HETEROCYCLES, Vol. 98, No. 5, 2019, pp. 650 - 665. © 2019 The Japan Institute of Heterocyclic Chemistry
Received, 8th March, 2019, Accepted, 5th April, 2019, Published online, 22nd April, 2019
DOI: 10.3987/COM-19-14071

FACILE SODIUM METABISULFITE MEDIATED SYNTHESIS OF 1,2-DISUBSTITUTED BENZIMIDAZOLES AND CYTOTOXICITY EVALUATION

Bui Thi Buu Hue,[1]* Hien Minh Nguyen,[2] Mai Van Hieu,[1] Danh La Duc Thanh,[1] Nguyen Hoang Son,[1] Tran Quang De,[1] and Hiroyuki Morita[2]*

[1] Department of Chemistry, College of Natural Sciences, Can Tho University, Viet Nam. Email: btbhue@ctu.edu.vn

[2] Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama 930-0194, Japan. Email: hmorita@inm.u-toyama.ac.jp

Abstract – A library of twenty-four, structurally diverse, 2-substituted and 1,2-disubstituted benzimidazole derivatives (**4a-x**) was designed and synthesized. The benzimidazole derivatives were constructed by a one-pot condensation reaction between 1,2-phenylenediamines and aromatic aldehydes under mild oxidation conditions utilizing inexpensive and non-toxic inorganic salt sodium metabisulfite. These compounds were assayed for cytotoxicities against five cancer cell lines; cervical (HeLa), breast cancer (MCF7), lung cancer (A549), stomach cancer (GSU) and Kato III cell lines *in vitro*. Most of the compounds had slightly inhibitory effects against all or limited tested cell lines. Among them, compound **4q** showed moderate cytotoxicity against HeLa, MCF-7, A549, and Kato III cell lines with IC_{50} values of 28.4 μM, 28.3 μM, 30.7 μM, and 28.5 μM, respectively. The structure-activity relationship analysis suggested that the substructure combination of benzimidazole and naphthalene bearing the free hydroxy group at C-1 and the three methoxy groups at the C-6, C-7, and C-8 of the naphthalene ring may exhibit a synergistic effect and an improved anticancer activity.

INTRODUCTION

Benzimidazole is the isostere of a purine-based nucleic acid. This compound and its derivatives are useful building blocks for the development of bioactive molecules. In particular, benzimidazole is the most prominent heterocycle with cytotoxic properties against different types of cancer cell lines.[1] Compound I

(Figure 1) is an example with the best cytotoxic activity against colon (HCT-116), breast (MDA-MB-468), and blood-leukaemia (CCRF-CEM) cancer cell lines at a 50 μM concentration.[2] Compound II also reportedly possessed good antitumor activity against the two MCF-7 and MDA-MB-468 breast cancer cell lines,[3] and compound III has exhibited significant cytotoxicity against the human lung A549 cancer cell line, with an IC_{50} value of 1.08 μM.[4] Furthermore, previous studies indicated that the 2-substituted and 1,2-disubstituted benzimidazoles play an important role in not only enhancing the cytotoxicity against cancer cell lines, but also producing diverse therapeutic activities, including antituberculosis,[5] antibacterial,[6] anti-inflammatory,[7] and antihistaminic activities.[8]

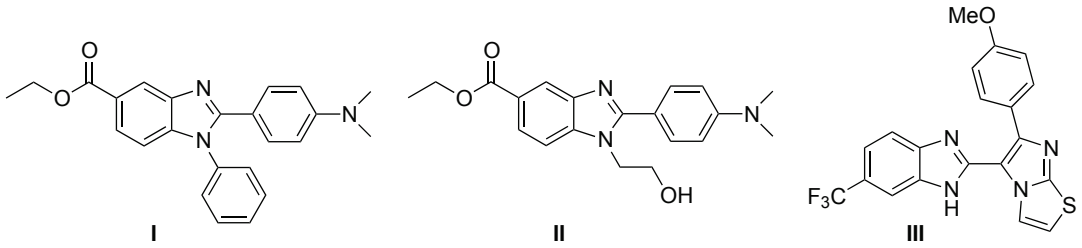


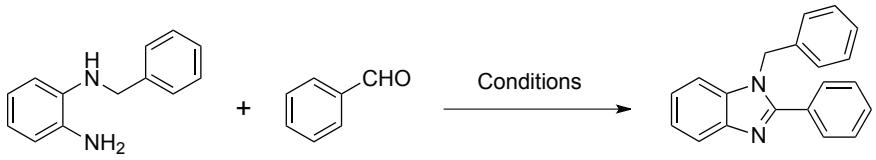
Figure 1. The structures of some cytotoxic benzimidazoles

One of the common methods for the construction of the 2-substituted and 1,2-disubstituted benzimidazoles is the oxidative condensation of *o*-phenylenediamines and *N*-substituted 1,2-phenylenediamine, respectively, with aldehydes.[9] Several reagents, including $NaHSO_3$,[2,10] $Na_2S_2O_3$,[11] $Na_2S_2O_4$,[12] and Oxone,[13] have been utilized to promote this type of condensation. In particular, the use of sodium metabisulfite, a nontoxic and inexpensive inorganic salt, in the synthesis of 2-arylbenzimidazoles has also been reported.[14] Despite its availability, there are only a few reports on the use of $Na_2S_2O_5$ for the construction of disubstituted benzimidazoles with two different functional groups at positions 1 and 2 of the benzimidazole ring.[3,15] Notably, the protocols developed in these reports required the use of a toxic and high boiling point solvent such as DMF, at a high temperature and for a prolonged reaction time. In a previous study, we reported the synthesis of twelve 2-arylbenzimidazoles, using sodium metabisulfite under microwave irradiation conditions, and their cytotoxicities.[16] In our ongoing research for the development of new cytotoxic benzimidazole derivatives, we have focused on establishing an ideal, mild reaction procedure to construct 2-substituted and especially 1,2-disubstituted benzimidazole derivatives using inexpensive sodium metabisulfite as the reagent. Herein we report a facile one-pot protocol for the construction of twenty-four 2-substituted and 1,2-disubstituted benzimidazole derivatives, including fourteen new ones, and their cytotoxic activities against five human cancer cell lines, as well as the structure-activity relationships of the synthesized benzimidazole derivatives.

RESULTS AND DISCUSSION

The condensation reaction between *N*-benzyl-*o*-phenylenediamines and substituted aromatic aldehydes was designed in this study, in order to synthesize benzimidazoles bearing diverse substituents at positions 1 and 2 of the benzimidazole ring. To set up the reaction conditions, the simple and readily available *N*-benzyl-1,2-phenylenediamine **1a** and the commercially available benzaldehyde **2a** were selected as the model substrates for the construction of the 1,2-disubstituted benzimidazole **4a**. Two reaction conditions using air (entries 1 and 2) and three sulfur reagents including sodium bisulfite ($NaHSO_3$), sodium hydrosulfite ($Na_2S_2O_4$), and sodium metabisulfite ($Na_2S_2O_5$), were evaluated in this study (entries 3-13). The results are presented in Table 1.

Table 1. Optimization of the reaction conditions[a]



1a **2a** **4a**

Entry	Oxidant	Molar ratio 1a:2a	Solvent, Temp.	Time	Yield[a]
1	air	1:1	DMF:H_2O (1:1), 100 °C	20 h	75%[b]
2	air	1:1	DMF, 100 °C	20 h	78%[b]
3	$NaHSO_3$ (3 eq)	1:1	EtOH, 80 °C	5 h	86%
4	$Na_2S_2O_4$ (3 eq)	1:1	EtOH, 80 °C	1 h	90%
5	$Na_2S_2O_5$ (3 eq)	1:1	EtOH, 80 °C	1 h	94%
6	$Na_2S_2O_4$ (3 eq)	1:1	DMSO, 100 °C	2 h	80%
7	$Na_2S_2O_5$ (3 eq)	1:1	DMSO, 100 °C	2 h	82%
8	$Na_2S_2O_5$ (3 eq)	1:1	DMF, 100 °C	2 h	88%
9	$Na_2S_2O_5$ (3 eq)	1:1	90% EtOH, 80 °C	1 h	57%
10	$Na_2S_2O_5$ (2 eq)	1:1	EtOH, 80 °C	1 h	89%
11	$Na_2S_2O_5$ (1 eq)	1:1	EtOH, 80 °C	1 h	72%
12	$Na_2S_2O_5$ (3 eq)	1:1	EtOH, rt	24 h	77%
13	$Na_2S_2O_5$ (3 eq)	1:1.2	EtOH, 80 °C	1 h	95%

[a] Conditions: **1a** (0.3 mmol); solvent (1 mL) in 10 mL septum seal tube, 80 °C (oil bath). Isolated yield.
[b] Open to air.

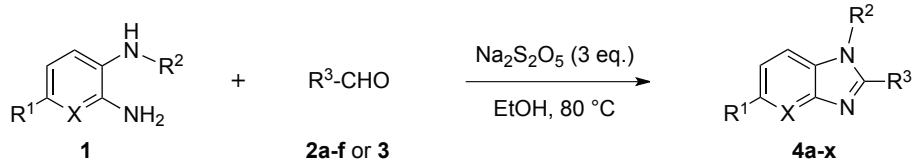
As expected, all combinations of the substrates and the tested oxidants led to the successful construction of the 1,2-disubstituted benzimidazole skeleton. In contrast to the previous studies where air served as a good oxidant for the synthesis of 2-arylbenzimidazoles and simple 1,2-disubstituted benzimidazoles,[17] higher temperatures and prolonged reaction times were required in such reactions (entries 1 and 2) to

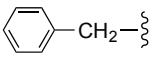
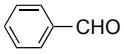
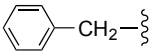
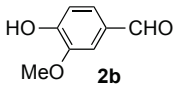
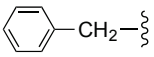
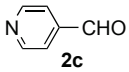
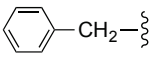
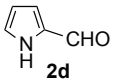
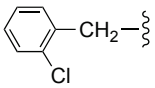
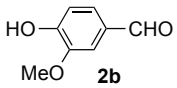
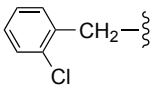
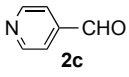
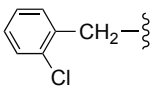
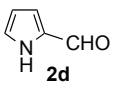
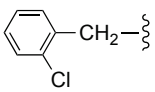
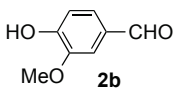
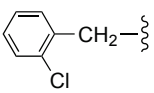
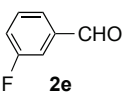
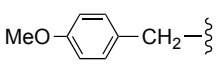
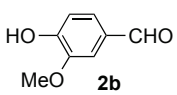
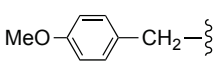
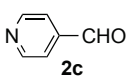
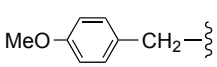
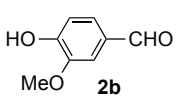
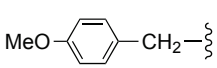
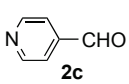
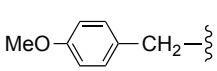
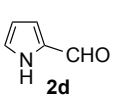
obtain desired product in 75-78% yields. Quantitative amount of the starting materials together with the imine product still remained in the reaction mixture, as observed in thin layer chromatography, when the reaction was run at the temperature below 100 ºC or for less than 20 h. However, the use of $NaHSO_3$ in the condensation reaction dramatically reduced the reaction time to 5 h with significant improvements of the yield of the desired product to 86% and a lower reaction temperature of 80 °C (entry 3). Moreover, the uses of $Na_2S_2O_4$ and $Na_2S_2O_5$ under the identical reaction conditions to entry 3 reduced the reaction time to 1 h and increased the product yields to 90% and 94%, respectively (entries 4 and 5). However, changing the solvent from ethanol to DMSO and increasing the reaction temperature from 80 °C to 100 °C, in the case of the use of $Na_2S_2O_4$ and $Na_2S_2O_5$, led to a decrease in the yields of the desired product (entries 6 and 7). Based on the observation of the highest yield, the lowest temperature, and the shortest reaction time for the production of the desired products in the above reaction conditions, we selected $Na_2S_2O_5$ as the reagent for the condensation reaction, and tested further different reaction conditions, including the use of DMF as a solvent at 100 °C (entry 8) and 90% ethanol as the solvent at 80 °C (entry 9), decreasing the amount of $Na_2S_2O_5$ in ethanol at 80 °C (entries 10 and 11), and performing the reaction at room temperature in ethanol (entry 12). However, lower yields were observed in all of these cases. In particular, the use of 90% ethanol as the solvent considerably reduced the yield of the product (57%, entry 9). Finally, we also tested the effect of the molar ratio between the aldehyde **2a** and the diamine **1a**. However, an increase of the ratio from 1:1 to 1.2:1 only showed a 1% increase in the yield of the desired product over that of entry 5 (entry 13).

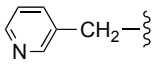
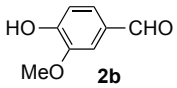
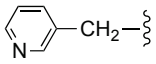
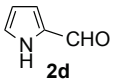
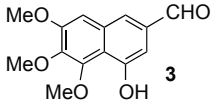
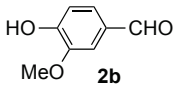
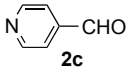
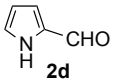
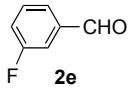
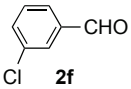
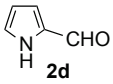
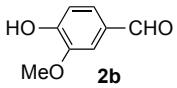
Thus, using the optimized conditions of entry 5, the substrate scope of this facile method to construct 1,2-disubstituted and 2-substituted benzimidazoles was examined, and we obtained twenty-four structurally diverse 2-substituted (**4a-p**) and 1,2-disubstituted benzimidazole derivatives (**4q-x**), including fourteen new ones (**4b-e**, **4g-p**). The substrate scope is presented in Table 2, in which the diamines (**1h-k**) and the aldehydes (**2a-f**) are commercially available, while the diamines (**1a-g**) were prepared according to the previously reported procedures.[12,18] The aldehyde **3** was prepared via a tandem Stobbe reaction and cyclization, starting from the commercially available 3,4,5-trimethoxybenzaldehyde.[16] In all cases, the reactions were proceeded smoothly and were completed within 2 h to generate the desired products with excellent isolated yields, ranging from 73% to 94%. It is worth noting that the protocols developed in the previous work required refluxing phenylenediamine with the previously prepared bisulfite adducts of the aldehydes in ethanol[19] or in DMF overnight.[3,15a,15b,20] In contrast, we found an efficient, environmentally benign, one-pot route by the combination of *N*-benzyl-*o*-phenylenediamines, substituted aromatic aldehydes, and sodium metabisulfite that afforded the 1,2-disubstituted benzimidazole derivatives in excellent yields. For industrial applications, the reasonable cost of chemicals, the applicability of reaction conditions, and the environmental consequences must be considered. This general, simple, fast, and clean

synthesis of the 2-substituted and 1,2-disubstituted benzimidazoles could be an effective method even for large-scale reaction because $Na_2S_2O_5$ is inexpensive and readily available.

Table 2. Substrate scope of the synthesis of benzimidazole derivatives (**4a-x**)[a]



Entry	Substrate 1				Aldehyde	Product	Time	Yield (%)
		X	R^1	R^2				
1	**1a**	C	H		 **2a**	**4a**	1 h	94
2	**1b**	C	CF_3		 **2b**	**4b**	45 min	85
3	**1b**	C	CF_3		 **2c**	**4c**	1 h	84
4	**1b**	C	CF_3		 **2d**	**4d**	1 h	90
5	**1c**	C	H		 **2b**	**4e**	1 h	83
6	**1c**	C	H		 **2c**	**4f**	1 h	87
7	**1c**	C	H		 **2d**	**4g**	1 h	85
8	**1d**	C	CF_3		 **2b**	**4h**	1.5 h	85
9	**1d**	C	CF_3		 **2e**	**4i**	1 h	88
10	**1e**	C	H		 **2b**	**4j**	1 h	81
11	**1e**	C	H		 **2c**	**4k**	1 h	87
12	**1f**	C	CF_3		 **2b**	**4l**	1.5 h	84
13	**1f**	C	CF_3		 **2c**	**4m**	1 h	85
14	**1f**	C	CF_3		 **2d**	**4n**	1.5 h	83

15	**1g**	C	CF_3		 **2b**	**4o**	1 h	86
16	**1g**	C	CF_3		 **2d**	**4p**	1 h	83
17	**1h**	C	H	H	 **3**	**4q**	1.5 h	92
18	**1i**	C	Cl	H	 **2b**	**4r**	1.5 h	80
19	**1i**	C	Cl	H	 **2c**	**4s**	2 h	75
20	**1i**	C	Cl	H	 **2d**	**4t**	1 h	82
21	**1i**	C	Cl	H	 **2e**	**4u**	1 h	85
22	**1i**	C	Cl	H	 **2f**	**4v**	1 h	85
23	**1j**	C	NO_2	H	 **2d**	**4w**	2 h	82
24	**1k**	N	H	H	 **2b**	**4x**	2 h	73

[a] Conditions: Aldehyde (0.3 mmol); diamine (0.3 mmol); solvent (1 mL), 10 mL septum seal tube, 80 °C (oil bath). Isolated yield.

All of the synthesized 2-substituted and 1,2-disubstituted benzimidazole derivatives **4a-x** were tested for their cytotoxicities against five human cancer cell lines, including cervical cancer (HeLa), breast cancer (MCF7), lung cancer (A549), and stomach cancer (GSU and Kato III) cell lines. Their cytotoxicities against each tested cancer cell line are summarized in Table 3. Among the tested compounds, compounds **4j** and **4o** did not show any cytotoxicities against all tested cancer cell lines. Similar results were also found for the cytotoxicities of compounds **4i** and **4s**. Compounds **4f**, **4p**, **4r** and **4x** showed weak activities against some of the cancer cell lines. Remarkably, compound **4q** exhibited the best activities among the tested compounds and had moderate cytotoxicities against all tested cancer cell lines, with IC_{50} values ranging from 28.4 to 35.4 μM. The other compounds showed weak or very weak cytotoxicities against all tested cell lines.

To further clarify the effects of the substitutions on the enhancement of the cytotoxic activities of the benzimidazole derivatives against cancer cell lines, structure-activity comparisons were performed. In the cases of the 1,2-disubstituted benzimidazole derivatives **4a-p**, the introduction of the 3-fluorophenyl group at the C-2 position did not enhance the activities, as verified by the cytotoxicity of compound **4i**.

Furthermore, as deduced from the assay results of compounds **4o** and **4p**, the presence of the 3-pyridinylmethyl group at N-1, in combination with the vanillin moiety at C-2, was also not required to increase the activities. Similar results were also observed for compound **4j**, with the *p*-methoxybenzyl group occupying the N-1 position. Remarkably, the incorporation of the trifluoromethyl ($–CF_3$) group at C-5, in conjunction with the vanillin or the *N*-heterocyclic moieties, especially the pyrrole moiety at C-2, led to approximately 1.5- to 2-fold increases of the activities as compared to the simplest 1,2-disubstituted derivative **4a**, as displayed for the cytotoxicities of compounds **4b**, **4c**, **4g**, **4m**, and **4n** against the Hela, compounds **4e**, **4g**, and **4n** against MCF-7, compounds **4b**, **4e**, **4h**, **4g**, and **4k** against A549, compounds **4h** and **4l** against GSU, and compound **4k** against Kato III cell lines. It is well known that the $–CF_3$ group is relatively highly lipophilic[21] and can permeate the cell membrane easily. Thus, the 1,2-disubstituted 5-trifluoromethylbenzimidazole derivatives are likely to be more active, as compared to the rest of the tested compounds. In addition, the *N*-heterocycle or the vanillin moieties at C-2 can facilitate enzyme or receptor binding via hydrogen bonds or hydrophobic interactions. Combining these structural features may have synergistic advantages and might be responsible for the observed activities.

Table 3. Cytotoxicities of compounds (**4a-x**) against a panel of five human cancer cell lines

Compounds	Cell lines[a] (IC_{50} μM)				
	HeLa	MCF7	A549	GSU	Kato III
4a	84.4	71.8	93.5	80	75.0
4b	50.5	68.4	48.0	86.4	91.0
4c	50.9	60.6	81.3	75.6	68.3
4d	53.6	56.1	62.9	62.5	58.5
4e	59.4	48.5	46.0	54.2	52.6
4f	62.3	51.1	>100	71.3	65.2
4g	43.8	44.4	43.1	79.4	78.2
4h	54.7	50.5	50.5	52.0	62.2
4i	>100	>100	>100	>100	88.2
4j	>100	>100	>100	>100	>100
4k	84.6	56.3	51.2	77.8	47.3
4l	57.3	55.5	56.6	49.4	76.9
4m	51.5	51.6	69.9	69.1	78.3
4n	49.3	45.5	60.6	87.7	96.0
4o	>100	>100	>100	>100	>100
4p	67.7	82.4	72.5	>100	>100
4q	28.4	28.3	30.7	35.4	28.5
4r	47.7	69.0	>100	68.3	72.5
4s	93.4	>100	>100	>100	85.8
4t	64.6	55.4	72.7	52.3	78.6

4u	58.3	60.3	64.3	61.5	65.2
4v	54.8	58.2	67.1	49.4	65.3
4w	59.8	51.7	78.8	91.5	87.7
4x	69.3	>100	>100	>100	79.7
5-Fluorouracil[b]	6.52	5.41	6.10	3.12	3.68

[a] HeLa, human cervix cancer; MCF7, breast cancer; A549, human lung cancer; GSU, Kato III, human stomach cancer. [b] Positive control.

Further structure-activity relationship studies for the 2-substituted benzimidazole derivatives **4q-x** revealed that the combination of the benzimidazole and naphthalene with the free hydroxy group at C-1 and the three methoxy groups at C-6, C-7, and C-8 is the most effective to improve the cytotoxicities of the benzimidazole derivatives, as verified from the comprehensive assessment of the activities of all tested compounds. This observation is in good agreement with our previous work[16] and strongly confirms the cytotoxicity-induced role of the benzimidazole-naphthalene hybrid structure bearing the free hydroxy group at C-1 and the three methoxy groups at C-6, C-7, and C-8 of the naphthalene ring. The presence of the additional chloro (–Cl) or methoxy (–OMe) groups at C-5(6) of the benzimidazole heterocycle undoubtedly contributes to the dramatically increased cytotoxicity against the MCF-7 cell line, as reported in our previous work. Furthermore, this result is also in accordance with the finding that a 3,4,5-trimethoxyphenyl ring was essential for potent antitumor activity.[22] Thus, the substructure combination of benzimidazole and naphthalene or small *N*-heterocycles with the $–CF_3$ or –Cl groups at C-5(6) of the benzimidazole ring may exhibit a synergistic effect and improved anticancer activity.

In conclusion, we have reported an efficient, environmentally friendly, one-pot route to synthesize twenty-four 2-substituted and 1,2-disubstituted benzimidazoles and their *in vitro* cytotoxicities against the HeLa, MCF7, A549, GSU, and Kato III human cancer cell lines. This general, simple, fast, and clean synthetic protocol using inexpensive and easily available $Na_2S_2O_5$ could be an effective method for large-scale synthesis. Furthermore, the present work clearly demonstrated that the combined benzimidazole and naphthalene bearing the free hydroxy group at C-1 and the three methoxy groups at C-6, C-7, and C-8 of the naphthalene ring represent the most effective structural features for enhancing the cytotoxicities of benzimidazoles. Further evaluations of the structure-activity relationships of benzimidazole-naphthalene hybrid analogues might contribute to the development of structurally interesting anti-cancer agents.

EXPERIMENTAL

Reactions were monitored by thin-layer chromatography (TLC) on 0.2-mm precoated silica-gel 60 F254 plates (Merck). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Avance 300, 500, and 600 MHz spectrometers in which chemical shifts are given in parts per million (ppm) relative to TMS and *J*

values are given in Hertz. MS were recorded on 1100 series LC-MSD-Trap-LS Aligent spectrometer by electrospray impact (ESI). FT-IR was acquired on Thermo Nicolet 6800 using KBR pellet method. Melting point was measured on Stuart SMP3 without calibration. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (Me_4Si, $\delta = 0$); J values are given in Hertz. All the chemicals and solvents used in this study were of analytical grade.

General procedure for the synthesis of benzimidazole derivatives (4a-x): A mixture of aldehyde **1** (0.3 mmol), diamine **2** (0.3 mmol) and $Na_2S_2O_5$ (0.9 mmol, 3 equiv.) was suspended in EtOH (1.0 mL) in a round bottom flask and sealed with septum. The resulting mixture was stirred in a 80 °C oil bath for appropriate time. After the completion as indicated by TLC, water (5 mL) was added to dissolve the excess $Na_2S_2O_5$ followed by treating with saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 and solvent was evaporated under reduced pressure. Purification by column chromatography afforded the desired benzimidazole products (**4a-x**).

1-Benzyl-2-phenyl-1*H*-benzo[*d*]imidazole (4a): White solid; mp 124−126 °C. IR (KBr) ν_{max}: 3059, 3030, 1604, 1469, 1392, 1362, 737, 699 cm^{-1}; HR-ESI-MS found *m/z* 285.1386 $[M+H]^+$ (calcd. 285.1386, $C_{20}H_{17}N_2$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 7.73-7.72 (m, 3H), 7.53-7.51 (m, 3H), 7.46-7.45 (m, 1H), 7.29-7.21 (m, 5H), 6.99 (d, J = 7.0 Hz, 2H), 5.58 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 153.2, 142.6, 136.9, 135.8, 130.1, 129.8, 129.0, 128.7, 127.4, 126.0, 122.7, 122.2, 119.2, 111.1, 47.4.

4-(1-Benzyl-5-trifluoromethyl-1*H*-benzo[*d*]imidazol-2-yl)-2-methoxyphenol (4b): White solid; mp 200−202 °C; IR (KBr) ν_{max}: 2923, 2853, 1488, 1431, 1333, 1281, 1212, 1155, 1109, 806 cm^{-1}; HR-ESI-MS found *m/z* 399.1314 $[M+H]^+$ (calcd. 399.1315, $C_{22}H_{18}N_2F_3O_2$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 9.61 (brs, 1H), 8.05 (s, 1H), 7.65 (*d*, J = 8.0 Hz, 1H), 7.54 (dd, J = 1.0, 8.5 Hz, 1H), 7.32 (t, J = 7.5 Hz, 2H), 7.26 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.0 Hz, 1H), 7.04 (d, J = 7.5 Hz, 2H), 6.91 (d, J = 8.0 Hz, 1H), 5.65 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 155.9, 148.7, 147.6, 142.1, 138.4, 136.7, 128.8, 127.5, 126.1, 125.9, 123.9, 123.1, 122.8, 122.2, 120.1, 118.9, 116.2, 115.6, 112.9, 111.8, 55.4, 47.8.

1-Benzyl-2-(pyridin-4-yl)-5-(triflouromethyl)-1*H*-benzo[*d*]imidazole (4c): White solid; mp 127−129 °C; IR (KBr) ν_{max}: 3037, 1606, 1442, 1413, 1331, 1230, 1161, 1118, 811, 727 cm^{-1}; HR-ESI-MS found *m/z* 354.1210 $[M+H]^+$ (calcd. 354.1213, $C_{20}H_{15}N_3F_3$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 8.75 (dd, J = 4.5 Hz, 2H), 8.17 (s, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.75 (d, J = 4.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 1H), 7.28 (m, 3H), 7.00 (d, J = 7, 2H), 5.74 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 153.0, 150.3, 141.9, 138.4, 136.8, 136.2, 128.9, 127.7, 126.1, 123.7, 123.5, 123.2, 120.0, 117.2, 112.5, 47.8.

1-Benzyl-2-(1*H*-pyrrol-2-yl)-5-(triflouromethyl)-1*H*-benzo[*d*]imidazole (4d): White solid; mp 117−119 °C; IR (KBr) ν_{max}: 3403, 3190, 2930, 1623, 1498, 1432, 1330, 1119, 1050, 933, 872, 808, 724

cm^{-1}; HR-ESI-MS found *m/z* 342.1204 $[M+H]^+$ (calcd. 342.1213, $C_{19}H_{15}F_3N_3$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 11.97 (brs, 1H), 7.94 (s, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.53 (t, *J* = 4.25 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.27 (d, *J* = 7.5 Hz, 1H), 7.08 (d, *J* = 7.5 Hz, 2H), 7.02 (s, 1H), 6.57 (s, 1H), 6.18 (d, *J* = 5.5 Hz, 1H), 5.79 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 149.3, 142.1, 138.6, 136.5, 129.0, 127.4, 126.0, 123.1, 122.9, 122.5, 119.9, 118.6, 115.1, 111.0, 110.9, 109.7, 47.3.

4-(1-(2-Chlorobenzyl)-1*H*-benzo[*d*]imidazol-2-yl)-2-methoxyphenol (4e): White solid; mp 164−166 °C; IR (KBr) ν_{max}: 3061, 2951, 1606, 1585, 1484, 1430, 1290, 743 cm^{-1}; HR-ESI-MS found *m/z* 365.1044 $[M+H]^+$ (calcd. 365.1051, $C_{21}H_{18}ClN_2O_2$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 9.56 (s, 1H), 7.73 (d, *J* = 7.5 Hz, 1H), 7.55 (dd, *J* = 0.5, 8.0 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.27-7.20 (m, 3H), 7.11 (d, *J* = 2.0 Hz, 1H), 7.06 (dd, *J* = 2.0, 8.0 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.62 (d, *J* = 7.0 Hz, 1H), 5.58 (s, 2H), 3.61 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_H 153.6, 148.3, 147.5, 142.5, 136.0, 134.4, 131.2, 129.7, 129.3, 127.8, 126.9, 122.5, 122.2, 121.8, 120.6, 119.0, 115.6, 112.5, 110.5, 55.2, 45.9.

1-(2-Chlorobenzyl)-2-(pyridin-4-yl)-1*H*-benzo[*d*]imidazole (4f): White solid; mp 124−126 °C; IR (KBr) ν_{max}: 3031, 1604, 1474, 1446, 1414, 1392, 1328, 1278, 1045, 825, 739, 697 cm^{-1}; HR-ESI-MS found *m/z* 320.0946 $[M+H]^+$ (calcd. 320.0949, $C_{19}H_{15}N_3Cl$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H 8.72 (d, *J* = 5.7 Hz, 2H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.57 (d, *J* = 6.0 Hz, 2H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.28-7.41 (m, 3H), 7.15-7.22 (m, 2H), 6.72 (d, *J* = 7.5 Hz, 1H), 5.55 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 150.7, 150.2, 142.5, 137.3, 136.1, 133.7, 131.4, 129.7, 129.4, 127.7, 127.4, 123.6, 122.9, 122.8, 119.8, 111.1, 45.9.

1-(2-Chlorobenzyl)-2-(1*H*-pyrrol-2-yl)-1*H*-benzo[*d*]imidazole (4g): White solid; mp 140−142 °C; IR (KBr) ν_{max}: 3141, 2924, 2860, 1504, 1454, 1401, 1136, 1044, 743 cm^{-1}; HR-ESI-MS found *m/z* 308.0939 $[M+H]^+$ (calcd. 308.0949, $C_{18}H_{15}ClN_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H 10.97 (brs, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.51 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.27-7.34 (m, 2H), 7.23-7.25 (m, 2H), 7.10 (td, *J* = 7.5, 1.2 Hz, 1H), 7.04 (d, *J* = 0.9 Hz, 1H), 6.67 (d, *J* = 7.8 Hz, 1H), 6.33 (m, 1H), 6.26 (m, 1H), 5.69 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 147.1, 142.5, 136.0, 134.1, 131.3, 129.7, 129.2, 127.7, 126.4, 122.2, 122.1, 121.78, 120.5, 118.3, 109.9, 109.4, 109.2, 45.3.

4-(1-(2-Chlorobenzyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)-2-methoxyphenol (4h): White solid; mp 180−182 °C; IR (KBr) ν_{max}: 3069, 1590, 1627, 1430, 1486, 1332, 1279, 750 cm^{-1}; HR-ESI-MS found *m/z* 433.0930 $[M+H]^+$ (calcd. 433.0925, $C_{22}H_{17}ClF_3N_2O_2$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 9.66 (s, 1H), 8.09 (s, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.56-7.54 (m, 2H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 7.13 (d, *J* = 1.5 Hz, 1H), 7.10 (dd, *J* = 2.0, 8.5 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.64 (d, *J* = 7.5 Hz, 1H), 5.66 (s, 2H), 3.62 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 155.9, 148.7, 147.6, 142.1, 138.5, 133.9, 131.3, 129.7, 129.4, 127.9, 127.0, 126.0, 123.9, 123.3, 123.0, 122.1, 119.9, 119.1, 116.3,

115.7, 112.5, 111.7, 55.2, 46.3.

1-(2-Chlorobenzyl)-2-(3-fluorophenyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (4i): White solid; mp 164−166 °C; IR (KBr) ν_{max}: 2924, 2852, 1618, 1585, 1463, 1439, 1377, 1331, 1267, 1236, 1163, 1109, 1047, 926, 755 cm^{-1}; HR-ESI-MS found *m/z* 405.0783 $[M+H]^+$ (calcd. 405.0776, $C_{21}H_{14}ClF_4N_2$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 8.15 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 6.30 (dd, J = 8.4, 1.5 Hz, 1H), 7.48-7.58 (m, 4H), 7.41 (tm, 1H), 7.31 (td, J = 7.5, 1.2 Hz, 1H), 7.21 (td, J = 7.8, 1.2 Hz, 1H), 6.67 (d, J = 7.5 Hz, 1H), 5.71 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 162.8, 160.8, 154.3, 141.8, 138.3, 133.4, 131.5, 131.42, 131.38, 131.1, 131.0, 129.7, 127.8, 127.5, 125.05, 125.03, 123.32, 119.7, 117.4, 117.2, 116.9, 115.9, 115.7, 112.2, 46.1.

2-Methoxy-4-[1-(4-methoxybenzyl)-1*H*-benzo[*d*]imidazol-2-yl]phenol (4j): White solid; mp 207−209 °C; IR (KBr) ν_{max}: 3037, 2922, 1608, 1514, 1411, 1341, 1244, 1182, 1158, 1107, 812 cm^{-1}; HR-ESI-MS found *m/z* 361.1550 $[M+H]^+$ (calcd. 361.1547, $C_{20}H_{18}N_2O_3$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 9.52 (s, 1H), 7.66 (dd, J = 2.0, 6.5 Hz, 1H), 7.42 (dd, J = 2.5, 6.75 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 7.22-7.17 (m, 2H), 7.15 (dd, J = 2.0, 8.0 Hz, 1H), 6.97 (d, J = 8.5 Hz, 2H), 6.89 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 8.5 Hz, 2H), 5.49 (s, 2H), 3.71 (s, 3H), 3.69 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 158.5, 153.5, 148.2, 147.6, 142.6, 135.9, 128.9, 127.4, 122.2, 121.9, 120.9, 118.8, 115.5, 114.1, 113.0, 110.8, 55.5, 55.0, 46.9.

1-(4-Methoxybenzyl)-2-pyridin-4-yl-1*H*-benzo[*d*]imidazole (4k): White solid; mp 134−136 °C; IR (KBr) ν_{max}: 3041, 2999, 1607, 1513, 1392, 1290, 1247, 1178, 1026, 829, 749 cm^{-1}; HR-ESI-MS found *m/z* 316.1455 $[M+H]^+$ (calcd. 316.1444, $C_{20}H_{18}N_3O$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 8.73 (d, J = 5.5 Hz, 2H), 7.77-7.74 (m, 3H), 7.57-7.55 (m, 1H), 7.30-7.28 (m, 2H), 6.92 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 5.59 (s, 2H), 3.67 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 158.6, 150.5, 150.2, 142.6, 137.6, 136.1, 128.5, 127.5, 123.4, 123.2, 122.6, 119.7, 114.2, 111.4, 55.0, 47.0.

2-Methoxy-4-[1-(4-methoxybenzyl)-5-trifluoromethyl-1*H*-benzo[*d*]imidazol-2-yl]phenol (4l): White solid; mp 202−204 °C; IR (KBr) ν_{max}: 3021, 1515, 1479, 1430, 1328, 1253, 1118, 1029, 818 cm^{-1}; HR-ESI-MS found *m/z* 429.1423 $[M+H]^+$ (calcd. 429.1421, $C_{23}H_{20}N_2O_3F_3$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 9.62 (s, 1H), 8.03 (s, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.25 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.0 Hz 1H), 6.97 (d, J = 9.0 Hz, 2H), 6.92 (d, J = 8.0 Hz, 1H), 6.86 (d, J = 9.0 Hz, 2H), 5.57 (s, 2H), 3.72 (s, 3H), 3.69 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 158.6, 155.9, 148.7, 147.7, 142.1, 138.3, 128.5, 127.4, 126.1, 123.9, 123.1, 122.8, 122.2, 120.2, 118.9, 116.1, 115.6, 114.2, 113.0, 111.9, 55.5, 55.1, 47.3.

1-(4-Methoxybenzyl)-2-pyridin-4-yl-5-trifluoromethyl-1*H*-benzo[*d*]imidazole (4m): White solid; mp 143−145 °C; IR (KBr) ν_{max}: 3037, 1608, 1514, 1411, 1341, 1292, 1182, 1158, 1107 cm^{-1}; HR-ESI-MS found *m/z* 384.1322 $[M+H]^+$ (calcd. 384.1318, $C_{21}H_{17}N_3F_3O$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 8.77 (d, J = 6.0 Hz, 2H), 8.15 (s, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.76 (dd, J = 1.5, 4.5 Hz, 2H), 7.63 (d, J = 9.0

Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 5.65 (s, 2H), 3.68 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 158.7, 152.9, 150.3, 141.9, 138.3, 136.9, 127.9, 127.6, 125.9, 123.7, 123.6, 123.4, 123.2, 119.9, 117.1, 117.0, 114.2, 112.6, 55.0, 47.3.

1-(4-Methoxybenzyl)-2-(1*H*-pyrrol-2-yl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (4n): White solid; mp 150−152 °C. IR (KBr) ν_{max}: 3384, 2922, 2852, 1614, 1581, 1515, 1483, 1431, 1334, 1248, 1152, 1098, 1050, 934, 809, 745, 600 cm^{-1}; HR-ESI-MS found *m/z* 372.1828 $[M+H]^+$ (calcd. 372.1318, $C_{20}H_{17}F_3N_3O$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 11.94 (brs, 1H), 7.93 (s, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.51 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.01-7.03 (m, 3H), 6.87-6.90 (m, 2H), 6.63 (m, 1H), 6.20 (m, 1H), 5.71 (s, 2H), 3.69 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 158.4, 149.3, 142.1, 138.4, 128.2, 127.3, 126.1, 123.0, 122.8, 122.4, 120.0, 118.5, 115.1, 114.2, 111.1, 110.9, 109.7, 55.0, 46.8.

2-Methoxy-4-(1-(pyridin-3-ylmethyl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)phenol (4o): White solid; IR (KBr) ν_{max}: 3450, 2928, 1727, 1589, 1494, 1330, 1284, 1218, 1119, 1040, 876, 799 cm^{-1}; HR-ESI-MS found *m/z* 400.1271 $[M+H]^+$ (calcd. 400.1267, $C_{21}H_{17}F_3N_3O_2$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 9.65 (s, 1H), 8.45 (d, *J* = 3.5 Hz, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.29-7.34 (m, 2H), 7.23 (s, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 6.92 (d, *J* = 8.0 Hz, 1H), 5.73 (s, 2H), 3.72 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 155.9, 148.8, 148.7, 147.8, 147.7, 142.1, 138.2, 133.9, 132.3, 123.8, 122.2, 120.0, 119.0, 116.3, 116.2, 115.7, 113.0, 111.7, 55.5, 45.6.

1-(Pyridin-3-ylmethyl)-2-(1*H*-pyrrol-2-yl)-5-(trifluoromethyl)-1*H*-benzo[*d*]imidazole (4p): White solid; mp 138−140 °C; IR (KBr) ν_{max}: 3145, 2919, 1616, 1577, 1501, 1456, 1401, 1321, 1250, 1133, 931, 834, 746 cm^{-1}; HR-ESI-MS found *m/z* 343.1898 $[M+H]^+$ (calcd. 343.1165, $C_{18}H_{14}F_3N_4$); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 11.96 (s, 1H), 8.47 (dd, *J* = 4.2, 1.2 Hz, 1H), 8.43 (d, *J* = 1.8 Hz, 1H), 7.95 (s, 1H), 7.78 (d, *J* = 9.0 Hz, 1H), 7.53 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.32 (m, 1H), 7.03 (m, 1H), 6.65 (m, 1H), 6.21 (dd, *J* = 6.0, 2.4 Hz, 1H), 5.87 (s, 2H); ^{13}C-NMR (150 MHz, DMSO-d_6): δ_C 149.0, 148.6, 147.7, 141.0, 111.0, 110.9, 109.5, 45.0.

3-(1*H*-Benzo[*d*]imidazol-2-yl)-6,7,8-trimethoxynaphthalen-1-ol (4q): White solid; mp 253−255 °C; IR (KBr) ν_{max}: 3325, 2936, 1733, 1615, 1580, 1481, 1422, 1380, 1261, 1182, 1113, 1087, 1021, 746 cm^{-1}; HR-ESI-MS found *m/z* 351.1343 $[M+H]^+$ (calcd. 351.1339, $C_{20}H_{17}N_2$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 12.90 (s, 1H), 9.65 (s, 1H), 8.07 (d, *J* = 1.5 Hz, 1H), 7.66 (d, *J* = 8 Hz, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 1.5 Hz, 1H), 7.21-7.18 (m, 3H), 4.01 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 153.7, 153.2, 151.2, 148.2, 143.8, 140.8, 135.0, 132.4, 128.0, 122.5, 121.7, 118.8, 116.1, 113.8, 111.3, 106.0, 104.1, 62.3, 60.9, 55.8.

4-(5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)-2-methoxyphenol (4r): Pale brown solid; mp 125−127 °C; IR (KBr) ν_{max}: 3280, 2933, 1601, 1500, 1429, 1273, 1215, 1128, 1030, 926, 813 cm^{-1}; HR-ESI-MS found *m/z* 275.0584 $[M+H]^+$ (calcd. 275.0587, $C_{14}H_{12}ClN_2O_2$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 9.63 (s, 1H),

7.73 (d, *J* = 1.8 Hz, 1H), 7.59-7.62 (m, 2H), 7.55 (d, *J* = 8.7 Hz, 1H), 7.19 (d, *J* = 8.4, 1.8 HZ, 1H), 6.92 (dd, *J* = 8.1 Hz, 1H). 3.89 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 153.2, 149.0, 147.9, 121.8, 120.9, 119.9, 115.7, 112.2, 110.5, 55.7.

5-Chloro-2-(pyridin-4-yl)-1*H*-benzo[*d*]imidazole (4s): Pale brown solid; mp 285−287 °C; IR (KBr) ν_{max}: 3043, 2962, 1864, 1699, 1612, 1425, 1304, 1001, 829, 800, 691 cm^{-1}; HR-ESI-MS found *m/z* 230.0461 $[M+H]^+$ (calcd. 230.0480, $C_{12}H_9ClN_3$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 13.5 (brs, 1H), 8.78 (dd, *J* = 4.5, 1.5 Hz, 2H), 8.08 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.73 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.30 (dd, *J* = 8.7, 2.1 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 150.5, 136.6, 123.6, 122.7, 120.7, 120.4, 118.8, 113.2, 111.4.

5-Chloro-2-(1*H*-pyrrol-2-yl)-1*H*-benzo[*d*]imidazole (4t): Pale brown solid; mp 163−165 °C; IR (KBr) ν_{max}: 3298, 3174, 2926, 1706, 1632, 1516, 1466, 1392, 1339, 1274, 1040, 987, 928, 807, 732, 590 cm^{-1}; HR-ESI-MS found *m/z* 218.0477 $[M+H]^+$ (calcd. 218.0480, $C_{11}H_9ClN_3$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 12.66 (brs, 1H), 11.84 (brs, 1H), 7.55 (m, 1H), 7.45 (m, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.95 (m, 1H), 6.86 (s, 1H), 6.20 (dd, *J* = 5.7, 2.4 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6; mixture of tautomers) δ_C 148.2, 147.8, 144.8, 142.6, 135.3, 133.3, 128.6, 125.5, 122.1, 121.8, 121.5, 118.8, 117.0, 111.8, 11.03, 109.6, 109.2.

5-Chloro-2-(3-fluorophenyl)-1*H*-benzo[*d*]imidazole (4u): White solid; mp 92−94 °C; IR (KBr) ν_{max}: 3142, 2924, 1680, 1589, 1482, 1457, 1271, 1207, 1059, 989, 928, 788, 718 cm^{-1}; HR-ESI-MS found *m/z* 247.0435 $[M+H]^+$ (calcd. 247.0433, $C_{13}H_9ClFN_2$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 13.21 (brs, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.95 (m, 1H), 7.58-7.65 (m, 3H), 7.39 (dt, *J* = 8.7, 2.7 Hz, 1H), 7.25 (dd, *J* = 8.4, 1.5 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 171.9, 163.3, 161.4, 151.9, 131.97, 131.90, 131.18, 131.11, 122.63, 122.61, 116.98, 116.81, 113.20, 113.02.

5-Chloro-2-(3-chlorophenyl)-1*H*-benzo[*d*]imidazole (4v): Pale brown solid; mp 143−145 °C; IR (KBr) ν_{max}: 3025, 2952, 1575, 1440, 1378, 1341, 1118, 1062, 926, 808, 721, 683 cm^{-1}; HR-ESI-MS found *m/z* 263.0137 $[M+H]^+$ (calcd. 263.0137, $C_{13}H_9Cl_2N_2$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 13.11 (brs, 1H), 8.21 (d, *J* = 0.9 Hz, 1H), 8.09-8.16 (m, 1H), 7.58-7.67 (m, 4H), 7.24 (dd, *J* = 8.7, 2.1 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 151.1, 133.8, 131.7, 131.0, 129.9, 126.1, 125.1, 122.7.

6-Nitro-2-(1*H*-pyrrol-2-yl)-1*H*-benzo[*d*]imidazole (4w): Orange solid; mp 267−269 °C; IR (KBr) ν_{max}: 3298, 3221, 2924, 1692, 1631, 1599, 1510, 1460, 1329, 1287, 1134, 1067, 725 cm^{-1}; HR-ESI-MS found *m/z* 229.0716 $[M+H]^+$ (calcd. 229.0720, $C_{11}H_9N_4O_2$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 13.19 (brs, 1H), 12.03 (s, 1H), 8.33 (s, 1H), 8.08 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 1.2 Hz, 1H), 6.98 (s, 1H), 6.25 (dd, *J* = 5.7, 2.4 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 171.9, 122.9, 121.5, 117.6, 111.0, 109.7.

4-(1*H*-Imidazo[4,5-*b*]pyridin-2-yl)-2-methoxyphenol (4x): Yellow solid; mp 246−248 °C; IR (KBr) ν_{max}: 3443, 3083, 2921, 2852, 1600, 1495, 1436, 1412, 1364, 1286, 1260, 1215, 1033, 970, 814, 760, 713, 644 cm^{-1}; HR-ESI-MS found *m/z* 242.0925 $[M+H]^+$ (calcd. 242.0924, $C_{13}H_{12}N_3O_2$); ^{1}H-NMR (300 MHz, DMSO-d_6): δ_H 13.21 (brs, 1H), 9.68 (brs, 1H), 8.26 (s, 1H), 7.95 (m, 1H), 7.80 (s, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.19 (dd, J = 8.1, 5.1 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 3.89 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 149.1, 147.9, 142.9, 125.4, 120.8, 120.1, 116.5, 115.7, 110.6, 55.7.

Cytotoxicity assay: α-Minimum essential medium with D-(+)-glucose, L-glutamine, phenol red, and sodium pyruvate (α-MEM, Nacalai Tesque) was used for the HeLa, A549, and MCF7 cell cultures, while RPMI 1640 (Nacalai Tesque) with D-(+)-glucose, L-glutamine, and phenol red was used for GSU and Kato III cell cultures. All media were supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% antibiotic-antimycotic solution (Sigma). Briefly, each cell line was seeded in 96-well plates (2×10^3 per well) and incubated in appropriate medium at 37 °C, under a 5% CO_2 and 95% humidity. After 24 h, the cells were washed with PBS (Nissui Pharmaceuticals) and serial dilutions of the tested samples were added to the wells. After 72 h of incubation, 10 μL of MTT working solution (5 mg/mL in phosphate buffer solution) was subsequently added to each well and the plate was incubated for 3 h at 37 °C in a CO_2 incubator, and then the medium was aspirated. The formed formazan crystals were solubilized with 100 μL of DMSO per well for 30 min at 37 °C in a CO_2 incubator, and the absorbance at 540 nm was measured. The concentrations of the serial dilutions of the tested samples were 100–0.1 μM for the isolated compounds and the positive control, 5-fluorouracil, respectively. Cell viability was calculated from the mean values of data from three wells by using the following equation, and cytotoxicity was expressed as the IC_{50} (50% inhibitory concentration) value.

$$(\%)\ \text{Cell viability} = 100 \times [\{Abs_{(\text{test samples})} - Abs_{(\text{blank})}\}/\{Abs_{(\text{control})} - Abs_{(\text{blank})}\}]$$

ACKNOWLEDGEMENTS

This research was supported by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2018.51, and partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (JSPS KAKENHI Grant Nos. JP15H05138, JP17H02203, and JP17H05435).

REFERENCES

1. N. Shrivastava, M. J. Naim, M. J. Alam, F. Nawaz, S. Ahmed, and O. Alam, *Arch. Pharm.*, 2017, **350**, e201700040.
2. Y. K. Yoon, M. A. Ali, A. C. Wei, A. N. Shirazi, K. Parang, and T. S. Choon, *Eur. J. Med. Chem.*, 2014, **83**, 448.

3. Y. K. Yoon, M. A. Ali, A. C. Wei, T. S. Choon, H. Osman, K. Parang, and A. N. Shirazi, *Bioorg. Med. Chem.*, 2014, **22**, 703.
4. M. F. Baig, V. L. Nayak, P. Budaganaboyina, K. Mullagiri, S. Sunkari, J. Gour, and A. Kamal, *Bioorg. Chem.*, 2018, **77**, 515.
5. K. Y. Yeong, C. W. Ang, M. A. Ali, H. Osman, and S. C. Tan, *Med. Chem. Res.*, 2017, **26**, 770.
6. D. Song and S. Ma, *ChemMedChem*, 2016, **11**, 646.
7. M. K. Kim, H. Shin, K. Park, H. Kim, J. Park, K. Kim, J. Nam, H. Choo, and Y. Chong, *J. Med. Chem.*, 2015, **58**, 7596.
8. Y. Bansal and O. Silakari, *Bioorg. Med. Chem.*, 2012, **20**, 6208.
9. S. I. Alaqeel, *J. Saudi Chem. Soc.*, 2017, **21**, 229.
10. K. Y. Yeong, M. A. Ali, C. W. Ang, S. C. Tan, and H. Osman, *Tetrahedron Lett.*, 2014, **55**, 4697.
11. H. Siddiqui, R. Farooq, B. P. Marasini, R. Malik, N. Syed, S. T. Moin, and M. I. Choudhary, *Bioorg. Med. Chem.*, 2016, **24**, 3387.
12. P. Roy and A. Pramanik, *Tetrahedron Lett.*, 2013, **54**, 5243.
13. J. R. Gillard and P. L. Beaulieu, *Org. Synth.*, 2012, **89**, 131.
14. (a) M. Taha, N. H. Ismail, S. Imran, H. Rashwan, W. Jamil, S. Ali, S. M. Kashif, F. Rahim, U. Salar, and K. M. Khan, *Bioorg. Chem.*, 2016, **65**, 48; (b) N. K. N. A. Zawawi, M. Taha, N. Ahmat, N. H. Ismail, A. Wadood, and F. Rahim, *Bioorg. Chem.*, 2017, **70**, 184; (c) P. Sharma, T. S. Reddy, N. P. Kumar, K. R. Senwar, S. K. Bhargava, and N. Shankaraiah, *Eur. J. Med. Chem.*, 2017, **138**, 234; (d) S. R. Archie, B. K. Das, M. S. Hossain, U. Kumar, and A. S. S. Rouf, *Int. J. Pharm. Pharm. Sci.*, 2017, **9**, 308; (e) N. T. Chandrika, S. K. Shrestha, H. X. Ngo, and S. Garneau-Tsodikova, *Bioorg. Med. Chem.*, 2016, **24**, 3680; (f) A. Kamal, A. B. Shaik, S. Polepalli, G. B. Kumar, V. S. Reddy, R. Mahesh, S. Garimella, and N. Jain, *Bioorg. Med. Chem.*, 2015, **23**, 1082; (g) Y. Yin, Y.-Q. Zhang, B. Jin, S. Sha, X. Wu, C. B. Sangani, S.-F. Wang, F. Qiao, A.-M. Lu, P.-C. Lv, and H.-L. Zhu, *Bioorg. Med. Chem.*, 2015, **23**, 1231; (h) S. Aslam, S. Zaib, M. Ahmad, J. M. Gardiner, A. Ahmad, A. Hameed, N. Furtmann, M. Gütschow, J. Bajorath, and J. Iqbal, *Eur. J. Med. Chem.*, 2014, **78**, 106; (i) K. M. Khan, Z. Shah, V. U. Ahmad, N. Ambreen, M. Khan, M. Taha, F. Rahim, S. Noreen, S. Perveen, M. I. Choudhary, and W. Voelter, *Bioorg. Med. Chem.*, 2012, **20**, 1521.
15. (a) Y. K. Yoon, M. A. Ali, A. C. Wei, T. S. Choon, K.-Y. Khaw, V. Murugaiyah, H. Osman, and V. H. Masand, *Bioorg. Chem.*, 2013, **49**, 33; (b) Y. K. Yoon, M. A. Ali, A. C. Wei, T. S. Choon, and R. Ismail, *Eur. J. Med. Chem.*, 2015, **93**, 614; (c) S. Ahn, Y.-B. Cha, M. Kim, K.-H. Ahn, and Y. C. Kim, *Synth. Met.*, 2015, **199**, 8; (d) D. Karthik, K. R. J. Thomas, J.-H. Jou, and Y.-L. Chen, *Dyes Pigm.*, 2016, **133**, 132.
16. H. T. B. Bui, Q. T. K. Ha, W. K. Oh, D. D. Vo, Y. N. T. Chau, C. T. K. Tu, E. C. Pham, P. T. Tran,

L. T. Tran, and H. Van Mai, *Tetrahedron Lett.*, 2016, **57**, 887.

17. (a) M. P. Singh, S. Sasmal, W. Lu, and M. N. Chatterjee, *Synthesis*, 2000, 1380; (b) S. Lin and L. Yang, *Tetrahedron Lett.*, 2005, **46**, 4315.
18. D. N. Kommi, P. S. Jadhavar, D. Kumar, and A. K. Chakraborti, *Green Chem.*, 2013, **15**, 798.
19. H. F. Ridley, R. G. W. Spickett, and G. M. Timmis, *J. Heterocycl. Chem.*, 1965, **2**, 453.
20. H. Göker, C. Kuş, D. W. Boykin, S. Yildiz, and N. Altanlar, *Bioorg. Med. Chem.*, 2002, **10**, 2589.
21. (a) W. Schmiegel, *ACS Monogr.*, 1995, **187**, 1101; (b) G. J. Koomen, *Recl. Trav. Chim. Pays-Bas*, 1991, **110**, 391.
22. J. A. Hadfield, S. Ducki, N. Hirst, and A. T. McGown, *Prog. Cell Cycle Res.*, 2003, **5**, 309.