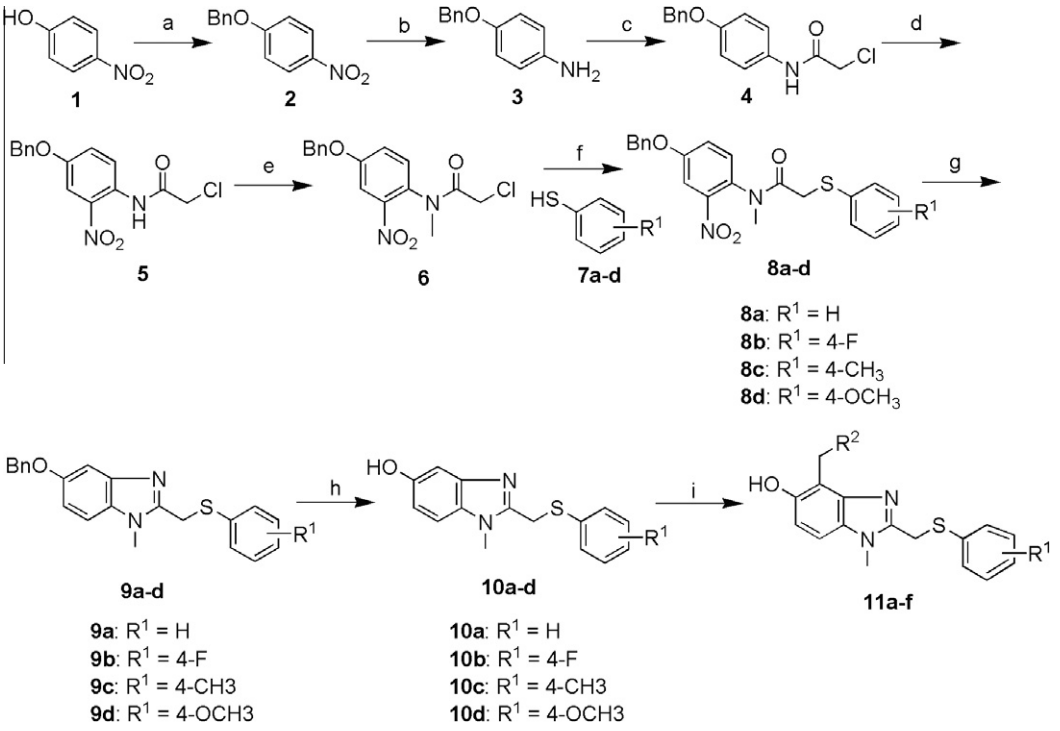
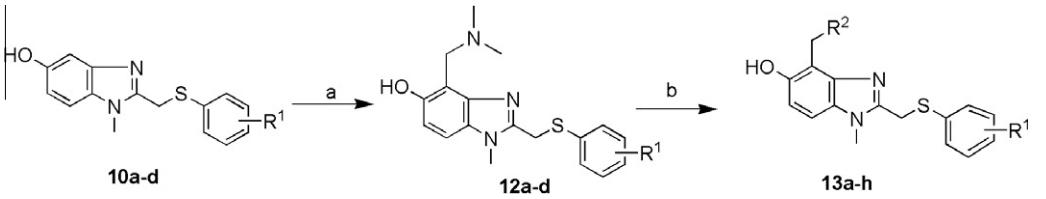
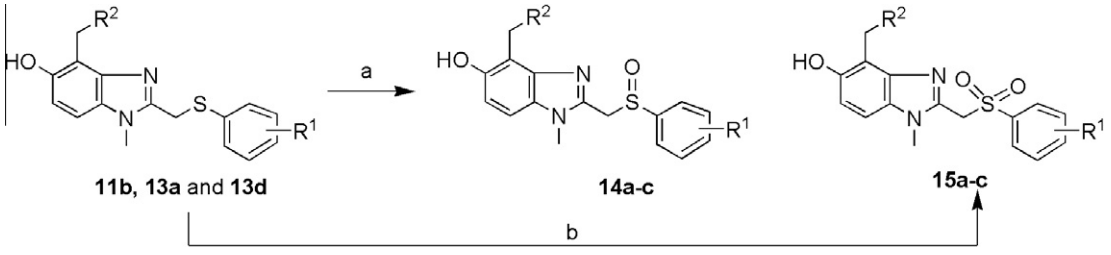


Scheme 1. Reagents and conditions: (a) Benzyl chloride, K_2CO_3, DMF, 80 °C, 3 h, 95%; (b) 80% hydrazine hydrate, $FeCl_3{\cdot}6H_2O$, activated carbon, 95% EtOH, reflux, 5 h, 75%; (c) 2-chloroacetyl chloride, pyridine, CH_2Cl_2, −10 °C, 0.5 h, rt, 3 h, 82%; (d) HNO_3, H_2SO_4, $CHCl_3$, 10 °C, 2 h, 68%; (e) $(CH_3)_2SO_4$, NaH, DMF, 0 °C, 0.5 h, rt, 1 h, 86%; (f) KOH, MeOH, rt, 6 h, 70–85%; (g) Fe, NH_4Cl (catalytic), AcOH, 95% EtOH, reflux, 6 h, 50–66%; (h) HCl, AcOH, argon, 80 °C, 8 h, 90%; (i) alkyl secondary amine, 37% HCHO, MeOH, rt, 10 h, 65–80%.



Scheme 2. Reagents and conditions: (a) Dimethylamine, 37% HCHO, MeOH, rt, 10 h, 70–80%; (b) imidazole or 2-methylimidazole, HCl, 1,4-dioxane, 80 °C, 2–3 h, 65–75%.



Scheme 3. Reagents and conditions: (a) $NaBO_3{\cdot}4H_2O$, AcOH, 50 °C, 2–3 h, 68–79%; (b) H_2O_2, Na_2WO_4, MeOH, 5 °C, 4–6 h, 70–86%.

the target compounds are in full agreement with the proposed structures.[14]

These synthesized 1-methyl-1*H*-benzimidazol-5-ols were evaluated for their anti-HBV activity and cytotoxicity with the antiviral drug lamivudine as reference control in HepG2.2.15 cells.[15] The results, expressed as IC_{50}, CC_{50} and SI, are illustrated in Table 1.

As shown in Table 1, six compounds (**13a–e** and **13g**) demonstrated more potent inhibition of HBV DNA replication than lamivudine with IC_{50} values between 7.8 and 44.5 μM. Of them, **13a**, **13b** and **13c** possessed SI from 6.9 to 13.0, which were comparable to or higher than that of lamivudine (SI = 9.1). In addition, compounds **11d**, **11f**, **14a–c** and **15a–c** exhibited moderate to good inhibitory effect on the secretion of HBsAg.

The subseries of compounds **11a–d**, **13a**, and **13b** has different Mannich side chains on 4-position of the benzimidazole core. Imidazolyl derivatives **13a** and **13b** showed remarkable inhibition of HBV DNA replication (IC_{50} = 9.1 and 7.8 μM, respectively). However, replacement of the imidazolyl group of **13a** (or **13b**) with dimethylamino, pyrrolidinyl, morpholinyl, or 4-methyl piperazinyl groups resulted in a complete loss of inhibitory activity, as seen in compounds **11a–d**. This result suggests that the imidazolyl group at the R^2 position played an important role on anti-HBV DNA activity. Besides, 2-methylimidazol-1-yl analogues showed less toxic than imidazol-1-yl analogues (**13a** vs **13b**, **13d** vs **13e**, **13f** vs **13g**).

The antiviral activity of these compounds appeared to be related to the electronic effect of the substituents on the phenyl ring

Table 1
The substituents, cytotoxicity, and anti-HBV activity of target compounds in vitro[e]

Compd	R^1	R^2	CC_{50}[a] (μM)	HBsAg		HBV DNA replication	
				IC_{50}[b] (μM)	SI[c]	IC_{50} (μM)	SI
11a	H	Dimethylamino	245.1 ± 4.8	—[d]	—	—	—
11b	H	Pyrrolidin-1-yl	227.0 ± 4.1	—	—	—	—
11c	H	Morpholino	86.9 ± 3.6	—	—	—	—
11d	H	4-Methylpiperazin-1-yl	209.8 ± 4.7	27.0 ± 3.5	7.8	—	—
11e	4-F	Pyrrolidin-1-yl	74.8 ± 3.9	—	—	—	—
11f	4-F	4-Methylpiperazin-1-yl	64.5 ± 2.8	10.7 ± 1.4	6.0	—	—
13a	H	Imidazol-1-yl	76.3 ± 3.3	—	—	9.1 ± 2.0	8.4
13b	H	2-Methylimidazol-1-yl	101.2 ± 3.8	—	—	7.8 ± 1.5	13.0
13c	4-F	Imidazol-1-yl	80.1 ± 3.5	—	—	11.6 ± 1.8	6.9
13d	4-Me	Imidazol-1-yl	60.8 ± 4.6	—	—	32.6 ± 0.9	1.9
13e	4-Me	2-Methylimidazol-1-yl	91.2 ± 3.5	—	—	36.1 ± 2.0	2.5
13f	4-OMe	Imidazol-1-yl	64.5 ± 4.2	—	—	—	—
13g	4-OMe	2-Methylimidazol-1-yl	163.9 ± 4.4	—	—	44.5 ± 2.1	3.7
13h	3-OMe	2-Methylimidazol-1-yl	102.7 ± 3.3	—	—	—	—
14a	H	Morpholino	900.5 ± 5.3	400.6 ± 3.0	2.2	—	—
14b	H	Imidazol-1-yl	556.9 ± 1.8	189.2 ± 3.2	2.9	—	—
14c	4-Me	Imidazol-1-yl	757.8 ± 4.3	175.6 ± 2.3	4.3	—	—
15a	H	Morpholino	739.9 ± 3.6	325.7 ± 3.5	2.3	—	—
15b	H	Imidazol-1-yl	622.8 ± 3.4	70.2 ± 2.8	8.9	—	—
15c	4-Me	Imidazol-1-yl	506.3 ± 3.2	62.6 ± 1.6	8.1	—	—
Lamivudine			2183.1 ± 4.1	—	—	240.0 ± 2.2	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: CC_{50}/IC_{50}).
[d] Means no antiviral activity at the concentration lower than its CC_{50}.
[e] Data are represented by mean values and standard deviations for three separate experiments.

of 2-position. It has been observed that compounds **13a–c** with electron-withdrawing groups such as F atom or with no substitution showed more potent inhibitory activity than compounds **13d–h** with electron-donating groups. With the conversion of sulfur into sulfinyl and sulfonyl group, the cellular toxicity was obviously reduced, whereas a complete loss in anti-HBV DNA activity was also noted (**13a** vs **14b** vs **15b**, **13d** vs **14c** vs **15c**). 2-Sulfinyl analogues **14a–c** and 2-sulfonyl analogues **15a–c** showed moderate activity against HBsAg with IC_{50} values ranging from 62.6 to 400.6 μM. Moreover, 2-sulfonyl analogues were more active than the 2-sulfinyl ones (**14a** vs **15a**, **14b** vs **15b**, and **14c** vs **15c**). Compounds **11a**–**15c** were also investigated for inhibition of HBeAg secretion in HepG2.2.15 cells and none of them possessed activity up to their 50% cytotoxic concentration.

In summary, a series of novel 1-methyl-1*H*-benzimidazol-5-ol derivatives were synthesized and assessed for their anti-HBV activity and cytotoxicity in vitro, using lamivudine as reference control. Six compounds showed significant inhibition of HBV DNA replication with IC_{50} values less than 45 μM. Of them, compound **13b**, with an IC_{50} value of 7.8 μM and a SI value of 13.0, was found to be more potent than lamivudine. The preliminary SAR provided useful indications for guiding the further design of new 1*H*-benzimidazol-5-ols as more active HBV inhibitors.

Acknowledgments

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14. *Spectroscopic data of selected compounds*: **11d**: mp 140–142 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.17 (s, 3H), 2.35 (m, 8H), 3.71 (s, 3H), 3.99 (s, 2H), 4.50 (s, 2H), 6.67 (d, J = 8.6 Hz, 1H), 7.22 (m, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.46 (d, J = 7.5 Hz), 10.49 (s, 1H). IR (KBr) cm^{-1}: 3445.1, 2944.4, 2795.7, 1616.1, 1585.4, 1467.1, 1208.5, 804.7, 741.3. MS (ESI) m/z: 383.0 (M+H). *Compound* **13a**: mp 135–137 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.74 (s, 3H), 4.56 (s, 2H,), 5.31 (s, 2H), 6.74(s, 1H), 6.81 (d, J = 8.7 Hz, 1H), 6.99 (s, 1H), 7.18–7.37 (m, 4H), 7.48 (d, J = 7.5 Hz, 2H), 7.60 (s, 1H), 9.44 (s, 1H). IR (KBr) cm^{-1}: 3417.9, 2663.6, 1602.0, 1506.5, 1480.5, 1424.0, 1395.1, 1224.6, 1051.7, 803.3, 730.6. MS (ESI) m/z: 351.2 (M+H). *Compound* **13b**: mp 141–143 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.45 (s, 3H), 3.75 (s, 3H), 4.55 (s, 2H), 5.22 (s, 2H), 6.62 (s, 1H), 6.82 (d, J = 8.5 Hz, 1H), 6.96 (s, 1H), 7.19–7.33 (m, 4H), 7.47 (d, J = 7.4 Hz, 2H), 9.46 (s, 1H). IR (KBr) cm^{-1}: 3427.8, 2924.6, 1594.2, 1499.1, 1418.9, 1383.9, 1269.8, 1147.1, 807.3. MS (ESI) m/z: 365.1 (M+H). *Compound* **14b**: mp 200–202 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.64 (s, 3H), 4.61 (d, J = 13.3 Hz, 1H), 4.73 (d, J = 13.4 Hz, 1H), 5.30 (s, 2H), 6.76 (s, 1H), 6.84 (d, J = 8.7 Hz, 1H), 6.98 (s, 1H), 7.30 (d, J = 8.7 Hz, 1H), 7.51–7.66 (m, 6H), 9.50 (s, 1H). IR (KBr) cm^{-1}: 3429.8, 2926.4, 2643.0, 1603.5, 1475.2, 1428.9, 1282.0, 1227.8, 1050.9, 802.8. MS (ESI) m/z: 367.2 (M+H). *Compound* **15b**: mp 209–211 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.77 (s, 3H), 5.21 (s, 2H), 5.25 (s, 2H), 6.78 (s, 2H), 6.87 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 8.7 Hz, 1H), 7.51 (s, 1H), 7.62 (t, J = 7.6 Hz, 2H), 7.79 (d, J = 7.7 Hz, 3H), 9.53 (s, 1H). IR (KBr) cm^{-1}: 3420.1, 2950.9, 1604.2, 1481.8, 1427.0, 1308.5, 1223.4, 1151.9, 1083.0, 803.4, 729.5. MS (ESI) m/z: 383.0 (M+H).
15. In vitro anti-HBV assays: 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. Cultures were treated with eight consecutive daily doses of the test compounds and lamivudine. 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.[16,17] Intracellular HBV DNA levels were measured by quantitative Southern blot hybridization.

The IC_{50} and selected index of the evaluated compounds and lamivudine were calculated, respectively. Cytotoxicity assay: 2.2.15 cells were grown to confluence in 96-well flat-bottomed tissue culture plates and treated with tested 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 cells' 510 nm absorbance relative to untreated cells, after the treatment of consecutive 9 days.

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