PAPER

Synthesis and insight into the structure–activity relationships of 2-phenylbenzimidazoles as prospective anticancer agents†

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In order to explore and develop new anticancer agents, three series of 2-phenylbenzimidazoles, **15–46**, were condensed under simple and mild conditions using sodium metabisulfite as an oxidation agent and another series, **47–55**, were obtained *via* a reduction reaction using sodium borohydride. All the compounds synthesized were evaluated for their *in vitro* anticancer activities against three human cancer cell lines. The novel compound **38** was found to be the most potent multi cancer inhibitor against A549, MDA-MB-231, and PC3 cell lines (IC$_{50}$ values 4.47, 4.68 and 5.50 μg mL^{-1}, respectively). In addition, compound **40** exhibited the best IC$_{50}$ value of 3.55 μg mL^{-1} against the MDA-MB-231 cell line. The results demonstrated that introducing a new substituent to compounds **37–55** could improve their antiproliferative activities.

1. Introduction

Cancer is a phenomenon that involves abnormal cell growth invading or spreading to other parts of the organism. There are more than 100 types of cancers which affect humans and that has led to the discovery of many options for cancer treatment such as chemotherapy, surgery, radiation therapy, targeted therapy, immunotherapy, and so on. In particular, chemotherapy is required in the treatment, either alone or in combination with the other therapies depending on type of cancer, its location, and its grade. For example, chemotherapy could reduce the size of an inoperable tumor, which could then be operated on in the future. However, the increase in multidrug resistance (MDR) to chemotherapy is a contributing factor that influences the treatment of cancer[1] and side effects can also limit the effectiveness of anticancer drugs such as nausea, arthralgia, fatigue, osteoporosis, and so on.[2] Therefore, the exploration and development of new anticancer drugs that have better efficacy and fewer side effects are required.

Benzimidazole structures are well-known heterocyclic compounds that possess a wide range of antiviral, antihistaminic, antifungal, antiallergic, anticoagulant,

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0ra02282a

antihypertensive, and antiparasitic properties.[3–12] In particular, some of the commercial anticancer drugs contain the benzimidazole scaffold (Fig. 1), so benzimidazole is one of the most promising structures used against several human cancer cell lines. Many studies have reported that benzimidazole derivatives that contain functional groups at the 1, 2, 5 and/or 6-positions on their skeleton show the most efficient anticancer activity.[13–17] However, previous studies[18–21] that evaluated the antiproliferative activity of 2-phenylbenzimidazoles had a small number of desired products which led to a lack of conclusion on the structure–activity relationship (SAR), the effect of the presence of substituents into both the C-5/6 and C-2 positions on benzimidazole structure and also the bioactivity. In addition, there are two general pathways to synthesize benzimidazole derivatives. The first method involves the condensation of *o*-phenylenediamines and carboxylic acids (or their derivatives such as nitriles, chlorides and orthoesters) in the presence of acid as we have previously reported.[22] The second one is conducted by using *o*-phenylenediamines in combination with aldehydes instead of the carboxylic acids and oxidation agents that should have been required for this process.[23–30] According to our latest publication, a large number of benzimidazoles were synthesized from *o*-phenylenediamines and aldehydes under simple and mild conditions using sodium metabisulfite (Na$_2$S$_2$O$_5$) as the oxidative reagent and a solvent which was a mixture of ethanol : water (9 : 1 v/v) in high yields.[30] Thus, it was recognized that this pathway is convenient to synthesize benzimidazoles that contain a variety of substituents on the C-2, C-5 and C-6 positions of the benzimidazole scaffold without undesired products. Continuing with our plans to develop new anti-tumor agents,[22,31,32] in this research, we planned to
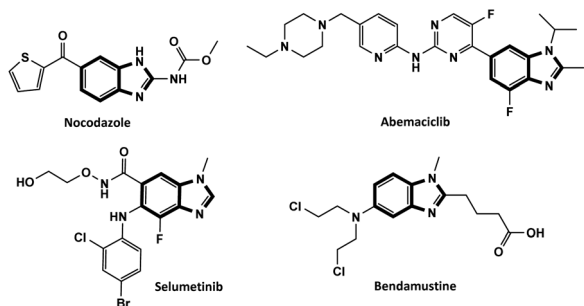
Fig. 1 Some anticancer drugs that contain the benzimidazole structure.

synthesize a large number of new 2-phenylbenzimidazole derivatives with a diverse range of substituents on the C-2, C-5 and C-6 positions *via* condensation of *o*-phenylenediamines and various aldehydes under the conditions mentioned previously and then to elucidate their SAR against three cancer cell lines: A549, MDA-MB-231, and PC3. The aim was that the presence of substituents such as carbonyl and hydroxyl groups in the C-5 position together with substituents at the C-2 position would affect the bioactivity of targeted products and their presence could significantly enhance the anticancer activity of the novel compounds.

## 2. Results and discussion

2.1. Chemistry

The preparation of the target 2-phenylbenzimidazoles 15–55 is shown in Scheme 1. Firstly, the *ortho*-phenylenediamine derivatives were sequentially condensed with various benzaldehydes that contain different substituents (–OH, –OCH$_3$, –NO$_2$, –N(CH$_3$)$_2$, –CF$_3$, –I, –O-CH$_2$-Ph). This reaction was carried out under mild conditions using a mixture of solvents: ethanol : water (ratio of 9 : 1 v/v) and Na$_2$S$_2$O$_5$ as an oxidation agent to produce a series of benzimidazoles, 15–46, that have substituent groups such as hydro, chloro, phenyl methanonyl at the 5, 6 positions, with yields ranging from 32% to 98%. Next, the

carbonyl group in compounds 37–46 was reduced to a corresponding hydroxyl group in the presence of NaBH$_4$ in methanol to give the desired benzimidazole derivatives 47–55 with yields of 45% to 98%. The structures of the synthesized compounds are shown in the Table 1. All the synthesized compounds 15–55 were carefully characterized using FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopic and ESI-HRMS spectrometric methods. The FTIR bands at 3256, 3025, 1613 cm^{-1} confirmed the presence of –NH, C–H and C=N functionalities, respectively, of the most potent compound 38. The ^{1}H-NMR spectrum showed a multiplet at δ 6.95–7.94 ppm confirming the presence of aromatic protons and benzimidazole protons. Similarly, in the ^{13}C-NMR spectrum of compound 38, the carbonyl carbon was at δ 195.58 ppm and the remaining carbons all belonged to aromatic carbons which appeared in the range of δ 113.53–157.79 ppm. The fashion in the FTIR, ^{1}H-NMR, and ^{13}C-NMR spectra similar patterns were observed for the other compounds 37–46. Moreover, the appearance of carbon which was eliminated from a carbonyl group (compounds 37–46) to form a hydroxyl group (compounds 47–55) was recognized by ^{13}C-NRM high field signals at δ 73.77–74.58 ppm. The HRMS (ESI) spectra of all the compounds showed the pseudo-molecular ion [M + H]$^+$ or [M − H]$^-$ peaks corresponding to their respective molecular weights. The spectroscopic data (FTIR, ^{1}H-NMR, ^{13}C-NMR and ESI-HRMS) of all the newly synthesized benzimidazoles 24, 35, 39, 41, 43–45 and 47–55 were in agreement with the corresponding structures illustrated in the Experimental section.
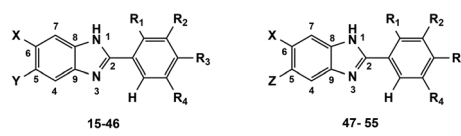
2.2. *In vitro* anticancer activities and structure–activity relationships (SAR)

All the synthesized benzimidazole derivatives 15–55 were tested for their cytotoxicity *in vitro* against human cancer cell lines: A549 (human lung adenocarcinoma epithelial cell line), MDA-MB-231 (human breast cancer cell line) and PC3 (human prostate cancer cell line), using camptothecin as a standard drug and the IC$_{50}$ values (μg mL^{-1}) are detailed in Table 2. Notably, the tested compounds showed a moderate to good antiproliferative activity on selected cancer cell lines. From the
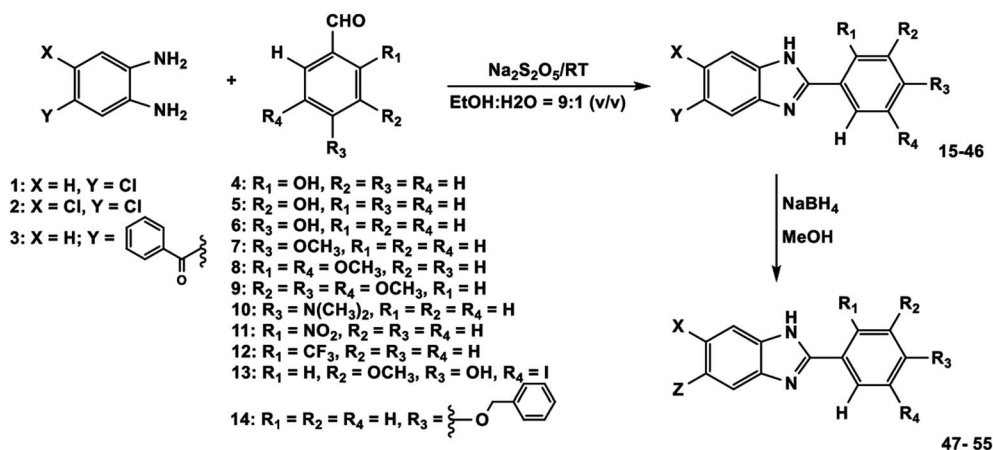
Scheme 1 Synthesis of different 2-phenyl benzimidazole derivatives 15–55.

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Table 1 Structures of the different substituents (X, Y, Z, R$_1$, R$_2$, R$_3$, R$_4$) used for the synthesis of benzimidazoles **15–55** and their yields (%)

Cpd no.	X	Y	Z	R$_1$	R$_2$	R$_3$	R$_4$	Yield (%)
15	H	Cl	—	OH	H	H	H	68
16	H	Cl	—	H	OH	H	H	54
17	H	Cl	—	H	H	OH	H	41
18	H	Cl	—	H	H	OCH$_3$	H	63
19	H	Cl	—	OCH$_3$	H	H	OCH$_3$	84
20	H	Cl	—	H	OCH$_3$	OCH$_3$	OCH$_3$	81
21	H	Cl	—	H	H	N(CH$_3$)$_2$	H	81
22	H	Cl	—	NO$_2$	H	H	H	67
23	H	Cl	—	CF$_3$	H	H	H	83
24	H	Cl	—	H	OCH$_3$	OH	I	98
25	H	Cl	—	H	H	–O-CH$_2$-Ph	H	48
26	Cl	Cl	—	OH	H	H	H	85
27	Cl	Cl	—	H	OH	H	H	78
28	Cl	Cl	—	H	H	OH	H	78
29	Cl	Cl	—	H	H	OCH$_3$	H	87
30	Cl	Cl	—	OCH$_3$	H	H	OCH$_3$	82
31	Cl	Cl	—	H	OCH$_3$	OCH$_3$	OCH$_3$	90
32	Cl	Cl	—	H	H	N(CH$_3$)$_2$	H	73
33	Cl	Cl	—	NO$_2$	H	H	H	32
34	Cl	Cl	—	CF$_3$	H	H	H	52
35	Cl	Cl	—	H	OCH$_3$	OH	I	85
36	Cl	Cl	—	H	H	–O-CH$_2$-Ph	H	68
37	H	Ph-CO–	—	OH	H	H	H	75
38	H	Ph-CO–	—	H	OH	H	H	78
39	H	Ph-CO–	—	H	H	OH	H	84
40	H	Ph-CO–	—	H	H	OCH$_3$	H	84
41	H	Ph-CO–	—	H	OCH$_3$	OCH$_3$	OCH$_3$	83
42	H	Ph-CO–	—	H	H	N(CH$_3$)$_2$	H	98
43	H	Ph-CO–	—	NO$_2$	H	H	H	54
44	H	Ph-CO–	—	CF$_3$	H	H	H	53
45	H	Ph-CO–	—	H	OCH$_3$	OH	I	98
46	H	Ph-CO–	—	H	H	–O-CH$_2$-Ph	H	76
47	H	—	Ph-CH(OH)–	OH	H	H	H	56
48	H	—	Ph-CH(OH)–	H	OH	H	H	72
49	H	—	Ph-CH(OH)–	H	H	OH	H	45
50	H	—	Ph-CH(OH)–	H	H	OCH$_3$	H	84
51	H	—	Ph-CH(OH)–	H	OCH$_3$	OCH$_3$	OCH$_3$	92
52	H	—	Ph-CH(OH)–	H	H	N(CH$_3$)$_2$	H	64
53	H	—	Ph-CH(OH)–	CF$_3$	H	H	H	53
54	H	—	Ph-CH(OH)–	H	OCH$_3$	OH	I	98
55	H	—	Ph-CH(OH)–	H	H	–O-CH$_2$-Ph	H	57

overall results, there were 11 compounds such as **16, 19, 21, 22, 23, 24, 28, 38, 45, 47,** and **49** which displayed IC$_{50}$ values less than 100 µg mL^{-1} on the three cancer cell lines. There were five compounds (**23, 25, 28, 38, 39**), two compounds (**23, 38**) and one compound (**38**) that displayed IC$_{50}$ values of less than 8 µg mL^{-1} on the MDA-MB-231, PC3, and A549 cell lines, respectively. From a scientific point of view, the MDA-MB-231 human breast cancer cell line was found to be more sensitive towards the synthesized 2-phenylbenzimidazoles than the PC3 and A549 cell lines.

The difference in the electron donating and electron withdrawing groups in the 2-phenyl ring affected the activity of the synthesized compounds and this effect was found to be related to the substituents in the C-5 position. To clarify this point, the activities of compounds containing the hydroxyl group (electron donating group) were compared to those of compounds containing the trifluoromethyl group (electron withdrawing group) at the R$_1$-position such as **15** *vs.* **23** (containing one chloro atom at the C-5 position), **26** *vs.* **34** (containing two chloro atoms at the C-5 and C-6 positions), **37** *vs.* **44** (containing a carbonyl group at the C-5 position) and **47** *vs.* **53** (containing a hydroxyl

Table 2 Anti-proliferative activity (IC$_{50}$, µg mL^{-1}) of 41 benzimidazole derivatives against three cancer cell lines, determined by MTT assay

Cpd no.	IC$_{50}$ ± SD (µg mL^{-1})		
	A549[a]	MDA-MB-231[b]	PC3[c]
15	>100	43.65 ± 1.58	>100
16	12.02 ± 0.56	22.39 ± 1.05	18.20 ± 1.85
17	31.62 ± 1.46	>100	36.56 ± 1.67
18	>100	>100	>100
19	44.67 ± 1.69	12.02 ± 0.75	85.11 ± 2.67
20	11.48 ± 0.74	>100	21.88 ± 1.59
21	9.12 ± 0.55	10.72 ± 1.11	18.62 ± 0.46
22	38.02 ± 1.89	30.2 ± 1.53	33.11 ± 1.43
23	45.71 ± 1.75	7.08 ± 0.86	6.92 ± 0.62
24	39.81 ± 1.03	37.15 ± 1.73	39.26 ± 1.59
25	>100	6.61 ± 0.83	19.95 ± 1.08
26	>100	>100	>100
27	>100	>100	>100
28	64.57 ± 2.68	6.92 ± 0.98	60.26 ± 2.12
29	>100	>100	>100
30	85.11 ± 2.12	>100	69.18 ± 2.09
31	>100	83.18 ± 2.05	>100
32	52.48 ± 2.34	>100	60.26 ± 2.42
33	>100	>100	>100
34	>100	45.71 ± 1.56	>100
35	ND	ND	ND
36	60.26 ± 1.25	>100	>100
37	>100	44.67 ± 1.69	>100
38	4.47 ± 0.33	4.68 ± 0.42	5.50 ± 0.28
39	>100	>100	>100
40	>100	3.55 ± 0.35	>100
41	>100	66.07 ± 2.12	>100
42	>100	>100	>100
43	>100	>100	>100
44	>100	>100	>100
45	42.66 ± 1.62	46.77 ± 1.50	54.95 ± 1.96
46	>100	>100	>100
47	14.13 ± 0.57	14.79 ± 1.08	17.78 ± 1.45
48	12.88 ± 0.46	>100	17.38 ± 0.78
49	38.90 ± 2.17	30.2 ± 2.04	33.88 ± 1.98
50	53.70 ± 1.68	>100	>100
51	22.39 ± 1.07	>100	44.67 ± 1.77
52	>100	>100	>100
53	47.86 ± 1.73	54.95 ± 1.84	>100
54	>100	61.66 ± 1.83	79.43 ± 1.62
55	>100	15.85 ± 0.56	22.91 ± 0.24
Camptothecin[d]	0.2 ± 0.06	0.47 ± 0.04	0.87 ± 0.11

[a] Human lung adenocarcinoma epithelial cell line. [b] Human breast cancer cell line. [c] Human prostate cancer cell line. [d] Positive contrast drug. All the values are showed as mean ± SD in which each treatment was calculated from at least three independent experiments. ND = not determined.

group at the C-5 position) on the MDA-MB-231 cell line and it was realized that the activity of compounds **15/26** and **23/34** also showed the same trend. The appearance of a hydroxyl group as an electron donating group at the R$_1$-position decreased the activity of compound **15/26** in comparison to that of **23/34** containing trifluoromethyl as a withdrawing electron group (IC$_{50}$ values of compounds **15**, **26**, **23** and **34** were 43.65 µg mL^{-1}, >100, 7.08 and 45.71 µg mL^{-1}, respectively). In contrast to this, introducing a hydroxyl group into the R$_1$-position boosted

the anticancer effect of **37/47** more than that of **44/53** which consisted of a trifluoromethyl group (IC$_{50}$ values of compounds **37**, **47**, **44** and **53** were 44.67, 14.79, >100 and 54.95 µg mL^{-1}, respectively).

To demonstrate that the chloro atoms on the benzimidazole skeleton make a significant contribution to the anticancer activity, compounds **15–25** and **26–36** were synthesized that contain one and two chloro atoms in the 5- and 6-positions, respectively. The compounds containing one chloro atom at the 5-position such as **21**, **23** and **25** reached IC$_{50}$ values of 10.7, 7.08 and 6.61 µg mL^{-1} with the MDA-MB-231 cell line, respectively. Unfortunately, for all three cancer cell lines of compounds, the anti-tumor activity of compounds that have two chloro atoms in the 5-, and 6-positions was considerably decreased and was less than that of compounds with one chloro atom in the 5-position, for example, **21** *vs.* **32**, **23** *vs.* **34** and **25** *vs.* **36**, but excluding compound **28**, which had an IC$_{50}$ value of 6.92 µg mL^{-1} for the MDA-MB-231 cell line. In the further studies, other halogen atoms (such as fluoro or bromo atoms) will be investigated at the 5-, and 6-positions in the benzimidazole structure so that those products may improve the antiproliferative activity, for example, against A549 cell lines, as reported previously in the literature.[21]

To find prospective anticancer agents, the phenyl methanonyl group was introduced into the benzene ring at the 5-position and compounds **37–46** were obtained. Nevertheless, this replacement lead to a decrease in the activity, compounds **39** and **42–46**, when compared to other compounds that had one chloro atom in the 5-position (compounds **17** and **21–25**). Surprisingly, there was a remarkable increase in the antiproliferative activity of benzimidazoles **38** and **40**. In detail, the IC$_{50}$ value of compound **40** with the MDA-MB-231 cell line reached 3.55 µg mL^{-1} which had decreased dramatically from more than 100 µg mL^{-1} – the IC$_{50}$ value of compound **18** (without a phenyl methanonyl group but with a chloro atom at 5-position). Specifically, compound **38** acted as a potential multi cancer inhibitor against A549, MDA-MB-231 and PC3 cell lines with IC$_{50}$ of 4.47 µg mL^{-1}, 4.68 µg mL^{-1} and 5.50 µg mL^{-1}, respectively.

To explore the effect of the presence of a carbonyl group in compounds **37–42** and **44–46** on their bioactivity, the carbonyl group was converted to a hydroxyl group by a reduction reaction and produced the novel compounds **47–55**. Basically, the bioactivity on A549 and PC3 cell lines of compounds **47–55** was better than that of compounds **37–46**. This was shown by the fact that there were six compounds (**47–51** and **53**) which displayed IC$_{50}$ values less than 100 µg mL^{-1} on the A549 cell line instead of only two compounds (**38** and **45**). Conversely, that modification gave no considerable change of the activity of compounds **50–54**, but compound **55** had an IC$_{50}$ of 15.85 µg mL^{-1} with the MDA-MB-231 cell line.

In detail, the IC$_{50}$ values of compound **48** on the three cancer cell lines were slightly increased, in contrast to that of compound **38** which did not have carbonyl group reduced to a hydroxyl group. Considering the SARs of compound **47**, **37**, **26** and **15**, the presence of substituent containing hydroxyl group at the R$_1$-position greatly increased the antiproliferative effect of

compound **47**, in comparison to those of the remained compounds (IC$_{50}$ values ranged from 43.65 µg mL^{-1} to more than 100 µg mL^{-1} on the three cancer cell lines). This was shown by the fact that the IC$_{50}$ values of compound **47** dropped to 14.13, 14.79 µg mL^{-1} and 17.78 µg mL^{-1} with the A549, MDA-MB-231 and PC3 cell lines, respectively. After the analysis of the SARs, it was hypothesized that it was necessary to introduce the carbonyl group into the benzimidazole structure at C-5 position and this adjustment markedly improved the anticancer activity of compounds **38** and **40**.

3. Conclusion

In summary, 41 2-phenylbenzimidazoles with various substituents in the 5-, 6-positions and a 2-phenyl ring, including 17 novel derivatives, were synthesized under mild conditions using a mixture of solvents: ethanol : water (9 : 1 v/v) to increase the solubility of reactants (step 1) and NaBH$_4$ in methanol (step 2) and the products were obtained in high yields. All of synthesized compounds were evaluated the antiproliferative activity against A549, MDA-MB-231 and PC3 human cancer cell lines. Compound **40** expressed a high selectivity towards the breast cancer cell line MDA-MB-231 (IC$_{50}$ = 3.55 µg mL^{-1}). Whereas compound **38** with the most potent anticancer activity showed IC$_{50}$ values of 4.47, 4.68 and 5.50 µg mL^{-1} for the A549, MDA-MB-231, and PC3 cell lines, respectively, and shows promise as a new multi-cancer inhibitor. Benzimidazoles and the 2-phenylbenzimidazole derivatives are also topoisomerase I inhibitors.[18,33–35] The studies to clarify the modes of action of the synthesized 2-phenylbenzimidazoles binding on the topo I and DNA complex are currently in progress.

4. Experimental section

4.1. Materials

All reagents and solvents were purchased from Acros Organics (Belgium), Sigma-Aldrich (USA) and Xilong (China) and used without further purification.

4.2. Characterization of synthesized benzimidazoles

Reactions were monitored by thin-layer chromatography (TLC) which was performed on silica gel 60 F$_{254}$ plates (Merck). The synthesized compounds were visualized by UV light (254 nm). All of the melting points (MPs) were determined on an IA 9000 series digital melting point apparatus (Electrothermal) and were uncorrected. The FTIR spectra were recorded using KBr pellets with an Equinox 55 IR spectrometer (Bruker, Germany) and the absorption bands were expressed in wavenumbers (cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C-NMR) on an AM0 FT-NMR spectrometer (Bruker) in (CD$_3$)$_2$SO. Tetramethylsilane (TMS) was used as an internal standard and the chemical shifts were expressed in δ (ppm) and the coupling constants (*J*) in hertz (Hz). The signal multiplicities were expressed by standard abbreviations as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, m = multiplet. High-resolution mass spectra

were recorded using electrospray ionization on a X500R QTOF quadrupole time-of-flight (QTOF) system (Sciex).

4.3. General procedure for the synthesis of compounds 15–46

A mixture of *o*-phenylenediamines **1–3** (2 mmol) and benzaldehyde derivatives **4–14** (2 mmol) were stirred in 10–20 mL of ethanol and water (9 : 1 v/v) (until both dissolved) and then Na$_2$S$_2$O$_5$ (2 mmol) was added. The suspension was stirred constantly at room temperature for 2 h and the reaction was monitored by TLC. After completion of the reaction, the precipitate was removed by filtering and the filtrate was evaporated under reduced pressure to obtain the crude product. The crude product was washed three times with water and *n*-hexane, and then dried in a vacuum at 80 °C to achieve the final product. The purification was performed on a column chromatography system or by crystallization from solvents with the desired method as listed in the information for each product.

2-(5-Chloro-1*H*-benzoimidazol-2-yl)-phenol (**15**): isolated by silica gel column chromatography with chloroform : methanol (98 : 2, v/v) to obtain a light yellow powder; yield: 68%; mp (°C): 240–241; FTIR (KBr, ν (cm^{-1})): 3330 (N–H), 3057 (C–H), 1633 (C=N), 1489 (C=C), 1257 (C–N), 738 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.02–8.06 (m, 7H, CH$_{Ar}$), 12.74 (s, 1H, OH), 13.23 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 112.51–157.79 (CH$_{Ar}$); ESI-HRMS (DMSO): *m/z* = 245.0463 [M + H]$^+$.

3-(5-Chloro-1*H*-benzoimidazol-2-yl)-phenol (**16**): light yellow powder; yield: 54%; mp (°C): 236–237; FTIR (KBr, ν (cm^{-1})): 3250 (N–H), 3060 (C–H), 1591 (C=N), 1466 (C=C), 1231 (C–N), 784 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.93–7.22 (m, 7H, CH$_{Ar}$), 9.75 (s, 1H, OH), 12.99 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 113.42–157.77 (CH$_{Ar}$); ESI-HRMS (DMSO): *m/z* = 245.0479 [M + H]$^+$.

4-(5-Chloro-1*H*-benzoimidazol-2-yl)-phenol (**17**): slightly brown powder; yield: 41%; mp (°C): 259–260; FTIR (KBr, ν (cm^{-1})): 3227 (N–H), 3070 (C–H), 1609 (C=N), 1461 (C=C), 1251 (C–N), 729 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.93–8.00 (m, 7H, CH$_{Ar}$), 10.04 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 114.55–159.53 (CH$_{Ar}$); ESI-HRMS (DMSO): *m/z* = 245.0482 [M + H]$^+$.

5-Chloro-2-(4-methoxy-phenyl)-1*H*-benzoimidazole (**18**): slightly yellow powder; yield: 63%; mp (°C): 174–175; FTIR (KBr, ν (cm^{-1})): 3007 (C–H), 1611 (C=N), 1492 (C=C), 1258 (C–N), 1180 (O–CH$_3$), 738 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.38 (s, 3H, O–CH$_3$), 6.67–7.67 (m, 7H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 55.30 (O–CH$_3$), 114.38–160.88 (CH$_{Ar}$); ESI-HRMS (DMSO): *m/z* = 259.0633 [M + H]$^+$.

5-Chloro-2-(2,5-methoxy-phenyl)-1*H*-benzoimidazole (**19**): slightly yellow powder; yield: 84%; mp (°C): 139–140; FTIR (KBr, ν (cm^{-1})): 3218 (N–H), 3008 (C–H), 1614 (C=N), 1490 (C=C), 1223 (C–N), 1176 (O–CH$_3$), 740 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.80 (s, 3H, O–CH$_3$), 3.98 (s, 3H, O–CH$_3$), 7.22–7.86 (m, 6H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 55.50, 56.17 (O–CH$_3$), 113.50–153.18 (CH$_{Ar}$); ESI-HRMS (DMSO): *m/z* = 289.0734 [M + H]$^+$.

5-Chloro-2-(3,4,5-trimethoxy-phenyl)-1*H*-benzoimidazole (**20**): slightly gray powder; yield: 81%; mp (°C): 233–234; FTIR (KBr, ν (cm^{-1})): 3063 (C–H), 1588 (C=N), 1464 (C=C), 1240 (C–N), 1127 (O–CH$_3$), 731 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.74 (s, 3H, O–CH$_3$), 3.90 (s, 6H, 2 × O–CH$_3$), 7.23–7.64 (m, 5H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.04, 60.11 (O–CH$_3$), 104.00–153.21 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 319.08466 [M + H]$^+$.

4-(5-Chloro-1*H*-benzoimidazol-2-yl)-*N*,*N*-dimethylaniline (**21**): light yellow powder; yield: 81%; mp (°C): 233–234; FTIR (KBr, ν (cm^{-1})): 3074 (C–H), 1611 (C=N), 1501 (C=C), 1367 (C–N), 1127 (N–(CH$_3$)$_2$), 740 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.00 (s, 6H, 2 × CH$_3$), 6.84–7.99 (m, 7H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 79.11 (CH$_3$), 111.74–153.55 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 272.0949 [M + H]$^+$.

5-Chloro-2-(2-nitro-phenyl)-1*H*-benzoimidazole (**22**): slightly yellow powder; yield: 67%; mp (°C): 103–104; FTIR (KBr, ν (cm^{-1})): 3197 (N–H), 3078 (C–H), 1613 (C=N), 1529 (C=C), 1348 (C–N), 753 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.28–8.04 (m, 7H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 122.78–148.83 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 274.0387 [M + H]$^+$.

5-Chloro-2-(2-trifluoromethyl-phenyl)-1*H*-benzoimidazole (**23**): slightly gray powder; yield: 83%; mp (°C): 173–174; FTIR (KBr, ν (cm^{-1})): 3053 (C–H), 1620 (C=N), 1552 (C=C), 1433 (C–N), 1129 (C–F), 769 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.26–7.95 (m, 7H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 120.41–150.8 (CF$_3$, CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 297.04084 [M + H]$^+$.

4-(5-Chloro-1*H*-benzoimidazole-2-yl)-2-iodo-6-methoxy-phenol (**24**): slightly gray powder; yield: 98%; mp (°C): 204–205; FTIR (KBr, ν (cm^{-1})): 3453 (OH), 3098 (C–H), 1624 (C=N), 1539 (C=C), 1464 (C–N), 1275 (O–CH$_3$), 724 (C–Cl), 593 (C–I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.93 (s, 3H, O–CH$_3$), 7.21–8.1 (m, 5H, CH$_{Ar}$), 10.14 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.20 (O–CH$_3$), 84.59 (C$_{Ar}$–I), 110.03–151.57 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 400.9527 [M + H]$^+$.

2-(4-(Benzyloxy)phenyl)-5-chloro-1*H*-benzoimidazole (**25**): brown powder; yield: 48%, mp (°C): 170–171; FTIR (KBr, ν (cm^{-1})): 3030 (C–H), 1620 (C=N), 1552 (C=C), 1314 (C–N), 1129 (C–O–C ether), 769 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.2 (s, 2H, O–CH$_2$), 7.19–7.60 (m, 12H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 69.37 (O–CH$_2$), 115.22–159.93 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 335.09497 [M + H]$^+$.

5,6-Dichloro-2-(4-trimethoxy-phenyl)-1*H*-benzoimidazole (**26**): brown powder; yield: 85%; mp (°C): 228–229; FTIR (KBr, ν (cm^{-1})): 3331 (OH), 3098 (C–H), 1636 (C=N), 1488 (C=C), 1369 (C–N), 752 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.02–8.07 (m, 6H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 112.48–157.64 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 279.0069 [M + H]$^+$.

3-(5,6-Dichloro-1*H*-benzoimidazol-2-yl)phenol (**27**): white powder; yield: 78%; mp (°C): 260–261, FTIR (KBr, ν (cm^{-1})): 3228 (OH), 3032 (C–H), 1650 (C=N), 1469 (C=C), 1232 (C–N), 7.18 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.94–7.82 (m, 6H, CH$_{Ar}$), 9.76 (s, 1H, OH), 13.13 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 113.53–157.77 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 279.0088 [M + H]$^+$.

4-(5,6-Dichloro-1*H*-benzoimidazol-2-yl)phenol (**28**): yellow powder; yield: 78%; mp (°C): 209–210; FTIR (KBr, ν (cm^{-1})): 3200 (OH), 3032 (C–H), 1609 (C=N), 1454 (C=C), 1250 (C–N), 743 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.93–8.00 (m, 6H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 115.76–159.69 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 279.0088 [M + H]$^+$.

5,6-Dichloro-2-(4-methoxy-phenyl)-1*H*-benzoimidazole (**29**): slightly yellow powder; yield: 87%; mp (°C): 200–201; FTIR (KBr, ν (cm^{-1})): 3019 (C–H), 1611 (C=N), 1493 (C=C), 1261 (C–N), 1181 (O–CH$_3$), 734 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.85 (s, 3H, O–CH$_3$), 7.13–8.11 (m, 6H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 55.39 (O–CH$_3$), 114.49–161.14 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 293.02468 [M + H]$^+$.

5,6-Dichloro-2-(2,5-dimethoxyphenyl)-1*H*-benzo[*d*]imidazole (**30**): yellow powder; yield: 82%; mp (°C): 173–174; FTIR (KBr, ν (cm^{-1})): 3086 (C–H), 1620 (C=N), 1483 (C=C), 1307 (C–N), 1169 (O–CH$_3$), 731 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.80 (s, 3H, O–CH$_3$), 3.98 (s, 3H, O–CH$_3$), 7.10–7.86 (m, 5H, CH$_{Ar}$), 12.29 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 55.52, 56.25 (O–CH$_3$), 104.18–153.79 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 323.0368 [M + H]$^+$.

5,6-Dichloro-2-(3,4,5-trimethoxy-phenyl)-1*H*-benzoimidazole (**31**): yellow powder; yield: 90%; mp (°C): 252–253; FTIR (KBr, ν (cm^{-1})): 3290 (N–H), 2990 (C–H), 1672 (C=N), 1412 (C=C), 1375 (C–N), 1127 (O–CH$_3$), 763 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, O–CH$_3$), 3.90 (s, 6H, 2 × O–CH$_3$), 7.51–7.92 (m, 4H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.07, 60.13 (O–CH$_3$), 104.18–153.79 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 353.04543 [M + H]$^+$.

4-(5,6-Dichloro-1*H*-benzo[*d*]imidazol-2-yl)-*N*,*N*-dimethylaniline (**32**): yellow powder; yield: 73%; mp (°C): 265–266; FTIR (KBr, ν (cm^{-1})): 3422 (NH), 3033 (C–H), 1612 (C=N), 1503 (C=C), 1372 (C–N), 1208 (N–(CH$_3$)$_2$), 735 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.01 (s, 6H, 2 × CH$_3$), 6.84–7.96 (m, 6H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 39.33 (CH$_3$), 111.72–154.68 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 306.0568 [M + H]$^+$.

5,6-Dichloro-2-(2-nitrophenyl)-1*H*-benzoimidazole (**33**): orange powder; yield: 32%; mp (°C): 218–219; FTIR (KBr, ν (cm^{-1})): 2924 (C–H), 1612 (C=N), 1526 (C=C), 1349 (C–N), 784 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.80–8.07 (m, 6H, CH$_{Ar}$), 13.38 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 123.62–150.09 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 307.9926 [M + H]$^+$.

5,6-Dichloro-2-(2-trifluoromethyl-phenyl)-1*H*-benzoimidazole (**34**): slightly yellow powder; yield: 52%; mp (°C): 266–267; FTIR (KBr, ν (cm^{-1})): 3007 (C–H), 1632 (C=N), 1582 (C=C), 1314 (C–N), 1134 (C–F), 772 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.84–7.98 (m, 6H, CH$_{Ar}$), 13.14 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 115.13–150.68 (CF$_3$, CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 331.0035 [M + H]$^+$.

4-(5,6-Dichloro-1*H*-benzoimidazol-2-yl)-2-iodo-6-methoxy-phenol (**35**): isolated by silica gel column chromatography with chloroform : methanol (98 : 2) as a slightly yellow powder; yield: 85%; mp (°C): 226–227; FTIR (KBr, ν (cm^{-1})): 3454 (OH), 3224 (NH), 2999 (C–H), 1699 (C=N), 1481 (C=C), 1279 (C–N), 1100 (O–CH$_3$), 731 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.93 (s, 3H, O–CH$_3$), 7.76–8.11 (m, 4H, CH$_{Ar}$), 10.17 (s, 1H, OH), 13.08 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.20 (O–

CH$_3$), 84.60 (C$_{Ar}$–I), 110.09–152.86 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 434.91745 [M + H]$^+$.

2-(4-Benzyloxy-phenyl)-5,6-dichloro-1H-benzoimidazole (36): isolated by silica gel column chromatography with chloroform : methanol (98 : 2) as a slightly yellow powder; yield: 68%; mp (°C): 262–263; FTIR (KBr, ν (cm^{-1})): 3291 (N–H), 3031 (C–H), 1608 (C=N), 1494 (C=C), 1252 (C–N), 1174 (C–O–C ether), 743 (C–Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.20 (s, 2H, O–CH$_2$), 7.22–8.11 (m, 11H, CH$_{Ar}$), 13.06 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 69.39 (O–CH$_2$), 112.34–160.18 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 369.05330 [M + H]$^+$.

[2-(2-Hydroxy-phenyl)-1H-benzoimidazol-5-yl]-phenyl-methanone (37): slightly yellow powder; yield: 75%; mp (°C): 262–263; FTIR (KBr, ν (cm^{-1})): 3296 (N–H), 3025 (C–H), 1613 (C=N), 1446 (C=C), 1294 (C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.06–8.08 (m, 12H, CH$_{Ar}$), 12.68 (s, 1H, OH), 13.38 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 112.51–195.45 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 315.11349 [M + H]$^+$.

[2-(3-Hydroxy-phenyl)-1H-benzoimidazol-5-yl]-phenyl-methanone (38): yellow powder; yield: 78%; mp (°C): 254–255; FTIR (KBr, ν (cm^{-1})): 3445 (OH), 3256 (NH), 3025 (C–H), 1613 (C=N), 1461 (C=C), 1276 (C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.95–7.94 (m, 12H, CH$_{Ar}$), 9.79 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 113.53–195.58 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 315.11315 [M + H]$^+$.

[2-(4-Hydroxy-phenyl)-1H-benzoimidazol-5-yl]-phenyl-methanone (39): slightly yellow powder; yield: 84%; mp (°C): 295–296; FTIR (KBr, ν (cm^{-1})): 3421 (OH), 3250 (NH), 3063 (C–H), 1607 (C=N), 1454 (C=C), 1282 (C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.96–8.02 (m, 12H, CH$_{Ar}$), 12.77 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 115.44–195.20 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 315.11300 [M + H]$^+$.

[2-(4-Methoxy-phenyl)-1H-benzoimidazol-5-yl]-phenyl-methanone (40): slightly yellow powder; yield: 84%; mp (°C): 295–296; FTIR (KBr, ν (cm^{-1})): 3273 (NH), 3061 (C–H), 1640 (C=N), 1493 (C=C), 1318 (C–N), 1177 (O–CH$_3$); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.85 (s, 3H, O–CH$_3$), 7.16–8.16 (m, 12H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 55.39 (O–CH$_3$), 114.53–195.53 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 329.12917 [M + H]$^+$.

Phenyl-[2-(3,4,5-trimethoxy-phenyl)-1H-benzoimidazol-5-yl]-methanone (41): slightly brown powder; yield: 83%; mp (°C): 220–221; FTIR (KBr, ν (cm^{-1})): 3094 (C–H), 1639 (C=N), 1585 (C=C), 1293 (C–N), 1177 (O–CH$_3$); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.74 (s, 3H, OCH$_3$), 3.90 (s, 6H, OCH$_3$), 7.53–7.95 (m, 10H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.09–60.18 (O–CH$_3$), 104.22–195.54 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 389.15027 [M + H]$^+$.

[2-(4-Dimethylamino-phenyl)-1H-benzoimidazol-5-yl](phenyl)methanone (42): yellow powder; yield: 98%; mp (°C): 216–217; FTIR (KBr, ν (cm^{-1})): 3302 (NH), 3062 (C–H), 1607 (C=N), 1502 (C=C), 1287 (C–N), 1198 (N–(CH$_3$)$_2$); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.01 (s, 6H, CH$_3$), 6.86–8.03 (m, 12H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 39.33 (CH$_3$), 111.79–195.54 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 342.16067 [M + H]$^+$.

[2-(2-Nitrophenyl)-1H-benzoimidazol-5-yl](phenyl)methanone (43): isolated by silica gel column chromatography with chloroform : methanol (98 : 2) as a slightly yellow powder;

yield: 54%; mp (°C): 194–195; FTIR (KBr, ν (cm^{-1})): 3216 (NH), 3062 (C–H), 1600 (C=N), 1537 (C=C), 1286 (C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.57–8.09 (m, 12H, CH$_{Ar}$), 13.44 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 123.92–195.66 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 344.1011 [M + H]$^+$.

Phenyl(2-(2-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-5-yl)methanone (44): recrystallized from ethyl acetate to obtain a brown solid; yield: 53%; mp (°C): 114–115; FTIR (KBr, ν (cm^{-1})): 3279 (NH), 3076 (C–H), 1650 (C=N), 1554 (C=C), 1311 (C–N), 1128 (C–F); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.58–7.99 (m, 12H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 122.55–195.65 (CF$_3$, CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 367.1052 [M + H]$^+$.

(2-(4-Hydroxy-3-iodo-5-methoxyphenyl)-1H-benzoimidazol-5-yl)(phenyl)methanone (45): yellow powder; yield: 98%; mp (°C): 164–165; FTIR (KBr, ν (cm^{-1})): 3491 (OH), 3256 (NH), 3060 (C–H), 1616 (C=N), 1544 (C=C), 1281 (C–N), 1116 (O–CH$_3$), 582 (C–I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.95 (s, 3H, O–CH$_3$), 7.57–8.15 (m, 10H, CH$_{Ar}$), 10.17 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.21 (O–CH$_3$), 84.65 (C–I), 110.12–195.46 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 471.02093 [M + H]$^+$.

(2-(4-(Benzyloxy)phenyl)-1H-benzoimidazol-5-yl)(phenyl)methanone (46): slightly yellow powder; yield: 76%; mp (°C): 198–199; FTIR (KBr, ν (cm^{-1})): 3246 (NH), 3060 (C–H), 1612 (C=N), 1495 (C=C), 1248 (C–N), 1179 (C–O–C ether); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.23 (s, 2H, O–CH$_2$), 7.24–8.17 (m, 2H, CH$_{Ar}$, 17H), 13.11 (s, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 69.41 (O–CH$_2$), 115.31–195.53 (CH$_{Ar}$); HRMS (m/z): 405.16112 [M + H]$^+$.

4.4. General procedure for the synthesis of the final compounds 47–55

The benzimidazoles 37–46 (1 mmol) in 5 mL of methanol were stirred in a 2-neck round-bottom flask until they were completely dissolved, then 2 mmol of NaBH$_4$ was added. The reaction mixture was stirred at room temperature for 1 hand monitored by TLC using a mixture of ethyl acetate : hexane (95 : 5 or 9 : 1 v/v) as eluting solvent. Then, the solvent was evaporated under reduced pressure and the residue was washed with water to obtain the product. The purification was performed on a column chromatography system or by crystallization from solvents using the desired method, as listed in the information for each product.

2-(5-(Hydroxy(phenyl)methyl)-1H-benzo[d]imidazol-2-yl) phenol (47): isolated by silica gel column chromatography with chloroform : methanol (98 : 2) as a white powder; yield: 56%; mp (°C): 161–162; FTIR (KBr, ν (cm^{-1})): 3361 (NH), 3056 (C–H), 1593 (C=N), 1486 (C=C), 1255 (C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.86 (s, 1H, C–H), 5.94 (s, 1H, OH), 7.03–8.03 (m, 12H, CH$_{Ar}$), 13.12 (s, 2H, NH and OH); 13C-NMR (125 MHz, DMSO-d_6, δ ppm): 74.41 (C–H), 112.61–157.92 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 315.1128 [M – H]$^-$.

3-(5-(Hydroxy(phenyl)methyl)-1H-benzo[d]imidazol-2-yl) phenol (48): isolated by silica gel column chromatography with chloroform : methanol (98 : 2) as a white powder; yield: 72%; mp (°C): 146–147; FTIR (KBr, ν (cm^{-1})): 3240 (NH), 3061 (C–H), 1600 (C=N), 1472 (C=C), 1232 (C–N); ^{1}H-NMR (500 MHz,

DMSO-d_6, δ ppm): 5.81 (s, 1H, C–H), 5.85 (s, 1H, OH), 6.88–7.55 (m, 12H, CH$_{Ar}$), 12.65 (s, 1H, NH), 9.66 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 74.46 (C–H), 108.85–157.67 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 315.1136 [M − H]$^-$.

4-(5-(Hydroxy(phenyl)methyl)-1H-benzo[d]imidazol-2-yl) phenol (**49**): isolated by silica gel column chromatography with chloroform : methanol (98 : 2) as a soft white powder; yield: 45%; mp (°C): 147–148; FTIR (KBr, ν (cm^{-1})):3212 (NH), 3031 (C–H), 1612 (C=N), 1466 (C=C), 1278 (C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.81 (s, 1H, CH), 5.84 (s, 1H, OH), 6.91–7.97 (m, 12H, CH$_{Ar}$), 12.53 (s, 1H, NH), 9.91 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 74.58 (C–H), 115.60–158.99 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 315.1134 [M − H]$^-$.

[2-(4-Methoxy-phenyl)-1H-benzoimidazol-5-yl]-phenyl-methanol (**50**): recrystallized from methanol to obtain a slightly yellow solid; yield: 84%; mp (°C):157–158; FTIR (KBr, ν (cm^{-1})): 3410 (NH), 3082 (C–H), 1608 (C=N), 1485 (C=C), 1252 (C–N), 1177 (O–CH$_3$); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.81 (m, 1H, C–H), 5.83 (m, 1H, OH), 3.83 (s, 3H, O–CH$_3$), 7.10–8.08 (m, 12H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 55.29 (O–CH$_3$), 74.56 (C–H), 114.31–160.51(CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 329.1291 [M − H]$^-$.

Phenyl-[2-(3,4,5-trimethoxy-phenyl)-1H-benzoimidazol-5-yl]-methanol (**51**): recrystallized from methanol to obtain a white solid; mp (°C): 231–232; yield: 92%, FTIR (KBr, ν (cm^{-1})): 3359 (NH), 3094 (C–H), 1632 (C=N), 1593 (C=C), 1263 (C–N), 1131 (O–CH$_3$); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.79–3.94 (s, 9H, O–CH$_3$), 5.93 (s, 1H, C–H), 7.22–7.88 (m, 10H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.62–60.28 (O–CH$_3$), 73.77 (C–H), 106.00–153.40 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 391.1660 [M + H]$^+$.

[2-(4-Dimethylamino-phenyl)-1H-benzoimidazol-5-yl]-phenyl-methanol (**52**): recrystallized from methanol to obtain a slightly yellow solid; yield: 64%; mp (°C): 269–270; FTIR (KBr, ν (cm^{-1})): 3312 (NH), 3010 (C–H), 1604 (C=N), 1514 (C=C), 1220 (C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.06 (s, 6H, CH$_3$), 5.89 (s, 1H, C–H), 6.93–8.15 (m, 12H, CH$_{Ar}$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 73.83 (CH$_3$), 108.39–153.11 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 342.1743 [M − H]$^-$.

Phenyl(2-(2-(trifluoromethyl)phenyl)-1H-benzo[d]imidazol-5-yl)methanol (**53**): recrystallized from ethyl acetate to obtain a soft white powder; yield: 53%; mp (°C): 154–155; FTIR (KBr, ν (cm^{-1})): 3357 (NH), 3057 (C–H), 1593 (C=N), 1486 (C=C), 1255 (C–N), 1018 (C–F); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.84 (s, 1H, C–H), 5.87 (s, 1H, OH), 7.21–7.93 (m, 12H, CH$_{Ar}$), 12.65 (s, 1H, NH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 74.54 (C–H), (CF$_3$, CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 369.1207 [M + H]$^+$.

4-[5-(Hydroxy-phenyl-methyl)-1H-benzoimidazol-2-yl]-2-iodo-6-methoxy-phenol (**54**): recrystallized from methanol to obtain a soft white solid; yield: 98%; mp (°C): 228–229; FTIR (KBr, ν/cm^{-1}: 3389 (NH), 3032 (C–H), 1629 (C=N), 1485 (C=C), 1294 (C–N), 511 (C–I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.98 (s, 3H, O–CH$_3$), 5.92 (s, 1H, OH), 7.22–8.33 (m, 10H, CH$_{Ar}$), 10.81 (s, 1H, OH); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.85 (O–CH$_3$), 73.78 (C–H), 84.97 (C–I), 110.54–150.86 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 471.0206 [M − H]$^-$.

[2-(4-Benzyloxy-phenyl)-1H-benzoimidazol-5-yl]-phenyl-methanol (**55**): recrystallized from methanol to obtain a slightly brown solid; yield: 57%; mp (°C): 126–127; FTIR (KBr, ν (cm^{-1})): 3353 (NH), 3030 (C–H), 1610 (C=N), 1492 (C=C), 1249 (C–N), 1130 (C–O–C ether); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.19 (s, 2H, O–CH$_2$), 5.82 (s, 1H, OH), 7.20–8.1 (m, 17H); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 69.35 (O–CH$_2$), 74.58 (C–H), 115.13–159.55 (CH$_{Ar}$); ESI-HRMS (DMSO): m/z = 405.1595 [M − H]$^-$.

4.5. Cytotoxicity assay

All of the target compounds **15–55** were tested against A549 (human lung adenocarcinoma epithelial cell line), MDA-MB-231 (human breast cancer cell line) and PC3 (human prostate cancer cell line). Dr Jeong-Hyung Lee (College of Natural Sciences, Kangwon National University) provided the A549, MDA-MB-231 and PC3 cell lines to the Vietnam Academy of Sciences and Technology (VAST). The benzimidazole derivatives **15–55** were dissolved in DMSO to obtain various concentrations. Campto-thecin and DMSO were used as the reference compound and blank controls, respectively. All the cancer cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin in a 5% CO$_2$ atmosphere for 48 h. Next, the cells were suspended in the culture medium added to well plates at a concentration of 104 cells/well. After 24 h of incubation, the cells were treated with various concentrations of the test compounds for a period of 72 h. Then, 0.5 mg mL^{-1} of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added and incubated at 37 °C, in 5% CO$_2$ for 4 h followed by the procedure described by Mosmann.[36] The formazan crystals were dissolved by adding acidified isopropanol and mixed thoroughly to produce the purple solution. This solution was spectrophotometrically measured using a Multiskan™ microplate reader (Thermo-Fisher Scientific) at 570 nm. Each concentration of tested benzimidazoles was examined in triplicate. The percentage of cell survival (CS) was calculated using the formula: CS (%) = 100 × (OD$_{sample}$ − OD$_{day\ 0}$)/(OD$_{DMSO}$ − OD$_{day\ 0}$). The IC$_{50}$ values (μg mL^{-1}) were calculated for tested compounds, which had CS values < 50% in the initial screening, using the non-linear regression analysis.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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