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Inhibitory properties of 2-substituent-1*H*-benzimidazole-4-carboxamide derivatives against enteroviruses

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ABSTRACT

A series of novel benzimidazole derivatives were designed, synthesized, and evaluated for their activities against four kinds of enteroviruses, that is, Coxsackie virus A16, B3, B6 and Enterovirus 71 in VERO cells. Strong activities against enterovirus replication and low cytotoxicities were observed in these benzimidazoles generally. The most promising compound was (L)-2-(pyridin-2-yl)-*N*-(2-(4-nitrophenyl)pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (**16**), with a high antiviral potency (IC_{50} = 1.76 μg/mL) and a remarkable selectivity index (328). These compounds were selected for further evaluation as novel enterovirus inhibitors.

1. Introduction

Benzimidazole compounds were known as promising biologically active agents. The fact that 5,6-dimethylbenzimidazole was a component of natural vitamin B_{12} had triggered the interest of researchers toward benzimidazole derivatives. Benzimidazole compounds had proven abilities to suppress bacterial growth[1] and proton pump function.[2] Additionally, many antihelminthic benzimidazoles were used in the veterinary and medical practice.[3] Due to the ability to interact with DNA, benzimidazole unit became an effective precursor for a wide array of drugs targeting DNA and DNA associated processes. The benzimidazole unit could interact with DNA in the minor groove to interfere with DNA processing enzymes, such as DNA polymerase, RNA polymerase, and topoisomerases I and II. For example, bisbenzimidazole derivatives were strong ligands to inhibit the activity of the ribozyme of *Escherichia coli* RnaseP, effecting the maturation of tRNA.[4]

Enteroviruses were a genus of (+)ssRNA viruses associated with several human and mammalian diseases. Conventionally, enteroviruses were classified into polioviruses, Coxsackie A viruses (CVA), Coxsackie B viruses (CVB), echoviruses, and enteroviruses 68–71 (EV68-71).[5] Coxsackie A viruses tended to infect the skin and mucous membranes, causing herpangina, acute hemorrhagic conjunctivitis and hand-foot-and-mouth disease (HFMD). Coxsackie B viruses tended to infect the heart, pleura, pancreas, and liver, causing pleurodynia, myocarditis, pericarditis, and hepatitis. As one of the major causative agents for HFMD, Enterovirus 71 was associated with severe central nervous system diseases sometimes. In recent years, numerous disease outbreaks worldwide have been caused by enteroviruses. In the spring of 2008, a large HFMD outbreak caused by EV71 in China resulted in a high aggregation of fatal cases.[6] HFMD outbreaks caused by CVA16 also occurred in Asia and all over the world.[7,8] Currently, as no efficient drug was found for the clinical treatment of enteroviruses, there is a tremendous clinical need to develop novel classes of antiviral agents for the treatment of enterovirus infection.

After the host cell was infected, the genome of enterovirus was translated in a cap-independent manner into a single polyprotein, and processed by virus-encoded proteases into structural capsid proteins and nonstructural proteins subsequently. Both kinds of proteins were mainly involved in the replication of virus.[9] Due to

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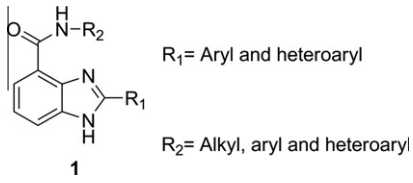


Figure 1. General structure of synthesized compounds.

the special structure of benzimidazole, specific hydrogen bonded interactions could be formed with the genome, interfering the translating process. Therefore, a series of molecules were designed based on the benzimidazole system to inhibit enterovirus.

In our previous work,[10] a series of 2-pyridyl-1*H*-benzimidazole-4-carboxamide derivatives were synthesized, screened and indentified as modest inhibitors of CVB3. In view of its novel structural template, which differed from those of all reported anti-enterovirus agents, we were interested to study further the structure–activity relationships of the related class of compounds. It was believed that a lead compound could be found effective against picornavirus. More benzimidazole derivatives were designed based on these compounds and their biological activities were tested (compounds **14–53** with the general structure shown in Fig. 1). Inhibitory activities of these benzimidazole derivatives were tested against CVA16, CVB3, CVB6, and EV71. There were no active clinical drugs against enterovirus. Therefore, a relatively effective drug, that is, ribavirin (RBV),[11] was selected as the positive control drug. These benzimidazole derivatives were found to exhibit good inhibitory activities against four kinds of enteroviruses. Hopefully, these 2-substituent-1*H*-benzimidazole-4-carboxamide derivatives might be effective against all the enteroviruses, even the entire pico-RNA-virus family, because of their similar inhibitory mechanisms.

2. Chemistry

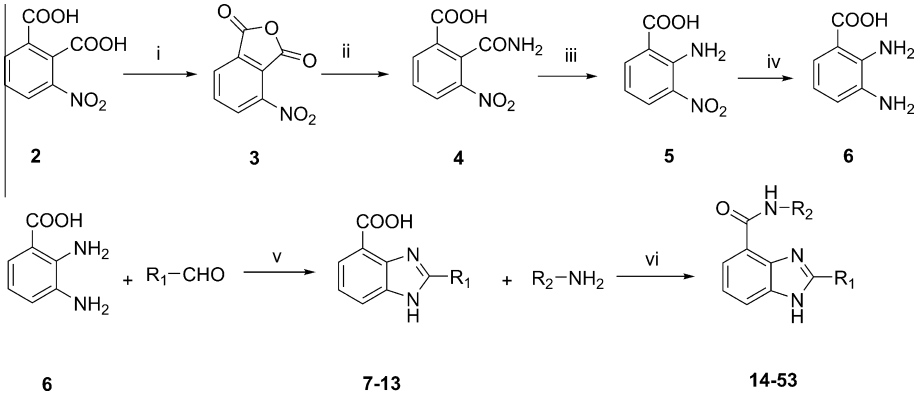
Starting from 3-nitrophthalic acid (**2**), the synthetical route of the 2-substituent-1*H*-benzimidazole-4-carboxamide derivatives (**14**–**53**) is shown in Scheme 1. The 3-nitrophthalic anhydride (**3**) was obtained in 90% yield by the anhybridization of 3-nitrophthalic acid (**2**) in acetic anhydride.[12] Compound **3** was transformed to 3-nitrophthalamic acid (**4**) by the treatment of aqueous ammonia,[10] and compound **4** was converted into 2-amino-3-nitro-benzoic acid (**5**) by Hofmann rearrangement.[13] The nitro group was reduced with hydrazine hydrate/methanol catalyzed by Raney nickel, and the obtained 2,3-diaminobenzoic acid (**6**) was isolated as bis-hydrochloride salt.[10]

Cyclization of 2,3-diaminobenzoic acid (**6**) with compounds containing aldehyde group provided the corresponding 2-(substituent)-1*H*-benzimidazole-4-carboxylic acids (compounds **7–13**) in about 80% yield respectively. 2-Substituent-1*H*-benzimidazole-4-carboxamide derivatives (compounds **14–53**) were synthesized by the amidation of corresponding 2-R_1-1*H*-benzimidazole-4-carboxylic acids (**7–13**) and amines under regular EDC/HOBt conditions.

3. Result and discussion

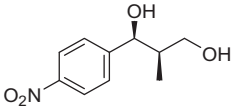
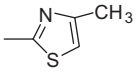
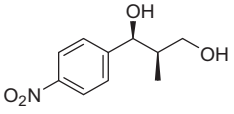
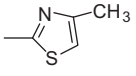
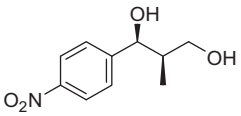
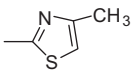
The potential anti-enterovirus activities and cytotoxicities of these synthesized benzimidazoles were evaluated in VERO cells against CVB3, CVA16, CVB6, and EV71. As there were no active clinical drugs against enterovirus and RBV was the recommended clinical antiviral drug in China, the IC_{50} values of RVB were provided as comparable data. These compounds were tested in different batches with various test items respectively. Pyridyl derivatives (**14–30**) and furyl derivatives (**31–36**) were evaluated against CVB3, and phenyl derivatives (**37–53**) were evaluated against CVB3, CVA16, CVB6, and EV71. From the activities of phenyl derivatives (**37–53**) against different enteroviruses, it was inferred that if effective against one kind of enterovirus, a compound could be effective against others. Therefore, activities against Cox A16, Cox B6, and Ev71 of pyridyl and furyl derivatives were not tested in our following studies. The results are summarized in Tables 1 and 2. The anti-enterovirus activity of each compound was expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) of enterovirus growth. The cytotoxicity of each compound was expressed as the concentration of compound required to kill 50% (TC_{50}) of the VERO cells. As a major pharmaceutical parameter for possible future clinical development, the selectivity index (SI) was determined as the ratio of TC_{50} to IC_{50}. The bioactivity of each compound was evaluated by the combination of its IC_{50} and SI.

The antiviral activities of these compounds against CVB3 are summarized in Tables 1 and 2. As shown in Table 1, compounds **14–30** with moieties of 2-pyridyl, 3-pyridyl and 4-pyridyl at the R_1 position generally exhibited good antiviral potency against CVB3 with IC_{50}s of less than 150 μM, much better than RVB with IC_{50} of 1690 μM. IC_{50}s of compounds **16**, **17**, **18**, **19**, and **23** were even less than 5 μM. As the most potent compound in this subseries, compound **16** (IC_{50} = 4.06 ± 0.6 μM and SI = 328) was 67 times more selective than RVB (SI = 4.9). Assay for the activity against EV71 of compound **16** had been carried out. It was found that the activity against EV71 of compound 16 was also significant (this result is presented in the Supplementary data). IC_{50}s of compounds **17**, **18**, **19**, and **23** (IC_{50} = 0.459 ± 0.1, 1.63 ± 0.2, 1.76 ± 0.2, and



Scheme 1. Synthetical route of 2-substituent-1*H*-benzimidazole-4-carboxamide derivatives (**14**–**53**). Reagents and conditions: (i) acetic anhydride, reflux; (ii) NH_3(aq), 0 °C; (iii) NaClO, NaOH, H_2O, 0–80 °C; (iv) NaOH, CH_3OH, Raney Ni, $N_2H_4{\cdot}H_2O$, reflux; (v) O_2, DMF, 80 °C; (vi) EDC·HCl, HOBt, Et_3N, DMF, 0–25 °C.

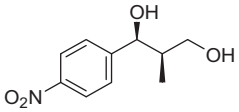
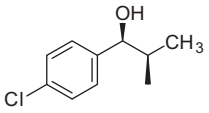
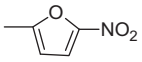
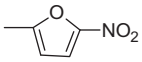
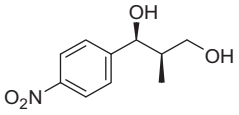
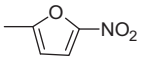
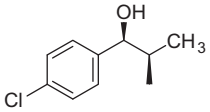
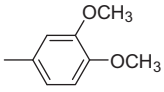
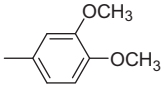
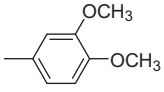
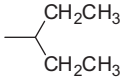
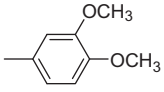
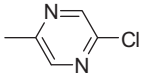
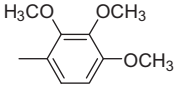
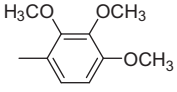
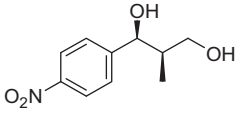
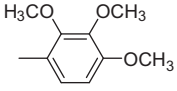
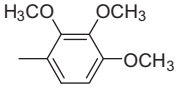
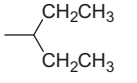
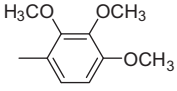
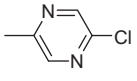
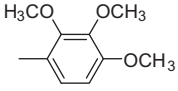
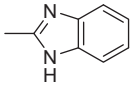
Table 1
Activity of benzimidazole derivatives against Coxsackie virus B3 in VERO cells

Compd	R_1	R_2	TC_{50}[a] (μM)	IC_{50}[b] (μM)	SI[c]
14		H	224 ± 33	30.6 ± 4.6	7.3
15		$-CH_2CH_2OH$	568 ± 84	131 ± 19.6	4.3
16			1332 ± 72	4.06 ± 0.6	328
17			17.9 ± 2.7	0.459 ± 0.1	38.9
18			54.7 ± 8.2	1.63 ± 0.2	28.4
19			17.7 ± 3.2	1.76 ± 0.2	10
20		H	77.7 ± 11.7	26 ± 3.9	3
21		$-CH_2CH_2OH$	682 ± 102	90.7 ± 13.6	7.5
22			148 ± 18	28.6 ± 4.3	5.2
23			34.2 ± 5.1	3.72 ± 0.6	9.2
24			54.7 ± 8.2	9.73 ± 1.5	3.2
25			111 ± 16.6	16.2 ± 2.4	6.8
26		$-CH_2CH_2OH$	498 ± 74	NT[d]	–
27			123 ± 18.4	30.2 ± 4.5	4.1
28			49.8 ± 7.5	12.3 ± 1.8	4
29			111 ± 16.6	37.2 ± 5.6	3
30			111 ± 16.6	21.3 ± 3.2	5.2
RVB	–	–	8190 ± 1228	1690 ± 254	4.9

[a] Cytotoxic concentration required to inhibit VERO cell growth by 50%.
[b] Concentration required to inhibit Coxsackie virus B3 growth by 50%.
[c] Selectivity Index values equaled to TC_{50}/IC_{50}.
[d] Not tested.

3.72 ± 0.6 μM, respectively) were better than compound **16**. IC_{50} of compound **18** was best in this subseries. However, compounds **17**, **18**, **19**, and **23** had pronounced cytotoxicity (TC_{50} = 17.9 ± 2.7, 54.7 ± 8.2, 17.7 ± 3.2, and 34.2 ± 5.1 μM, respectively) resulting in relatively small selectivity indices (SI = 38.9, 28.4, 10, and 9.2, respectively). Compounds **15** and **21** with hydroxyethyl at the R_2 position showed higher IC_{50}s (IC_{50} = 131 ± 19.6 and 90.7 ± 13.6 μM, respectively) than other compounds in this subseries (IC_{50}s less than 45 μM), but compounds **15**, **21**, and **26** appeared to be less toxic (TC_{50} = 568 ± 82, 682 ± 102 and 498 ± 74 μM, respectively) than most of the other compounds (TC_{50} less than 300 μM except compound **16**). It was indicated that both antiviral activity and cytotoxicity could be reduced due to the introduction of hydroxyethyl in the R_2 position. Generally, 2-pyridyl derivatives were more efficient than 3-pyridyl and 4-pyridyl derivatives. Structurally, the main difference between 2-pyridyl

Table 2
Activity of benzimidazole derivatives against Coxsackie virus B3 in VERO cells

Compd	R_1	R_2	TC_{50}[a] (μM)	IC_{50}[b] (μM)	SI[c]
31			25 ± 3.8	3.3 ± 0.5	7.5
32			152 ± 22.8	37.7 ± 5.6	4
33			162 ± 24.3	13.4 ± 2	12.1
34			75.9 ± 11.4	25.3 ± 3.8	3
35			68.7 ± 10.3	22.9 ± 3.4	3
36			48.6 ± 7.3	16.2 ± 2.4	3
37			24.5 ± 3.7	3.5 ± 0.5	7
38		$-CH_2CH_2CH_2OH$	46 ± 6.9	17.2 ± 2.6	2.7
39			2.23 ± 0.3	0.7 ± 0.1	3
40			78.3 ± 11.7	18.4 ± 2.8	4.3
41			37.8 ± 5.7	9.8 ± 1.5	3.9
42			9.85 ± 1.5	4.73 ± 0.7	2
43		$-CH_2CH_2CH_2OH$	40 ± 6	19.5 ± 2.9	2.1
44			32.3 ± 4.8	18.7 ± 2.8	1.7
45			65.2 ± 9.8	17.4 ± 2.6	3.8
46			29 ± 4.3	16.9 ± 2.5	1.7
RVB	—	—	8190 ± 1228	1690 ± 254	4.9

[a] Cytotoxic concentration required to inhibit VERO cell growth by 50%.
[b] Concentration required to inhibit Coxsackie virus B3 growth by 50%.
[c] Selectivity Index values equaled to TC_{50}/IC_{50}.

and 3-/4-pyridyl derivatives was that a hydrogen bond could be formed between H in position 1 of benzimidazole and N in 2-pyridyl, while it was not achieved in 3-pyridyl and 4-pyridyl derivatives. The reason why 2-pyridyl derivatives were more efficiently than 3-pyridyl and 4-pyridyl compounds might rely on this structural difference.

Compounds **31–46** had groups of furyl and phenyl at the R_1 position. As shown in Table 2, these derivatives were found to have excellent IC_{50}s (IC_{50}s less than 45 μM), much better than RVB with IC_{50} of 1690 ± 153 μM. The 2-fluorophenyl derivatives showed good IC_{50}s (IC_{50}s of compound **31**, **37**, and **41** less than 12 μM) except compound **34**, with a slightly higher IC_{50} of 25.3 ± 3.8 μM. The chiral substituent compounds **32**, **33**, **35**, **36**, and **42** showed different activities (IC_{50} = 37.7 ± 5.6, 13.4 ± 2, 22.9 ± 3.4, 16.2 ± 2.4, and 4.73 ± 0.7 μM, respectively). It was found that the (erythro)-1-(4-chlorophenyl)-1-hydroxypropan-2-yl derivatives pronounced better antiviral activities than the (L)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl derivatives did. The pentan-3-yl compounds **39** and **44** expressed better IC_{50}s (IC_{50}s = 0.7 ± 0.1 and 18.7 ± 2.8 μM, respectively) than the corresponding 3-hydroxypropyl compounds **38** and **43** (IC_{50}s = 17.2 ± 2.6 and 19.5 ± 2.9 μM) and the pentan-3-yl compounds were more toxic than the 3-hydroxypropyl compounds (TC_{50}s of compounds **39** and **44** less than 35 μM and TC_{50}s of compounds **38** and **43** more than 35 μM), resulting in similar selective indices of compounds **38**, **39**, **43**, and **44** (SI = 2.7, 3, 2.1, and 1.7, respectively). Generally, the antiviral regularity of this series was inconspicuous. However, it could still be found that furyl derivatives pronounced better anti-CVB3 activities than 5-nitrofuryl derivatives did, and 3,4-dimethoxyphenyl derivatives performed better than 2,3,4-trimethoxyphenyl derivatives.

Compounds with phenyl group at the R_1 position were also screened against CVA16, CVB6, and EV71. The results are presented in Supplementary data. IC_{50}s of most of these benzimidazole compounds against CVA16 were less than 33 μM except compounds **48** and **49**. Compound **48** had relatively moderate activity (IC_{50} = 102 ± 15.3 μM) and compound **49** was not active. The most selective compound against CVA16 was compound **37**, with SI of 7 and IC_{50} of 3.5 ± 0.5 μM. IC_{50} of compound **39** against CVA16, CVB6, and EV71 was the best (IC_{50} = 0.736 ± 0.1 μM), but an insignificant selectivity (SI = 3) was caused by the cytotoxicity (TC_{50} = 2.23 ± 0.3 μM). Comparing these results with those shown in Table 2, it was found that the similarity existed among antiviral activities against CVB3, CVA16, CVB6, and EV71 of a certain compound, indicating that a compound seemed to be effective against other virus if it was effective against one virus of the same family.

4. Conclusion

In summary, a series of novel benzimidazole analogues based on **1** was synthesized and assessed for their anti-enterovirus activities in VERO cells. Most were proved to be potential enterovirus inhibitors. Compounds **16**, **17**, **18**, and **19** displayed optimal profiles against CVB3, with IC_{50}s of 0.4–4 μM and SIs of 10–328. The most promising result was observed for compound **16** with a potent antiviral activity (IC_{50} = 4.06 ± 0.6 μM) and an extraordinarily high selectivity (SI = 328). Such activity and cytotoxicity profiles and their ease of preparation made them attractive candidate compounds for further assessment in vivo as anti-enterovirus agents.

5. Experimental

5.1. Chemistry

The starting materials and reagents, purchased from commercial suppliers, were used without further purification. All final compounds had a purity of >95% as assessed by analytical HPLC. HPLC analyses were conducted on Shimazu Prominence LC-20A system using a YMC-PACK ODS-A 150 × 4.6 nm, 5 μm column with UV 220 and 245 nm detection. The mobile phase consisted of acetonitrile–methanol–water (45:45:10) with the flow rate of 1 mL/min. ^{1}H NMR spectra of DMSO-d_6 solutions were recorded on a Bruker DPX400 spectrometer. Elementary analyzes were performed on a Vario ELIII instrument within ±0.5% of the theoretical values.

5.1.1. General procedure A: synthesis of 2-substituent-1*H*-benzimidazole-4-carboxylic acids (compounds 7–13)

Appropriate aldehyde (10.5 mmol) was added to the solution of 2,3-diaminobenzoic acid **6** (1.52 g, 10 mmol) in DMF (15 mL). The solution was heated to 80 °C and then stirred for about 120 h. Then, the solution was cooled to room temperature. The precipitates were filtered, washed with ethanol and dried.

5.1.2. General procedure B: synthesis of 2-substituent-1*H*-benzimidazole-4-carboxamide derivatives (compounds 14–53)

A mixture of appropriate 2-substituent-1*H*-benzimidazole-4-carboxylic acid (1 mmol), EDC·HCl (0.29 g, 1.5 mmol), HOBt (0.20 g, 1.5 mmol) and Et_3N (0.15 g, 1.5 mmol) in DMF (10 mL) was stirred for 1 h at 0 °C. Then appropriate amine (1.05 mmol) was added to the solution. The solution was heated up to 25 °C, stirred for 12 h and then evaporated to dryness. The solid was washed with water and dried. The products were purified on silica gel column using 10:1 ethyl acetate/ethanol.

5.1.3. 2-(Pyridin-2-yl)-1*H*-benzimidazole-4-carboxylic acid (7)

Compound **7** was synthesized from picolinaldehyde and 2,3-diaminobenzoic acid **6** using general procedure A as a grayish-white solid. Yield = 85%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.36 (t, J = 8 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 8 Hz, 1H), 8.04 (t, J = 8.4 Hz, 1H), 8.46 (d, J = 8 Hz, 1H), 8.76 (d, J = 7.2 Hz, 1H). Anal. Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.77; N, 17.57. Found: C, 65.54; H, 3.68; N, 17.40.

5.1.4. 2-(Pyridin-3-yl)-1*H*-benzimidazole-4-carboxylic acid (8)

Compound **8** was synthesized from nicotinaldehyde and 2,3-diaminobenzoic acid **6** using general procedure A as a grayish-white solid. Yield = 83%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.36 (t, J = 8 Hz, 1H), 7.61 (d, J = 8.8 Hz, 1H), 7.75 (d, J = 8 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 8.57 (t, J = 7.2 Hz, 1H), 8.72 (d, J = 8 Hz, 1H), 9.45 (s, 1H). Anal. Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.77; N, 17.57. Found: C, 65.01; H, 3.80; N, 17.62.

5.1.5. 2-(Pyridin-4-yl)-1*H*-benzimidazole-4-carboxylic acid (9)

Compound **9** was synthesized from isonicotinaldehyde and 2,3-diaminobenzoic acid **6** using general procedure A as a grayish-white solid. Yield = 83%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.36 (t, J = 8 Hz, 1H), 7.86 (d, J = 7.2 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 8.27 (d, J = 7.6 Hz, 2H), 8.75 (d, J = 7.6 Hz, 2H). Anal. Calcd for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.77; N, 17.57. Found: C, 65.15; H, 3.82; N, 17.72.

5.1.6. 2-(Furan-2-yl)-1*H*-benzimidazole-4-carboxylic acid (10)

Compound **10** was synthesized from furan-2-carbaldehyde and 2,3-diaminobenzoic acid **6** using general procedure A as a grayish-white solid. Yield = 80%. ^{1}H NMR (DMSO, 400 MHz) δ: 6.90 (d, J = 8 Hz, 1H), 7.26 (t, J = 8 Hz, 1H), 7.32 (t, J = 8.4 Hz, 1H), 7.68 (d, J = 8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H). Anal. Calcd for $C_{12}H_8N_2O_3$: C, 63.16; H, 3.53; N, 12.28. Found: C, 62.97; H, 3.57; N, 12.35.

5.1.7. 2-(5-Nitrofuran-2-y)-1*H*-benzimidazole-4-carboxylic acid (11)

Compound **11** was synthesized from 5-nitrofuran-2-carbaldehyde and 2,3-diaminobenzoic acid **6** using general procedure A as a grayish-white solid. Yield = 82%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.30 (t, J = 8 Hz, 1H), 7.82 (d, J = 8 Hz, 1H), 7.88 (d, J = 8 Hz, 3H). Anal. Calcd for $C_{12}H_7N_3O_5$: C, 52.76; H, 2.58; N, 15.38. Found: C, 52.84; H, 2.63; N, 15.27.

5.1.8. 2-(3,4-Dimethoxyphenyl)-1*H*-benzimidazole-4-carboxylic acid (12)

Compound **12** was synthesized from 3,4-dimethoxybenzaldehyde and 2,3-diaminobenzoic acid **6** using general procedure A as a grayish-white solid. Yield = 81%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.83 (s, 3H), 3.88 (s, 3H), 7.10 (d, J = 8.4 Hz, 1H), 7.28 (t, J = 7.2 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.85–7.93 (m, 3H), 12.22 (s, 1H), 13.23 (s, 1H). Anal. Calcd for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.35; H, 4.77; N, 9.42.

5.1.9. 2-(2,3,4-Trimethoxyphenyl)-1*H*-benzimidazole-4-carboxylic acid (13)

Compound **13** was synthesized from 2,3,4-dimethoxybenzaldehyde and 2,3-diaminobenzoic acid **6** using general procedure A as a grayish-white solid. Yield = 85%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.83 (s, 3H), 3.88 (s, 3H), 3.99 (s, 3H), 7.04 (d, J = 9.2 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 8 Hz, 1H), 8.05 (d, J = 8.8 Hz, 1H), 11.61 (s, 1H), 13.42 (s, 1H). Anal. Calcd for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.53. Found: C, 62.40; H, 4.81; N, 8.47.

5.1.10. 2-(Pyridin-2-yl)-1*H*-benzimidazole-4-carboxamide (14)

Compound **14** was synthesized from ammonium hydroxide and compound **7** using general procedure B as a white solid. Yield = 90%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.36 (t, J = 8 Hz, 1H), 7.57 (d, J = 6.8 Hz, 1H), 7.72 (d, J = 8 Hz, 1H), 7.82 (d, J = 2.8 Hz, 1H), 7.88 (d, J = 7.6 Hz, 1H), 8.02 (t, J = 8 Hz, 1H), 8.44 (d, J = 8 Hz, 1H), 8.77 (t, J = 4.8 Hz, 1H), 9.27 (d, J = 2 Hz, 1H), 13.59 (s, 1H). Anal. Calcd for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.81; H, 4.21; N, 23.32.

5.1.11. 2-(Pyridin-2-yl)-*N*-ethylol-1*H*-benzimidazole-4-carboxamide (15)

Compound **15** was synthesized from 2-aminoethanol and compound **7** using general procedure B as a white solid. Yield = 86%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.54 (q, J = 5 Hz, 2H), 3.65 (q, J = 5.2 Hz, 2H), 4.99 (t, J = 4.8 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.57 (d, J = 8 Hz, 1H), 7.70 (d, J = 9.2 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 8.04 (t, J = 8 Hz, 1H), 8.46 (d, J = 7.6 Hz, 1H), 8.76–8.77 (d, J = 4.8 Hz, 1H), 10.14–10.16 (t, J = 5.2 Hz, 1H), 13.62 (s, 1H). Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.87; H, 4.89; N, 19.72.

5.1.12. (L)-2-(Pyridin-2-yl)-*N*-(2-(4-nitrophenyl)pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (16)

Compound **16** was synthesized from (L)-2-amino-1-(4-nitrophenyl)propane-1,3-diol and compound **7** using general procedure B as a white solid. Yield = 85%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.58 (q, J = 5.2 Hz, 1H), 3.68 (q, J = 5.2 Hz, 1H), 4.21 (q, J = 5.2 Hz, 1H), 5.07 (q, J = 6.4 Hz, 1H), 5.28 (d, J = 3.2 Hz, 1H), 6.26 (d, J = 4 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.58 (d, J = 8 Hz, 1H), 7.67–7.73 (m, 4H), 8.02–8.06 (m, 3H), 8.57 (d, J = 8 Hz, 1H), 8.78 (d, J = 4.8 Hz, 1H), 10.46 (d, J = 8.4 Hz, 1H), 13.62 (s, 1H). Anal. Calcd for $C_{22}H_{19}N_5O_5$: C, 60.97; H, 4.42; N, 16.16. Found: C, 60.92; H, 4.50; N, 16.07.

5.1.13. 2-(Pyridin-2-yl)-*N*-phenyl-1*H*-benzimidazole-4-carboxamide (17)

Compound **17** was synthesized from aniline and compound **7** using general procedure B as a slightly yellow solid. Yield = 88%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.16 (t, J = 8 Hz, 1H), 7.41–7.47 (m, 4H), 7.65 (d, J = 8 Hz, 1H), 7.92–8.00 (m, 3H), 8.28 (d, J = 7.6 Hz, 1H), 8.46 (d, J = 7.6 Hz, 1H), 8.68 (d, J = 5.6 Hz, 1H), 11.07 (s, 1H), 12.11 (s, 1H). Anal. Calcd for $C_{19}H_{14}N_4O$: C, 72.60; H, 4.49; N, 17.82. Found: C, 72.43; H, 4.47; N, 17.95.

5.1.14. 2-(Pyridin-2-yl)-*N*-(2-fluorophenyl)-1*H*-benzimidazole-4-carboxamide (18)

Compound **18** was synthesized from 2-fluoroaniline and compound **7** using general procedure B as a slightly yellow solid. Yield = 87%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.16 (t, J = 8 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 7.40–7.48 (m, 2H), 7.61 (t, J = 7.6 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 8.14 (t, J = 7.6 Hz, 1H), 8.46 (d, J = 8 Hz, 1H), 8.66 (t, J = 8 Hz, 1H), 8.80–8.81 (d, J = 4.8 Hz, 1H), 12.52 (s, 1H), 13.84 (s, 1H). Anal. Calcd for $C_{19}H_{14}FN_4O$: C, 68.67; H, 3.94; N, 16.86. Found: C, 68.75; H, 4.06; N, 16.74.

5.1.15. 2-(Pyridin-2-yl)-*N*-(4-methylthiazol-2-yl)-1*H*-benzimidazole-4-carboxamide (19)

Compound **19** was synthesized from 4-methylthiazol-5-amine and compound **7** using general procedure B as a slightly yellow solid. Yield = 87%. ^{1}H NMR (DMSO, 400 MHz) δ: 2.34 (s, 3H), 6.87 (s, 1H), 7.47 (t, J = 7.6 Hz, 1H), 7.63 (t, J = 7.6 Hz, 1H), 7.85 (d, J = 8 Hz, 1H), 8.03 (d, J = 8 Hz, 1H), 8.17 (t, J = 8 Hz, 1H), 8.36 (d, J = 8 Hz, 1H), 8.81 (d, J = 4.8 Hz, 1H), 12.22 (s, 1H), 13.89 (s, 1H). Anal. Calcd for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88. Found: C, 60.87; H, 3.92; N, 20.75.

5.1.16. 2-(Pyridin-3-yl)-1*H*-benzimidazole-4-carboxamide (20)

Compound **20** was synthesized from ammonium hydroxide and compound **8** using general procedure B as a white solid. Yield = 90% ^{1}H NMR (DMSO, 400 MHz) δ: 7.37 (t, J = 8 Hz, 1H), 7.61 (d, J = 8 Hz, 1H), 7.76–7.80 (m, 2H), 7.89 (d, J = 7.6 Hz, 1H), 8.57 (t, J = 8 Hz, 1H), 8.71 (d, J = 4.4 Hz, 1H), 9.26 (s, 1H), 9.40 (s, 1H), 13.60 (s, 1H). Anal. Calcd for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.55; H, 4.17; N, 23.48.

5.1.17. 2-(Pyridin-3-yl)-*N*-ethylol-1*H*-benzimidazole-4-carboxamide (21)

Compound **21** was synthesized from 2-aminoethanol and compound **8** using general procedure B as a white solid. Yield = 89%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.54 (q, J = 5.6 Hz, 2H), 3.64 (q, J = 5.2 Hz, 2H), 4.92–4.84 (t, J = 4.8 Hz, 1H), 7.36 (t, J = 8 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.75 (d, J = 8 Hz, 1H), 7.90 (t, J = 7.6 Hz, 1H), 8.57 (d, J = 7.6 Hz, 1H), 8.72 (d, J = 4.8 Hz, 1H), 9.45 (s, 1H), 10.12 (t, J = 5.2 Hz, 1H), 13.57 (s, 1H). Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.94; H, 4.95; N, 19.74.

5.1.18. (L)-2-(Pyridin-3-yl)-*N*-(2-(4-nitrophenyl)pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (22)

Compound **22** was synthesized from (L)-2-amino-1-(4-nitrophenyl)propane-1,3-diol and compound **8** using general procedure B as a white solid. Yield = 82%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.58 (q, J = 5.2 Hz, 1H), 3.68 (q, J = 5.2 Hz, 1H), 4.22 (q, J = 4 Hz, 1H), 5.10 (q, J = 4 Hz, 1H), 5.27 (d, J = 3.2 Hz, 1H), 6.30–6.31 (d, J = 4 Hz, 1H), 7.27 (t, J = 7.6 Hz, 1H), 7.64 (d, J = 8 Hz, 1H), 7.70–7.75 (m, 4H), 8.00–8.06 (m, 2H), 8.65 (d, J = 8 Hz, 1H), 8.74 (d, J = 7.6 Hz, 1H), 9.56 (d, J = 4.8 Hz, 1H), 10.45 (d, J = 8.4 Hz, 1H), 13.87 (s, 1H). Anal. Calcd for $C_{22}H_{19}N_5O_5$: C, 60.97; H, 4.42; N, 16.16. Found: C, 60.88; H, 4.39; N, 16.09.

5.1.19. 2-(Pyridin-3-yl)-*N*-phenyl-1*H*-benzimidazole-4-carboxamide (23)

Compound **23** was synthesized from aniline and compound **8** using general procedure B as a slightly yellow solid. Yield = 91%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.14 (t, J = 8 Hz, 1H), 7.42–7.48 (m, 3H), 7.68 (d, J = 8 Hz, 1H), 7.84–7.90 (m, 3H), 8.00–8.02 (d, J = 7.2 Hz, 1H), 8.63 (t, J = 4 Hz, 1H), 8.76–8.77 (d, J = 3.6 Hz, 1H), 9.47 (s, 1H), 12.12 (s, 1H), 13.80 (s, 1H). Anal. Calcd for $C_{19}H_{14}N_4O$: C, 72.60; H, 4.49; N, 17.82. Found: C, 72.84; H, 4.48; N, 17.74.

5.1.20. 2-(Pyridin-3-yl)-*N*-(2-fluorophenyl)-1*H*-benzimidazole-4-carboxamide (24)

Compound **24** was synthesized from 2-fluoroaniline and compound **8** using general procedure B as a slightly yellow solid. Yield = 86%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.14 (t, J = 7.6 Hz, 1H), 7.24 (d, J = 8 Hz, 1H), 7.38–7.42 (m, 2H), 7.68 (d, J = 7.6 Hz, 1H), 7.86 (d, J = 8 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 8.58–8.67 (m, 3H), 9.50 (d, J = 4 Hz, 1H), 12.52 (s, 1H), 13.82 (s, 1H). Anal. Calcd for $C_{19}H_{14}FN_4O$: C, 68.67; H, 3.94; N, 16.86. Found: C, 68.78; H, 4.01; N, 16.77.

5.1.21. 2-(Pyridin-3-yl)-*N*-(4-methylthiazol-2-yl)-1*H*-benzimidazole-4-carboxamide (25)

Compound **25** was synthesized from 4-methylthiazol-5-amine and compound **8** using general procedure B as a slightly yellow solid. Yield = 80%. ^{1}H NMR (DMSO, 400 MHz) δ: 2.33 (s, 3H), 6.86 (s, 1H), 7.47 (t, J = 8 Hz, 1H), 7.69 (t, J = 8 Hz, 1H), 7.90 (d, J = 8 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 8.54 (d, J = 8 Hz, 1H), 8.76 (d, J = 3.2 Hz, 1H), 9.43 (s, 1H), 13.29 (s, 1H), 13.83 (s, 1H). Anal. Calcd for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88. Found: C, 60.75; H, 3.98; N, 20.79.

5.1.22. 2-(Pyridin-4-yl)-*N*-ethylol-1*H*-benzimidazole-4-carboxamide (26)

Compound **26** was synthesized from 2-aminoethanol and compound **9** using general procedure B as a white solid. Yield = 75%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.54–3.57 (q, J = 5.2 Hz, 2H), 3.64 (q, J = 5.2 Hz, 2H), 4.97 (t, J = 4.8 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 7.78 (d, J = 8 Hz, 1H), 7.92 (d, J = 7.6 Hz, 1H), 8.18 (d, J = 7.2 Hz, 2H), 8.80 (s, 1H), 10.10 (t, J = 4.8 Hz, 1H), 13.75 (s, 1H). Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.96; H, 5.02; N, 19.78.

5.1.23. (L)-2-(Pyridin-4-yl)-*N*-(2-(4-nitrophenyl)pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (27)

Compound **27** was synthesized from (L)-2-amino-1-(4-nitrophenyl)propane-1,3-diol and compound **9** using general procedure B as a white solid. Yield = 87%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.58 (q, J = 5.2 Hz, 1H), 3.68 (q, J = 5.2 Hz, 1H), 4.22 (q, J = 4.4 Hz, 1H), 5.09 (q, J = 4.4 Hz, 1H), 5.28 (d, J = 3.2 Hz, 1H), 6.32 (d, J = 4 Hz, 1H), 7.35 (t, J = 8 Hz, 1H), 7.71–7.77 (m, 4H), 8.00–8.07 (m, 2H), 8.27–8.29 (m, 2H), 8.80–8.82 (m, 2H), 10.45 (d, J = 8.4 Hz, 1H), 13.78 (s, 1H). Anal. Calcd for $C_{22}H_{19}N_5O_5$: C, 60.97; H, 4.42; N, 16.16. Found: C, 60.85; H, 4.47; N, 16.10.

5.1.24. 2-(Pyridin-4-yl)-*N*-phenyl-1*H*-benzimidazole-4-carboxamide (28)

Compound **28** was synthesized from aniline and compound **9** using general procedure B as a slightly yellow solid. Yield = 68%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.15 (t, J = 7.6 Hz, 1H), 7.41–7.50 (m, 3H), 7.88 (t, J = 8 Hz, 3H), 8.02 (d, J = 7.2 Hz, 1H), 8.21 (d, J = 5.6 Hz, 2H), 8.84 (d, J = 4.4 Hz, 2H), 12.05 (s, 1H). Anal. Calcd for $C_{19}H_{14}N_4O$: C, 72.60; H, 4.49; N, 17.82. Found: C, 72.43; H, 4.42; N, 17.92.

5.1.25. 2-(Pyridin-4-yl)-*N*-(2-fluorophenyl)-1*H*-benzimidazole-4-carboxamide (29)

Compound **29** was synthesized from 2-fluoroaniline and compound **9** using general procedure B as a slightly yellow solid. Yield = 75%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.16 (t, J = 7.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 7.40–7.51 (m, 2H), 7.88 (d, J = 8 Hz, 1H), 8.04 (d, J = 7.6 Hz, 1H), 8.19–8.21 (m, 2H), 8.62–8.66 (t, J = 7.6 Hz, 1H), 8.85 (d, J = 5.6 Hz, 2H), 13.93 (s, 1H). Anal. Calcd for $C_{19}H_{14}FN_4O$: C, 68.67; H, 3.94; N, 16.86. Found: C, 68.62; H, 3.89; N, 16.81.

5.1.26. 2-(Pyridin-4-yl)-*N*-(4-methylthiazol-2-yl)-1*H*-benzimidazole-4-carboxamide (30)

Compound **30** was synthesized from 4-methylthiazol-5-amine and compound **9** using general procedure B as a slightly yellow solid. Yield = 82%. ^{1}H NMR (DMSO, 400 MHz) δ: 2.34 (s, 3H), 6.87 (s, 1H), 7.50–7.54 (t, J = 8 Hz, 1H), 7.93 (d, J = 8 Hz, 1H), 8.05 (d, J = 7.2 Hz, 1H), 8.13 (d, J = 5.2 Hz, 2H), 8.88 (d, J = 5.2 Hz, 2H), 13.16 (s, 1H), 14.05 (s, 1H). Anal. Calcd for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88. Found: C, 60.74; H, 3.85; N, 20.93.

5.1.27. 2-(Furan-2-yl)-*N*-(2-fluorophenyl)-1*H*-benzimidazole-4-carboxamide (31)

Compound **31** was synthesized from 2-fluoroaniline and compound **10** using general procedure B as a slightly yellow solid. Yield = 90%. ^{1}H NMR (DMSO, 400 MHz) δ: 6.86 (d, J = 7.6 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 7.24 (t, J = 8 Hz, 1H), 7.35 (m, J = 8 Hz, 1H), 7.38–7.41 (m, 2H), 7.43–7.49 (m, 2H), 7.77–7.82 (t, J = 8 Hz, 1H), 8.00 (d, J = 7.6 Hz, 1H), 8.04–8.06 (m, 2H), 8.61 (d, J = 8 Hz, 1H), 13.75 (s, 1H). Anal. Calcd for $C_{18}H_{12}FN_3O_2$: C, 67.29; H, 3.76; N, 13.08. Found: C, 67.47; H, 3.64; N, 13.11.

5.1.28. (L)-2-(Furan-2-yl)-*N*-(2-(4-nitrophenyl)pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (32)

Compound **32** was synthesized from (L)-2-amino-1-(4-nitrophenyl) propane-1,3-diol and compound **10** using general procedure B as a white solid. Yield = 90%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.58 (q, J = 5.2 Hz, 1H), 3.66 (q, J = 5.2 Hz, 1H), 4.17 (q, J = 5.2 Hz, 1H), 5.18 (s, 1H), 5.25 (d, J = 8.8 Hz, 1H), 6.12 (d, J = 4.4 Hz, 1H), 6.87 (d, J = 3.6 Hz, 1H), 7.27 (t, J = 8 Hz, 1H), 7.27–7.36 (m, 2H), 7.65–7.76 (m, 4H) 8.05 (d, J = 8 Hz, 2H), 10.18 (d, J = 8.4 Hz, 1H), 13.55 (s, 1H). Anal. Calcd for $C_{21}H_{18}N_4O_6$: C, 59.71; H, 4.30; N, 13.26. Found: C, 59.82; H, 4.33; N, 13.17.

5.1.29. (Erythro)-2-(furan-2-yl)-*N*-(1-amino-2-hydroxy-2-(4-nitrophenyl)ethyl)-1*H*-benzimidazole-4-carboxamide (33)

Compound **33** was synthesized from (erythro)-2-amino-1-(4-nitrophenyl) propan-1-ol and compound **10** using general procedure B as a slightly yellow solid. Yield = 91%. ^{1}H NMR (DMSO, 400 MHz) δ: 1.04 (d, J = 6.8 Hz, 3H), 1.20 (t, J = 8 Hz, 1H), 4.29 (q, J = 6 Hz, 1H), 4.83 (d, J = 4.4 Hz, 1H), 5.79 (s, 1H), 6.79 (s, 1H), 7.21 (t, J = 8 Hz, 1H), 7.29–7.47 (m, 6H), 7.65 (d, J = 7.6 Hz, 1H), 7.84 (d, J = 8 Hz, 1H), 8.02 (s, 1H), 10.02 (d, J = 8 Hz, 1H), 13.42 (s, 1H). Anal. Calcd for $C_{21}H_{18}ClN_3O_3$: C, 63.72; H, 4.58; N, 10.62. Found: C, 63.84; H, 4.47; N, 10.57.

5.1.30. 2-(5-Nitrofuran-2-yl)-*N*-(2-fluorophenyl)-1*H*-benzimidazole-4-carboxamide (34)

Compound **34** was synthesized from 2-fluoroaniline and compound **11** using general procedure B as a slightly yellow solid. Yield = 92%. ^{1}H NMR (DMSO, 400 MHz) δ: 7.15 (t, J = 8 Hz, 1H), 7.24 (t, J = 8 Hz, 1H), 7.38 (t, J = 8 Hz, 1H), 7.50–7.54 (m, 2H), 7.86 (d, J = 8 Hz, 1H), 7.94 (d, J = 3.6 Hz, 1H), 8.06 (d, J = 7.6 Hz, 1H), 8.59 (t, J = 8 Hz, 1H), 12.20 (d, J = 2.8 Hz, 1H), 14.25 (s, 1H). Anal. Calcd for $C_{18}H_{11}FN_4O_4$: C, 59.02; H, 3.03; N, 15.30. Found: C, 59.23; H, 2.98; N, 15.27.

5.1.31. (ι)-2-(5-Nitrofuran-2-yl)-*N*-(2-(4-nitrophenyl)pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (35)

Compound **35** was synthesized from (ι)-2-amino-1-(4-nitrophenyl)propane-1,3-diol and compound **11** using general procedure B as a white solid. Yield = 92%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.60 (q, J = 5.2 Hz, 1H), 3.66 (q, J = 5.2 Hz, 1H), 4.18 (q, J = 5.2 Hz, 1H), 5.07 (s, 1H), 5.27 (d, J = 2.4 Hz, 1H), 6.16 (d, J = 3.6 Hz, 1H), 7.37 (t, J = 8 Hz, 1H), 7.53 (d, J = 4.4 Hz, 1H), 7.72–7.78 (m, 4H), 8.00 (d, J = 4.4 Hz, 1H), 8.06 (d, J = 9.6 Hz, 3H), 10.05 (d, J = 8.4 Hz, 2H), 14.05 (s, 1H). Anal. Calcd for $C_{21}H_{17}N_5O_8$: C, 53.96; H, 3.67; N, 14.98. Found: C, 54.17; H, 3.56; N, 14.85.

5.1.32. (Erythro)-2-(5-nitrofuran-2-yl)-*N*-(1-amino-2-hydroxy-2-(4-nitrophenyl)ethyl)-1*H*-benzimidazole-4-carboxamide (36)

Compound **36** was synthesized from (erythro)-2-amino-1-(4-nitrophenyl)propan-1-ol and compound **11** using general procedure B as a slightly yellow solid. Yield = 90%. ^{1}H NMR (DMSO, 400 MHz) δ: 1.06–1.08 (d, J = 6.4 Hz, 3H), 4.29 (q, J = 4 Hz, 1H), 4.85 (d, J = 3.2 Hz, 1H), 5.73 (d, J = 5.2 Hz, 1H), 7.29 (d, J = 8.4 Hz, 2H), 7.37 (t, J = 8 Hz, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8 Hz, 1H), 7.72 (d, J = 7.6 Hz, 1H), 7.85 (d, J = 4 Hz, 1H), 7.92 (d, J = 8 Hz, 1H), 9.90 (d, J = 2 Hz, 1H), 13.93 (s, 1H). Anal. Calcd for $C_{21}H_{17}ClN_4O_5$: C, 57.22; H, 3.89; N, 12.71. Found: C, 57.44; H, 3.76; N, 12.65.

5.1.33. 2-(3,4-Dimethoxyphenyl)-*N*-(2-fluorophenyl)-1*H*-benzimidazole-4-carboxamide (37)

Compound **37** was synthesized from 2-fluoroaniline and compound **12** using general procedure B as a slightly yellow solid. Yield = 76%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.86 (s, 3H), 3.93 (s, 3H), 7.14 (t, J = 5.2 Hz, 1H), 7.20–7.27 (m, 2H), 7.37–7.41 (m, 2H), 7.78 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.95–7.98 (m, 2H), 8.69 (t, J = 8 Hz, 1H), 12.68 (s, 1H), 13.42 (s, 1H). Anal. Calcd for $C_{22}H_{18}FN_3O_3$: C, 67.51; H, 4.64; N, 10.74. Found: C, 67.40; H, 4.69; N, 10.82.

5.1.34. 2-(3,4-Dimethoxyphenyl)-*N*-(3-hydroxypropyl)-1*H*-benzimidazole-4-carboxamide (38)

Compound **38** was synthesized from 3-aminopropan-1-ol and compound **12** using general procedure B as a white solid. Yield = 80%. ^{1}H NMR (DMSO, 400 MHz) δ: 1.78 (q, J = 6.4 Hz, 2H), 3.53 (q, J = 5.2 Hz, 2H), 3.63 (q, J = 5.2 Hz, 2H), 3.84 (s, 3H), 3.89 (s, 3H), 4.60 (t, J = 6 Hz, 1H), 7.16 (d, J = 8 Hz, 1H), 7.279 (t, J = 8 Hz, 1H), 7.67 (d, J = 8 Hz, 1H), 7.81–7.83 (m, 3H), 10.02 (t, J = 5.2 Hz, 1H), 13.22 (s, 1H). Anal. Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 64.38; H, 5.84; N, 11.74.

5.1.35. 2-(3,4-Dimethoxyphenyl)-*N*-(pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (39)

Compound **39** was synthesized from pentan-3-amine and compound **12** using general procedure B as a yellow solid. Yield = 83%. ^{1}H NMR (DMSO, 400 MHz) δ: 0.97 (t, J = 8 Hz, 6H), 1.58–1.66 (m, 4H), 3.84 (s, 3H), 3.86 (s, 3H), 3.96 (q, J = 6 Hz, 1H), 7.18 (d, J = 9.2 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.67 (d, J = 8 Hz, 1H), 7.76–7.83 (m, 3H), 9.93 (d, J = 8.4 Hz, 1H), 13.24 (s, 1H). Anal. Calcd for $C_{21}H_{25}N_3O_3$: C, 68.64; H, 6.86; N, 11.44. Found: C, 68.80; H, 6.94; N, 11.27.

5.1.36. 2-(3,4-Dimethoxyphenyl)-*N*-(5-chloropyrazin-2-yl)-1*H*-benzimidazole-4-carboxamide (40)

Compound **40** was synthesized from 5-chloropyrazin-2-amine and compound **12** using general procedure B as a yellow solid. Yield = 75%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.86 (s, 3H), 3.93 (s, 3H), 7.21 (d, J = 8 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.82–7.87 (m, 2H), 7.92 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 8.64 (s, 1H), 9.45 (s, 1H), 13.23 (s, 1H), 13.51 (s, 1H). Anal. Calcd for $C_{20}H_{16}ClN_5O_3$: C, 58.61; H, 3.94; N, 17.09. Found: C, 58.74; H, 3.88; N, 17.15.

5.1.37. 2-(2,3,4-Trimethoxyphenyl)-*N*-(2-fluorophenyl)-1*H*-benzimidazole-4-carboxamide (41)

Compound **41** was synthesized from 2-fluoroaniline and compound **13** using general procedure B as a slightly yellow solid. Yield = 75%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.85 (s, 3H), 3.91 (s, 3H), 3.99 (s, 3H), 7.09–7.17 (m, 2H), 7.25 (t, J = 7.6 Hz, 1H), 7.36–7.42 (m, 2H), 7.87 (d, J = 8 Hz, 1H), 7.97 (d, J = 7.6 Hz, 1H), 8.22 (d, J = 8.8 Hz, 1H), 8.66 (t, J = 8 Hz, 1H), 12.58 (s, 1H), 12.59 (s, 1H). Anal. Calcd for $C_{23}H_{20}FN_3O_4$: C, 65.55; H, 4.78; N, 9.97. Found: C, 65.67; H, 4.82; N, 9.85.

5.1.38. (ι)-2-(2,3,4-Trimethoxyphenyl)-*N*-(2-(4-nitrophenyl)-pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (42)

Compound **42** was synthesized from (ι)-2-amino-1-(4-nitrophenyl)propane-1,3-diol and compound **13** using general procedure B as a white solid. Yield = 80%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.55 (q, J = 6.8 Hz, 1H), 3.67 (q, J = 7.6 Hz, 1H), 3.85 (s, 3H), 3.90 (s, 3H), 4.00 (s, 3H), 4.23 (q, J = 3.6 Hz, 1H), 5.04 (q, J = 2 Hz, 1H), 5.25 (d, J = 4 Hz, 1H), 6.20 (d, J = 4 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 7.22 (t, J = 8 Hz, 1H), 7.66–7.74 (m, 4H), 8.05 (d, J = 9.2 Hz, 2H), 8.34 (d, J = 8.8 Hz, 1H), 10.50 (d, J = 8.8 Hz, 1H), 12.40 (s, 1H). Anal. Calcd for $C_{26}H_{26}N_4O_8$: C, 59.77; H, 5.02; N, 10.72. Found: C, 59.92; H, 5.09; N, 10.61.

5.1.39. 2-(2,3,4-Trimethoxyphenyl)-*N*-(3-hydroxypropyl)-1*H*-benzimidazole-4-carboxamide (43)

Compound **43** was synthesized from 3-aminopropan-1-ol and compound **13** using general procedure B as a white solid. Yield = 80%. ^{1}H NMR (DMSO, 400 MHz) δ: 1.76 (q, J = 6.8 Hz, 2H), 3.51 (q, J = 6.4 Hz, 1H), 3.58 (q, J = 5.2 Hz, 1H), 3.83 (s, 3H), 3.89 (s, 3H), 3.94 (s, 3H), 4.58 (t, J = 5.2 Hz, 1H), 7.04 (d, J = 8.8 Hz, 1H), 7.28 (t, J = 8 Hz, 1H), 7.75 (d, J = 8 Hz, 1H), 7.84 (d, J = 6.8 Hz, 1H), 8.04 (d, J = 8.8 Hz, 1H), 10.00 (t, J = 5.6 Hz, 1H), 12.49 (s, 1H). Anal. Calcd for $C_{20}H_{23}N_3O_5$: C, 62.33; H, 6.01; N, 10.90. Found: C, 62.12; H, 6.11; N, 10.85.

5.1.40. 2-(2,3,4-Trimethoxyphenyl)-*N*-(pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (44)

Compound **44** was synthesized from pentan-3-amine and compound **13** using general procedure B as a yellow solid. Yield = 80%. ^{1}H NMR (DMSO, 400 MHz) δ: 0.95 (t, J = 8 Hz, 6H), 1.53–1.69 (m, 4H), 3.83 (s, 3H), 3.88 (s, 3H), 3.94–4.00 (m, 4H), 7.08 (d, J = 8.8 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.75 (d, J = 8 Hz, 1H), 7.813 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 9.88 (d, J = 8.4 Hz, 1H), 12.50 (s, 1H). Anal. Calcd for $C_{22}H_{27}N_3O_4$: C, 66.48; H, 6.85; N, 10.57. Found: C, 66.54; H, 6.78; N, 10.68.

5.1.41. 2-(2,3,4-Trimethoxyphenyl)-*N*-(5-chloropyrazin-2-yl)-1*H*-benzimidazole-4-carboxamide (45)

Compound **45** was synthesized from 5-chloropyrazin-2-amine and compound **13** using general procedure B as a yellow solid. Yield = 75%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.85 (s, 3H), 3.91 (s, 3H), 3.98 (s, 3H), 7.11 (d, J = 9.2 Hz, 1H), 7.41 (t, J = 8 Hz, 1H), 7.90 (d, J = 8 Hz, 1H), 7.99–8.05 (m, 2H), 8.63 (s, 1H), 9.46 (s, 1H), 12.74 (s, 1H), 12.96 (s, 1H). Anal. Calcd for $C_{21}H_{18}ClN_5O_4$: C, 57.34; H, 4.12; N, 15.92. Found: C, 57.16; H, 4.08; N, 16.13.

5.1.42. 2-(2,3,4-Trimethoxyphenyl)-*N*-(1*H*-benzimidazol-2-yl)-1*H*-benzimidazole-4-carboxamide (46)

Compound **46** was synthesized from 1*H*-benzimidazol-2-amine and compound **13** using general procedure B as a slightly yellow solid. Yield = 69%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.84 (s, 3H), 3.88 (s, 3H), 4.02 (s, 3H), 7.05 (d, J = 9.2 Hz, 1H), 7.31–7.37 (m, 3H),

7.46 (d, J = 7.6 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8 Hz, 1H), 8.05 (d, J = 9.2 Hz, 1H), 11.55 (s, 1H), 12.14 (s, 1H), 12.39 (s, 1H). Anal. Calcd for $C_{24}H_{21}N_5O_4$: C, 65.00; H, 4.77; N, 15.79. Found: C, 64.75; H, 4.83; N, 15.87.

5.1.43. (ι)-2-(3,4-Dimethoxyphenyl)-*N*-(2-(4-nitrophenyl)pentan-3-yl)-1*H*-benzimidazole-4-carboxamide (47)

Compound **47** was synthesized from (ι)-2-amino-1-(4-nitrophenyl)propane-1,3-diol and compound **12** using general procedure B as a white solid. Yield = 80%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.60 (q, J = 5.2 Hz, 1H), 3.69 (q, J = 5.2 Hz, 1H), 3.85 (s, 3H), 3.88 (s, 3H), 4.23 (q, J = 5.6 Hz, 1H), 5.07 (q, J = 4.8 Hz, 1H), 5.28 (d, J = 4.4 Hz, 1H), 6.20 (d, J = 4.4 Hz, 1H), 7.16 (d, J = 8.8 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.63–7.67 (m, 2H), 7.73 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 8.03–8.08 (m, 3H), 10.53 (d, J = 8.8 Hz, 1H), 12.21 (s, 1H). Anal. Calcd for $C_{25}H_{24}N_4O_7$: C, 60.97; H, 4.91; N, 11.38. Found: C, 61.16; H, 4.87; N, 11.25.

5.1.44. 2-(3,4-Dimethoxyphenyl)-*N*-(1*H*-imidazol-4-yl)-1*H*-benzimidazole-4-carboxamide (48)

Compound **48** was synthesized from 1*H*-imidazol-4-amine and compound **12** using general procedure B as a slightly yellow solid. Yield = 63%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.86 (s, 3H), 3.93 (s, 3H), 7.23 (d, J = 8.4 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.83 (d, J = 8 Hz, 1H), 7.87 (d, J = 8 Hz, 1H), 7.93 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 8.43 (d, J = 2.4 Hz, 1H), 8.49 (t, J = 2.8 Hz, 1H), 9.64 (s, 1H), 13.10 (s, 1H), 13.49 (s, 1H). Anal. Calcd for $C_{19}H_{17}N_5O_3$: C, 62.80; H, 4.72; N, 19.27. Found: C, 62.63; H, 4.86; N, 19.37.

5.1.45. 2-(3,4-Dimethoxyphenyl)-*N*-(1*H*-imidazol-2-yl)-1*H*-benzimidazole-4-carboxamide (49)

Compound **49** was synthesized from 1*H*-imidazol-2-amine and compound **12** using general procedure B as a slightly yellow solid. Yield = 65%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.83 (s, 3H), 3.88 (s, 3H), 7.14–7.21 (m, 2H), 7.29 (t, J = 8 Hz, 1H), 7.40 (s, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.78–7.81 (m, 2H), 7.91 (t, J = 6.8 Hz, 1H), 9.34 (s, 1H), 13.27 (s, 1H). Anal. Calcd for $C_{19}H_{17}N_5O_3$: C, 62.80; H, 4.72; N, 19.27. Found: C, 62.69; H, 4.76; N, 19.35.

5.1.46. 2-(3,4-Dimethoxyphenyl)-*N*-(4,5-dicyano-1*H*-imidazol-2-yl)-1*H*-benzimidazole-4-carboxamide (50)

Compound **50** was synthesized from 4,5-dicyano-1*H*-imidazol-2-amine and compound **12** using general procedure B as a slightly yellow solid. Yield = 60%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.83 (s, 3H), 3.88 (s, 3H), 7.15 (d, J = 8.8 Hz, 1H), 7.29 (t, J = 7.6 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.69 (d, J = 8 Hz, 1H), 7.82 (t, J = 8 Hz, 1H), 7.91 (d, J = 6.8 Hz, 1H), 9.34 (s, 1H), 13.20 (s, 1H). Anal. Calcd for $C_{21}H_{15}N_7O_3$: C, 61.01; H, 3.66; N, 23.72. Found: C, 61.25; H, 3.57; N, 23.68.

5.1.47. 2-(3,4-Dimethoxyphenyl)-*N*-(pyrazin-2-yl)-1*H*-benzimidazole-4-carboxamide (51)

Compound **51** was synthesized from pyrazin-2-amine and compound **12** using general procedure B as a slightly yellow solid. Yield = 60%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.83 (s, 3H), 3.88 (s, 3H), 7.10 (d, J = 8.4 Hz, 1H), 7.28 (t, J = 8 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.85 (s, 1H), 7.88–7.93 (m, 3H), 12.27 (s, 1H), 12.55 (s, 1H). Anal. Calcd for $C_{20}H_{17}N_5O_3$: C, 63.99; H, 4.56; N, 18.66. Found: C, 63.78; H, 4.62; N, 18.74.

5.1.48. 2-(3,4-Dimethoxyphenyl)-*N*-(1*H*-benzimidazol-2-yl)-1*H*-benzimidazole-4-carboxamide (52)

Compound **52** was synthesized from 1*H*-benzimidazol-2-amine and compound **12** using general procedure B as a slightly yellow solid. Yield = 70%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.86 (s, 3H), 3.94 (s, 3H), 7.09–7.12 (m, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.89–7.91 (m, 3H), 8.02 (d, J = 7.6 Hz, 2H), 12.34 (s, 1H), 13.28 (s, 1H), 13.52 (s, 1H). Anal. Calcd for $C_{23}H_{19}N_5O_3$: C, 66.82; H, 4.63; N, 16.94. Found: C, 66.74; H, 4.71; N, 17.02.

5.1.49. 2-(3,4-Dimethoxyphenyl)-*N*-(3-hydroxypyridin-2-yl)-1*H*-benzimidazole-4-carboxamide (53)

Compound **53** was synthesized from 3-hydroxypyridin-2-amine and compound **12** using general procedure B as a slightly yellow solid. Yield = 62%. ^{1}H NMR (DMSO, 400 MHz) δ: 3.85 (s, 3H), 3.92 (s, 3H), 7.14–7.20 (m, 3H), 7.40 (t, J = 7.6 Hz, 1H), 7.83 (d, J = 8 Hz, 1H), 7.89–7.91 (m, 2H), 7.96–7.98 (m, 2H), 11.14 (s, 1H), 13.10 (s, 1H), 13.48 (s, 1H). Anal. Calcd for $C_{21}H_{18}N_4O_4$: C, 64.61; H, 4.65; N, 14.35. Found: C, 64.78; H, 4.58; N, 14.40.

5.2. Antiviral activity evaluation in vitro

The virus strains of CVA16, CVB3, CVB6, and EV71 were provided by American Type Culture Collection (ATCC). The positive control drug, RBV was produced by Hubei Keyi Pharmaceutical Factory. The newly synthesized benzimidazole compounds (**14–53**) were dissolved in DMSO and diluted with the culture medium. VERO cells were planted in 96-well culture plates. After 24 h the plates were placed in the corresponding virus bulk for 2 h. Then the solutions of benzimidazole compounds and RBV were added in the plates and cell wells and virus wells were set simultaneously. When the cytopathic effect (CPE) of virus wells was over 4, the CPE of cell wells was observed. The concentration required to inhibit virus growth by 50% (IC_{50}) was determined by the Reed–Muench method.[14]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.007. These data include MOL files and InChiKeys of the most important compounds described in this article.

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