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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY FOR SOME BENZOTRIAZOLE SUBSTITUTED WITH N-PHENYLACETAMIDE AND ACETYLCARBAMIC ACID DERIVATIVES

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ABSTRACT

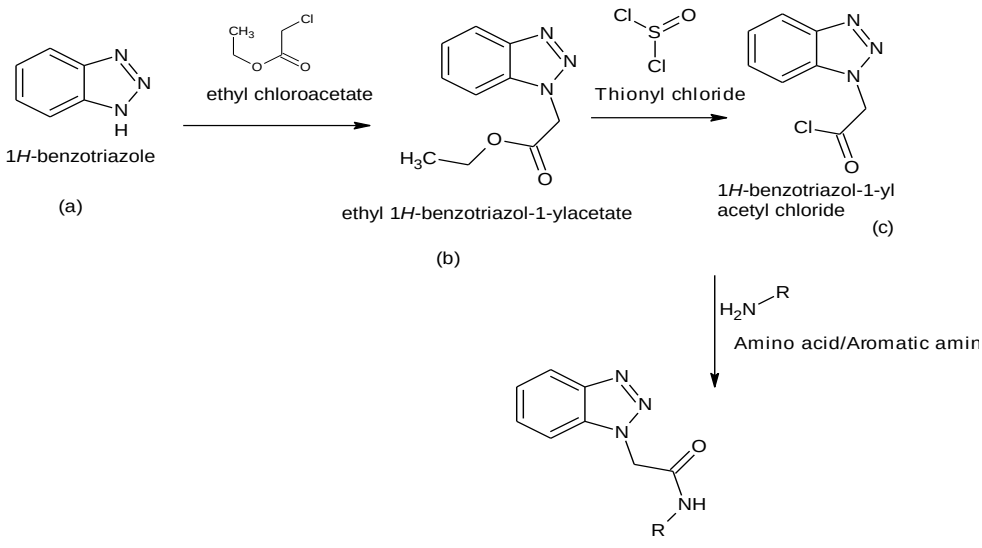
The objective of the present study was to evaluate antioxidant activity of a series of ten Benzotriazole Substituted with N-Phenylacetamide (Ia to Va), acetylcarbamic acid (Ib to Vb) Derivatives. The derivatives were synthesized with planned synthetic pathway and screened for antioxidant activity by adopting Griess reaction assay method. Sodium Nitroprusside was used to produce nitric oxide which oxidizes sulphonilamide present in Griess reagent to form diazonium salt and on further react with N-(naphthalene-1-yl)ethylenediamine (NED) to chromophore. The nitrogen scavenging activity of the derivatives was measured by absorption of chromophore formed with spectrophotometer at 548nm using ascorbic acid as standard. IIa, IIIa and IIb derivatives showed highest antioxidant activity and rest of the derivatives were having medium activity. The data obtained was expressed statistically.

Keywords: Antioxidants, Scavenging activity, Benzotriazole derivatives, Griess reagent.

INTRODUCTION

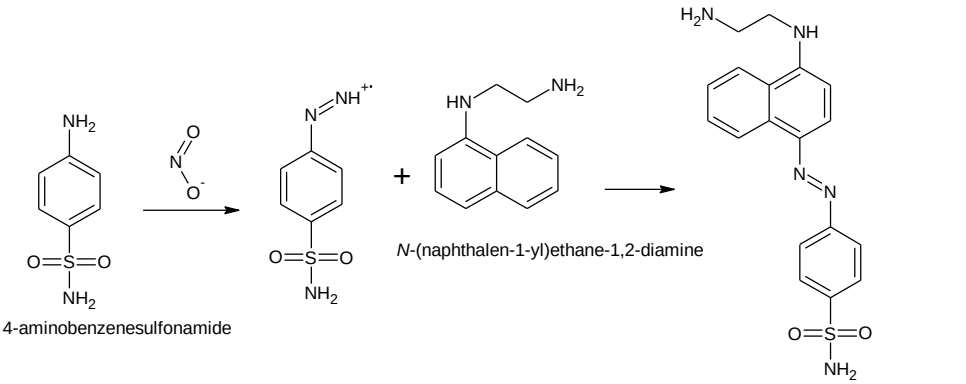
Antioxidants are the reducing agents which used to stabilize some free radicals which produced as result of cellular metabolism. Some of these free radicals or Reactive Oxygen Species (ROS) are destructive to cell and stabilization of these radicals is necessary for proper functioning or protection of the cell. The antioxidants can be promising prophylactic agents in pathogenesis [1]. Some food items, vegetables, and fruits act as antioxidants [2-6]. Antioxidant activity reported for imidazolylbenzamides[7], Aminothiozoles[8], stilbene derivatives[9], hetero-chitooligosaccharides[10], 1-acyl-4-cycloalkyl/arylsemicarbazides and 1-acyl-5-benzyloxy/ hydroxy carbamoylcarbazides[11] and herbal extracts [12-21]. Measurement of Nitric oxide in biological systems using Griess reaction assay was reported [22]. There are different in vivo and in vitro methods available to measure the scavenging activites[23]. The derivatives of Benzotriazole endowed with analgesic, antibacterial, antifungals activities [24-30]. Benzotriazole also reported for anticonvulsant and anti-inflammatory [31], antitumor [32] activities, and literature study reveals the antiviral activity[33]. The methods of synthesis of Benzotriazole derivatives with different techniques have been reported [34].

The Benzotriazole derivatives of N-Phenylacetamide (Ia to Va) and acetylcarbamic acid (Ib and Vb) were synthesized (Scheme 1) and antimicrobial activity was reported [35-39].



Scheme 1: Synthetic pathway for Benzotriazole derivatives (Ia to Va and Ib to Vb)

Present study was focused on evaluation of 2-(1*H*-1,2,3-benzotriazol-1-yl)-*N*-phenylacetamide and [(1*H*-benzotriazol-1-ylacetyl)amino]acetic acid derivatives (Table 1)for antioxidant activity following Radical activity of Nitric oxide or Griess reaction assay (Scheme 2). The nitroprussde used in the reaction produce nitric oxide at physiological pH which interacts with oxygen to produce nitrite ion. The synthesized derivatives contain carbonyl and amine functional groups which interact with nitric oxide resulting in reduced production of nitrite ion, a strong oxidizing agent. The remaining nitrite ion oxidizes the sulphonilamde to form diazonium salt which couples with N-(naphthalene-1-yl)ethylenediamine (NED) to form 4-[(E)-{4-[(2-aminoethyl)amino]naphthalene-11-yl}diazenyl]benzenesulfonamde a purple colored complex. Thus formed complex was measured spectrophotometrically at 548nm.



4-[(*E*)-{4-[(2-aminoethyl)amino]naphthalen-1-yl}diazenyl]benzenesulfonamide

Scheme 2: Reaction pathway of 4-[(*E*)-{4-[(2-aminoethyl) amino] naphthalen-1-yl}diazenyl]benzenesulfonamide complex formation with Griess reagent

MATERIALS AND METHODS

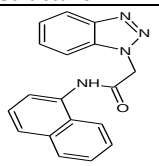
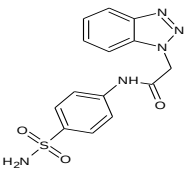
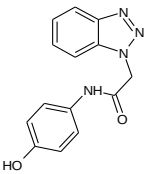
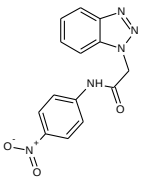
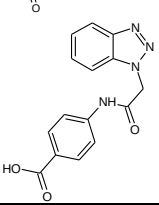
All the reagents used were analytical grade. DMSO was used as solvent, Griess Reagent Prepared with 0.2% naphthylenediamine dihydrochloride, and 2% sulphonilamide in 5% phosphoric acid. Phosphate buffer saline (PBS) and Sodium nitroprusside (10mM) were prepared. All absorbance was measured with Digital Spectrophotometer Model VSI- SP1. Absorption of radical scavenging activity were recorded at 548nm using DMSO as blank and Ascorbic acid as standard or control using formula given below.

$$\% \text{ Nitric Oxide Scavenging Activity} = \frac{A_{Control} - A_{Test}}{A_{Control}} \times 100$$

Procedure

Test solutions of Benzotriazole derivatives were prepared in range of 25µgml -100µg/ml in DMSO. 1ml of test solution of each concentration transferred to 10ml volumetric flasks. 5ml of Phosphate Buffer Salt (PBS) solution was added and 2ml of Sodium Nitroprusside (SNP) solution was added. Similarly Standard Ascorbic acid was treated with PBS and SNP in volumetric flask. All the mixtures were incubated at 25°C for 150 minutes. The Greiss reagent of 3ml was added to all the solutions. Chromophore developed was measured spectrophotometrically. For each concentration minimum three observations were recorded and mean of the observations were used for the calculation.

Table 1: Chemistry of the Benzotriazole derivatives (Ia to Va and Ib to Vb)

Derivative	Mol. Formula	Derivative Name	Structure	Mol. Weight
Ia	$C_{18}H_{14}N_4O$	2-(1*H*-benzotriazol-1-yl)-*N*-(naphthalen-1-yl)acetamide		302.32
IIa	$C_{14}H_{13}N_5O_3S$	2-(1*H*-benzotriazol-1-yl)-*N*-(4-sulfamoylphenyl)acetamide		334.34
IIIa	$C_{14}H_{12}N_4O$	2-(1*H*-benzotriazol-1-yl)-*N*-(4-hydroxyphenyl)acetamide		268.27
IVa	$C_{14}H_{11}N_5O_3$	2-(1*H*-benzotriazol-1-yl)-*N*-(4-nitrophenyl)acetamide		297.26
Va	$C_{15}H_{12}N_4O_3$	4-[(1*H*-benzotriazol-1-ylacetyl)amino]benzoic acid		296.28

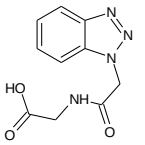
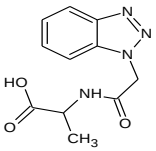
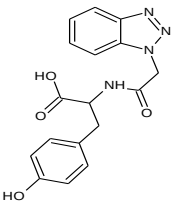
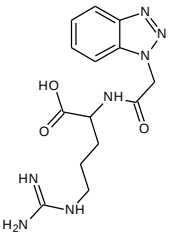
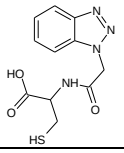
Ib	C10H10N4O3	[(1H-benzotriazol-1-ylacetyl)amino]acetic acid		234.21
IIb	C11H12N4O3	2-[(1H-benzotriazol-1-ylacetyl)amino]propanoic acid		248.23
IIIb	C17H16N4O4	2-[(1H-benzotriazol-1-ylacetyl)amino]-3-(4-hydroxyphenyl)propanoic acid		340.33
IVb	C14H19N7O3	2-[(1H-benzotriazol-1-ylacetyl)amino]-5-carbamimidamidopentanoic acid		333.34
Vb	C11H12N4O3S	2-[(1H-benzotriazol-1-ylacetyl)amino]-3-sulfanylpropanoic acid		280.30

Table 2: Antioxidant activity Benzotriazole derivatives (Ia to Va and Ib to Vb)

Derivative	*Conc. 25µg/ml	*Conc. 50µg/ml	*Conc. 75µg/ml	*Conc. 100µg/ml	Average	#STDEV	SEM
I a	40.37736	53.58491	45.31722	52.67176	47.98781	6.28007	±3.140035
II a	43.57143	55.10949	50.27473	62.42424	52.84497	7.947646	±3.973822
III a	47.68212	53.7594	50.54645	61.49068	53.36966	5.955992	±2.977996
IV a	43.57143	52.50965	47.53623	57.24138	50.21467	5.942734	±2.971367
V a	42.54546	53.93258	46.2908	54.74453	49.37834	5.937404	±2.968701
I b	44.5614	52.32558	45.31722	52.67176	48.71899	4.377572	±2.188786
II b	42.12454	53.23194	49.1573	61.00629	51.38002	7.888789	±3.944394
III b	43.36918	51.5748	51.34409	50	49.07202	3.864789	±1.932394
IV b	42.12454	53.05344	50	48.33333	48.37783	4.604284	±2.302141
V b	45.51724	49.17355	50.41096	57.53425	50.659	5.032387	±2.516193
Ascorbic	48.02632	55.7554	53.82653	64.77273	55.59524	6.944274	±3.472137

* Average of three values of % Scavenging activity at different concentrations. SEM is Standard Error of the Mean.

STDEV is standard deviation.

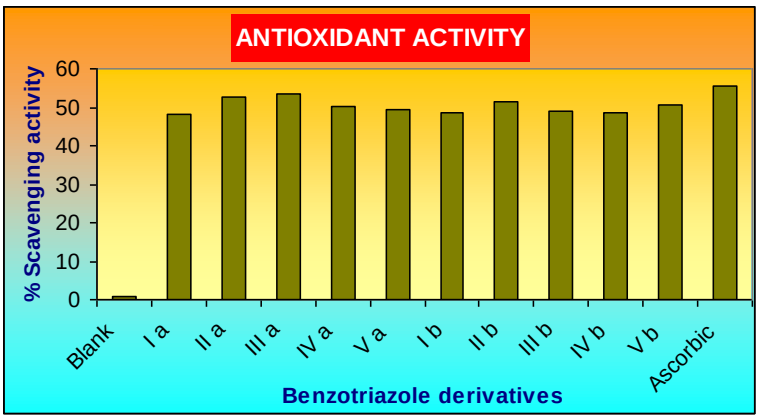


Fig. 1: Graphical representation of % Scavenging activity of the Benzotriazole derivatives.

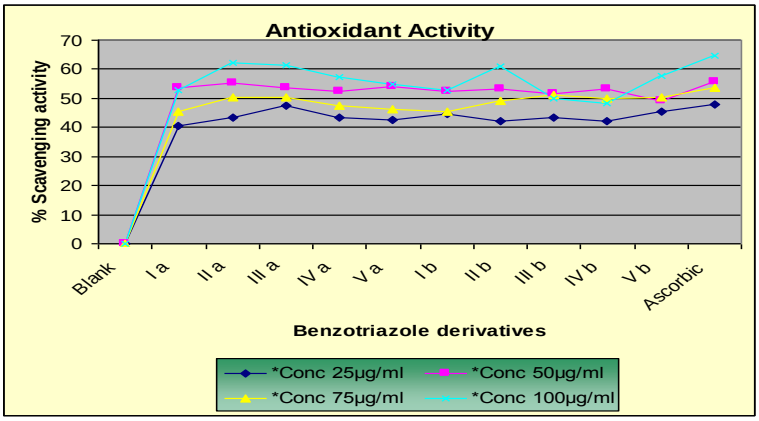


Fig. 2: Graphical representation of % Scavenging activity of the Benzotriazole derivatives at different concentrations.

RESULTS AND DISCUSSION

The percentage of scavenging activity of derivatives was calculated with Absorption of different concentrations. The derivatives like IIa, IIIa and IIb showed remarkable Scavenging activity when compared to ascorbic acid (Figure 1 & 2). Greater the values of scavenging activity better the antioxidant activity (Table 2), Standard Error of the Mean (SEM) was calculated for each derivative and found to be within the normal range.

CONCLUSION

The Benzotriazole derivatives are like II a, III a and IIb showed comparable percentage of nitric oxide scavenging activity with standards reference ascorbic acid. The other derivatives show medium activity.

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