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REGULAR ARTICLE

A facile and efficient synthesis of benzimidazole as potential anticancer agents

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Abstract. This study reports a simple process to synthesize and separate of 2-(substituted-phenyl) benzimidazole derivatives with high yield and efficiency. Specifically, by reacting ortho-phenylenediamines with benzaldehydes using sodium metabisulphite as an oxidation agent in a mixture of solvent under mild condition, twenty-three compounds of benzimidazoles were obtained and separated easily using hexane and water to wash, respectively. The structure of all obtained compounds was identified by FTIR, NMR and HRMS. The SAR analysis of synthesized benzimidazoles on human lung (A549), breast (MDA-MB-231) and prostate (PC3) cancer cell lines showed that the presence of methyl group at 5(6)-position on benzimidazole scaffold was a contributing factor influencing the anticancer activity. The presence of electron-donating groups (OH, OMe, $–NMe_2$, $–O–CH_2–C_6H_5$) also caused significant increase of anticancer activity, while the presence of electron-withdrawing groups ($–NO_2$, $–CF_3$) on the phenyl group at 2-position of benzimidazole ring decreased the ability of inhibition of synthesized benzimidazoles. The compounds **2f** and **2g** displayed the significant anticancer activity on both A549 and PC3 cell lines.

Keywords. Benzimidazole; condensation reaction; $Na_2S_2O_5$; anticancer agent.

1. Introduction

Benzimidazole is an important heterocyclic organic compound which possess an extensive range of therapeutic applications such as anti-inflammatory, antibacterial,[1] antifungal,[2] antiviral,[3] and analgesic.[4] Since its structure is analogized with the nucleotides found in human body, benzimidazole derivatives have been intensively studied to use as a new generation of anticancer agent.[5] The bioactivities of benzimidazole compounds can be further improved by changing its functional groups on the core structure. This is the most popular method to promote new drugs to treat cancer, produced many commercially available anticancer drugs based on the benzimidazole skeleton such as osimertinib, navelbine, alectinib, nocodazole, abermaciclib, and vinblastine. As of now, research on benzimidazole is a main focus for many laboratories in the world including our lab to prepare better anticancer drugs.[6–8]

The synthesis of benzimidazole derivatives typically involvedin the condensation of the benzene rings possessed nitrogen-containing functional groups at ortho position with various reagents. In our previous study, four 2-alkyl-1*H*-benzimidazoles were synthesized successfully through the reaction between *o*-phenylenediamine and mono carboxylic acids using two different methods (Scheme 1).[8] However, the reaction mixture after completion had to undergo the neutral process and crystallization to obtain the final products, led to a decrease in reaction yield. Besides, it took a period of time to own the desire products for

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further experiments in spite of short reaction time. So, the development of fast, convenient and high yielding approach is still desirable.

In another way, *o*-phenylenediamine derivatives and aldehydes are frequently used as oxidizing agents to generate the benzimidazole core directly (Figure 1). The conditions for the oxidation reaction to happen are varied, it can be done with cupric salts in water or alcoholic medium,[9] sodium metabisulphite in DMF at 130 °C,[10] sodium metabisulphite ($Na_2S_2O_5$) or sodium hydrosulfite under microwave assistance,[11] lanthanum chloride (10 mol%) in acetonitrile at room temperature,[12] sodium hexafluoroaluminate at 50 °C,[13] dioxane dibromide under mild condition,[14] zinc triflate in ethanol solvent at reflux temperature,[15] iodine at 80–90 °C,[16] nickel acetate in chloroform at room temperature,[17] and sodium dodecyl sulfate (10 mol%) at room temperature.[18] Sodium metabisulphite is the most use in directed condensation of benzimidazole from *o*-phenylenediamine and benzaldehyde since it is a low-cost material, allows high reaction yield with easy to separate products. However, due to $Na_2S_2O_5$ low solubility in organic solvents, extreme temperature is needed throughout the reaction to dissolve the salts, thus the reaction can be hard to control,[11,19] In this research, we present a facile and efficient method to synthesize a series of benzimidazoles with different substitutions using $Na_2S_2O_5$ in a solvent mixture of ethanol–water (9:1 v/v). The presence of water helped increase the solubility of $Na_2S_2O_5$, thus the reaction can happen at mild condition with increased performance and yield.

2. Experimental

2.1 *Materials and physical measurements*

The *o*-phenylenediamines and benzaldehyde derivatives were bought from Acros (Belgium) and Sigma-Aldrich (USA) and used as recieved. The ethanol and hexane were purchased from Xilong and used without further purification. The melting points of synthesized benzimidazoles were determined on Electrothermal IA 9000 series and are uncorrected. Merck silica gel 60 F_{254} plates were used for thin-layer chromatography (TLC). FTIR spectra were obtained with an Equinox 55 IR-Bruker (Germany) spectrometer and the absorption bands were recorded in wave number (cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $(CD_3)_2SO$ on a Bruker AM0 FT-NMR Spectrometer at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C-NMR). The chemical shifts were expressed in δ (ppm) relative to tetramethylsilane (TMS) as internal standard. Data were reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet), and coupling constants (J, Hz) in Hz and position. The ESI–MS were performed on aSciex X500R QTOF instrument.

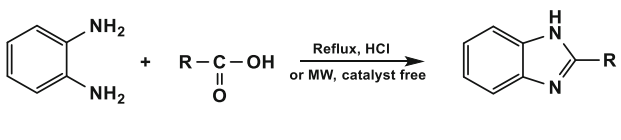
2.2 *General procedure for the preparation of 2-(substituted-phenyl) benzimidazole derivatives*

O-phenylenediamines (2 mmol), benzaldehydes (2 mmol) and sodium metabisulfite (4 mmol) were added in 20 mL solution of ethanol and water (9:1 v/v) and the mixture was stirred constantly at room temperature for 2 h. The reaction progress was monitored by TLC. After the reaction was completed, the reaction mixture was filtered and the filtrate was then concentrated in vacuum. The obtained solid residue was washed with water and n-hexane, respectively, and dried at 80 °C under reduced pressure to achieve the product.

2-(1*H*-benzoimidazol-2-yl)-phenol(**1a**) : white powder, m.p.= 230–231 °C; yield: 73 %, FTIR (KBr), ν/cm^{-1} 3457, 3324, 3056, 1631, 1589, 1261; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.03 (2H, m), 7.29 (2H, m), 7.38 (1H, dt, *J*=1.5, *J*=8.5 Hz), 7.61 (1H, d, *J* =6.5 Hz), 7.72 (1H, d, *J*=6 Hz), 8.06 (1H, dd, *J*=1.5, *J*=7.5 Hz), 13.17–13.14 (2H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 111.45, 112.53, 117.11, 117.89, 123.21, 131.66, 133.10, 140.80, 151.63, 157.95; HRMS (m/z): 211.08771 $[M+H]^+$, 211.08714 calcd $[M+H]^+$ for $C_{13}H_{10}N_2O$.

3-(1*H*-Benzoimidazol-2-yl)-phenol(**1b**) : brown powder, m.p.= 266–267 °C; yield: 81 %, FTIR (KBr), ν/cm^{-1} 3278, 3058, 1754, 1590, 1219; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.90 (1H, dd, *J*=1.5, *J*=8 Hz), 7.20 (2H, dd *J*=3, *J*=5.5 Hz), 7.34 (1H, t, *J*=7.5 Hz), 7.60 (4H, m), 9.69 (1H, s), 12.78 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 113.28, 116.88, 117.14, 121.94, 129.89, 131.34, 151.28, 157.68; HRMS (*m/z*): 211.08723 $[M+H]^+$, 211.08714 calcd $[M+H]^+$ for $C_{13}H_{10}N_2O$.

4-(1*H*-Benzoimidazol-2-yl)-phenol(**1c**) : brown powder, m.p.= 269–270 °C; yield: 90 %; FTIR (KBr), ν/cm^{-1} 3408, 3242, 3057, 1609, 1378, 1250; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 6.92 (2H, d, *J*= 8.5 Hz), 7.16 (2H, dd,



Scheme 1. Condensation of 2-alkyl benzimidazoles from *o*-phenylenediamine and mono carboxylic acids.

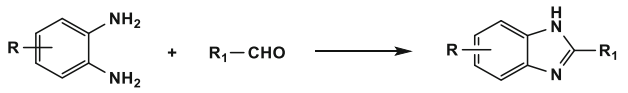


Figure 1. Condensation of benzimidazole from *o*-phenylenediamines and benzaldehydes.

J=3, *J*=6 Hz), 7.54 (2H, dd, *J*=6, *J*=3 Hz), 8.01(2H, d, *J*=8.5 Hz), 9.93 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 115.62, 121.05, 121.56, 128.10, 151.70, 159.08; HRMS (*m/z*): 211.08807 $[M+H]^+$, 211.08714 calcd $[M+H]^+$ for $C_{13}H_{10}N_2O$.

2-(4-Methoxy-phenyl)-1*H*-benzoimidazole(**1d**) : Light yellow powder, m.p.= 170–171 °C; yield: 82 %, FTIR (KBr), ν/cm^{-1} 3384, 3056, 1609, 1505, 1254, 1181; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 3.86 (3H, s), 7.18 (2H, d, *J*=8.5 Hz), 7.30 (2H, dd, *J* =3.5 Hz, *J*=6), 7.64 (2H, dd, *J* =3.5 Hz, *J* =6 Hz), 8.14 (2H, d, *J*=8.5 Hz); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 55.47, 114.41, 114.63, 120.25, 122.98, 127.51, 128.58, 130.69, 136.96, 150.61, 161.43; HRMS (*m/z*): 225.10393 $[M+H]^+$, 225.10279 calcd $[M+H]^+$ for $C_{14}H_{12}N_2O$.

2-(2,5-Dimethoxyphenyl)-1*H*-benzoimidazole(**1e**) : white crystalline needle, m.p.= 212–213 °C; FTIR (KBr), ν/cm^{-1} 3217, 3012, 1614, 1493, 1230, 1133; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.80 (3H, s), 3.97 (3H, s), 7.05 (1H, dd, J = 9.0, 3.5 Hz), 7.23 – 7.14 (m, 3H), 7.64 (2H, dd, J = 5.5, 3.5 Hz), 7.89 (1H, d, J = 3.5 Hz), 12.12 (1H, s, N-H); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 55.49, 56.11, 113.41, 113.61, 117.07, 118.51, 148.72, 151.05, 153.18; HRMS (*m/z*): 255.1111 $[M+H]^+$, 255.1133 calcd $[M+H]^+$ for $C_{15}H_{14}N_2O_2$.

2-(3,4,5-Trimethoxy-phenyl)-1*H*-benzimidazole(**1f**) : white powder, m.p.= 252–254 °C; yield: 73 %, FTIR (KBr), ν/cm^{-1} 3451, 3095, 1676, 1462, 1242,1128; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 3.74–3.90 (9H, s), 7.23 (2H, m), 7.54 (3H, m), 7.66 (1H, d, *J*=8 Hz), 12.82 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 56.06, 60.16, 103.88, 111.15, 118.67, 122.46, 125.43, 134.94, 138.96, 143.70, 151.21, 153.23; HRMS (*m/z*): 285.12706 $[M+H]^+$, 285.12391 calcd $[M+H]^+$ for $C_{16}H_{16}N_2O_3$.

[4-(1*H*-benzoimidazol-2-yl)-phenyl]-dimethyl-amine(**1g**) : light yellow powder, m.p.= 262–263 °C; yield: 97 %, FTIR (KBr), ν/cm^{-1} 3414, 3052, 1610, 1470, 1200; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.01 (3H, s), 6.85 (2H, d, *J*=8.5 Hz),7.17 (2H, dd, *J* = 3, *J* = 6 Hz), 7.53 (2H, q, *J*= 3 Hz), 7.98 (2H, d, *J* =9 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 39.33, 111.82, 121.71, 127.68, 151.45, 151.96; HRMS (*m/z*): 238.13661 $[M+H]^+$, 238.13442 calcd $[M+H]^+$ for $C_{15}H_{15}N_3$.

2-(2-Nitro-phenyl)-1*H*-benzoimidazole(**1h**) : yellow powder, m.p. = 255–256 °C; yield: 73 %, FTIR (KBr), ν/cm^{-1} 3424, 3063, 1612, 1525, 1277; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 7.26 (2H, d, *J* =6.5 Hz), 7.58 (1H, s), 7.65 (1H, s), 7.76 (1H, td, *J*=1, *J*=7.5 Hz), 7.88 (1H, td, *J*=1, *J*=7.5 Hz), 7.98 (1H, dd, *J* =1, *J* = 8 Hz), 8.04 (1H, dd, *J* =0.5, *J* = 8 Hz), 13.02 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 111.59, 119.18, 124.19, 124.22, 130.82, 130.86, 132.56, 132.56, 132.56, 134.57, 143.57, 147.24, 148.91; HRMS (*m/z*): 240.08088 $[M+H]^+$, 240.07730 calcd $[M+H]^+$ for $C_{13}H_9N_3O_2$.

2-(2-Trifluoromethyl-phenyl)-1*H*-benzoimidazole(**1i**) : brown powder, m.p.= 170–171 °C; yield: 82 %; FTIR (KBr), ν/cm^{-1} 3431, 3047, 1650, 1543, 1312, 1180; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 7.28(2H, m), 7.55 (1H, d, *J*= 7.5 Hz), 7.70 (1H, d, *J*= 8 Hz), 7.80 (2H, q, *J*= 8 Hz), 7.84 (1H, t, *J*=7.5 Hz), 7.95 (1H, d, *J*= 8 Hz), 12.74 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 124.76, 119.11, 111.40, 126.50, 126.54, 130.12, 130.25, 132.13, 132.30, 134.45, 143.39, 149.31; HRMS (*m/z*): 263.07919 $[M+H]^+$, 263.07961 calcd $[M+H]^+$ for $C_{14}H_9F_3N_2$.

4-(1*H*-Benzoimidazol-2-yl)-2-iodo-6-methoxy-phenol(**1j**) : Light yellow powder; m.p.= 183–184 °C; yield: 97 %, FTIR (KBr), ν/cm^{-1} 3064, 1629, 1589, 1358, 1227, 512; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.94 (3H, s), 7.22 (2H, q, *J*=3 Hz), 7.58 (2H, q, *J*=3 Hz), 7.78 (1H, d, *J*= 1.5 Hz), 8.12 (1H, d, *J*= 1.5 Hz), 10.09 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 56.20, 84.61, 109.98, 115.00, 122.12, 122.62, 128.39, 147.19, 148.12, 150.06; HRMS (*m/z*): 366.99413 $[M+H]^+$, 366.99435 calcd $[M+H]^+$ for $C_{14}H_{11}IN_2O_2$.

2-(4-Benzyloxy-phenyl)-1*H*-benzoimidazole(**1k**) : yellow powder, m.p.= 262–263 °C; yield: 77 %, FTIR (KBr), ν/cm^{-1} 3422, 3052, 1609, 1499, 1245, 1172; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 5.20 (2H, s), 7.20 (4H, m), 7.35 (1H, t, J=7.5 Hz), 7.43 (2H, t, *J*=7.5 Hz), 7.50 (2H, d, *J*= 7.5 Hz), 7.61 (1H, s), 8.11 (2H, d, *J*= 8.5 Hz), 12.71 (1H, s); ^{13}C-NMR(125 MHz, DMSO-d_6, δ ppm): 69.35, 110.96, 115.16, 118.44, 121.40, 122.88, 127.71, 127.87, 127.95, 128.42, 136.75, 151.23, 159.65; HRMS (*m/z*): 301.13531 $[M+H]^+$, 301.13408 calcd $[M+H]^+$ for $C_{20}H_{16}N_2O$.

2-Phenyl-1*H*-benzoimidazole(**1l**) : light yellow powder, m.p.= 248–249 °C; yield: 97 %; FTIR (KBr), ν/cm^{-1} 3649, 3047, 1620, 1409, 1274; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.90 (1H, s, N-H), 8.20 (2H, m), 7.69 (1H, d, J=8.5 Hz), 7.55 (3H, m), 7.50 (1H, t, J=7.5), 7.21 (1H, s); ^{3}C-NMR (125 MHz, DMSO-d_6, δ ppm): 111.25, 118.83, 121.60, 122.46, 126.38, 128.87, 129.75, 130.13, 134.98, 143.76, 151.18; HRMS (*m/z*): 195.0949 $[M+H]^+$, 195.0922 calcd $[M+H]^+$ for $C_{13}H_{10}N_2$.

2-(5(6)-Methyl-1*H*-benzoimidazol-2-yl)-phenol(**2a**) : white powder, m.p.= 248–249 °C; yield: 96 %; FTIR (KBr), ν/cm^{-1} 3448, 3237, 3083, 1639, 1598, 1261; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.46 (3H, s), 7.04 (2H, m), 7.12 (1H, s), 7.39 (1H, dt, *J* = 1.5, *J* = 8 Hz), 7.51 (1H, s), 7.59 (1H, s), 8.04 (1H, dd, J=1.5 *J* = 7.5 Hz), 13.05 (1H, s), 13.19 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.20, 111.12, 112.64, 118.98, 123.90, 125.96, 131.47, 157.89;

HRMS (*m/z*): 225.10314 $[M+H]^+$, 225.10279 calcd $[M+H]^+$ for $C_{14}H_{12}N_2O$.

3-(5(6)-Methyl-1*H*-benzoimidazol-2-yl)-phenol(**2b**) : light brown powder, m.p.= 259–260 °C; yield: 98 %; FTIR (KBr), ν/cm^{-1} 3285, 2920, 1589, 1440, 1221; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.42 (3H, s), 6.88 (1H, ddd, *J* = 1, J=2.5, J= 8 Hz), 7.02 (1H, dd, *J* = 1, *J* = 8),7.35 (2H, m), 7.46 (1H, d, *J* = 7.5), 7.57 (1H, m), 9.66 (1H, s), 12.66 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.25, 113.16, 116.71, 117.02, 123.43, 129.83, 131.41, 150.93, 157.65; HRMS (*m/z*): 225.10349 $[M+H]^+$, 225.10279 calcd $[M+H]^+$ for $C_{14}H_{12}N_2O$.

4-(5(6)-methyl-1*H*-benzoimidazol-2-yl)phenol(**2c**) : brown powder, m.p.= 262–263 °C; yield: 89 %; FTIR (KBr), ν/cm^{-1} 3417, 3246, 3030, 1609, 1455, 1251; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.41 (3H, s), 6.91 (2H, d, *J* = 9), 7.00 (1H, dd, *J* = 1, *J* = 8.5 Hz), 7.31 (1H, s), 7.42 (1H, d, *J* = 8), 7.98 (2H, d, *J* = 9), 9,91 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.25, 115.60, 123.13, 128.02, 151.28, 159.04; HRMS (*m/z*): 225.10349 $[M+H]^+$, 225.10279 calcd $[M+H]^+$ for $C_{14}H_{12}N_2O$.

2-(4-Methoxy-phenyl)-5(6)-methyl-1*H*-benzoimidazole(**2d**) : soft white powder, m.p.= 262–263 °C; yield: 89 %; FTIR (KBr), ν/cm-1 2990, 1610, 1492, 1254, 1148,; 1H-NMR (500 MHz, DMSO-d6, δ ppm): 2.42 (3H, s), 3.83 (3H, s), 7.01 (1H, dd, J = 1, J = 8 Hz), 7.11 (2H, d, J = 9 Hz), 7.33 (1H, s), 7.44 (1H, d, J = 8 Hz), 8.10 (2H, d, J = 9 Hz); 13C-NMR (125 MHz, DMSO-d6, δ ppm): 21.27, 55.30, 114.31, 122.63, 123.24, 127.88, 130.97, 150.94, 160.51; HRMS (*m/z*): 239.12039 $[M+H]^+$, 239.11844 calcd $[M+H]^+$ for $C_{15}H_{14}N_2O$.

2-(2,5-Dimethoxyphenyl)-5(6)-methyl-1H-benzo[d]imidazole(**2e**) : soft white powder; m.p.= 163–164 °C, yield: 83%; FTIR (KBr), *ν*/cm^{-1} 3212, 3010, 1620, 1365, 1209; ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.51 (s, 3H), 3.81 (s, 3H), 3.97 (s, 3H), 7.03 (m, 2H), 7.16 (1H, d, J = 9.0 Hz), 7.43 (1H, s), 7.52 (1H, d, J = 8 Hz), 7.88 (1H, d, J = 3 Hz), 11.99 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 21.35, 55.47, 56.08, 113.36, 113.50, 116.82, 118.67, 123.35, 130.89, 148.38, 150.93,153.16; HRMS (*m/z*): 269.1290 $[M+H]^+$, 269.1278 calcd $[M+H]^+$ for $C_{16}H_{16}N_2O_2$.

5(6)-methyl-2-(3,4,5-trimethoxyphenyl)-1*H*-benzo[d]imidazole(**2f**) : Pale gold powder, m.p.= 203–204 °C; yield: 93 %; FTIR (KBr), ν/cm-1 3320, 3098, 2996, 1589, 1464, 1239, 1128; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.43 (3H, s), 3.73-3.90 (9H, s), 7.03 (1H, d, J = 8 Hz), 7.49 (2H, m), 7.49 (2H, m), 12.65 (1H, s); 13C-NMR (125 MHz, DMSO-d6, δ ppm): 21.28, 56.00, 60.08, 103.70, 110.73, 118.25, 125.58, 138.76, 150.93, 153.14; HRMS (*m/z*): 299.14233 $[M+H]^+$, 299.13956 calcd [M+H]+ for $C_{17}H_{18}N_2O_3$.

Dimethyl-[4-(5(6)-methyl-1*H*-benzoimidazol-2-yl)-phenyl]-amine(**2g**) : light orange powder, m.p.= 228–229 °C; yield: 97 %; FTIR (KBr), ν/cm^{-1} 3421, 2917, 1610, 1440, 1276, 1226; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.42 (1H, s), 3.00 (3H, s) 6.85 (2H, d, *J* = 9 Hz), 7.01 (1H, dd, *J* =1, *J* = 8 Hz), 7.32 (1H, s), 7.42 (1H, d, *J* = 8 Hz), 7.98 (2H, d, *J* = 9 Hz);^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 39.67, 21.18, 111.74, 113.55, 116.15, 123.15, 127.56, 130.93, 151.36, 151.50; HRMS (*m/z*): 252.15027 $[M+H]^+$, 252.15007 calcd $[M+H]^+$ for $C_{16}H_{17}N_3$.

5(6)-Methyl-2-(2-nitro-phenyl)-1*H*-benzoimidazole(**2h**) : light brown powder, m.p.= 199–200 °C; yield: 88 %; FTIR (KBr), ν/cm^{-1} 3420, 3237, 3064, 1623, 1526, 1277; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.45 (3H, d, *J* = 13.5), 7.11 (1H, dd, *J* =8, *J*=28.5), 7.53 (2H, m), 7.75 (1H, t, *J* = 7.5 Hz), 7.86 (1H, t, *J* = 7.5 Hz), 8.01 (2H, dd, *J* = 7.5 Hz), 12.87 (1H, d, *J* = 14.5); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.21, 111.08, 111.19, 123.42, 124.15, 124.47, 130.62, 130.70, 132.45, 132.62, 134.83, 141.76, 147.05, 148.87; HRMS (*m/z*): 254.09427 $[M+H]^+$, 254.09295 calcd $[M+H]^+$ for $C_{14}H_{11}N_3O_2$.

5(6)-Methyl-2-(2-trifluoromethyl-phenyl)-1*H*-benzoimidazole(**2i**) : light yellow powder, m.p. = 207–208 °C; yield: 91 %; FTIR (KBr), ν/cm^{-1} 3421, 3062, 1610, 1584, 1313, 1133; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.44 (3H, s), 7.06 (1H, d, *J* = 8.5 Hz), 7.39 (1H, s), 7.50 (1H, d, *J* = 7 Hz), 7.78 (1H, m) 7.94 (1H, d, *J* = 8 Hz), 12.61 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.22, 122.58, 124.76, 126.44, 126.49, 126.53, 130.02, 130.33, 132.08, 132.28, 148.93; HRMS (*m/z*): 277.09756 $[M+H]^+$, 277.09525 calcd $[M+H]^+$ for $C_{15}H_{11}F_3N_2$.

2-Iodo-6-methoxy-4-(5(6)-methyl-1*H*-benzoimidazol-2-yl)-phenol(**2j**) : brown powder, m.p.= 165–166 °C; yield: 98 %; FTIR (KBr), ν/cm^{-1} 3448, 3060, 1629, 1463, 1228, 1107, 518; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.44 (3H, s), 3.95 (3H, s), 7.04 (1H, d, *J* = 8 Hz), 7.31 (1H, s), 7.46 (1H, d, *J* = 8 Hz), 7.76 (1H, s), 8.10 (1H, s), 10.07 (1H, s); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.28, 56.23, 84.61, 109.93, 113.50, 123.68, 128.29, 131.51, 147.21, 148.05, 149.72; HRMS (*m/z*): 381.00984 $[M+H]^+$, 381.01000 calcd $[M+H]^+$ for $C_{15}H_{14}IN_2O_2$.

2-(4-Benzyloxy-phenyl)-5(6)-methyl-1*H*-benzoimidazole(**2k**) : white powder, m.p.= 205–206 °C; yield: 95 %; FTIR (KBr), ν/cm^{-1} 3412, 3029, 1609, 1422, 1252, 1184; ^{1}H-NMR (500 MHz, DMSO-d6, δ ppm): 2.42 (3H, s), 5.20 (2H, s), 7.00 (1H, dd, *J* = 1, *J* = 8 Hz), 7.18 (1H, d, *J* = 9 Hz),7.34 (2H, m), 7.42 (3H, m), 7.48 (2H, d, *J* = 7 Hz), 8.08 (2H, d, *J* = 7 Hz); ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.25, 69.34, 115.12, 122.96, 123.17, 127.71, 127.82, 128.41, 130.98, 136.76, 150.89, 159.53; HRMS (*m/z*): 315.15122 $[M+H]^+$, 315.14974 calcd $[M+H]^+$ for $C_{21}H_{18}N_2O$.

2.3 *Assay of breast anticancer activity*

The synthesized benzimidazoles were dissolved in DMSO 0.1% (v/v) to obtain the different

concentrations. Camptothecin was used as the reference compound and DMSO (0.1% (v/v)) was used as blank controls. All cells (A549, MDA-MB-231, PC3 cell lines) were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/ml of penicillin and 100 µg/ml of streptomycin in a 5% CO_2 atmosphere for 48 h. After that, the cells were seeded in 96-well plates at concentration of 10^4 cells/well. After 24 h, the cells were treated with culture medium containing tested compounds at concentration ranges. After 72 h, cell viability was evaluated as mitochondrial succinate dehydrogenase (SDH) activity using 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test as a marker of viable cells and incubated at 37 °C, 5% CO_2 for 4 h followed the procedure described by Mosmann (1983),[20] Acidified isopropanol was added to all wells and mixed thoroughly to dissolve the formazan crystals. The produced purple solution was spectrophotometrically measured at 570 nm using Multikan^{TM} microplate reader. Each concentration of synthesized benzmidazoles was tested in triplicate.

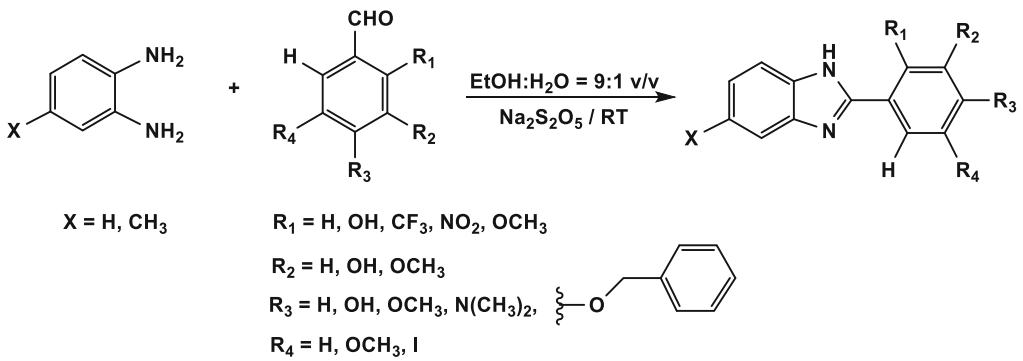
3. Results and Discussion

3.1 *Synthesis and characterization*

The use of EtOH:H_2O mixture (9:1 v/v) as the medium for the synthesis process of benzimidazole derivatives allows $Na_2S_2O_5$ to be dissolved easily, thus the oxidation reaction to produce twenty-three of 2-phenyl benzimidazole derivatives (**1a-l** and **2a-k**) can happen at room temperature as shown in Scheme 2.

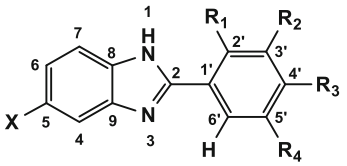
The synthesized benzimidazoles contained various substituents on the phenyl group at 2-position (-OH, -OCH_3, -$N(CH_3)_2$, -NO_2, -CF_3, -I, -O-CH_2-C_6H_5) along with the replacement of hydrogen atom by a methyl group at 5-position on the benzimidazole scaffold. The tautomerization that takes place in cases of 5-substituted benzimidazole derivatives such as 5-methyl- 1*H*-benzimidazoles 2a-k makes them indistinguishable for their 6-methyl positional isomers. Table 1 shows that most of the reactions for 2-phenylbenzimidazoles **1a-l**, **2a-k** have a high yield of more than 73%. The presence of water in reaction medium helped increase the solubility of $Na_2S_2O_5$, thus leads to an increment in the reaction rate and production yield than using only ethanol. These results confirmed our hypothesis that heating does not play a crucial role in the condensed efficiency of 2-phenyl benzimidazoles derivatives in this research. Besides, taking a look at the yields of obtained benzimidazoles, all of compounds **2a-k** that owned the yields better than that of **1a-k** despite the appearance of electron-withdrawing or donating groups on the phenyl group at 2-position in their structures. As an evidence, **1a/2a** had the hydroxyl group as the electron-donating group at the 2'-position and **1h/2h** and **1i/2i** owned the electron-withdrawing groups which are nitro and trifluoromethyl groups at this position, in those cases, the yields of **1a**, **1h** and **1i** were 73%, 73% and 82%, respectively, less than that of **2a**, **2h** and **2i** had 96%, 88% and 90% yield, respectively. From the scientific point of view, the difference in yield between products **1** and **2** is caused by the presence of electron-donating group (methyl group) in 4-methyl-*o*-phenylenediamine structure that leads to increase both the nucleophilicity of the amine moiety and the ability of condense benzimidazole. While lack of electron providing group in *o*-phenylendiamine structure (only owning hydrogen atoms) decreased the yield in the cyclization reaction which produced the products **1**.

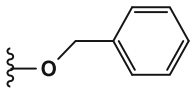
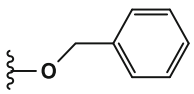
The structures of all synthesized benzimidazoles were confirmed using FTIR, ^{1}H-NMR, ^{13}C-NMR and HRMS spectroscopy. Novel compound **2j** was obtained as a brown amorphous powder. The IR spectrum of **2j**



Scheme 2. The synthesis of 2-(substituted-phenyl) benzimidazole derivatives.

Table 1. The structures and yield of synthesized benzimidazoles.



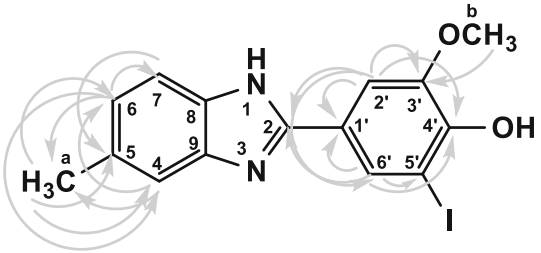
Compound	X	R_1	R_2	R_3	R_4	Yield %
1a	H	OH	H	H	H	73
1b	H	H	OH	H	H	81
1c	H	H	H	OH	H	91
1d	H	H	H	OCH_3	H	82
1e	H	OCH_3	H	H	OCH_3	74
1f	H	H	OCH_3	OCH_3	OCH_3	73
1g	H	H	H	$N(CH_3)_2$	H	97
1h	H	NO_2	H	H	H	73
1i	H	CF_3	H	H	H	82
1j	H	H	OCH_3	OH	I	97
1k	H	H	H		H	77
1l	H	H	H	H	H	97
2a	CH_3	OH	H	H	H	96
2b	CH_3	H	OH	H	H	98
2c	CH_3	H	H	OH	H	89
2d	CH_3	H	H	OCH_3	H	89
2e	CH_3	OCH_3	H	H	OCH_3	83
2f	CH_3	H	OCH_3	OCH_3	OCH_3	93
2g	CH_3	H	H	$N(CH_3)_2$	H	97
2h	CH_3	NO_2	H	H	H	88
2i	CH_3	CF_3	H	H	H	90
2j	**CH_3**	**H**	**OCH_3**	**OH**	**I**	**98**
2k	CH_3	H	H		H	95

appeared absorptions of C=N, C=C (1629–1586 cm^{-1}) and C-N (1358 cm^{-1}) stretch. The NMR spectrum of **2j** which was described in Table 2 revealed that there were five aromatic protons at δ_H 7.31–8.10 ppm corresponded to aromatic carbons at δ_C 84.61–149.72 ppm. In the HSQC spectrum of **2j**, it showed correlation of the cross peaks between two methyl carbons at δ_C 21.28 (C-a) and 56.23 (C-b) and methyl protons at δ_H 2.44 (3H, *s*, H-a) and 3.95 (3H, *s*, H-b), respectively. The HMBC spectrum signified correlations between aromatic protons at δ_H 7.76 (1H, *s*, H-2') and 8.10 (1H, *s*, H-6') and same quaternary carbons at δ_C 149.72 (C-2), 148.05 (C-1') and 147.21 (C-4'); between methyl proton at δ_H 2.44 (3H, *s*, H-a) and carbons at δ_C 113.50 (C-4(7)), 131.51 (C-5(6)) and 123.68 (C-6(5)). Notably, because of high electron density of the iodine atom which was attached at C-5' position, the ^{13}C shift of C-5' carbon was moved to upfield region at δ_C 84.61 ppm instead of aromatic region. Molecular formula of **2j** was demonstrated as $C_{15}H_{13}IN_2O_2$ by HR-ESI-MS data with the pseudo-molecular ion $[M+H]^+$ *m/z* 381.00984 (calcd. For $C_{15}H_{14}IN_2O_2$, 381.01000). Based on spectral data above, the structure of **2j** was indicated as 2-iodo-6-methoxy-4-(5(6)-methyl-1*H*-benzoimidazol-2-yl)-phenol.

3.2 *Anti-proliferative activity*

All the synthesized benzimidazoles **1a–l** and **2a–k** were evaluated for their inhibitory effect on growth of three cancer cell lines [human lung adenocarcinoma epithelial cell line (A549), human breast cancer cell line (MDA-MB-231), human prostate cancer cell line (PC3)] with positive contrast drug Camptothecin. Cells were treated with different concentrations of the listed compounds, and the viabilities were measured by MTT assay. The inhibitory results are presented as IC_{50} values and the mean (±SD) values were

Table 2. ^{1}H, ^{13}C NMR spectral data and HSQC and HMBC correlations of compound **2j**.



Position	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)	Correlations	
			HSQC	HMBC
2		149.72	–	–
4(7)	7.31 (1H, s)	113.50	C-4	C-6, C-a
5(6)	–	131.51	–	–
6(5)	7.04 (1H, d, 8 Hz)	123.68	C-6	C-4, C-7, C-a
7(4)	7.46 (1H, d, 8 Hz)	113.50	C-7	C-5
8/9/1’	–	148.05	–	–
2’	7.76 (1H, s)	109.93	C-2’	C-2, C-1’, C-3’, C-4’, C-6’
3’/4’	–	147.21	–	–
5’	–	84.61	–	–
6’	8.10 (1H, s)	128.29	C-6’	C-2, C-1’, C-2’, C-4’, C-5’
A	2.44 (3H, s)	21.28	C-a	C-4, C-5, C-6
B	3.95 (3H, s)	56.23	C-b	C-3’
NH/OH	10.07 (1H, s)	–	–	–

calculated from at least three independent experiments. The results in Table 3 indicate that the 23 benzimidazole derivatives showed the potential antiproliferative activity against all of the tested tumor cell lines, especially for MDA-MB-231 cell line. Indeed, ten compounds, seven compounds and ten compounds showed IC_{50} values higher than 100 μg/mL on the A549, MDA-MB-231 and PC3 cell lines, respectively. Moreover, two compounds (**2f** and **2g**) showed an IC_{50} in the range of 11.75–12.88 μg/mL on the A549 cell line; IC_{50} values of two compounds (**1a** and **1k**) were 19.5 μg/mL on MDA-MB-231 and the IC_{50} values of three compounds (**2e-g**) for the PC3 cell line showed between 16.22 and 18.20 μg/mL. Specifically, there was no appearance of any substitutions on the benzimidazole skeleton and 2-phenyl ring system in **1l** compound leads to no anticancer activity on all three cell lines ($IC_{50} > 100$ μg/mL).

The differences in the IC_{50} values may be correlated to factors such as electron-withdrawing and donating groups on the benzimidazole scaffold, the functionality of the phenyl ring system at 2-position, and the biochemical characteristics of cell lines. To elaborate more details about the effect on the inhibitory activity of the electron-donating and withdrawing group on the phenyl ring at 2-position as well as on benzimidazole frame, we synthesized other benzimidazole derivatives with different substituents on the 2-phenyl ring system including -OH, -NO_2, -CF_3, -I, -OMe, -NMe_2 and -O-CH_2-C_6H_5 in parallel with replacement hydrogen atom by methyl group at 5(6)-position. Surprisingly, the bioactivities of the compounds **2** were better than those of compounds on A549 and PC3 cell lines; for instance, **2b** > **1b**, **2c** > **1c**, **2e** > **1e**, **2f** > **1f**, **2g** > **1g**, **2h** > **1h**, **2j** > **1j** and **2k** > **1k**, which demonstrated that methyl group at 5-position plays an important role to contribute to the bioactivities of these compounds. Besides, the more the electron-donating substituted groups in the 2-phenyl ring system were, the better the anticancer activity of synthesized benzimidazoles were, and this could be illustrated from the comparison of bioactivity of compounds on the A549 cell line: **2f** > **2e** > **2d**. In addition, taking a look at the ability of inhibition of synthesized benzimidazoles on PC3 cell line, the compounds that showed electron-donating groups (such as OH, OMe, NMe_2, -O-CH_2-C_6H_5) on the 2-phenyl ring were preferable to electron-withdrawing groups (such as NO_2, CF_3), which observed

Table 3. Cytotoxic effect of benzimidazole derivatives against A549, MDA-MB-231 and PC3 cell lines.

	IC_{50} ± SD (μg/mL)		
Compound	A549	MDA-MB-231	PC3
1a	>100	19.5±1.39	>100
1b	36.31±1.42	>100	44.67±1.23
1c	72.44±1.94	34.2±2.06	52.48±1.54
1d	30.2±2.06	38.99±1.45	37.15±1.94
1e	>100	>100	>100
1f	>100	22.91±1.18	34.67±1.27
1g	>100	>100	>100
1h	>100	28.84±1.67	>100
1i	>100	>100	>100
1j	32.36±1.34	60.26±1.57	69.18±1.28
1k	>100	19.5±1.05	>100
1l	>100	>100	>100
2a	>100	32.36±1.93	>100
2b	33.11±1.67	24.55±1.48	32.36±1.83
2c	28.18±2.11	>100	33.88±1.53
2d	57.54±1.89	44.67±1.35	42.66±1.58
2e	21.88±1.66	21.38±1.9	17.78±1.04
2f	11.75±0.35	>100	18.20±0.67
2g	12.88±0.45	93.33±1.93	16.22±1.23
2h	41.69±1.34	72.44±1.78	>100
2i	>100	44.67±1.68	>100
2j	26.3±1.26	29.51±1.63	39.17±1.97
2k	29.51±1.03	83.18± 2.53	44.67±2.13
Camptothecin	0.2±0.06	0.47±0.04	0.87±0.11

from the IC_{50} values of **2c** (33.88 μg/mL), **2d** (42.66 μg/mL), **2g** (16.22 μg/mL), **2k** (44.67 μg/mL) in comparison to that of **2h** and **2i** (>100 μg/mL).

According to these analyses mentioned above, some structure-activity relationship (SAR) could be concluded: the presence of methyl group at 5(6)-position on benzimidazole scaffold was a contributing factor influencing the anticancer activity. The presence of electron-donating groups (OH, OMe, -NMe_2, -O-CH_2-C_6H_5) also caused a significant increase of anticancer activity, while the presence of electron-withdrawing groups (-NO_2, -CF_3) on the phenyl group at 2-position of benzimidazole ring decreased the ablility of inhibition of synthesized benzimidazoles.

4. Conclusions

In summary, we present the first attempt of synthesizing twenty-three 2-(substituted)-phenyl benzimidazoles from *ortho*-phenylenediamines and benzaldehyde derivatives in a mixture of ethanol and water (9:1 v/v) using $Na_2S_2O_5$ as the oxidizing agent. The results showed that the presence of water in the reaction mixture helped increase the solubility of $Na_2S_2O_5$, thus faster reaction rate and higher yield without the need for extreme temperature compared to other methods. We also successfully synthesized a new benzimidazole derivative **2j**, and the compound had been thoroughly characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and HRMS spectroscopy. All obtained compounds inhibition capabilities against human lung adenocarcinoma epithelial cell line A549, breast cancer cell line MDA-MB-231 and prostate cancer cell line PC3 were evaluated by IC_{50} values. Some of the synthesized benzimidazoles exhibited a significant anti-proliferative activity against A549, MDA-MB-231 and PC3 cells. The SAR analysis showed that the derivatives bearing electron-donating groups have significantly higher inhibition than that of benzimidazole derivatives bearing electron-withdrawing groups on the phenyl rings at 2-position. Notably, the appearance of the methyl group at 5(6)-position was a crucial role in growth of inhibition of synthesized compounds. Other studies in our group to expand the method for the synthesis of other benzimidazole derivatives by varying the substitutions at 5(6)-position and 2-position is in progress.

5. Supplementary Information (SI)

Supplementary information (characterization data such as HRMS, 1D and 2D NMR spectra for the synthesized compounds) associated with this article are available at www.ias.ac.in/chemsci.

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