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Original article

Identification of 1-isopropylsulfonyl-2-amine benzimidazoles as a new class of inhibitors of hepatitis B virus

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Abstract

A series of 1-isopropylsulfonyl-2-amine benzimidazole derivatives were synthesized and evaluated for their anti-hepatitis B virus (HBV) activity and cytotoxicity in the HepG2.2.15 cell line. In general, these derivatives are potent HBV inhibitors ($IC_{50} < 4$ μM) with high selectivity indices (SIs > 40). Compounds **5b–e**, **g**, **j**, and **9a** were among the most prominent compounds, with IC_{50}s of 0.70–2.0 μM and SIs of 41–274. The potent anti-HBV activity and safety profiles of the most promising compounds **5d** and **j** (IC_{50}s = 0.70 μM, SIs > 120) demonstrate the potential of this series of benzimidazoles for the development of new anti-HBV drugs.

Keywords: Benzimidazoles; Synthesis; Anti-HBV activity

1. Introduction

Hepatitis B virus (HBV) infection is the world's ninth leading cause of death, and responsible for both acute and chronic hepatitis [1]. An estimated 350–400 million people are chronically infected by HBV throughout the world with 0.5–1.2 million global deaths per year. Chronic HBV infection can lead to cirrhosis, liver failure, and hepatocellular carcinoma [2,3]. Only a few agents have been approved for the clinical treatment of HBV infections: α-interferon (IFN-α), lamivudine (3-TC), adefovir dipivoxil, and entecavir (Fig. 1). Interferon-α, an immunomodulator and the first therapeutic agent developed for HBV carries since the early 1980s, suffers from low cure rate (only effective for 30–40% patients under this treatment) and serious side effects [4]. The form of i.v. administration and expensive price limit its application. Lamivudine and adefovir dipivoxil are two nucleoside analogues with excellent activity and oral bioavailability. The mechanism of their functions is believed to mainly act by inhibition of HBV polymerase activity resulting in quantitative decrease in viral replication with few side effects in most patients [5]. However, lamivudine has the potential of inducing drug resistant HBV after 6–12 months of therapy, and causes an associated risk of increase of viremia during the therapy in HBV patients. Further, after cessation of this treatment, relapse rates are reported high: long-lasting virological and biochemical responses have been maintained in approximately 20% of patients, and a 74% relapse rate has been reported at 12 months after therapy withdrawal [4,6]. Adefovir dipivoxil has proven to be

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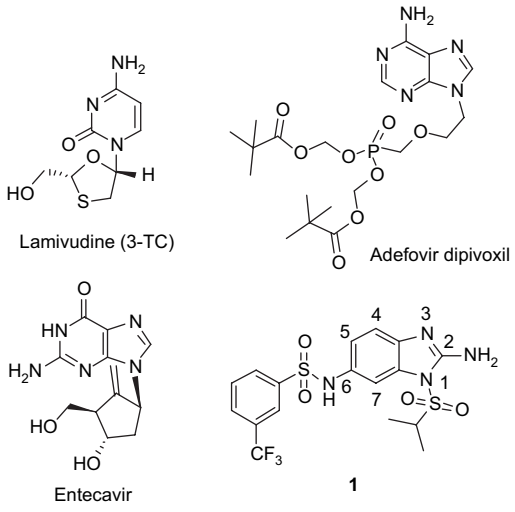


Fig. 1.

effective in the treatment of infections with lamivudine-resistant HBV in vitro and in vivo [7]. It might be used as a therapeutic option in view of its good tolerability and few resistant HBV mutants. However, similar to lamivudine, relapse rates are also high because the nucleoside analogues cannot eradicate the covalently closed circular (ccc) DNA pool of HBV. Entecavir, which was approved by the FDA in 2005 for the treatment of chronic hepatitis B infection, was found to be more potent than lamivudine and effective to lamivudine-resistant HBV mutant [8,9]. However, it may exert its effects via the same biological mechanism and encounter the same problems as lamivudine and adefovir dipivoxil because of their similar chemical structures.

Although the combination therapy of lamivudine with adefovir dipivoxil, or antiviral drugs together with immunomodulators such as IFN-α might be more effective and safer in the therapeutic process [4], it is still limited [10]. Therefore, there is an urgent need for the development of novel classes of anti-HBV agents with new molecular structures and optimal pharmacological profiles for the chemotherapy of HBV infection.

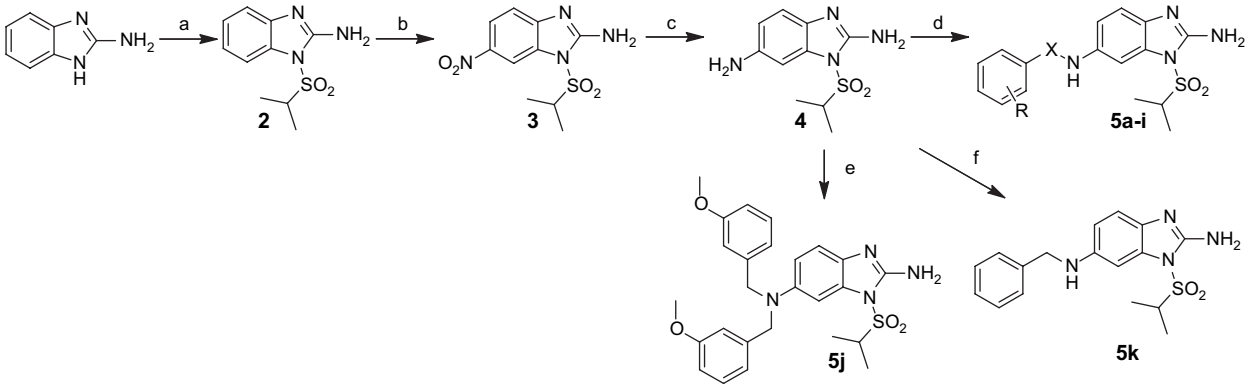
In an ongoing effort to identify such compounds [11], we continuously screen our in-house collection of compounds for active leads. *N*-(2-Amino-1-(isopropylsulfonyl)-1*H*-benzo[*d*]imidazol-6-yl)-3-(trifluoromethyl)benzenesulfonamide (**1**, Fig. 1), with an IC_{50} of 4 μM in inhibiting HBV DNA replication and selectivity index (SI) of 27 in vitro, was identified from our sample collection. To date, 1-isopropylsulfonyl-2-amine benzimidazole derivatives have not been reported to exhibit activity against HBV, even though a set of structurally related analogues has been reported to have potent broad spectrum antiviral activity against both rhinoviruses and enteroviruses [12–14].

Herein, we chose compound **1** as a new lead compound and developed a series of related benzimidazoles, which were mainly modified at positions 5 and 6 on the fused phenyl ring of the benzimidazole core, with significant anti-HBV activities.

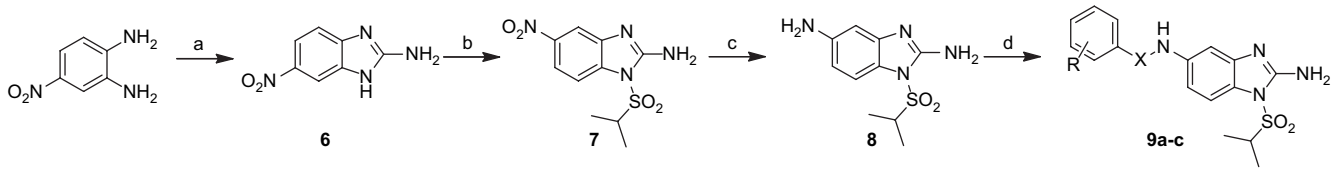
2. Chemistry

The syntheses of this class of benzimidazoles are shown in Schemes 1 and 2. Treatment of commercially available 2-aminobenzimidazole with isopropylsulfonyl chloride in the presence of NaOH in a mixture of acetonitrile and water led to the formation of compound **2** [15]. Nitration of **2** with 67% nitric acid in concentrated sulfuric acid afforded a mixture of 6-nitro (**3**) and 5-nitro (**7**) analogues. After recrystallization the mixture from ethanol twice, the major product 6-nitro compound **3** was obtained as yellow crystals in 65% yield [16]. Compound **3** was subsequently reduced with 10% Pd/C under hydrogen atmosphere in acidic conditions, to give the corresponding 6-amine derivative **4**. Treatment of compound **4** with various benzoyl, benzenesulfonyl and benzyl chlorides furnished derivatives **5a–k**.

The isomeric position 5 benzimidazole derivatives were prepared by another route (Scheme 2). Starting from 4-nitro-1,2-phenylenediamine, benzimidazole **6** was synthesized in good yield. N-Sulfonylation of the free benzimidazole nitrogen of **6** with isopropylsulfonyl chloride in the presence of K_2CO_3 in acetone at room temperature produced the



Scheme 1. Synthetic pathway to substituted 1-(isopropylsulfonyl)-2,6-diamine benzimidazole analogs **5a–k**. *Reagents and conditions*: (a) $(CH_3)_2CHSO_2Cl$, CH_3CN/H_2O, NaOH, rt; (b) 67% HNO_3, concd. H_2SO_4, 0 °C to rt; (c) 10% Pd/C, H_2, 2 N HCl, rt; (d) R-benzoyl or R-benzenesulfonyl chlorides, dry pyridine, rt; (e) 3-methoxybenzyl chloride, Na_2CO_3, EtOH, reflux; (f) benzyl chloride, $NaHCO_3$, $EtOH/H_2O$, reflux.



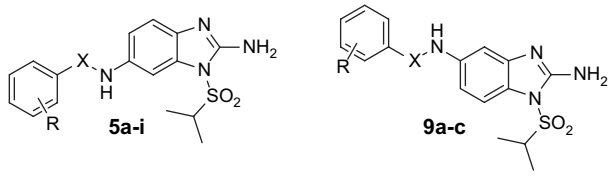
Scheme 2. Synthetic pathway to substituted 1-(isopropylsulfonyl)-2,5-diamine benzimidazole analogs **9a–c**. *Reagents and conditions*: (a) BrCN, H_2O, reflux; (b) $(CH_3)_2CHSO_2Cl$, acetone, K_2CO_3, rt; (c) 10% Pd/C, H_2, 2 N HCl, rt; (d) R-benzoyl or R-benzenesulfonyl chlorides, dry pyridine, rt.

5-nitro derivative **7** with a minor amount of the 6-nitro analogue **3**. Compound **7** was obtained as a pure product after crystallization of the product mixture from acetonitrile and recrystallization from methanol subsequently. Compound **7** was then reduced to afford the corresponding 5-amine derivative **8**. Derivatives **9a–c** were prepared by the reaction of compound **8** with appropriate benzoyl and benzenesulfonyl chlorides.

3. Results and discussion

The synthesized benzimidazole analogues were evaluated for their anti-HBV activity and cytotoxicity with the antiviral drugs lamivudine and adefovir as reference controls in HepG2.2.15 cells, and the results are summarized in Table 1.

Table 1
Anti-HBV activity and cytotoxicity of 1-isopropylsulfonyl-2-amine benzimidazole analogues in vitro



Compds.	X	R	IC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
5a	CO	H	33.7	>500	>15
5b	CO	2-F	0.94	48	51
5c	CO	4-F	1.2	67	56
5d	CO	2,6-DiF	0.70	192	274
5e	CO	2-Cl	0.82	88	107
5f	CO	4-CH_3	4.2	>500	>119
5g	SO_2	H	1.5	62	41
5h	SO_2	4-CH_3	4.8	80	17
5i	SO_2	3-NO_2	10.5	57	5
5j	–	–	0.70	86	123
5k	–	–	NA	98	–
9a	CO	H	2.0	>500	>250
9b	CO	4-F	3.7	34	9
9c	SO_2	4-CH_3	16	151	9
Lamivudine	–	–	0.38	>1000	>2632
Adefovir	–	–	1.7	57	34

NA, not active.
[a] Concentrations of compounds achieving 50% inhibition of cytoplasmic HBV DNA synthesis.
[b] Concentrations of compounds required for 50% extinction of HepG2.2.15 cells.
[c] Selectivity index (SI) was determined as the CC_{50}/IC_{50} value.

As we investigated the substituents on the benzoyl ring, several derivatives with substituents of different electronic, steric, lipophilic, and substituted position characters on the phenyl were prepared (Scheme 1, **5a–f**). We found that the unsubstituted derivative **5a** had only a weak antiviral activity ($IC_{50} = 33.7$ μM) and was significantly less potent than the substituted benzoyl derivatives **5b–f**. This result suggests that the substituent on the benzoyl ring is an important feature in conferring relatively potent inhibitory activity. Among derivatives **5b–f**, the halide substituted analogues **5b–e** demonstrated IC_{50} values between 0.7 and 1.2 μM, and were more active than the methyl substituted analogue **5f** ($IC_{50} = 4.2$ μM). Changing the position of the F group from *ortho* position (**5b**, $IC_{50} = 0.94$ μM, SI = 51) to *para* position (**5c**, $IC_{50} = 1.2$ μM, SI = 56) could not improve the inhibitory activity, while introduction of another F atom at the *ortho* position (**5d**, $IC_{50} = 0.70$ μM, SI = 274) slightly increased the potency. Enlarging the bulk of the substituent from F (**5b**) to Cl (**5e**, $IC_{50} = 0.82$ μM, SI = 107) led to increase in both antiviral potency and SI. The bis(fluoro) derivative **5d** displayed more potent antiviral activity ($IC_{50} = 0.70$ μM) than other halide substituted analogues (**5b**, **c**, and **e**) and a 48-fold enhancement in antiviral potency relative to the unsubstituted benzoyl derivative **5a**, and demonstrated the best SI (274). Compound **5d** emerged as the optimal compound in this series.

To identify the effects of substituting the benzoyl moiety with benzenesulfonyl group, three benzenesulfonamide derivatives were prepared (Scheme 1, **5g–i**). The 6-benzenesulfonamide derivative **5g** ($IC_{50} = 1.5$ μM, SI = 41) showed anti-HBV potency comparable to that of the 4-F benzoyl derivative **5c** ($IC_{50} = 1.2$ μM, SI = 56) and adefovir ($IC_{50} = 1.7$ μM, SI = 34). An electron-donating methyl group at the 4-position (**5h**) and an electron-withdrawing nitro group at the 3-position (**5i**) were examined and they led to decrease in both the IC_{50} and SI values. As for the benzyl analogues, the bis(3-methoxybenzyl) derivative **5j** ($IC_{50} = 0.7$ μM, SI = 123) exhibited similar antiviral potency to that of the bis(fluoro) derivative **5d**. However, compound **5k**, which bears no substituent on the phenyl, proved to be virtually inactive.

With the purpose of investigating the anti-HBV effects of regioisomers, we prepared three 5-position substituted derivatives (Scheme 2, **9a–c**). As seen in Table 1, the 5-benzoyl derivative **9a** exhibited a potency that was nearly 17-fold greater than its corresponding 6-benzoyl derivative **5a**. No significant toxicities were observed for **9a** at the highest tested concentration of 500 μM, which led to a high selectivity index (>250). However, compounds **9b** and **c** were less potent than their 6-position isomers.

4. Conclusions

In conclusion, a series of 1-isopropylsulfonyl-2-amine benzimidazole derivatives based on compound **1** were synthesized and assessed for their anti-HBV activity and cytotoxicity in vitro, using lamivudine and adefovir as reference controls. Most of them proved to be potential HBV inhibitors ($IC_{50} < 4$ μM) with high selectivity indices ($SI > 40$). Compounds **5b–e**, **g**, **j**, and **9a** showed outstanding anti-HBV potency with IC_{50}s of 0.70–2.0 μM and SIs of 41–274. The most promising compounds **5d** and **j** were selected for further assessment in vivo. Further studies of structure–activity relationships and mechanisms of action of this new class of anti-HBV agents are going on in our group.

5. Experimental

5.1. Chemistry

^{1}H NMR spectral data were recorded in DMSO-d_6, or $CDCl_3$ on Varian Mercury 400 or 300 NMR spectrometer and ^{13}C NMR spectral data were recorded in DMSO-d_6, or $CDCl_3$ on Varian Mercury 400 NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm). Data are reported as follows: chemical shift, multiplicity (br s = broad singlet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, m = multiplet, s = singlet and t = triplet), coupling constants (Hz), integration. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded at an ionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. Pyridine was distilled from CaH_2. Column chromatography was carried out on silica gel (200–300 mesh).

5.1.1. 1-(Isopropylsulfonyl)-6-nitro-1H-benzo[d]imidazol-2-amine (3)

To a solution of **2** (6.0 g, 25.1 mmol) in 80 mL of concentrated sulfuric acid, which was cooled to 0 °C, was added dropwise 67% nitric acid (18.0 mL, 27.6 mmol). After that, the reaction mixture was stirred at room temperature for 3 h and then poured into crushed ice slowly. The resulting mixture was neutralized to pH 8 with 25% NH_4OH. The formed precipitate was filtered, washed with water, and dried to give the crude product, which was recrystallized from EtOH twice to give the title compound as yellow crystals (4.6 g, 65% yield); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (d, 1H, $J = 2.0$ Hz), 8.10 (dd, 1H, $J = 8.8$, 2.4 Hz), 7.68 (br s, 2H), 7.34 (d, 1H, $J = 9.2$ Hz), 4.00 (m, 1H), 1.29 (d, 6H, $J = 6.8$ Hz); ^{13}C NMR (400 MHz, DMSO-d_6) δ 156.36, 148.83, 140.34, 130.91, 121.21, 115.19, 107.67, 56.12, 15.58 (2C); MS (EI) *m/z* 284 (M^+), 178, 149, 57.

5.1.2. 1-(Isopropylsulfonyl)-1H-benzo[d]imidazole-2,6-diamine (4)

Compound **3** (680 mg, 2.4 mmol) was hydrogenated over 10% Pd/C (70 mg) in 2 N HCl (50 mL) at room temperature overnight. The reaction mixture was filtered, neutralized with 25% NH_4OH, and extracted with ethyl acetate. The organic extracts were washed with brine, dried over anhydrous $MgSO_4$ and concentrated to afford **4** (600 mg, 99% yield) as a brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, 1H, $J = 8.4$ Hz), 7.00 (s, 1H), 6.61 (dd, 1H, $J = 8.4$, 2.2 Hz), 3.65 (m, 1H), 1.41 (d, 6H, $J = 6.9$ Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 151.09, 141.59, 133.94, 132.13, 117.10, 112.75, 99.54, 55.67, 16.01 (2C); MS (EI) *m/z* 254 (M^+), 147, 120, 57.

5.1.3. 6-Nitro-1H-benzo[d]imidazol-2-amine (6)

A suspension of 4-nitro-*o*-phenylenediamine (1.4 g, 9.1 mmol) in a solution of BrCN (0.97 g, 9.2 mmol) in water (30 mL) was refluxed for 7 h, cooled, and neutralized with 25% NH_4OH to pH 10–11. The formed precipitate was filtered, washed with water and dried to give the title compound as a yellow solid (1.5 g, 92% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (d, 1H, $J = 2.4$ Hz), 7.89 (dd, 1H, $J = 8.7$, 2.4 Hz), 7.20 (d, 1H, $J = 8.4$ Hz), 7.01 (br s, 2H).

5.1.4. 1-(Isopropylsulfonyl)-5-nitro-1H-benzo[d]imidazol-2-amine (7)

To a reaction mixture containing **6** (2.0 g, 11.2 mmol) and K_2CO_3 (1.6 g, 11.6 mmol) in acetone (70 mL) was added dropwise 2-propanesulfonyl chloride (1.6 g, 11.2 mmol). The reaction mixture was stirred at room temperature for 4 h and treated with 1 N HCl (360 mL). The resulting mixture was stirred for 1 h at room temperature and filtered. The filtrate was neutralized to pH 6. The formed precipitate was filtered, crystallized from acetonitrile and recrystallized from methanol subsequently to give compound **7** as yellow crystals (0.66 g, 20% yield); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (d, 1H, $J = 2.1$ Hz), 7.97 (dd, 1H, $J = 8.4$, 2.1 Hz), 7.68 (d, 1H, $J = 8.7$ Hz), 7.39 (br s, 2H), 3.97 (m, 1H), 1.31 (d, 6H, $J = 6.6$ Hz); MS (EI) *m/z* 284 (M^+), 178, 148, 132, 105, 77.

5.1.5. 1-(Isopropylsulfonyl)-1H-benzo[d]imidazole-2,5-diamine (8)

The product was obtained from **7** using a procedure similar to that described for the preparation of **4** as a brown solid. Yield = 99%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, 1H, $J = 8.4$ Hz), 6.69 (d, 1H, $J = 2.4$ Hz), 6.45 (dd, 1H, $J = 8.4$, 2.0 Hz), 5.78 (br s, 2H), 3.60 (m, 1H), 1.36 (d, 6H, $J = 6.8$ Hz); MS (EI) *m/z* 254 (M^+), 147, 120, 105.

5.2. General procedure for the preparation of derivatives 5a–i and 9a–c

A stirred solution of compound **4** or compound **8** (1 equiv) in dry pyridine (12 mL/mmol) was treated in one portion with the appropriate acyl chloride (1 equiv). The reaction mixture was stirred at room temperature overnight and poured into water (30 mL/mmol). The resulting precipitate was filtered and dried to obtain the product.

*5.2.1. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)benzamide (**5a**)*

Compound **5a** was prepared from **4** and benzoyl chloride as a white solid. Yield = 85%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.26 (s, 1H), 8.11 (d, 1H, J = 2.1 Hz), 7.95 (d, 2H, J = 7.8 Hz), 7.61–7.49 (m, 4H), 7.21 (d, 1H, J = 9.0 Hz), 3.84 (m, 1H), 1.29 (d, 6H, J = 6.9 Hz); MS (EI) *m/z* 358 (M^+), 252, 105, 77; HRMS (EI): cal. for $C_{17}H_{18}N_4O_3S$ 358.1100, found 358.1108.

*5.2.2. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)-2-fluorobenzamide (**5b**)*

Compound **5b** was prepared from **4** and 2-fluorobenzoyl chloride as a white solid. Yield = 82%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.40 (s, 1H), 8.10 (d, 1H, J = 1.8 Hz), 7.69–7.48 (m, 3H), 7.37–7.30 (m, 2H), 7.20 (d, 1H, J = 8.7 Hz), 6.88 (br s, 2H), 3.84 (m, 1H), 1.28 (d, 6H, J = 6.6 Hz); MS (EI) *m/z* 376 (M^+), 270, 123; HRMS (EI): cal. for $C_{17}H_{17}FN_4O_3S$ 376.1005, found 376.0997.

*5.2.3. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)-4-fluorobenzamide (**5c**)*

Compound **5c** was prepared from **4** and 4-fluorobenzoyl chloride as a white solid. Yield = 81%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27 (s, 1H), 8.07–8.01 (m, 3H), 7.55 (dd, 1H, J = 8.8, 2.0 Hz), 7.37–7.32 (m, 2H), 7.19 (d, 1H, J = 8.7 Hz), 3.82 (m, 1H), 1.27 (d, 6H, J = 6.4 Hz); MS (EI) *m/z* 376 (M^+), 270, 123, 79; HRMS (EI): cal. for $C_{17}H_{17}FN_4O_3S$ 376.1005, found 376.1004.

*5.2.4. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)-2,6-difluorobenzamide (**5d**)*

Compound **5d** was prepared from **4** and 2,6-difluorobenzoyl chloride as a white solid. Yield = 80%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.79 (s, 1H), 8.04 (s, 1H), 7.56 (m, 1H), 7.46 (dt, 1H, J = 8.4, 1.8 Hz), 7.23 (m, 3H), 3.83 (m, 1H), 1.27 (d, 6H, J = 6.9 Hz); MS (EI) *m/z* 394 (M^+), 288, 141; HRMS (EI): cal. for $C_{17}H_{16}F_2N_4O_3S$ 394.0911, found 394.0897.

*5.2.5. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)-2-chlorobenzamide (**5e**)*

Compound **5e** was prepared from **4** and 2-chlorobenzoyl chloride as a white solid. Yield = 79%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, 1H, J = 1.8 Hz), 7.98 (s, 1H), 7.79 (dd, 1H, J = 7.4, 1.9 Hz), 7.48–7.34 (m, 5H), 5.74 (br s, 2H), 3.72 (m, 1H), 1.42 (d, 6H, J = 6.9 Hz); MS (EI) *m/z* 392 (M^+), 286, 139; HRMS (EI): cal. for $C_{17}H_{17}ClN_4O_3S$ 392.0710, found 392.0714.

*5.2.6. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)-4-methylbenzamide (**5f**)*

Compound **5f** was prepared from **4** and 4-methylbenzoyl chloride as a white solid. Yield = 88%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.85 (s, 1H), 7.78 (d, 2H, J = 8.1 Hz), 7.41–7.28 (m, 4H), 5.67 (br s, 2H), 3.70 (m, 1H), 2.43 (s, 3H), 1.42 (d, 6H, J = 6.6 Hz); ^{13}C NMR (400 MHz, DMSO-d_6) δ 164.98, 152.60, 141.39, 138.41, 132.74, 132.15, 131.07, 128.87 (2C), 127.63 (2C), 117.50, 115.46, 105.22, 55.44, 21.01, 15.65 (2C); MS (EI) *m/z* 372 (M^+), 266, 119, 79; HRMS (EI): cal. for $C_{18}H_{20}N_4O_3S$ 372.1256, found 372.1256.

*5.2.7. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)benzenesulfonamide (**5g**)*

Compound **5g** was prepared from **4** and benzenesulfonyl chloride as a white solid. Yield = 81%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65–7.48 (m, 5H), 7.22 (d, 1H, J = 2.1 Hz), 7.09 (d, 1H, J = 8.7 Hz), 6.89 (dd, 1H, J = 8.4, 2.1 Hz), 3.56 (m, 1H), 1.15 (d, 6H, J = 6.6 Hz); MS (EI) *m/z* 394 (M^+), 253, 147, 79, 52; HRMS (EI): cal. for $C_{16}H_{18}N_4O_4S_2$ 394.0769, found 394.0768.

*5.2.8. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)-4-methylbenzenesulfonamide (**5h**)*

Compound **5h** was prepared from **4** and tosyl chloride as a white solid. Yield = 85%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.53 (d, 2H, J = 8.1 Hz), 7.29 (d, 2H, J = 8.4 Hz), 7.25 (s, 1H), 7.08 (d, 1H, J = 8.4 Hz), 6.88 (m, 2H), 3.62 (m, 1H), 2.31 (s, 3H), 1.15 (d, 6H, J = 6.9 Hz); ^{13}C NMR (400 MHz, DMSO-d_6) δ 152.71, 142.96, 139.48, 136.32, 131.34, 130.40, 129.43 (2C), 126.80 (2C), 119.40, 115.88, 106.76, 55.64, 20.92, 15.54 (2C); MS (EI) *m/z* 408 (M^+), 253, 147, 91; HRMS (EI): cal. for $C_{17}H_{20}N_4O_4S_2$ 408.0926, found 408.0920.

*5.2.9. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-6-yl)-3-nitrobenzenesulfonamide (**5i**)*

Compound **5i** was prepared from **4** and 3-nitrobenzenesulfonyl chloride as yellow crystals. Yield = 85%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.2 (s, 1H), 8.45–8.41 (m, 2H), 8.04 (d, 1H, J = 7.9 Hz), 7.83 (t, 1H, J = 8.1 Hz), 7.26 (s, 1H), 7.12 (d, 1H, J = 8.3 Hz), 6.92–6.89 (m, 3H), 3.65 (m, 1H), 1.16 (d, 6H, J = 6.6 Hz); MS (EI) *m/z* 439 (M^+), 253, 147, 119; HRMS (EI): cal. for $C_{16}H_{17}N_5O_6S_2$ 439.0620, found 439.0626.

*5.2.10. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-5-yl)benzamide (**9a**)*

Compound **9a** was prepared from **8** and benzoyl chloride as an off-white solid. Yield = 88%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (s, 1H), 7.95 (m, 2H), 7.74 (s, 1H), 7.59–7.34 (m, 5H), 3.84 (m, 1H), 1.26 (d, 6H, J = 6.6 Hz); MS (EI) *m/z* 358 (M^+), 251, 105, 77; HRMS (EI): cal. for $C_{17}H_{18}N_4O_3S$ 358.1100, found 358.1107.

*5.2.11. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]-imidazol-5-yl)-4-fluorobenzamide (**9b**)*

Compound **9b** was prepared from **8** and 4-fluorobenzoyl chloride as an off-white solid. Yield = 55%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.22 (s, 1H), 8.06–8.02 (m, 2H), 7.74 (s, 1H), 7.46–7.34 (m, 4H), 3.85 (m, 1H), 1.25 (d, 6H, J = 6.6 Hz); ^{13}C NMR (400 MHz, DMSO-d_6) δ 164.25, 164.00 ($J_{C,F}$ = 247.1 Hz), 153.14, 142.42, 135.89, 131.47,

130.32 (2C), 127.53, 115.30 (2C), 113.37, 111.49, 108.49, 55.40, 15.62 (2C); MS (EI) *m/z* 376 (M^+), 269, 123, 83; HRMS (EI): cal. for $C_{17}H_{17}FN_4O_3S$ 376.1005, found 376.0996.

5.2.12. N-(2-Amino-1-(isopropylsulfonyl)-1H-benzo[d]imidazol-5-yl)-4-methylbenzenesulfonamide (**9c**)

Compound **9c** was prepared from **8** and tosyl chloride as a white solid. Yield = 73%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.60 (d, 2H, J = 8.1 Hz), 7.32 (m, 3H), 6.96 (s, 1H), 6.77 (dd, 1H, J = 8.7, 2.1 Hz), 3.82 (m, 1H), 2.31 (s, 3H), 1.23 (d, 6H, J = 6.9 Hz); MS (EI) *m/z* 408 (M^+), 301, 253, 149, 91; HRMS (EI): cal. for $C_{17}H_{20}N_4O_4S_2$ 408.0926, found 408.0942.

5.3. 1-(Isopropylsulfonyl)-N6,N6-bis(3-methoxybenzyl)-1H-benzo[d]imidazole-2,6-diamine$^{[N-6]}$ (**5j**)

A mixture of **4** (137 mg, 0.54 mmol) and anhydrous sodium carbonate (80 mg, 075 mmol) in EtOH (5 mL) was heated to reflux. 3-Methoxybenzyl chloride (248 mg, 1.58 mmol) was added and the resulting mixture was refluxed for 18 h. The reaction mixture was filtered while hot and washed with EtOH. The combined mixture was filtered to give **5j** (169 mg, 63% yield) as a pale yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25–7.14 (m, 3H), 6.95–6.71 (m, 8H), 5.52 (br s, 2H), 4.62 (s, 4H), 3.75 (s, 6H), 3.18 (m, 1H), 1.15 (d, 6H, J = 6.9 Hz); MS (EI) *m/z* 494 (M^+), 387, 267, 121; HRMS (EI): cal. for $C_{26}H_{30}N_4O_4S$ 494.1988, found 494.1986.

5.4. N6-Benzyl-1-(isopropylsulfonyl)-1H-benzo[d]imidazole-2,6-diamine $^{[N-6]}$ (**5k**)

To a solution of **4** (222 mg, 0.87 mmol) in EtOH (5 mL) were added sodium bicarbonate (15 mg, 0.18 mmol) and two drops of water. To this ice cooled mixture was added dropwise a solution of benzyl chloride (22 mg, 0.17 mmol) in EtOH (1 mL). The resulting mixture was stirred at room temperature for 0.5 h and then refluxed for 2.5 h, cooled to room temperature, filtered, and concentrated in vacuo. The residue was subjected to silica gel column chromatography ($CHCl_3$) to afford compound **5k** (53 mg, 91% yield) as an orange-yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.28 (m, 5H), 7.17 (d, 1H, J = 6.9 Hz), 6.90 (s, 1H), 6.58 (dd, 1H, J = 8.4, 2.1 Hz), 5.41 (br s, 2H), 4.35 (s, 2H), 3.50 (m, 1H), 1.31 (d, 6H, J = 6.9 Hz); MS (EI) *m/z* 344 (M^+), 237, 147, 91; HRMS (EI): cal. for $C_{17}H_{20}N_4O_2S$ 344.1307, found 344.1305.

5.5. Biological assays

5.5.1. Cell culture and antiviral assays

Details of the design of the antiviral procedure and the growth conditions for HepG2.2.15 cells have been previously described [17,18]. Briefly, confluent cultures in 96-well tissue culture plates were treated with various doses of antiviral compounds in minimal essential medium (MEM) supplemented with 10% fetal bovine serum. Fresh MEM with the same concentration of compounds was replaced on day 4, and the supernatants were harvested on day 8, then the extracellular (virion) HBV DNA was measured by real time fluorescent PCR.

5.5.2. Toxicity measurements

Cytotoxicity of compound was assessed by the MTT assay as previously described [19]. Briefly, HepG2.2.15 cells were cultured in triplicate of 96-well tissue culture plates for 8 days with various doses of test compounds. The cells with media alone were used as controls. MTT (5 g/L) reagent was added 4 h before the end of culture, and then cells were lysed with 10% sodium dodecyl sulfate (SDS), 50% *N*,*N*-dimethylformamide, pH = 7.2. O.D. values were read at 570 nm 6 h later and the cell death percent was calculated.

5.5.3. Real time fluorescent PCR

The supernatants of HepG2.2.15 cells were collected from 8 days' culture after the compounds were added. The HBV DNA in the supernatants was quantified by using fluorescent PCR [11]. Briefly, 50 μL of the supernatants were added into the extraction buffer, boiled for 10 min and centrifuged for 5 min, and then proper aliquots were used for the fluorescent PCR. PCR primers were: P1: 5′-ATCCTGCTGC TATGCCTCATCTT-3′, P2: 5′-ACAGTGGGGAAAGCCCTA CGAA-3′. The probe was 5′-TGGCTAGTTTACTAGTGC CATTTTG-3′. PCR reaction was run at MJ Research PTC-200, and results were analyzed by software OpticonMonitor v2.01.

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