

## EVALUATION OF ANTIOXIDANT ACTIVITY FOR SOME BENZOTRIAZOLE SUBSTITUTED WITH N-PHENYLACETAMIDE AND ACETYL CARBAMIC 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 IIIb derivatives showed highest antioxidant activity and rest of the derivatives were having medium activity. The data obtained was expressed statistically.

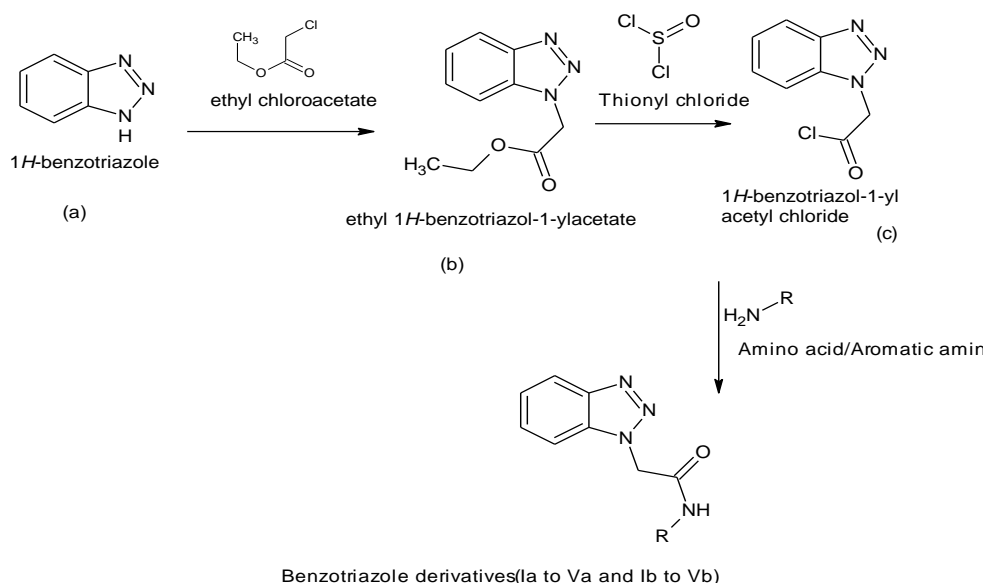
**Keywords:** Antioxidants, Scavenging activity, Benzotriazole derivatives, Griess reagent.

### 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-chitoooligosaccharides[10], 1-acyl-4-cycloalkyl/arylsemicarbazides and 1-acyl-5-benzyloxy/ hydroxy carbamoylcarbazines[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 activities[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].

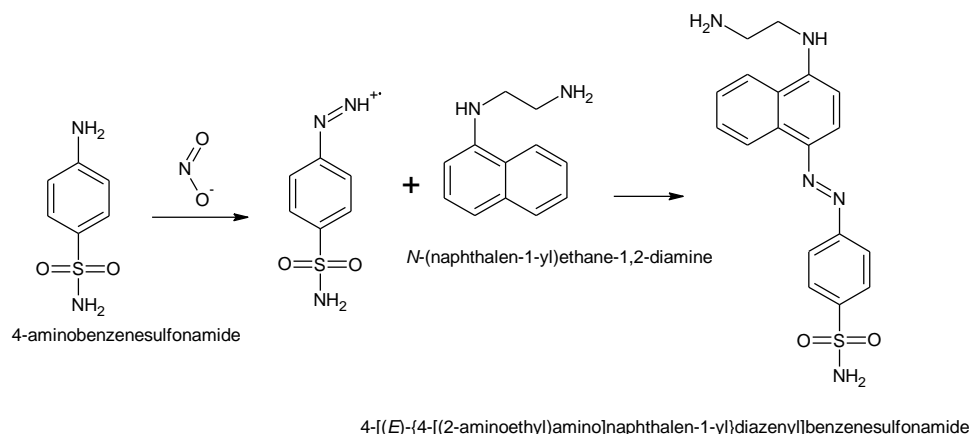
The Benzotriazole derivatives of N-Phenylacetamide (Ia to Va) and acetylcarbamic acid (Ib and Vb) were synthesized (Scheme 1) and antimicrobial activity was reported [35-39].



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

Present study was focused on evaluation of 2-(1H-1,2,3-benzotriazol-1-yl)-N-phenylacetamide and [(1H-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 nitroprusside 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 sulphonilamide to form diazonium salt which couples with N-(naphthalene-1-yl)ethylenediamine (NED) to form 4-[(E)-{4-[(2-aminoethyl)amino]naphthalene-11-yl}diazonyl]benzenesulfonamide a purple colored complex. Thus formed complex was measured spectrophotometrically at 548nm.



**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.

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

### Procedure

Test solutions of Benzotriazole derivatives were prepared in range of 25µg/ml -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 Griess 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 <sub>18</sub> H <sub>14</sub> N <sub>4</sub> O	2-(1 <i>H</i> -benzotriazol-1-yl)- <i>N</i> -(naphthalen-1-yl)acetamide		302.32
IIa	C <sub>14</sub> H <sub>13</sub> N <sub>5</sub> O <sub>3</sub> S	2-(1 <i>H</i> -benzotriazol-1-yl)- <i>N</i> -(4-sulfamoylphenyl)acetamide		334.34
IIIa	C <sub>14</sub> H <sub>12</sub> N <sub>4</sub> O	2-(1 <i>H</i> -benzotriazol-1-yl)- <i>N</i> -(4-hydroxyphenyl)acetamide		268.27
IVa	C <sub>14</sub> H <sub>11</sub> N <sub>5</sub> O <sub>3</sub>	2-(1 <i>H</i> -benzotriazol-1-yl)- <i>N</i> -(4-nitrophenyl)acetamide		297.26
Va	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>3</sub>	4-[(1 <i>H</i> -benzotriazol-1-yl)acetyl]amino]benzoic acid		296.28

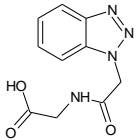
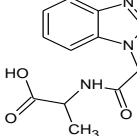
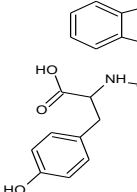
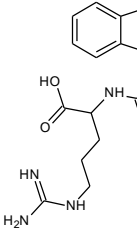
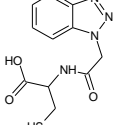
Ib	C10H10N4O3	[(1H-benzotriazol-1-ylacetyl)amino]acetic acid		234.21
IIb	C11H12N4O3	2-[(1H-benzotriazol-1-ylacetyl)amino]propanoic acid		248.23
IIIb	C17H16N4O4	2-[(1H-benzotriazol-1-ylacetyl)amino]-3-(4-hydroxyphenyl)propanoic acid		340.33
IVb	C14H19N7O3	2-[(1H-benzotriazol-1-ylacetyl)amino]-5-carbamimidamidopentanoic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

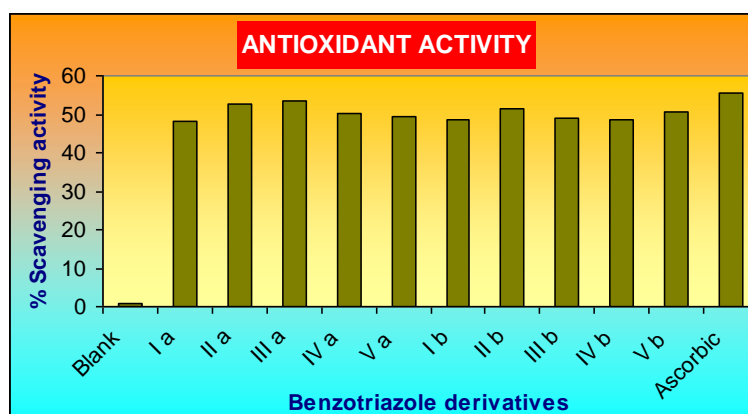


Fig. 1: Graphical representation of % Scavenging activity of the Benzotriazole derivatives.

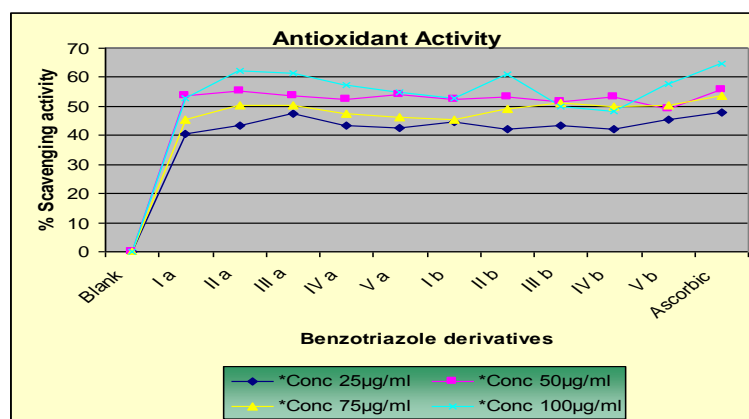


Fig. 2: Graphical representation of % Scavenging activity of the Benzotriazole derivatives at different concentrations.

## 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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## REFERENCES

- Ratnam DV, DD Ankola, Bhardwaj V, Sahana DK, Ravi Kumar MNV. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *J Control Release* 113 (2006) 189–207
- AA Hamid, OO Aiyelaagbe, LA Usman, OM Ameen, A Lawal. Antioxidants: Its medicinal and pharmacological Applications. *Afr J Pure Appl Chem* 2010; 4(8):142-51.
- Singh N, Singh G. Antioxidants from Natural Sources: Ray of Hope for Oxidative Damage. *J pharm Sci inno* 2012;1(1):14-17.
- McCarty MF. Scavenging of peroxynitrite-derived radicals by flavonoids may support endothelial NO synthase activity, contributing to the vascular protection associated with high fruit and vegetable intakes. *Med Hypotheses* 2008; 70:170–81.
- Almeida IMC, Barreira JCM, Oliveira MBPP, Ferreira ICFR. Dietary antioxidant supplements: Benefits of their combined use. *Food Chem Toxicol* 2011;49 : 3232–37.
- Marathe SA, V Rajalakshmi, Jamdar SN, Sharma A. Comparative study on antioxidant activity of different varieties of commonly consumed legumes in India. *Food Chem Toxicol* 2011;49 :2005–12
- Khan KM, Mughal UR, Omar I, Choudhary MI. Microwaves-Assisted Syntheses of Imidazolylbenzamides and their Antioxidant Activities. *Letters in Drug Design & Discovery* 2008;5:152-57.
- De S, Adhikari S, Jain JT, Menon VP, Devasagayam TPA. Antioxidant activity of an aminothiazole compound: Possible mechanisms. *Chem Bio Interact* 2008;173:215–23.
- Jung JC et al. Synthesis of novel trans-stilbene derivatives and evaluation of their potent antioxidant and neuroprotective effects. *Eur J Med Chem* 2009;44: 3166–74.
- Je YJ, Park PJ, Kim SK. Free radical scavenging properties of hetero-chitoooligosaccharides using an ESR spectroscopy. *Food Chem Toxicol* 2004; 42:381–87.
- Perkovic et al. Novel NSAID 1-acyl-4-cycloalkyl/arylsemicarbazides and 1-acyl-5-benzyloxy/hydroxyl carbamoylcarbazides as potential anticancer agents and antioxidants. *Eur J Med Chem* 2012; 51: 227-38.
- T. Yokozawa, CP Chen, T Tanaka. Direct scavenging of nitric oxide by traditional crude drugs. *Phytomedicine* 2000; 6(6):453-63.
- Balakrishnan N , Panda A B, Raj N R, Shrivastava A, Prathani R. The Evaluation of Nitric Oxide Scavenging Activity of *Acalypha Indica* Linn Root. *Asian J Research Chem* 2009; 2(2): 148-50.
- Ak T, Gulcin I. Antioxidant and radical scavenging properties of curcumin. *Chem Biol Interact* 2008; 174:27–37.
- Scandran I et al. Assessment of phenolic content, free-radical-scavenging capacity genotoxic and anti-genotoxic effect of aqueous extract prepared from *Moricandia arvensis* leaves. *Food Chem Toxicol* 2010; 48 : 710–715.
- Olabinri BM et al. In vitro Evaluation of Hydroxyl and nitric oxide Radical Scavenging Activites of Artemether. *Res J Biol Sci* 2010;5(1):102-05.
- Wahab SIA et al. Antimicrobial and free radical activities of the Dichloromethana Extract of *Goniathalamus umbrosus*. *Int J trop Med* 2009;4(1):32-36.
- Bergman M, Varshavsky L, Gottlieb HE, Grossman S. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. *Phytochemistry* 2001;58:143–52.
- Joshi R, Kamatb JP, Mukherjee T. Free radical scavenging reactions and antioxidant activity of embelin: Biochemical and pulse radiolytic studies. *Chem Biol Interact* 2007;167:125–34.
- Arora S, Kumar D, Shiba. Phytochemical, antimicrobial and antioxidant activity of methanol extract of leaves and flowers of *Ipomoea cairica*. *Int J Pharm Pharm Sci* 2013;5(1): 198-202.
- Sheik Sana, KR Chandrashekar. In Vitro Antimicrobial, Antioxidant, Antiarthritic, and Phytochemical Evaluation of *Psychotria flavida* Talbot, An endemic plant of Western Ghats. *Int J Pharm Pharm Sci* 2013;5(1): 214-218.
- Sun J, Zhang X, Broderick M, Fein H. Measurement of Nitric Oxide Production in Biological Systems by Using Griess Reaction Assay. *Sensors* 2003; 3:276-84.
- Alam MN, Nusrat Jahan Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm J* (2012). Article in press.
- Shukla DK, Srivastava SD. Synthesis of some new 5-[2-((1,2,3-benzotriazole)-1-yl-methyl)-1'-(4'-substituted aryl-3'-chloro-2'-oxo azetidine)]-amino-1,3,4-thiadiazoles: Antifungal and antibacterial agents. *Indian J Chem.*2008,47B;463-69.

25. Asati KC, Srivastav SK, Srivastav SD. Synthesis of some new 5-arylidene-2-aryl-3-(benzotriazoloacetamidyl)-1,3-thiazolidine-4-ones as analgesic and antimicrobial agents. Indian J Chem. 2006;45B:526-31.
26. R Ramachandran, M Rani, S Senthana, YT Jeong, S Kabilan. Synthesis, spectral, crystal structure and in vitro antimicrobial evaluation of imidazole/benzotriazole substituted piperidin-4-one derivatives. Eur J Med Chem 2011;46:1926-34.
27. Z Rezaei, S Khabnadideh, K Pakshir, Z Hossaini, F Amiri, El Assadpour. Design, synthesis, and antifungal activity of triazole and benzotriazole derivatives. Eur J Med Chem 2009;44:3064-67.
28. S Khabnadideh, Z Rezaei, K Pakshir, K Zomorodian, N Ghafari. Synthesis and antifungal activity of benzimidazole, benzotriazole and aminothiazole derivatives. Res Pharm Sci 2012; 7(2):1-8.
29. KP.Namdeo, VK.Singh, SK.Prajapati. Synthesis of Some 2-(Substituted)-5-[(N Benzotriazolomethyl)-1, 3,4-Thiadiazolyl]-4-Thiazolidinones for their Anti-Fungal Activity. Indian J Pharm Educ Res 2009;43(3):266-71.
30. BV Suma, NN Nitesh, CHS Venkataramana, Jays J, V Madhavan. Synthesis and antibacterial of some new 1,2,3-Benzotriazole derivatives containing pyrrolidinedione moieties. Int J Pharm Pharm Sci 2012;4(1): 169:73.
31. KM Dawood, HA Gawad, EA. Rageb, M Ellithey, HA Mohamed. Synthesis, anticonvulsant, and anti-inflammatory evaluation of some new benzotriazole and benzofuran-based heterocycles. Bioorg Med Chem 2006;14 :3672-80.
32. YA Al-Soud, NA Al-Masoudi, ARS Ferwanah. Synthesis and Properties of New Substituted 1,2,4-Triazoles: Potential Antitumor Agents. Bioorg Med Chem 2003;11:1701-08.
33. Bretne Maria et al. Synthesis and biological activity of 1*H*-benzotriazole and 1*H*-benzimidazole analogues – inhibitors of the NTPase/helicase of HCV and of some related *Flaviviridae*. Antivir Chem Chemoth 2005;16:315-26.
34. BV Suma, NN Natesh, V Madhavan. Benzotriazole in medicinal chemistry: An overview. J Chem Pharm Res 2011;3(6):375-81.
35. Jamkhandi CM, Disouza JI. Synthesis and Antimicrobial Evaluation of 2-(1*H*-1,2,3-Benzotriazol-1-yl)-*N*-Phenylacetamide derivatives. Res J Pharm Tech 2012;5(8): 1072-75.
36. Jamkhandi CM, Disouza JI. Synthesis and Antimicrobial Evaluation of [(1*H*-benzotriazol-1-yl)acetyl] amino] acetic acid Derivatives. Res J Pharm Tech 2012;5(9):1197-200.
37. Vogel's Text Book of Practical Organic chemistry; 4<sup>th</sup> Edn. Longman Group Ltd 1978; p124.
38. Gerard Vogel H. Drug Discovery and Evaluation. 2<sup>nd</sup> ed. Springer – Verlag Berlin Heidelberg New York 2002. p.49.
39. Bansal. RK. Heterocyclic Chemistry, 4<sup>th</sup> .New Age International Publishers.2005;p 475.