

Benzimidazole Derivatives: Active Class of Antioxidants

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Abstract:

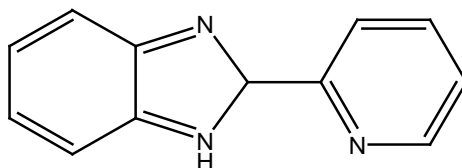
In this study, we prepared some new benzimidazole derivatives and investigated their antioxidant properties by determination of DPPH Radical scavenging, Superoxide Scavenging, Iron Chelating assays methods. Benzimidazoles are an important class of compounds with a wide spectrum of biological activity. The five membered heterocyclic moieties also confer for various biological activities. Hence a series of benzimidazole derivatives have been synthesized characterized by UV, IR and ¹H NMR spectral data and evaluated for their *in vitro* and antioxidant activity. The synthesized benzimidazole derivatives showed remarkable antioxidant activity Furthermore their effects on various antioxidant enzymes are discussed and evaluated from the perspective of structure- activity relationships.

Introduction

Benzimidazoles and its derivatives represent one of the most biologically active class of compounds, possessing a wide spectrum of activities and these are well-documented in literature because it is one of the components of naturally occurring compounds such as vitamin B₁₂. Benzimidazole compounds had proven quality exhibiting a broad range of pharmacological actions, including analgesic [1], anti-inflammatory [2], anticonvulsant [3], anticancer [4], antihypertensive [5], antimicrobial [6-9] anthelmintic [10], tranquilizing [11] immuno-suppression, antitumor and antiviral inhibition activities [12].

In the similar context, our group is engaged in the efforts to discover the lead molecule working on different heterocyclic compounds as heterocyclic compounds play pivotal role in the metabolism of all living cells and have many pharmacological applications [13-14]. Biological importance of five membered-rings fused with benzene moiety in the heterocyclic family got much importance due to their wide usage in different pharmacological disciplines [15-18].

Keeping this view in mind, 2-(2'-pyridyl)-benzimidazole has been selected as lead molecule from the heterocyclic family for the preparation of derivatives having biological potentials and their screening to find the probability of commercial exploitation.



2-(2'-pyridyl)-benzimidazole

Antioxidant Activity

Antioxidants are nutrients that help to protect cells from a normal but damaging physiological process known as "oxidative stress". Such nutrients are a part of the natural makeup of many types of food, particularly fruits and vegetables. They also have been added to some foods and are available in the form of dietary supplements. There has been growing demand for antioxidants due to report their effective defensive role against oxygen free radical toxicity in our body system. It has been determined that active oxygen molecules such as superoxide, hydroxyl and peroxy radicals play an important role in oxidative stress related to the pathogenesis of different diseases such as Alzheimer, Parkinson and cataracts, and DNA damage leads to carcinogenesis [19]. The application of antioxidant is widespread in the industry as well and they are in use in preventing polymers from oxidative degradation, synthetic and natural pigments from discoloration and as additives in cosmetics. The natural antioxidants are flavonoids, phenolic acids and nitrogen compounds such as alkaloids [20].

Reactive oxygen species (ROS) are generated when oxygen is supplied in excess and its reduction is insufficient. The best explored ROS are superoxide anion radical ($O_2^{\cdot-}$) its protonated form, the perhydroxyl radical, hydrogen peroxide and hydroxyl radical (HO) [21-22]. ROS are harmful for cells and are the cause of many disease, *e.g.*, ischemic/reperfusion "States", inflammatory disease, cancer, drug toxicity, degenerative processes associated with aging and neurodegenerative diseases such as Parkinson's disease, Alzheimer dementia and multiple sclerosis [23-26], cataracts, and DNA damage leads to carcinogenesis [27]. Several factors influence the efficiency of an antioxidant and require consideration of its bioavailability, the site of action, its stability, toxicity and the type of reactive oxygen species.

A variety of synthetic antioxidants are available, as well as natural ones, including vitamins C and E, and the bioflavonoids [28]. Derived from a wide range of sources, antioxidants play a major role in “preserving” food quality. Although what an antioxidant does is not exactly new, the ways their benefits have been promoted and subsequently reported on have certainly change over the years, moving from an emphasis on their functionality to one that includes health and the potential prevention of certain diseases [29].

Hence an attempt has been made to synthesize some novel compounds of benzimidazoles containing five membered-rings fused with benzene moiety in the heterocyclic family moiety and evaluate for their in vitro antioxidant activity.

MATERIALS AND METHODS

All the solvents were of analytical grade from E. Merck and were purified by distillation prior to use. Thin layer chromatography was monitored using pre-coated silica gel, GF-254. Spots were visualized under ultraviolet light at (254 nm and 366 nm) using HP-UV/Visible lamp (Dessaga Heidelberg). Melting points were determined on Gallen Kamp melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded in KBr (Pellet form) on a nicolet Avatar 330 fourier transform FTIR spectrophotometer and noteworthy absorption values (cm^{-1}) alone were listed. Mass spectra were determined under electron impact (EI) condition using varian Massen spectrometer MAT 312, MAT 113D MASPEC system. Proton NMR measurements were performed on a Bruker SF 300 and 400 MHz spectrometer. Samples were dissolved in CD_3OD and DMSO-d_6 with (TMS) using tetra methyl silane as internal reference. Chemical shifts were reported in δ (ppm). Selected data were reported as multiplicities and characterized by “s” (singlet), “d” (doublet), “dd” (double doublet), “t” (triplet), “m” (multiplet), “q” (quartet) and coupling constant, (j values) were reported in hertz

Experimental Procedure for the Synthesis of Benzimidazoles

2-(2'-pyridyl) benzimidazole (**Ia**) and corresponding substituted phenacyl halides in equimolar quantities (0.01mole) were dissolved in 15-20 mL acetone separately in conical flask and mixed together in a round bottom flask. The reaction mixture was stirred on magnetic stirrer for four hrs and then refluxed on water bath for about 5 to 6 hrs. Precipitates appeared either on mixing the reactants at once or after some hours on refluxing. Completion of reaction was monitored by TLC with solvent system CHCl_3 : MeOH (in varying proportions). As soon as the reaction completed, it was worked up. TLC plates were visualized under ultraviolet light at 254 nm and

366 nm for fluorescence quenching and fluorescent spot respectively on HP-UV/Visible Dessaga (Heidelberg). Iodine vapors were also employed somewhere for the detection of spots. The resulting precipitates of products were filtered and washed with warm acetone to remove the unreacted starting material. The precipitates of each product were re-crystallized at least three times to ensure purity and to improve color and shape of crystals. The pure compounds were dried in a vacuum desiccator over anhydrous calcium sulphate.

Experimental

Antioxidant activity was performed by the following methods:

1-Superoxide Scavenging Assay

Compounds of synthetic source can be assessed by the method used by Gaulejac, *et al* [30]. In aerobic reaction mixtures containing NADH (β -Nicotinamide adenine dinucleotide reduced, disodium salt trihydrate), Phenazine metho sulphate and Nitroblue tetrazolium, PMS (Phenazine Metho Sulphate) was reduced by NADH and then gave raise to O_2 , which in turn, reduced NBT (Nitro Blue tetrazolium Salt). On this basis, PMS has frequently been used to mediate O_2 . The reaction mixture comprised 40 μ L of 280 μ M β -nicotinamide adenine dinucleotide reduced from (NADH), 40 μ L of 80 μ M nitro blue tetrazolium (NBT), 20 μ L, 8 μ M phenazine methosulphate (PMS) of 10 μ L of 1 mM sample and 90 μ L of 0.1 M Phosphate buffer (pH 7.4). The reagents were prepared in buffer and sample in DMSO. The reaction was performed in 96-well microtitre plate at room temperature and absorbance was measured at 560 nm. The formation of superoxide was monitored by measuring the formation of water soluble blue formazan dye. A lower absorbance of reaction mixture indicated a higher scavenging activity of sample. Percent Radical scavenging activity (% RSA) by samples could be determined in comparison with a control in the following way:-

$$\% \text{ RSA} = 100 - [(\text{OD test compound} / \text{OD control}) \times 100]$$

2- DPPH Free Radical Scavenging Assay

Materials and Method

Test samples were allowed to react with stable free radical, 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) for half an hour at 37° C. The concentration of DPPH was kept as 300 μ M. The

test samples were dissolved in DMSO while the DPPH solution was prepared in ethanol [31-32]. After incubation, decrease in absorption was measured at 515 nm using multiplate reader (Spectra MAX-340). Percent radical scavenging activity of samples was determined in comparison with a DMSO treated control group by using the following formula:

$$\% \text{ RSA} = 100 - \{(\text{OD test compound} / \text{OD control}) \times 100\}$$

Where, RSA is radical scavenging activity, OD is the optical density

3-Iron chelating Assay

Materials and Method

i Ferrozine ii Ferrous Sulphate iii Dimethyl Sulfoxide (DMSO) iv Micro Plate reader

The Fe^{+2} chelating ability of diterpenoids were determined according to the modified method of Kexue, *et al* [33]. In the following way:

- The Fe^{+2} were monitored by measuring the formation of ferrous ion-ferrozine complex.
- The pure compound (0.5 mM) was mixed with 0.0625 mM FeSO_4 and 0.5mM ferrozine then mixture was shaken and left at room temperature for 10 min.
- The absorbance of resulting mixture was measured at 526 nm.
- A lower absorbance of reaction mixture indicated a higher Fe^{+2} chelating ability

Table 1: Physical data of the synthesized compounds

Compound Code	Physical State	Melting Point	Molecular Formula	% Yield	Solubility	Formula Wt
Ia	Light yellow powder	218 - 220°C	C ₁₂ H ₉ N ₃	-	Acetone, DMSO, methanol	195.22
1	Light cream color powder	250°C	C ₂₀ H ₁₄ N ₄ O ₃	52	Ethanol, DMSO, methanol	358
2	Grey powder	296°C decompose	C ₂₀ H ₁₄ N ₄ O ₃	30	20% DMSO, methanol	346
3	Brown crystal	276 - 280°C decompose	C ₂₀ H ₁₄ N ₄ O ₃	52	Ethanol, DMSO, methanol	348
4	Off white powder	210 - 212°C	C ₂₀ H ₁₄ N ₄ O ₃	54	Ethanol, DMSO, methanol	358
5	Golden yellow powder	245 - 250°C	C ₂₀ H ₁₄ N ₄ O ₃	50	DMSO	358
6	Off white shiny crystal	225 - 230°C	C ₂₀ H ₁₄ N ₃ OF	62	Ethanol, DMSO, methanol	331
7	Brown shine crystal	232 - 236°C	C ₂₀ H ₁₄ N ₃ OF ₂	30	Ethanol, DMSO, methanol	349
8	Light off white powder	205 - 210°C	C ₂₀ H ₁₅ N ₃ O	17	Ethanol, methanol	313
9	Dark off white	240 - 243°C	C ₂₆ H ₁₉ N ₃ O	50	Ethanol, methanol	389
10	Light grey powder	228 - 234°C	C ₂₁ H ₁₇ N ₃ O ₂	56	Ethanol, DMSO, methanol	343
11	Off white powder	202 - 205°C	C ₂₂ H ₁₉ N ₃ O ₂	22	Ethanol, methanol	373

Table 2

***In vitro* Antioxidant activities of compound 2-(2'-pyridyl) benzimidazole (1a) and its derivatives (1-11)**

Compound No.	DPPH Radical Scavenging Assay (<i>n</i> -Propyl gallate)	Superoxide Anion Assay (EDTA)	Iron chelating Assay (Ascorbic Acid)
	% R.S.A	% R.S.A	% R.S.A
Ia	N.D	N.D	0.892
1	54.14	N.D	52.33
2	N.D	35.6	95.29
3	N.D	7.4	34.44
4	61.01	N.D	59.44
5	66.46	N.D	60.63
6	58.74	N.D	31.74
7	N.T	N.T	N.T
8	N.D	N.D	21.15
9	45.33	N.D	34.98
10	N.D	3.31	26.44
11	N.D	1.61	20.95
Std. drug	90.13	91.00	98.00

Activity Key:

N.D = Not detected

NT = Not tested

Results and Discussion

All the newly synthesized compounds (1-11) were screened for their antioxidative potential along with the parent molecule (**1a**) employing the superoxide, iron chelating, and DPPH scavenging assay methods and the results were presented in **Table-2**

From the table, it was clearly shown that the parent molecule (**1a**) did not possess anti-oxidative activity while its derivatives expressed significant to moderate results. It was also observed that the % RSA was method dependent at the test concentration exhibiting variable results. Most of the derivatives were found to possess antioxidant activity by the Iron chelating assay method while the same compounds remained inactive by the other two aforementioned methods.

Compound 1-[2-(3',4'-dihydroxyphenyl)-2-oxo-ethyl]-2-(2'-pyridinyl)-1*H*-benzimidazol-1-ium chloride (**2**) displayed highly significant result (%RSA 95.29) by the Iron Chelating assay method as compared to the standard drug, Ascorbic acid (%RSA 98) while the same compound did not show remarkable activity by the other two methods.

Significant antioxidant results were given by the compounds 1-[2-(2'-nitrophenyl)-2-oxo-ethyl]-2-(2'-pyridinyl)-1*H*-benzimidazol-1-ium bromide (**4**), 1-[2-(4'-nitrophenyl)-2-oxo-ethyl]-2-(2'-pyridinyl)-1*H*-benzimidazol-1-ium bromide (**5**) and 1-[2-(4'-fluorophenyl)-2-oxoethyl]-2-(2'-pyridinyl)-1*H*-benzimidazol-1-ium bromide (**6**) through DPPH and iron chelating assay methods while remaining derivatives showed low level of % RSA.

During SAR, it was observed that the compounds having nitro groups at different positions in the phenyl ring possessed comparable activities in the significant range. It meant, nitro group imparted the radical scavenging ability to the parent molecule.

Among the halogenated derivatives (compounds 3 and 6), the fluoro group produced significant % RSA i.e., 58.74 as compared to the chloro group (%RSA 34.44).

In the methoxy derivatives it was found that the compound containing one methoxy group (compound 10) expressed low level of antioxidant activity (%RSA 26.44) which became even

more less by the introduction of one more methoxy group (%RSA 20.95). Similarly, the low levels of radical scavenging activity given by compounds 8 and 9 were also comparable. Therefore, it can be inferred that the introduction of phenyl, methoxy and/or halogens in the phenyl moiety did not play any important role in the inhibition of free radicals.

Highly significant antioxidant result (% RSA 95.29) was expressed by the derivative containing two hydroxyl groups at *ortho* and *para* positions in the phenyl ring (compound 2). It was concluded that phenolic group might be responsible for such activity. It was already investigated that flavonoids and phenolic acids were the major complementary compounds of propolis that had beneficial effects as natural antioxidants and prevent oxidative damage of DNA caused by reactive oxygen species [34]. Moreover, the antioxidant activity might be due to the combined effect radical scavenging and interaction with enzyme functions [35].

Conclusion:

It was concluded that the antioxidant activity might be due to the phenolic group and the results obtained would be method dependent as most of the derivatives demonstrated the positive results by iron chelating assay method.

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