Synthesis and Biological Evaluation of 2-methyl-1H-benzimidazole and 1H-benzimidazol-2-yl-methanol

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Received: June 05, 2016; Accepted: June 13, 2016; Published (web): June 20, 2016

ABSTRACT: Two benzimidazole derivatives namely 2-methyl-1H-benzimidazole **(1)** and 1H-benzimidazol-2-ylmethanol **(2)** were synthesized in high yields by condensing *O*-phenylenediamine with two different carboxylic acid derivatives: glacial acetic acid and glycolic acid, following a different synthetic protocol. Structures were elucidated by spectroscopic methods. Among the two benzimidazole derivatives synthesized, 2-methyl-1H-benzimidazole **(1)** showed moderate antioxidant activity with IC_{50} values of 144.84µg/ml whereas 1H-benzimidazol-2-ylmethanol **(2)** displayed mild antioxidant activity (IC_{50} value 400.42 µg/ml). Compound **1** also exhibited prominent cytotoxic activities with the LC_{50} value of 0.42 µg/ml when compared to the standard vincristine sulphate (LC_{50} value of 0.544 µg/ ml). It also demonstrated weak antimicrobial activity having 7-8 mm of zone of inhibition.

Key words: Benzimidazole, NMR spectroscopy, Antioxidant activity, Brine shrimp lethality, Antimicrobial.

INTRODUCTION

The search for new synthetic compounds for the treatment of different ailments has always attracted the scientists. The synthesis of benzimidazole is one of the successful outcomes of such relentless efforts. It has a heterocyclic ring structure which comprises benzene ring fused with an imidazole ring. The purpose of this type of synthesis of benzimidazole derivatives is that compounds containing these structural features have multifarious activities like antitumor, antifungal, antiviral, antiulcer, anticoagulant, antiallergic activities etc.[1] Moreover, benzimidazole derivatives are used as important drug candidates such as anthelmintics and proton-pump inhibitors (PPIs) like lansoprazole and omeprazole.[1] However, these drugs sometimes show side effects like diarrhea, frequent chest pain and heart burn for some patients. Now-a-days, drug resistance has also become one of the most alarming situations. As these drugs have got beneficial effect upon our body, their structural modification can be a helpful approach to enhance the pharmacological activity, reduce the drug side-effects, and compensate for drug resistant molecules.

There are different reported synthetic procedures for benzimidazole. One of these procedures is the condensation reaction between *O*-phenylenediamine and carboxylic acid containing moieties (Figure 1).[2] Following this condensation reaction, various types of benzimidazole derivatives can be synthesized depending on the type of carboxylic acid derivatives. In this work, two benzimidazole derivatives were synthesized following suitable and feasible synthetic pathways using *O*-phenylenediamine with glacial acetic acid and glycolic acid separately followed by studying their antioxidant, brine shrimp lethality and antimicrobial activities.

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MATERIALS AND METHODS

All reactions were carried out in well-dried glasswares using a reflux condenser. The solvents were removed under reduced pressure using rotary evaporator. Progress of all the reactions were monitored by thin layer chromatography (TLC), where the spots were visualized by spraying para-anisaldehyde followed by heating at 120°C for about 2 minutes. For column chromatography, silica gel 60 (0.06-0.2 mm, ROTH) was employed. ^{1}H NMR (400MHz) was acquired in $CDCl_3$ on JEOL alpha 400 instrument. FT-IR spectra were recorded with FT-IR 8400S Shimadzu spectrophotometer in the range of 4000-400 cm^{-1}. Melting points were measured by using WRS-1B (Digital MP apparatus) in capillary tube and are uncorrected. *O*-phenylenediamine was purchased from Sigma-Aldrich, Germany.

Typical procedure for the synthesis of benzimidazole derivatives. Appropriate amount of *O*-phenylenediamine was taken in dimethyl formamide and glycolic acid was added to it. The mixture was then refluxed at 90°C-100°C to optimize the reaction conditions. The reaction was monitored by TLC. After completion of reaction, the reaction mixture was diluted with water. Then $NaHCO_3$ solution was added to it to neutralize the residual acid, which was monitored by litmus paper. The mixture was then extracted with ethyl acetate. The organic layer was washed with brine solution and dehydrated over anhydrous sodium sulphate. The solvent was removed under reduced pressure and crude product was then subjected to column chromatography (Table 1).

Table 1. Summary of reaction conditions and products.

Entry	Reactant	Amount	Reactant	Amount	Milimole	Solvent	Products
1	*O*-phenylenediamine	300 mg	Glacial acetic acid	0.63 ml	18.5	Glacial acetic acid	2-methyl-1H-benzimidazole
2	*O*-phenylenediamine	500 mg	Glycolic acid	703.508 mg	9.2506	Dimethyl formamide	1H-benzi-midazol-2-ylmethanol

2-methyl-1H-benzimidazole (1). Yield: 50%; m.p.175-177°C; R_f: 0.28 (ethyl acetate: n-hexane = 3:1); IR (KBr, cm^{-1}): 800 (aromatic ring), 1280 (C-N of imidazole ring), 1600 (C=C of aromatic ring), 1100 (C-N), 1650 (N-H), 2960 (C-H), 3400 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$): 7.52 (m, 2H), 7.20 (m, 2H), 2.62 (s, 3H, CH_3).

1H-benzimidazol-2-ylmethanol (2). Yield: 72%; m. p.158.1-159.2°C; R_f: 0.27 (ethyl acetate: n-hexane = 3:1); IR (KBr, cm^{-1}): 3113.21 (C-H of aromatic ring), 1410.01, 1477.52 (C=C of aromatic ring), 1186.26, 1202.66, 1246.06 (C-N of imidazole ring), 3113.21 (O-H of alcohol); ^{1}H NMR (400 MHz, $CDCl_3$,): 8.157 (s, -NH-), 7.607 (dd, *J*=6Hz, 6Hz, 2H), 7.228 (dd, *J*=6Hz, 8.8Hz, 2H), 6.31(s, -CH_2-), 5.72 (s, -OH).

Pharmacological evaluation. The synthesized derivatives were subjected to antioxidant, antimicrobial and brine shrimp lethality bioassays.

Antioxidant activity. The antioxidant activity of the synthesized compounds was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by the method of Brand-Williams *et al.* using butylated hydroxytoluene (BHT) as the standard.[3,4] The inhibition of free radical DPPH in percent (I %) was calculated by using the following equation:

$$\mathbf{(I\ \%) = (1- A_{sample}/A_{blank}) \times 100}$$

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

Brine shrimp lethality bioassay. The cytotoxicity assay of compound **1** and **2** was performed on brine shrimp nauplii.[5] Here, vincristine sulphate dissolved in DMSO was used as the positive control. Ten brine shrimp nauplii were added in each of the test tubes marked as sample, positive control and negative control (simulated sea water).

Antimicrobial activity assay. Compound **1** and **2** were assayed for antimicrobial activities by the standardized disc diffusion method.[6] In vitro antimicrobial screening was done against gram positive and negative strains of bacteria and fungi. The obtained results were compared with standard antibiotic, ciprofloxacin.

RESULTS AND DISCUSSION

Synthesis and structure elucidation. The synthesis of compound **1** and **2** has been illustrated in scheme-1. *O*-phenylenediamine reacted with glacial acetic acid and glycolic acid to yield **1** and **2** having yields of 50% and 72% respectively. Compound **2** was synthesized in a synthetic pathway which is different than the reported protocol. The structures of the compounds were elucidated by analysis of their IR and NMR spectral data and confirmed by comparison with published values.[7,8]

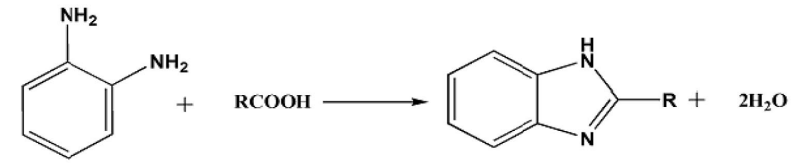
Pharmacological screenings

Antioxidant activity. The compounds were subjected to DPPH free radical scavenging assay by the method of Brand-Williams *et al*.[5] Of the synthesized compounds, **1** showed moderate antioxidant activity with the IC_{50} values of 144.84 µg/ml as compared to that of the standard BHT having IC_{50} values of 51.56 µg/ml (Table 2). On the other hand, **2** showed very weak antioxidant activity having IC_{50} of 400.42 µg/ml. Therefore, compound **1** might be an interesting lead compound for further development of potent antioxidant agent.

Brine shrimp lethality activity. According to the data presented in Table 3, **1** showed very prominent cytotoxic activities with the LC_{50} values of 0.42 µg/ml as compared to the standard vincristine sulphate having LC_{50} value of 0.544 µg/ml. In contrast, **2** only showed mild cytotoxic activity with very high LC_{50} value (Table 3).

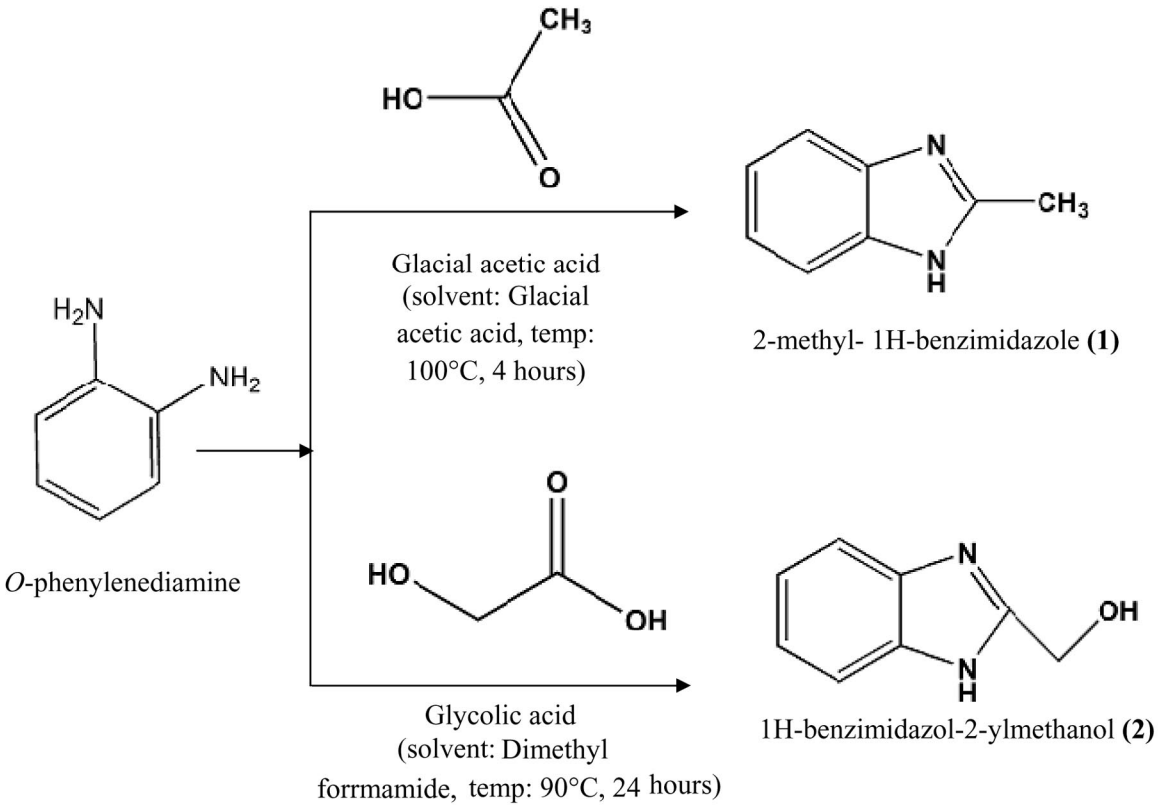
Table 2. Determination of IC_{50} value of standard and synthesized compounds.

Serial	Concentration (µg/ml)	ter-butyl-1-hydroxytoluene (BHT)		1		2	
		% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)	% inhibition	IC_{50} (µg/ml)
1	500	94.05	51.56	71.97	144.84	51.32	400.42
2	250	90.79		56.73		45.79	
3	125	83.42		49.78		42.89	
4	62.5	94.47		43.72		37.37	
5	31.25	93.95		43.27		35.53	
6	15.625	43.95		42.15		32.63	
7	7.813	22.89		41.26		31.32	
8	3.9	17.63		40.58		31.32	
9	1.953	14.74		40.36		27.1	
10	0.977	13.16		42.6		23.68	



O-phenylenediamine Carboxylic acid Carboxylic acid derivative of benzimidazole

Figure 1. General synthetic scheme for synthesis of substituted benzimidazole derivatives.



Scheme 1. Synthesis of benmidazole derivatives from *O*-phenylenediamine.

Table 3. Effect of standard and synthesized compounds on shrimp nauplii.

Serial no	Conc (µg/ml)	Vincristine sulphate		Conc (µg/ml)	1		2	
		% mortality	LC_{50} (µg/ml)		% mortality	LC_{50} (µg/ml)	% mortality	LC_{50} (µg/ml)
1	40	100	0.544	400	100	0.42	40	72.93
2	20	100		200	100		30	
3	10	100		100	100		20	
4	5	90		50	100		0	
5	2.5	80		25	100		0	
6	1.25	60		12.5	90		0	
7	0.625	50		6.25	80		0	
8	0.3125	40		3.125	70		0	
9	0.156	30		1.56	60		0	
10	0.078	20		0.781	40		0	

Antimicrobial activity. In the antimicrobial screening by disc diffusion method, compound **2** did not show any antimicrobial activity while standard ciprofloxacin showed a zone of inhibition of 41-45 mm. On the other hand, compound **1** showed very weak antimicrobial activity with the values of zone of inhibition ranging from 7-8 mm. Compound **1** was appeared as potent in terms of zone of inhibition and spectrum of activity when compared to those of compound **2**.

CONCLUSION

In conclusion, two benzimidazole derivatives were synthesized by condensation of *O*-phenylenediamine with two carboxylic acids: glacial acetic acid and glycolic acid. Both the synthesized derivatives were evaluated for their antioxidant, brine shrimp lethality bioassay, antimicrobial activity. Among the two derivatives, 2-methyl-1H-benzimidazole showed prominent antioxidant and cytotoxic activities. Further investigations and structural modifications of these derivatives might be helpful to generate more potent benzimidazole derivatives.

ACKNOWLEDGEMENTS

We greatly acknowledge the financial support (Grants no.39.009.002.01.00.020.2010/EAS-03/ 1673) from the Ministry of Science, Information and Communication Technology, Government of the People's Republic of Bangladesh.

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