Synthesis and Antioxidant Activity of Some Novel Benzimidazole Derivatives

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ABSTRACT: A series of 2-substituted-5-methylbenzimidazole derivatives (**3a-e**) were synthesized by reacting 4-methyl-1,2-phenylenediamine (**1**) with a number of *p*-substituted benzaldehydes (**2a-e**) in moderate yields (25.51-40.21%). The synthesized compounds (**3a-e)** were characterized by spectroscopic data and were evaluated for antioxidant activity using DPPH free radical scavenging assay. The compounds showed significant antioxidant activity having IC_{50} value of 1.054-19.05 µg/ml as compared to the standard BHT (26.96 µg/ml).

Key words: Benzimidazole, synthesis, antioxidant activity, DPPH, BHT

INTRODUCTION

Oxidative stress is a major contributing factor for developing degenerative diseases like atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer and so on.[1] Oxidative process results in the formation of reactive oxygen species (ROS) free radicals such as superoxide anion radical (O_2^-), hydroxyl radical (OH^-) and non-free-radical species like H_2O_2 and singlet oxygen in the cells.[2] These reactive oxygen species cause lipid peroxidation, protein peroxidation, DNA damage and cellular degeneration in the cells.[3,4] Therefore, free radical reactions have been implicated in the pathology of numerous diseases including brain disorders, platelet aggregation, inflammatory diseases and a variety of other disorders.[5,6] Antioxidants have the ability of curbing free radical chain reaction. So, the development of effective antioxidant agents needs enough attention in the field of drug design and discovery.

Heterocycles like benzimidazole derivatives can be promising candidates as antioxidants as these compounds are reported to exhibit a wide range of biological activities such as antimicrobial[7], anthelmintic[8], anticancer[9], anti-HIV[10], anticonvulsant[11], anti-inflammatory[12,13], antiulcer[14], antiviral[15], anti-hepatitis B[16] and antihypertensive[17] effects. Though, the earlier report of the antioxidant activity of these compounds appeared in 2004[18] and 2006[19], but the activity has not yet been extensively explored. By considering the biological activities of benzimidazole derivatives, we became interested to design and synthesize 2-substituted benzimidazole derivatives in order to investigate their antioxidant potential.

In our earlier report, we have synthesized few benzimidazole derivatives having promising antioxidant activity.[20] In continuation of our research, herein we report few more newly synthesized benzimidazole derivatives with antioxidant property.

EXPERIMENTAL

Materials and Methods. Melting points were determined in open capillaries with the help of WRS-1B (Germany) digital melting point apparatus and were corrected. UV-Vis data were taken using SHIMADZU UV-166V spectrophotometer and IR

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spectra (KBr) were recorded on SHIMADZU IR-470 spectrophotometer. ^{1}H NMR spectra were recorded by Bruker 400 Ultra Shield instrument using deutero-DMSO (*d*-DMSO) as solvent and tetramethylsilane (TMS) as internal reference standard. All chemical shifts (δ) are in ppm. The purities of the compounds were checked by thin layer chromatography (TLC) on silica gel-G plates. The major chemicals were purchased from Sigma-Aldrich Chemical Corporation and all other chemicals were of reagent/analytical grade.

General procedure for synthesis of 2-substituted 5-methylbenzimidazole derivatives. To a solution of 4-methyl-1,2-phenylenediamine **1** (1.0 equivalent) and the corresponding aldehydes **2** (1.5 equivalent) in absolute ethanol, sodium metabisulfite (4.0 equivalent) was added and the reaction mixture was stirred at room temperature for 4-6 hours. After completion of the reaction observed by TLC, the reaction mixture was evaporated in vaccum at 40^{o}C to dry and oily residue was obtained. To this residue, water was added and then extracted with ethyl acetate (3x10 ml). The organic solution was treated with brine solution and then separated. Afterwards, organic solution was dried by anhydrous sodium sulfate. The organic solution was further filtered, evaporated under vaccum to get an oily residue. The oily residue was purified by silica-gel column chromatography. Then the residue was dried and recrystallized. The compounds (**3a-e)** obtained were characterized by spectral analysis.

Synthesis of 2-(4-chloro-phenyl)-5-methyl-1H-benzimidazole (3a). To a solution of 4-methyl-1, 2-phenylenedimine (500 mg, 4.098 mmol) and 4-chloro-benzaldehyde **2a** (1.0 equiv, 575.82 mg, 4.098 mmol) in absolute ethanol (50 ml). $Na_2S_2O_5$ (1.0 equiv, 778.68 mg, 4.098 mmol) was added and the resulting mixture was stirred for 5-6 hours at room temperature. After checking completion of the reaction by TLC, distilled water was added to the reaction mixture and was extracted three times with ethyl acetate (3 x 20 ml). The organic portion collected together was washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dry. The crude mass was purified by silica-gel column chromatography using hexane: ethyl acetate (8:2) as the mobile phase and the product **3a** was obtained as a light brown amorphous powder. Yield 31.23%; melting point 179-180°C; UV-Vis ($CHCl_3$): λ_{max} 530 nm; IR (KBr) ν (cm^{-1}) 3437 (N-H), 2922 (=C-H), 1608 (aromatic C=C), 1429 (C=N), 813 (Ar-Cl); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.57-7.96 (m, 3H, benzimidazole ring), 7.08-7.52 (m, 4H, ArH), 5.35 (s, 1H, NH), 2.49 (s, 3H, Ar-CH_3).

Synthesis of 2-(4-bromo-phenyl)-5-methyl-1H-benzimidazole (3b). Synthetic procedure is same as described for **3a** except 4-bromobenzaldehyde **2b** (758.19 mg, 4.098 mmol) used in place of **2a**. The product was obtained as a dark brown amorphous solid. Yield: 29.13%; melting point 197-198 °C; UV-Vis ($CHCl_3$): λ_{max} 442 nm; IR (KBr) ν (cm^{-1}) 3441 (N-H), 2924 (=C-H), 1616 (aromatic C=C), 1429 (C=N), 486 (Ar-Br); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.53-7.89 (m, 3H, benzimidazole ring), 7.07-7.50 (m, 4H, ArH), 5.33 (s, 1H, NH), 2.43 (s, 3H, Ar-CH_3).

Synthesis of 2-(4-Fluoro-phenyl)-5-methyl-1H-benzimidazole (3c). Synthetic procedure is same as described for **3a** except 4-fluorobenzaldehyde **2c** (508.19 mg, 4.098 mmol) used in place of **2a**. The product was obtained as dark brown amorphous solid. Yield: 25.51%; melting point 181-182 °C; UV-Vis ($CHCl_3$): λ_{max}444 nm; IR (KBr) ν (cm^{-1}) 3442 (N-H), 2992 (=C-H), 1602 (aromatic C=C), 1435 (C=N), 1226 (Ar-F); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.57-8.06 (m, 3H, benzimidazole ring), 7.05-7.49 (m, 4H, ArH), 5.36 (s, 1H, NH), 2.41 (s, 3H, Ar-CH_3).

Synthesis of 2-(4-methoxy-phenyl)-5-methyl-1H-benzimidazole (3d). Synthetic procedure is same as described for **3a** except 4-anisaldehyde **2d** (557.37 mg, 4.098 mmol) used in place of **2a**. The product was obtained as a light brown amorphous solid. Yield: 27.95%; melting point 205-206°C; UV-Vis ($CHCl_3$): λ_{max}393 nm; IR (KBr) ν (cm^{-1}) 3439 (N-H), 2924 (=C-H), 1635, 1627 (aromatic C=C), 1427 (C=N), 1027 (Ar-OCH_3); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.45-8.04 (m, 3H, benzimidazole ring), 7.05-7.32 (m, 4H, ArH), 5.34 (s, 1H, NH), 2.40 (s, 3H, Ar-CH_3).

Synthesis of 2-(4-nitro-phenyl)-5-methyl-1H-benzimidazole (3e). Synthetic procedure is same as described for **3a** except 4-nitrobenzaldehyde **2e** (557.37 mg, 4.098 mmol) used in place of **2a**. The product was obtained as a light yellow amorphous solid. Yield: 40.21%; melting point 220-221 °C; UV-Vis ($CHCl_3$): λ_{max}482 nm; IR (KBr) ν (cm^{-1}) 3390 (N-H), 2931 (=C-H), 1512 (aromatic C=C), 1442 (C=N), 1336 (Ar-NO_2); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.43-7.57 (m, 3H, benzimidazole ring), 8.19-8.33 (m, 4H, NO_2-ArH), 2.50 (s, 3H, Ar-CH_3).

Antioxidant activity Test by DPPH free radical scavenging assay. The antioxidant potential of all the synthesized compounds was evaluated *in vitro* by free radical scavenging assay using DPPH (2, 2-diphenyl-1-picryl hydrazyl) reduction method.[21] Four mg of DPPH was dissolved in 4 ml of methanol. From this stock solution, dilutions were made to obtain solutions of concentrations 0.997 µg/ml, 1.953 µg/ml, 3.906 µg/ml, 7.813 µg/ml, 15.625 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, and 500 µg/ml. The absorbance values were recorded for these dilutions at 517 nm. The solution was prepared in the amber reagent bottle and kept in the light-proof box. *Tert*-butyl-1-hydroxytoluene (BHT), a potential antioxidant, was used as positive control. 2 mg of BHT was dissolved in methanol to get a mother solution having a concentration 1000 µg/ml. The test compounds (**3a-e**), each 2 mg in amount, was dissolved in methanol to prepare a stock solution whose concentration is 1000 µg/ml. The test samples were prepared from this stock solution by serial dilution with methanol to attain the concentrations similar to DPPH. 2.0 ml solution of the test compounds was mixed with 3.0 ml of DPPH solution (20µg/ml). The mixture was then shaken vigorously and allowed to stand at room temperature for 30 minutes in dark place and the absorbance was measured at 517 nm by UV-Spectrophotometer against methanol as blank.

Percent Inhibition of free radical DPPH (% of DPPH radical scavenging) was calculated by using the following equation:

Percent inhibition = $(1- A_{sample}/A_{blank})\times100$

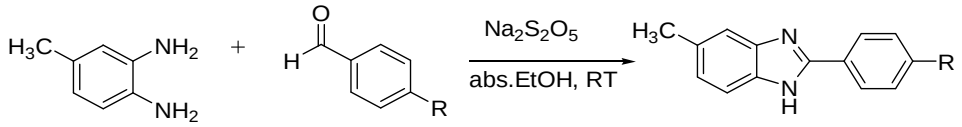
Where A_{blank} is the absorbance of control reaction (containing all reagents except the test material). Concentration of the compound providing 50% inhibition (IC_{50}) was calculated from the graph having the % inhibitions plotted against respective concentrations of the samples.

RESULTS AND DISCUSSION

Chemical Synthesis. Five new benzimidazole derivatives were synthesized by known procedures according to the reaction mentioned in **Scheme 1**. The synthesized compounds were characterized by various physicochemical parameters (TLC, melting point, solubility) and also by spectroscopic methods (UV-Vis, IR and NMR) for their structural elucidation. The purity of the synthesized compounds was confirmed by TLC and column chromatography. Compounds **3a-e** were synthesized by condensation of corresponding aldehydes i.e., 4-chlorobenzaldehyde (**2a**), 4-bromobenzaldehyde (**2b**), 4-fluorobenzaldehyde (**2c**), 4-anisaldehyde (**2d**) and 4-nitrobenzaldehyde (**2e**) with 4-methyl-1, 2-phynylenediamine (**1b**) respectively. The reaction was stirred at room temperature (RT) in absolute ethanol in presence of sodium metabisulfite for a period of 4 to 6 hours. The compounds were obtained in moderate yields (25.51- 40.21 %).

Antioxidant activity. The antioxidant activity was performed using DPPH radical scavenging method where BHT was used as a positive control for comparison. The results of antioxidant activity of the compounds **3a-e** are shown in table 1. The activity was assessed by measuring its electron donating ability to DPPH which was indicated by changes in absorbance of the solution of different concentrations at 517 nm. The DPPH radical scavenging activity of the compounds increased with an increase in concentration. The result of the radical scavenging was expressed in terms of half-inhibition concentration (IC_{50}) which denotes the concentration required to scavenge 50% of DPPH radicals.

Scheme 1 Synthesis of 2-subsituted-5-methylbenzimidazole derivatives



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2a (R=Cl)**, 2b** (R=Br)
2c (R=F)**, 2d** (R=OMe)
2e (R=NO_2)

3a-e

Entry	R	Product	Yield (%)
1	Cl	3a	31.23
2	Br	3b	29.13
3	F	3c	25.51
4	OMe	3d	27.95
5	NO_2	3e	40.21

Table 1. Antioxidant activity of 5-methyl-2-substituted benzimidazoles 3a-e.

Comds	% Inhibition of DPPH free radical at different concentrations										IC_{50} (µg/ml)
	0.997 µg/ml	1.953 µg/ml	3.906 µg/ml	7.813 µg/ml	15.62µg /ml	31.25 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	
3a	35.19	37.82	40.17	58.65	73.02	78.59	85.34	89.74	91.20	92.67	4.02
3b	24.05	25.80	39	60.99	63.34	71.26	74.19	77.42	78.88	79.76	8.34
3c	26.29	27.56	29.91	40.17	61.58	67.16	74.45	82.40	86.80	87.97	11.38
3d	26.69	26.98	28.15	36.95	43.99	60.99	65.98	71.26	73.02	80.35	19.05
3e	46.04	56.89	59.24	65.98	68.62	73.31	83.28	86.80	88.86	91.79	1.054
BHT	7.04	8.21	14.66	22.58	29.32	53.08	71.26	74.49	89.73	92.67	26.96

Compounds **3e** at IC_{50} of 1.054 µg /ml seemed to be most active which is assumed to be due to the presence of nitro substituent group in the molecule as the presence of nitro group in any organic molecule in general confers significant biological activity like metronidazole[22], nitrazepam[23], chloramphenicol[24] etc. On the other hand, compounds **3a, 3b** and **3c** also exhibited moderate to significant antioxidant activity (11.38-4.02 µg /ml) which is due to halogen substituents of which is found in many drug molecules.[25]

CONCLUSION

We have synthesized a series of 2-substituted-5-methyl benzimidazole derivatives. Among the synthesized benzimidazoles, compound with nitro substituent **(3e)** was found to have potential or higher antioxidant activity in comparison to standard compound BHT. Moreoever, compounds with halogen derivatives (**3a-3c**) and methoxy substituted benzimidazole (**3d**) also showed promising antioxidant activity. However, more extensive studies needed to confirm the preliminary results. Besides, pharmacodynamic studies are required to confer the effectiveness of this series of compounds.

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