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Evaluation of antioxidant potential of Some N-1-Substituted Imidazole Derivatives

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ABSTRACT

A series of N-1-Substituted imidazole derivatives (1a-1d, 2a-2d) has been synthesized and evaluated their antioxidant efficacy by *in- vitro*. The compounds were confirmed by FTIR, ^{1}HNMR, MASS and Elemental spectral data. The in-vitro antioxidant methods viz., DPPH radical scavenging activity and scavenging of hydrogen peroxide was used for this study at different concentrations of 05-30 µg/mL. The results (IC_{50} values) were compared with the standard antioxidants Butylatedhydroxyanisole (BHA), Ascorbic acid and α-Tocopherol. The test compounds 1b, 1c, 2b and 2d had shown significant ($P<0.05$) action and compound, 2c showed its higher significant ($P<0.01$) action with that of standard anti oxidants used in this assay.

KEY WORDS: N-1-Substituted Imidazoles, Antioxidant efficacy, BHA, Ascorbic acid and α-Tocopherol.

INTRODUCTION:

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in aerobic organisms as part of the normal physiological and metabolic processes. They are very important mediators of cell injury or death due to the damages they can inflict if they are produced in excessive concentrations or in wrong locations. The damages that ROS/RNS cause, essentially on biological macromolecules (membrane lipids, proteins, nucleic acids and etc), are directly or indirectly implicated in the pathogenesis of various disorders such as cardiovascular diseases, reperfusion injury, Alzheimer's and other neurodegenerative diseases, cancer development and progression, inflammation as well as in the aging process.[1–3] Therefore the interest for the protective role of antioxidants in medicine has been growing over the last 15 years. Antioxidants are considered as potential drugs due to their ability to reduce or inhibit the free radical reactions initiated by ROS/RNS.[4] Imidazole nucleus has proved to be a prolific source for a number of medicinal agents.[5]

The various activities associated with the imidazole nucleus are antiprotozoal, mutagenic properties, anticancer, antiviral, enzyme inhibitory activities, H_2-Antagonism, α- Adrenergic agonist and β-blocking, anticonvulsant, broad spectrum antibacterial and antifungal activities.[6-16] Therefore an attempt has been made to evaluate the antioxidant potential of some newly synthesized N-1-Substituted Imidazoles (1a-1d & 2a-2d).

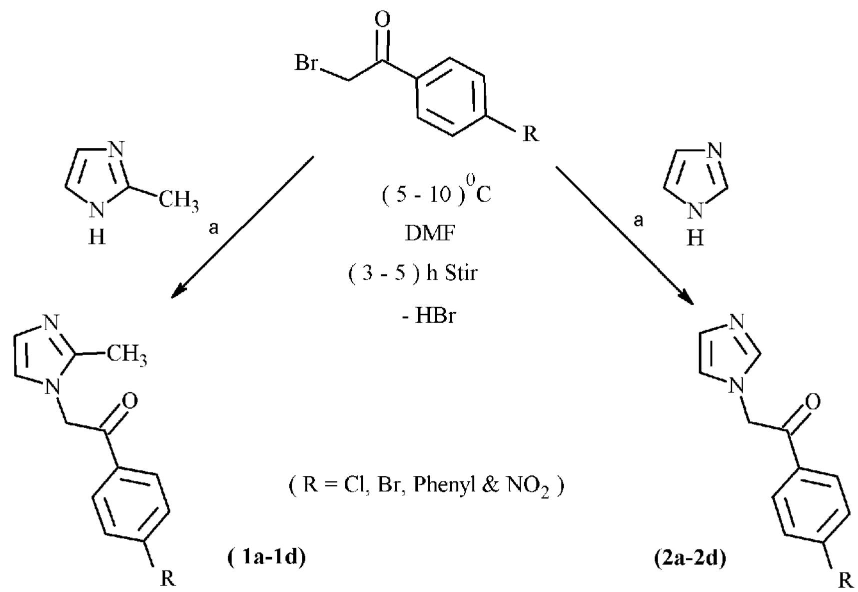
MATERIALS AND METHODS:

Butylatedhydroxyanisole (BHA), l-ascorbic acid, α-tocopherol (Toc), 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) were obtained from Sigma Aldrich, India. All other chemicals used were analytical grade and obtained from Merck, India.

The compounds were dissolved in ethanol to make a test solution of 05, 10, 15, 20, 25 and 30µg/mL. Standard solutions of 05, 10, 15, 20, 25 and 30 µg/mL of BHA, Toc and l-ascorbic acid were prepared.

General procedure for Synthesis of 1-substituted imidazoles (1a-1d & 2a-2d)

To a solution of Imidazole/2-methyl imidazole (0.03mol, 2.46 g) in dry DMF (10 ml) was added dropwise to a solution of appropriate para substituted phenacyl bromides (0.002 mol, 0.46 g) in DMF (10 ml) at a temperature of 5-10 ˚C with stirring. The stirring was continued for another 3-6 h at the same temperature. Then the mixture was poured into cold water (20ml) and stirred for further 1 h. The precipitate obtained was removed by filteration and the filterate was extracted with benzene. Upon evaporation of organic layer compounds 1a-1d & 2a-2d were obtained as crystalline mass and are recrystallised from benzene-ethanol. The purity of all compounds was established by single spot on the TLC plates.[17]



Scheme 1. Reagents: a) 2-methyl imidazole, Imidazole, P-substituted phenazyl bromides.

***In vitro* antioxidant activity:**

DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) free radical scavenging activity:

The free radical scavenging activity of the all the prototype (1a-1d, 2a-2d) was measured by DPPH˙ (1, 1-diphenyl-2-picryl-hydrazil) using the method described by Shimada et al.[18] A series of individual prototypes concentration in methanol was prepared (05, 10, 15, 20, 25 and 30 µg/ml). Then, 3 ml of prototypes from different concentrations was mixed with 1000 µl of 0.004 % DPPH in methanol. The disappearance of DPPH was read spectrophotometrically at 517 nm by UV-Visible spectrophotometer after 30 min of incubation at room temperature in the dark environment. A purple to yellow colour change is observed.

The same solvent was used as a control instead of prototypes. The same procedure was repeated with methanolic solutions of standard antioxidants butylatedhydroxyanisole (BHA) and l-Ascorbic acid as positive control. Methanol was used as blank. The measurements were performed in triplicate and the results were averaged.

$$\text{DPPH}^{\cdot}\ \text{scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard sample or individual compound (1a-1d, 2a-2d). The control contained DPPH$^{\cdot}$ in ethanol. From the obtained values, the IC_{50} (defined as the concentration of compounds at which 50% of maximum scavenging activity was recorded) was calculated for each compound.

Scavenging of Hydrogen Peroxide:

The potential of compounds (1a-1d, 2a-2d) to scavenge hydrogen peroxide was determined according to the method of Ruch et al.[19] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) and its concentration was determined spectrophotometrically at 230 nm. 1 mL of Individual prototypes (05 - 25 µg/mL) was added to the hydrogen peroxide solution (0.6 mL, 40 mM) and the absorbance at 230 nm was determined after 19 min against a blank solution in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of compounds (1a-1d, 2a-2d) and standards was calculated using the following equation:

$$\%\ H_2O_2\ \text{scavenged} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of prototypes (1a-1d, 2a-2d) or standards.

Statistical Analysis:

Experimental results were expressed as mean ± S.D of three parallel measurements. Analysis of variance was performed by ANOVA followed by Newmans-Keul multiple comparison test. P values less than 0.05, 0.01 and 0.001 were regarded.

Results and discussion:

All the newly synthesized compounds (1a-1d, 2a-2d) were screened for their *in vitro* antioxidant activity by DPPH radical scavenging activity and scavenging of hydrogen peroxide. The compounds were tested at various concentrations of 5, 10, 15, 20, 25 and 30 µg/mL and the IC_{50} values had been determined for each compound and compared with control as well as standard antioxidants. Butylatedhydroxyanisole (BHA), l- ascorbic acid (AA) and α-tocopherol (Toc) was used as the standard antioxidants.

In DPPH radical scavenging activity assay, the purple chromogen radical 2, 2-diphenyl-1-picryl hydrazyl (DPPH) is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine.[20] The scavenging capacity is generally evaluated in organic media by monitoring the absorbance decrease at 515–528nm until the absorbance remains constant or by electron spin resonance.[20] DPPH radical is reduced by antioxidants and causing absorbance decrease at 515nm is the principle of measurement of this assay.

The test compounds 1c, 2c and 2d significantly reduced the absorbance caused by DPPH free radical with the IC_{50} values of 27.66, 21.61 and 26.53 (µg/mL) at concentration dependent manner against the control group. In which compounds 1b and 2b showed significant activity ($P<0.01$) and their IC_{50} values are (18.85 and 18.11 µg/mL) comparable with that of standard anti oxidants Ascorbic acid and Butylated hydroxyanisole (13.14 and 14.25 µg/mL, $P<0.001$) used in this assay.

In Scavenging of hydrogen peroxide assay**,** Absorbance decrease at UV region due to consumption of H_2O_2 is inhibited by antioxidants is the principle of measurement of this assay. As the H_2O_2 concentration is decreased by scavenger compounds or antioxidants, the absorbance value at 230nm is also decreased.

The test compounds 1b, 1c, 2b and 2d significantly ($P<0.05$) reduced the absorbance caused by H_2O_2 with the IC_{50} values of 20.97, 18.94, 19.22 and 23.83 (µg/mL) when compared to the control group at various concentrations used from 05 - 25 µg/mL. The lone compound, 2c showed significant activity ($P<0.01$) and its IC_{50} value (17.85 µg/mL) was comparable with that of standard anti oxidants Ascorbic acid and α-Tocopherol (12.64 and 13.58 µg/mL, $P<0.001$) used in this assay.

All the test compounds showed significant action while increasing the concentration and that was comparable to the standard antioxidants used in both assays Butylatedhydroxyanisole (BHA), l- ascorbic acid (AA) and α-tocopherol (Toc). However the test compounds 1a and 2a showed comparatively less significant activity than all the other compounds.

The observations reveals that 2-methyl imidazole derivatives (1a-1d) were found to possess moderately less activity than imidazole derivatives (2a-2d) and their results were not significant when compared to the standards. Introduction of p-bromophenacyl, p-phenylphenacyl and p-nitrophenacyl at first position of the imidazole nucleus has significantly improved potency and showed higher activity in all the *in vitro* antioxidant models used in this screening.compare to standards

Fig I. Effect of prototypes (1a-1d), standards Ascorbic acid and Butylated hydroxy anisole on DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical scavenging activity in % at different concentrations.

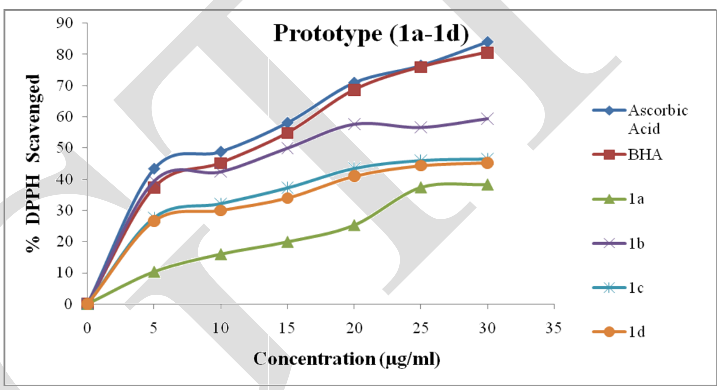


Fig II. Effect of prototypes (2a-2d), standards Ascorbic acid and Butylated hydroxy anisole on DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical scavenging activity in % at different concentrations

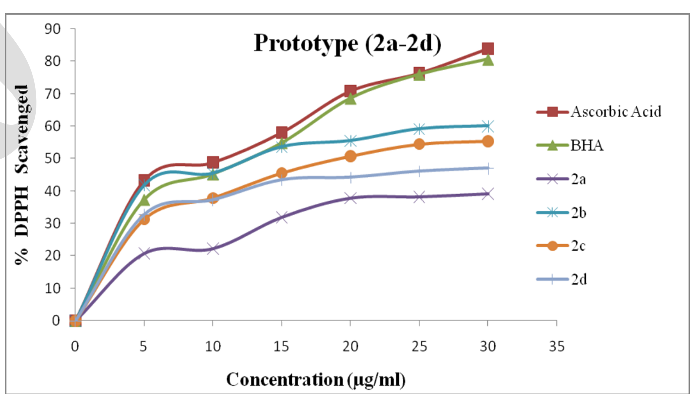
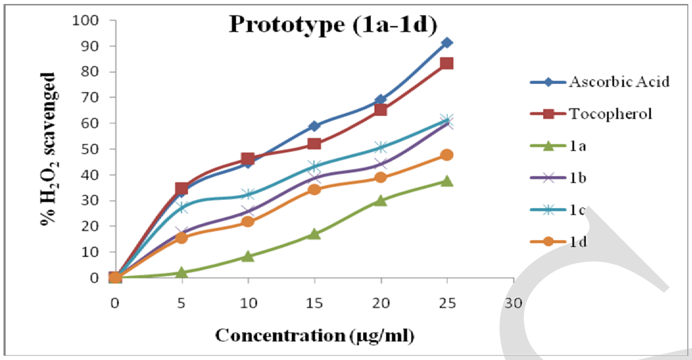


Fig III. Effect of prototypes (1a-1d), standards Ascorbic acid and α-Tocopherol on Scavenging of Hydrogen Peroxide in % at different concentrations



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