International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 9, Issue 1, 2017

Short Communication

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF 2-SUBSTITUTED-5-NITRO BENZIMIDAZOLE DERIVATIVES

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Received: 31 Aug 2016 Revised and Accepted: 05 Nov 2016

ABSTRACT

Objective: This study was conducted to synthesise some 2-substituted-5-nitro benzimidazole derivatives to evaluate their antioxidant activity.

Methods: The titled compounds were synthesised by sodium metabisulfite mediated reaction of 4-nitro-1, 2-phenylenediamine with a number of *para*-substituted benzaldehydes. The antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.

Results: All the compounds exhibited good antioxidant activity having IC_{50} values in the range of 3.17 to 7.59 µg/ml while that of standard butylated hydroxytoluene (BHT) was 18.42 µg/ml.

Conclusion: Among the synthesised compounds, compounds 3a-c have been found to possess the most prominent antioxidant activity which might be attributed due to the presence of chloro, bromo and fluoro substituents in the molecule. The compounds may act as the future lead(s) for the development of potential antioxidant compounds.

Keywords: Benzimidazole, Synthesis, Antioxidant, DPPH

DOI: http://dx.doi.org/10.22159/ijpps.2017v9i1.14972

Benzimidazole derivatives comprise a promising classes of heterocyclic compounds that exhibit a range of biological activities such as antimicrobial [1, 2], antiprotozoal [3], anthelmintic [4], antiproliferative [5], anti-HIV [6], anticonvulsant [7], anti-inflammatory [8], antineoplastic [9] and antiulcer [10] activity. A Recent report shows that two groups of substituted benzimidazoles, namely 5, 6-dinitro and 2-trifluoromethylderivatives are the promising candidates for antimicrobial drugs [11, 12]. Besides 2-mercapto-benzimdiazole compounds have been reported to have significant antimicrobial properties [13].

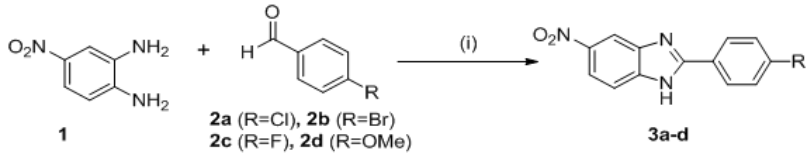
Therefore benzimidazole has drawn considerable interest as an important scaffold in drug discovery [14]. However, many of the activities of benzimidazole derivatives have not been extensively investigated like antioxidant activity. But the compounds having antioxidant and free radical scavenging properties are considered to be used for the prevention or treatment of human diseases like neurodegenerative disorders, atherosclerosis, rheumatoid arthritis and carcinogenesis, because oxygen-derived free radicals such as superoxide (O_2.-), nitric oxide (NO.), hydroxyl (OH.) and peroxy (ROO.) play an important role in causing these diseases [15]. N-substituted benzimidazole derivatives and 2-aryl substituted benzimidazole and benzothiazole derivatives have recently been reported to show significant antioxidant activity [16, 17]. Taking in mind all these recent observations regarding biological activities of benzimidazole derivatives we became interested in synthesising 2-substituted benzimidazole derivatives in order to investigate their anti-oxidant activity. In the present study, we had studied the antioxidant activity of 2-substituted-5-nitro benzimidazole derivatives 3 (scheme 1) and we herein report the results of synthesis as well as the anti-oxidant potential of these derivatives.

The major reagents for synthesis were purchased from Sigma-Aldrich Chemical Corporation and were used after being purified by standard procedures. The other chemicals used were of reagent/analytical grade. Melting points were determined by open capillary method with the help of WRS-1B (Germany) digital melting point apparatus and are uncorrected. All the reactions were monitored by TLC on silica gel thin layer plates. UV data were taken using Shimadzu UV-166V spectrophotometer. IR spectra were recorded by using the KBr disk on Shimadzu IR-470 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker 400 Ultra Shield instrument using deutero-DMSO (*d*-DMSO) as solvent and tetramethylsilane (TMS) as internal reference standard. All chemical shift (δ) values are expressed in ppm. The purities of the compounds were checked by thin layer chromatography (TLC) on silica gel-G plates.

Numerous methods are available for the synthesis of benzimidazole derivatives [14, 16, 17]. A simple method was applied for the synthesis of our target compounds. To a solution of 4-nitro-1, 2-phenylenediamine 1 (1.0 equivalent) and the corresponding aldehydes 2a-d (1.0 equivalent) in absolute ethanol (10 ml), sodium metabisulfite (4.0 equivalent) was added and the resulting mixture was heated to reflux (80-85 °C) for 4 h. After completion of the reaction observed by TLC, the reaction mixture was cooled to room temperature. Ethyl acetate (about 100 ml) was added, and the solid obtained was filtered off.

The crude product was attempted to recrystallize using dichloromethane-methanol, but it was failed. Then the crude product was purified by suspending in a mixture of hexane-ethyl acetate (1:1 ratio) several times, and the solid was collected by filtration and dried in the desiccator. The compounds were obtained in moderate to good yields (48-72%) which were characterized by various physicochemical parameters (TLC, melting point, solubility) and also by spectroscopic methods (UV, IR and NMR) for their structure elucidation. The purity of the synthesised compounds was confirmed by TLC and column chromatography.

Scheme 1: It shows the synthesis of 5-nitro-2-substituted benzimidazole derivatives 3a-d



Entry	R	Product	Yield (%)
1	Cl	3a	48.93
2	Br	3b	71.18
3	F	3c	54.88
4	OMe	3d	61.96

Reagents and conditions: (i) $Na_2S_2O_5$, absolute ethanol, reflux 4 h

After characterization, the synthesised compounds was evaluated *in vitro* for their antioxidant potential by free radical scavenging activity using DPPH (2, 2-diphenyl-1-picryl hydroxyl) reduction method [18, 19]. Four mg of DPPH was dissolved in 4 ml of methanol and from this stock solution, solutions of different concentrations i.e. 0.997 µg/ml, 1.953µg/ml, 3.906 µg/ml, 7.813 µg/ml, 15.625 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125µg/ml, 250µg/ml, and 500 µg/ml were prepared by serial dilution. The absorbance was recorded for these dilutions at 517 nm.

After that, 2 mg of BHT was dissolved in methanol to get a mother solution having a concentration 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 methanolic solution of the test compounds was mixed with 3.0 ml of DPPH solution (20 µg/ml). The mixture was then shaken vigorously and allowed to stand at room temperature in dark place for 30 minutes and the absorbance was measured at 517 nm against methanol as blank by UV-Spectrophotometer. Finally, BHT was used as positive control and the whole experiment was done in triplicate [20].The free radical scavenging was expressed as the percentage inhibition and was calculated using the formula:

$$\text{Percent Inhibition} = (1 - \frac{A_{sample}}{A_{blank}}) \times 100$$

Where: A_{blank} = Absorbance of control (containing all reagents except the test material).

A_{sample} = Absorbance of test or standard.

The percent inhibition was plotted against the sample of the standard concentration to obtain the amount of antioxidants necessary to decrease the initial concentration of DPPH to 50% (IC_{50}). IC_{50} values were calculated from the calibration curve. IC_{50} value is defined as the concentration of test compound required to achieve half-maximal inhibition, and lower IC_{50} value indicates greater antioxidant activity.

The results of antioxidant activity of the compounds 3a-d 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.

Table 1: It shows the results of free radical scavenging activity of 5-nitro-2-substituted benzimidazoles 3a-d and BHT

Code	Percent inhibition of DPPH free radical at different concentrations*										IC_{50} value µg/ml
	0.997 µg/ml	1.953 µg/ml	3.90µg/ml	7.81 µg/ml	15.62µg/ml	31.25 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	
3a	47.63	48.22	49.70	50	51.18	51.78	52.36	53.85	59.76	62.72	6.21
3b	32.25	38.46	41.72	50.88	58.28	65.38	67.75	70.71	86.98	93.19	7.41
3c	39.64	47.04	50.59	56.21	61.83	65.98	80.77	82.84	87.28	92.89	3.17
3d	35.79	41.42	46.15	50.59	54.14	57.39	60.65	65.38	72.78	81.95	7.59
BHT	14.29	29.59	33.43	39.05	48.23	52.96	60.06	71.30	79.88	91.72	18.42

*The percent inhibition values have been calculated by plotting the mean absorbance values against different concentrations mentioned. The regression coefficient (R^2) values for the compounds 3a-d are 0.8388, 0.9651, 0.9785, and 0.9768 respectively and of standard BHT is 0.9585.

A plethora of reports are available which reflects the diverse range of biological activities of benzimidazoles derivatives [1-10]. Of particular interest, we have chosen 5-nitro-2-substituted benzimidazoles derivatives as our target compounds because of the recent observations of biological activities of these derivatives especially antimicrobial activities [1]. But antioxidant activities of these types of derivatives have not been studied much. From the results of our study, all the compounds are found to be more active than the standard. A possible explanation for this result is that the biological activity of compounds may depend on the basic skeleton of the molecule as well as on the nature of substituents. But the compound **3c** seemed to be the most active of all which is assumed to be due to the presence of fluorine atom in the molecule as the presence of fluorine atom in benzimidazole compounds, in general, confers significant biological activity [21, 22]. The results obtained were statistically evaluated by regression analysis and the values indicate that the compounds possess significant antioxidant activity.

It can be concluded that all the synthesised benzimidazoles derivatives exhibited significant antioxidant activity. But the activity of 2(-4-fluoro-phenyl)-5-nitro-1H-benzimidazole (3c) was more promising than others. The study related to the synthesis of newer antioxidant benzimidazoles derivatives is on progress. Although several synthetic antioxidant agents are available like BHT, there is still a scarcity of safe and effective antioxidant. Therefore the successful implementation of the synthesis could help to produce molecules which might be potential leads for the development of drug molecules having antioxidant activity.

ACKNOWLEDGEMENT

We gratefully acknowledge the financial contribution of Higher Education Quality Enhancement Project (HEQEP), Window 2, Round 3, CP 3245, University Grants Commission of Bangladesh.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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How to cite this article

- Sabrina Rahman Archie, Biplab Kumar Das, MD Shahadat Hossain, Uttom Kumar, Abu Shara Shamsur Rouf. Synthesis and antioxidant activity of 2-substituted-5-nitro benzimidazole derivatives. Int J Pharm Pharm Sci 2017;9(1):308-310.