CHAPTER 7: PUBLICATIONS


Synthesis and Antimicrobial Evaluation of 2-(1H-1,2,3-Benzotriazol-1-yl)-
N-Phenylacetamide Derivatives

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ABSTRACT:
2-(1H-1,2,3-benzotriazol-1-yl)-N-phenylacetamide derivatives find useful as anti-infective agents and substitution of benzotriazol produces more potent anti-infective agents. Derivatives of benzotriazole substituted 2-(1H-1,2,3-benzotriazol-1-yl)-N-phenylacetamide are synthesized and anti microbial activities have been measured against Norfloroxacin and Ketoconazole as standards drugs. It is found that the derivatives from 1a to Va are shown to produce good anti microbial actions against S. Aureus, S. Pyogens, B. Subtilis, E.coli, P. Aerogenosa, C Albicanse, A Niger. The all the derivatives show satisfactory anti-microbial activities.

KEYWORDS: Antibacterials, Anti fungi, benzotriazoles, N-phenyl acetamide derivatives.

INTRODUCTION:
Treatment of human infections is challenging from time immemorial to present day, as many new infections have taken birth and some old infections are surfacing. Development of resistance to antibiotics and chemotherapeutic agents is observed in microorganisms. Due to these reasons there is need for continuous development of anti-infective agents. Synthetic compounds of derivatives of Benzotriazole have shown analgesic, antibacterial, antifungal activities, antifilarial activities, and Benzotriazole also reported for anticonvulsant and anti-inflammatory, antitumor activities. Literature study reveals the antiviral activity. The methods of synthesis of Benzotriazole derivatives with different techniques have been reported. Present study is focused on Synthesis of 2-(1H-1,2,3-benzotriazol-1-yl)-N-phenylacetamide derivatives following published procedures to get possible potent anti-infective derivatives.

EXPERIMENTAL METHODS:
Procedure of Synthesis
Synthesis of Benzotriazole(a): 10.8 gm of O-phenylenediamine is added to mixture of 12g (11.5 ml) of glacial acetic acid and 30 ml of water, which is cooled to 15°C, stir. Then solution of 7.5g of sodium nitrate in 15 ml water is added in portion. The temperature rises slowly to 85°C and then cools slowly. When temperature is 45°C the mixture is chilled at ice bath for 30 min. Pale brown solid separated by the filtration. The recrystallization is done using benzene as solvent.

Synthesis of ethyl 1H-benzotriazol-1-yl acetate(b): A mixture of Benzotriazole (0.1M), ethyl chloroacetate (0.1M) and 0.3g of K2CO3 in 60 ml of acetone was stirred for 10 hrs. The solvent was removed under reduced pressure. A solid mass was produced and then needle shaped brown crystals were obtained after recrystallization from the mixture of chloroform and ether (82%Y.Y). The yield obtained was 60% and M.P. was 40°C.

Synthesis of 1H-benzotriazol-1-yl acetyl chloride(c): A 250-mL, three-necked flask, equipped with a magnetic stirbar, condenser, thermometer, and addition funnel, is charged with 10.0 g Benzotriazole and 34 mL of chloroform (CHCl3). At 25°C, 22 mL (600 mmol) of thionyl chloride and 1 drop of dimethylformamide (DMF) are added, followed by heating the mixture at 68°C for 3 hr. After the initial suspension turns into a yellow solution, the heating source is removed and the acid chloride precipitates as a pale red solid. After cooling the reaction mixture to 25°C, the solid is collected via filtration using a Buchner funnel, washed with CHCl3, and dried in a vacuum desiccator for 15 hr to give 3.12 g (90%) of a white powder.
Synthesis of 2-(1H-benzo triazol-1-yl)-N-(napthalen-1-yl)acetamide (Ia): the compound (c) was treated with 1-amino naphthalene in equimolar concentration and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm-1): 2924.92 (IC Aromatic), 1597.54 (-N=N str), 1675.15 (-C=O), 1H-NMR (DMSO 16 ppm): 7.1-7.5 (7H, d, Ar), 1.9 (NH), m/z of m⁺ ion is 302.

Synthesis of 2-(1H-benzo triazol-1-yl)-N-(4-sulfamoylphenyl)acetamide (IIa): the compound (c) was treated with equimolar 4-aminobenzenesulfonylamine and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm-1): 2924.92 (IC Aromatic), 1598.89 (-N=N str), 1633.15 (-C=O), 1H-NMR (DMSO 16 ppm): 7.3 (4H, d, Ar), 1.9 (NH), 2.13 (2H, SOH, NH₂), m/z of m⁺ ion is 331.

Synthesis of 2-(1H-benzo triazol-1-yl)-N-(4-hydroxyphenyl)acetamide (IIa): the compound (c) was treated with equimolar 4-aminophenol and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm-1): 2854.33 (IC Aromatic), 1593.03 (-N=N str), 1416.50 (-C=N str), 3337.73 (-NH str), 1H-NMR (DMSO 16 ppm): 7.1-6.6 (7H, d, Ar), 1.9 (NH), m/z of m⁺ ion is 268.

Synthesis of 2-(1H-benzo triazol-1-yl)-N-(4-nitrophenyl)acetamide (IVa): the compound (c) was treated with equimolar 4-nitroaniline and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm-1): 2925.75 (IC Aromatic), 1602.45 (-N=N str), 1301.75 (-C=N str), 3355.07 (-NH str), 1H-NMR (DMSO 16 ppm): 7.1-6.7 (4H, d, Ar), m/z of m⁺ ion is 296.

Synthesis of 4-[(1H-benzo triazol-1-yl)acetamido] benzoic acid (V): the compound (c) was treated with equimolar 4-aminobenzoic acid and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm-1): 2924.00 (IC Aromatic), 1610.40 (-N=N str), 1704.78 (-C=O), 1317.98 (-C-N str), 1H-NMR (DMSO 16 ppm): 7.4-7.1 (4H, d, Ar), m/z of m⁺ ion is 297.

The melting points of the synthesized derivatives were determined by open capillary (LABHOSP) and were uncorrected. The purity of the compounds was checked using pre coated TLC plates (MERCK, 60F) using Benzene:chloroform:methanol (8:4:2) solvent system. The developed chromatographic plates were visualized under UV at 254nm. IR spectra were recorded using KBr on Shimadzu FTIR model 8400 spectrophotometer and Agilent Technologies Cary 630 FTIR. 1H NMR spectra in DMSO on a BRUKER FT-NMR instrument using TMS as internal standard. Fluorimetric determination was carried out using Elco Fluorimeter, model CL-53.

Synthetic Scheme for benzo triazole substituted N-Phenyl acetamide derivative (Scheme-1).

Antimicrobial Activity:
The synthesized compounds were screened for antibacterial and antifungal activity (Fig 1 and 2). The cup plate method was adopted for screening and Norfloxacin and Ketoconazole used as standard drugs in concentration of 1µg/ml. The bacterial strains of B. subtilis, S. aureus, E. coli, and S. pyri were used for antibacterial activity. For antifungal activity, A. niger and C. albicans strains were
used. The MICs and MBCs were determined. Overnight broth cultures were used in all MIC determinations. Agar dilution MICs were determined by using agar plates with an incorporated standard drug dilutions and samples with incubation at 37°C and were defined as the lowest antibiotic concentrations completely inhibiting growth. Broth dilution MICs were determined with overnight broth cultures of the strains to be tested.

Table 1: Physical properties of the Benzotriazole derivatives (Ia to Va)

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Mol. Weight</th>
<th>Derivative Name</th>
<th>R</th>
<th>% Yield</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>C₉H₆N₄O₂</td>
<td>2-(1H-benzo[d]imidazol-1-yl)-N-(2-naphthalen-1-yl)acetamide</td>
<td>NH₂</td>
<td>65</td>
<td>243°C</td>
</tr>
<tr>
<td>IIa</td>
<td>C₂₀H₁₈N₆O₈S</td>
<td>2-(1H-benzo[d]imidazol-1-yl)-N-(4-sulfonylphenyl)acetamide</td>
<td>NH₂</td>
<td>76</td>
<td>130°C</td>
</tr>
<tr>
<td>IIIa</td>
<td>C₂₀H₁₈N₆O₂</td>
<td>2-(1H-benzo[d]imidazol-1-yl)-N-(4-hydroxyphenyl)acetamide</td>
<td>NH₂</td>
<td>65</td>
<td>223°C</td>
</tr>
<tr>
<td>Iva</td>
<td>C₂₀H₁₈N₆O₃</td>
<td>2-(1H-benzo[d]imidazol-1-yl)-N-(4-nitrophenyl)acetamide</td>
<td>NH₂</td>
<td>68</td>
<td>260°C</td>
</tr>
<tr>
<td>Va</td>
<td>C₂₀H₁₈N₆O₃</td>
<td>4-[(1H-benzo[d]imidazol-1-ylacetamido)benzoic acid</td>
<td>NH₂</td>
<td>64</td>
<td>220°C</td>
</tr>
</tbody>
</table>

![ANTIBACTERIAL ACTIVITY](image)

Figure 1: Graphical representation Antimicrobial activity.
RESULTS AND DISCUSSION:
The derivatives synthesized are characterized by the physical properties (Table 1), chemical properties, spectral data like IR, NMR and Mass spectra.

CONCLUSION:
The synthesized derivatives are like 1a to 1c showed comparable Zone of inhibition with standards and have good antibacterial and antifungal activities.

ACKNOWLEDGEMENTS:
Authors are thankful to Shri. G. D. Patil Secretary Shree Wivash Shikshan Mandal, Warnaagar for providing laboratories facilities. Authors are thankful to Mrs. U. S. Choogule, Mr. Shrikish and Mr. Krishm Patnali, CIFC and Chemistry Dept of Shivaji University for kind assistance in microbial and spectral data.

REFERENCES:
RESEARCH ARTICLE

Synthesis and Antimicrobial Evaluation of [(1H-benzotriazol-1-ylacetyl) amino] Acetic Acid Derivatives

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ABSTRACT:
Five novel [(1H-benzotriazol-1-ylacetyl)amino]acetic acid derivatives were synthesized by substituting corresponding amino acids on a reaction intermediate 1H-benzotriazol-1-ylacetyl chloride. The derivatives were characterized by physical, chemical parameters, TLC and spectral data. Synthesized derivatives from 1b to 1v were screened for antimicrobial activity against S. Aureus, B. Subtilis, E. coli, S. typhi, C Albicans, A Niger. MIC was measured against norfloxacin and ketocazole. Present study confirms the synthesized derivatives endowed with effective antimicrobial activities.

KEY WORDS: Benzo triazole, Antimicrobial, Anti fungal, MIC, Benzo triazole derivatives

INTRODUCTION:
The derivatives of Benzotriazole are endowed with various biological activities such as analgesic, antibacterial, antifungal activities, and Benzotriazole derivatives reported for anticonvulsant and anti-inflammatory activities; literature study also reveals the antiviral activity. The methods of synthesis of Benzotriazole derivatives with different techniques have been reviewed. Use of Benzotriazole as synthetic auxiliary was studied. Syntheses of N-protected aminoacyl derivatives of the Benzotriazole were reported. Treatment of microbial infections is challenging task from time immemorial to present day, as many new infections have born and some old infections are resurfacing due to resistance to antibiotics and chemotherapeutic agents. To keep pace with genetic changes of microorganisms there is need for continuous development of anti-infective agents.

The Synthesis of [(1H-benzotriazol-1-ylacetyl)amino]acetic acid Derivatives was effected by substitution of corresponding amino acids on reaction intermediate 1H-benzotriazol-1-ylacetyl chloride which was synthesized by following reported procedures and following the below scheme (scheme 1) to get possible potent anti-infective derivatives.

MATERIAL AND METHODS:
Procedure of Synthesis
Synthesis of Benzotriazole (0.1L): 10.8 gm of O-phenylene diamine is added to mixture of 12gm (11.5 ml) of glacial acetic acid and 30 ml of water, which is cooled to 15°C, stir. Then solution of 7.5 of sodium nitrite in 15 ml water is added in portion. The temperature rises slowly to 85°C and then cools slowly. When temperature is 45°C the mixture is chilled at ice bath for 30 min. Pale brown solid separated by the filtration. The recrystallization is done using benzene as solvent.

Synthesis of ethyl 1H-benzotriazol-1-ylacetate (1b): A mixture of Benzotriazole (0.1M), ethyl chloroacetate (0.1M) and 0.3g of K2CO3 in 60 ml of acetone was stirred for 10 hrs. The solvent was removed under reduced pressure. A solid mass was produced and then needle shaped brown crystals were obtained after recrystallization from the mixture of chloroform and ether (8:29V/V). The yield obtained was 60% and M.P: was 40°C.

Synthesis of 1H-benzotriazol-1-ylacetyl chloride(s): A 250-ml three-necked flask, equipped with a magnetic stirrer, condenser, thermometer, and addition funnel, is charged with 10.0 g Benzotriazole and 34 mL of chloroform (CHCl3). At 25°C, 22 mL (600 mmol) of thionyl chloride and 1 drop of dimethylformamide (DMF) are added, followed by heating the mixture at 68°C for 3 hr. After the initial suspension turns into a yellow solution, the heating source is removed and the acid chloride precipitates as a pale red solid. After cooling the reaction mixture to 25°C, the solid is collected via filtration using a Buchner funnel, washed with CHCl3 and dried in a vacuum desiccator for 15 hr to give 5.12 g (90%) of as a white powder.
Figure: Scheme for synthesis of [(1H-benzotriazol-1-ylacetyl) amino] acetic acid derivatives

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\text{Figure 1: Reaction pathway}
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Substitution of amino acids on (c) to get the below derivatives.

**Synthesis of [(1H-benzotriazol-1-ylacetyl)amino]acetic acid (Ib)**: the compound (c) was treated with glacial acetic acid in equimolar concentration and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm⁻¹): 2835.93 (HC=O), 1493.93 (C=C=O), 1677.21 (C=C=O), 1H-NMR (DMSO 16 ppm): 6.9 (H, CONH=), 7.2 (H, d, 4Ar), 12.2 (H, COOH), m/z of m⁺ ion is 234.

**Synthesis of 2-[(1H-benzotriazol-1-ylacetyl)amino]propanoic acid (IIb)**: the compound (c) was treated with equimolar alamine and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm⁻¹): 3017.14 (HC=O), 1508.24 (N=N str), 1682.70 (C=O), 1H-NMR (DMSO 15 ppm): 1.4-1.5 (3H, -CH₃), 7.4 (1H, -NHCOCO), 7.30-7.66 (4H, d, 4Ar), m/z of m⁺ ion is 248.

**Synthesis of 2-[(1H-benzotriazol-1-ylacetyl)amino]-3-(4-hydroxyphenyl)propanoic acid (IIIb)**: the compound (c) was treated with equimolar tyrosine and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm⁻¹): 2926.32 (HC=O), 1559.59 (N=N str), 1357.48 (C-N str), 3024.48 (NH str), 3320.00 (Ar-OH), 1H-NMR (DMSO 15 ppm): 2.4 (H, -CH₂), 3.6 (H, -CH₂CO), 3.9 (H, Ar-OH), 6.6 (H, -NH), 7.2-7.3 (4H, d, 4Ar), 7.34 (H, -NHCOCO), m/z of m⁺ ion is 340.

Synthesis of 2-[(1H-benzotriazol-1-ylacetyl)]amino]-5-carbamimidamidopentanoic acid (IVb): the compound (c) was treated with equimolar arginine and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm⁻¹): 2857.39 (HC=O), 1539.25 (C=N str), 1671.54 (C=O str), 3271.38 (NH str), 1H-NMR (DMSO 16 ppm): 1.9-2.3 (CH₂), 2.8-2.6 (NH₂), 7.1-7.2 (4H, d, Ar), m/z of m⁺ ion is 353.

Synthesis of 4-[(1H-benzotriazol-1-ylacetyl)amino] benzoic acid (Vb): the compound (c) was treated with equimolar cysteine and refluxed for 4 hrs in benzene solvent. Spectral data FTIR (KBr cm⁻¹): 2925.12 (HC=O), 1507.42 (C=N str), 1578.68 (C=O), 1H-NMR (DMSO 15 ppm): 12.2 (H, -COOH), 7.1-7.2 (4H, d, Ar), m/z of m⁺ ion is 280.30.

The melting points of the synthesized derivatives were determined by open capillary (LABIOSP) and were uncorrected. The purity of the compounds was checked using pre coated TLC plates (MERCK, 60F) using Benzene: chloroform: methanol (8:4:2) solvent system.

The developed chromatographic plates were visualized under UV at 254nm. IR spectra were recorded using KBr on Shimadzu FTIR model 8400 spectrophotometer and Agilent Technologies Cary 60 FTIR FTIR 1H NMR spectra in DMSO on a BRUKER FT-NMR instrument using TMS as internal standard.
Table 1: Physicochemical properties of the Benzo-triazole derivatives (Ia to Vb)

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Mol. Weight</th>
<th>Derivative Name</th>
<th>R</th>
<th>% Yield</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ib</td>
<td>C₂₁H₂₆N₈O₇</td>
<td>1H-benzo-triazole-1-ylacetyl-L-amino-propionic acid</td>
<td>H₂O</td>
<td>68</td>
<td>182°C</td>
</tr>
<tr>
<td>IIb</td>
<td>C₂₁H₂₆N₈O₇</td>
<td>2-[1H-benzo-triazole-1-ylacetyl-L-amino-propionic acid]</td>
<td>C₂H₅</td>
<td>62</td>
<td>207°C</td>
</tr>
<tr>
<td>IIIb</td>
<td>C₂₁H₂₆N₈O₇</td>
<td>2-[1H-benzo-triazole-1-ylacetyl-L-amino-5-4-hydroxyphenylpropanoic acid]</td>
<td></td>
<td>66</td>
<td>227°C</td>
</tr>
<tr>
<td>IVb</td>
<td>C₂₁H₂₆N₈O₇</td>
<td>2-[1H-benzo-triazole-1-ylacetyl-L-amino-5-carboxylindolpropionic acid]</td>
<td>H₂O</td>
<td>63</td>
<td>210°C</td>
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<tr>
<td>Vb</td>
<td>C₂₁H₂₆N₈O₇</td>
<td>2-[1H-benzo-triazole-1-ylacetyl-L-amino-5-sulfanylpropanoic acid]</td>
<td>H₂O</td>
<td>67</td>
<td>202°C</td>
</tr>
</tbody>
</table>

**Antimicrobial Activity:**
The derivatives synthesized were evaluated for antibacterial and antifungal activity with agar diffusion method using Norfloxacin and Ketoconazole as standard drugs in concentration of 1μg/ml (Fig 1 and 2). The bacterial strains of *B. subtilis*, *S. aureus*, *E. coli*, and *S. typhi* were used for antibacterial activity and *A. niger* and *C. albicans* strains were used for antifungal activity. Overnight broth cultures were used for MIC determinations. Agar dilution MICs were determined by using agar plates with an incorporated standard drug dilutions and samples with incubation at 37°C and were defined as the lowest antibiotic concentrations completely inhibiting growth. Broth dilution MICs were determined with overnight broth cultures of the strains to be tested.

**RESULTS AND DISCUSSION:**
Substitution of amino acids in 1H-benzo-triazole-1-ylacetyl chloride was produced various derivatives (Ia-Vb). The derivatives synthesized were characterized by the physical properties (Table 1), chemical properties, spectral data like IR, NMR and Mass spectra. The synthesized derivatives were evaluated for their antimicrobial activity using Norfloxacin and Ketoconazole as standard.

**CONCLUSION:**
The synthesized derivatives like Ia to Vb showed comparable Zone of inhibition with standards and have good antibacterial and antifungal activities.

**ACKNOWLEDGMENTS:**
Authors are thankful to Shri. G. D. Patil, Secretary Shree Varana Vihang Shishkan Mandal Waranangur for providing laboratories facilities. Authors are thankful to Mrs. U. S. Chougule, and Mr. Krishnath Paymal, IISc Bangalore, CFC and Chemistry Dept. of Shivaji University Kolhapur for kind assistance in microbial and spectral data.

**REFERENCES:**
1. Shukla DK, Srivastava SD. Synthesis of some new 5-[2-[1,2,3-benzo-triazole-1-ylacetyl]-L-amino]-1,4-substituted ary1-3-chloro-2-oxo azetidin-1-one-1,4-thiazolides: Antifungal and antibacterial agents. Indian J Chem 2008;47B:463-69.


Benzotriazole Derivatives As Antimicrobial Agents

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Abstract: The saga of investigations on Benzotriazole derivatives reveals wide applicability of molecules having Benzotriazole moiety. The various derivatives synthesized showed antimicrobial activities such as antibacterial, antifungal, antiviral, antihelminthics, antiprotozoal and antinocobacterial activity. The present article throws light on Benzotriazole derivatives synthesized and their antimicrobial activity reported chronologically. This opens new avenues for researchers to work on promising molecules to develop into lead molecules.

Key Words: Benzotriazole, antimicrobial, antifungal, antiviral, Benzotriazole derivatives

INTRODUCTION:

As the micro organisms are rapidly undergoing genetic changes and developing resistance against many antibiotics and therapeutic agents for various diseases more quickly than new drugs are being made available so the war against the infectious diseases has become a never ending process.

History:

The history reveals the development of antibacterial agents, the scientific development of antimicrobial drugs can be observed in to three main stages. The first stage began with Erlich in the 1890’s, with the use of methylene blue for managing malaria, the organic arsenicals for trypansomiasis in 1904, and salvarsan 60 for syphilis in 1909. Atebrin was made in 1932 and used for prophylaxis of malaria. In second stage discovery of wonder drug Penicillin by Fleming in 1928 brought revolutionary change in therapeutics and effectively controlled gonorrhea, streptococcus throat infections, or pneumonia and wound healing. The third stage is observed as “Golden era of antimicrobial therapy”, with discovery of prontosil by Domagk in pyrogenic infection. It was later realised that the active moiety was para-amino benzene sulfonamide, and the dye part is not essential. The substituted heterocyclic structures like coumarines, quinolines, quinines, furans imidazoles, pyrimidines, triazoles and benzotriazoles are proved to be effective antimicrobial agents.

Importance of Antimicrobials: Infectious diseases raise awareness of our global vulnerability, the need for strong health care systems and the potentially broad and borderless impact of disease.

- Over 9.5 million people die each year due to infectious diseases – nearly all live in developing countries.
• Children are particularly vulnerable to infectious diseases. Pneumonia, diarrhea and malaria are leading causes of death among children under age 5; cerebral malaria can cause permanent mental impairment.

• Infectious diseases are also destructive to the health of adults, causing disability, a diminished quality of life, decreased productivity or death.

• Co-infection. People infected with one infectious disease become more susceptible to other diseases. Examples include: HIV/AIDS co-infection with tuberculosis or malaria co-infection with multiple neglected diseases.

• Some old infections are resurfacing which is really challenging task. Treatment of these infections with rapid development of resistance in organisms has added fuel to worsened situation.

• Some viral infections like Dengue, Hepatitis, Japanese Viral, and West Nile Viral Infections cause large scale deaths every year as epidemics.

The pharmaceutical researchers have to keep in pace with these changes to control the infections by exploring newer anti-infective agents as many as possible. The ability of mankind to synthetically prepare medicinally important molecules during the past century has allowed for a continued decrease in the mortality rate from numerous diseases. The gravity of situation made WHO to resolve to put special focus on antimicrobial resistance and its global spread particularly the HIV/AIDS, tuberculosis and malaria epidemics [1-2].

These therapeutics agents bear wide range of structural differences and many of these compounds are having heterocyclic ring as their part structure [3]. The literature survey reveals that heterocyclic compounds bearing benzotriazole as part of structure showed valuable biological activity particularly antibacterial and antifungal activity. Benztiazone derivatives have proven to be effective antimicrobials.

**Antimicrobial Activity of Benzo triazole Derivatives:** Benzotriazole is heterocyclic compound endowed with multiple biological activities. Extensive literature survey revealed antimicrobial activity like antibacterial, antifungal, and antiviral, antihelmintic and antiprotozoal action.

**Antibacterial and antifungal activity:** Antimicrobial activity screening for Benzotriazole derivatives started with antibacterial activity (M Purohit et al). Large number of investigations on Benzotriazole derivatives showed the both antibacterial and antifungal activity.

**Antimycobacterial activity:** Development of antitubercular agents is very tedious process therefore only one or two new drugs will arrive in the market from these efforts. The treatment of tuberculosis with combination of drugs has even not satisfactory in combating the disease due to bacterial resistance. There is need for effective antitubercular agents to win the battle against this millenary scourge [22], new class of benzotriazole derivatives triazoloquinolones were active against multi-drug resistant M. tuberculosis (MDR-Mtb) was reported by Carla Antonio et al 2011.

**Antiviral activity:** Antihelicase activity of Benzotriazole derivatives against Flaviviridae like hepatitis C, West Nile Virus, Dengue Virus, Japanese Encephalitis was reported by Maria B et al. Dialkylamine and ester derivatives were screened against respiratory syncitial virus and coronovirus.
**Antiprotozoal activity:** The 5, 6-dimethyl and 5,6-dibromo derivatives of Benzotriazole were reported active against Acanthamoeba castellani by Katarzyna K et al in 2004.

**Anthelmintic activity:** Benzotriazole derivatives of N-heteroaryl/diphenyl amino acetyl/propon were tested for anthelmintic activity. Apart from antimicrobial activity the Benzotriazole is importa as a synthetic auxiliary[4-6], in synthesis of peptides[7], acid azides[8], preparation of hydroxymethyl-2,3-dihydrobenzofurans and 3-hydroxymethylbenzofurans has been developed usin benzotriazole mediated benzofuran ring closure was reported[9]. Some derivatives of Benzotriazole a reported to have antiproliferative activity[10-14], pharmacological activities like analgesi, anticonvulsant, anti-inflammatory[15-16], inhibitors of human (CK2) protein kinase[17], agonist f 5-HT receptor[18], metal corrosion inhibitors[19], cytochalasin B-nimetic activity[20], synthesis and biological activities of Benzotriazole derivatives was reviewed by BV Suma et al[21]. T1 antimicrobial activity of Benzotriazole derivatives with reference and main investigator is arranged chronological order in Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Benzotriazole derivative synthesized</th>
<th>Author/Investigator/s</th>
<th>Reported activity</th>
<th>Ref No</th>
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<tbody>
<tr>
<td>1992</td>
<td>Chlorosubstituted phenoxy acetyl and propionyl benzotriazoles</td>
<td>M Purohit and SK Srivastava</td>
<td>Antibacterial, Antifungal</td>
<td>[23]</td>
</tr>
<tr>
<td>1994</td>
<td>Benzotriazole sulfonic acid derivatives</td>
<td>Peter Ackerman and Max Schellenbaum</td>
<td>Antifungal</td>
<td>[25]</td>
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<td>2000</td>
<td>Derivatives of 3-aryl substituted-2-(1H/(2H)) benzotriazol-1(2)-yl)acrylonitrile</td>
<td>Paolo Sanna</td>
<td>Antituberculosis</td>
<td>[26]</td>
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<tr>
<td>2002</td>
<td>Benzotriazole derivatives of 2-aminothiophene-3-carbonitrile, 2-thioxopyridine-3-carbonitrile, 1,8-naphthyridine-2-one, thieno[2,3-b]pyridine-5-carbonitrile and thieno[2,3-d]pyrimidine</td>
<td>Fatima Al-Omran et al</td>
<td>Antibacterial, Antifungal</td>
<td>[27]</td>
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<td>2003</td>
<td>Dialkylamino side chain substituted on the Benzotriazole</td>
<td>Kuo-Long Yu et al</td>
<td>Respiratory syncytial virus</td>
<td>[28]</td>
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<tr>
<td>Year</td>
<td>Title</td>
<td>Authors</td>
<td>Inhibitor</td>
<td>Reference</td>
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<td>2004</td>
<td>5,6-dimethyl-1Hbenzotriazole and and 5,6-dibromo-1H-benzotriazole</td>
<td>Katarzyna K et al</td>
<td>inhibitor of Acanthamoeba castellanii.</td>
<td>[29]</td>
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<td>2005</td>
<td>Oxazolidinone derivatives with positional and geometrical substitutions on benzotriazole</td>
<td>Jagattaran Das et al</td>
<td>Antibacterial activity</td>
<td>[30]</td>
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<td>2005</td>
<td>Benzotriazolyl oxazolidinone derivatives</td>
<td>Prasad PD and coworkers</td>
<td>Antibacterial activity</td>
<td>[31]</td>
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<td>2005</td>
<td>N-alkyl derivatives of 1Hbenzotriazole</td>
<td>Maria B. et al</td>
<td>Antihelicase activity against Flaviviridae</td>
<td>[32]</td>
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<td>2006</td>
<td>5-arylidene-2-aryl-3-(benzotriazolooacetamidyl)-1,3-thiazolidin-4-ones</td>
<td>KC Asati et al</td>
<td>Antibacterial activity</td>
<td>[15]</td>
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<td>2006</td>
<td>Derivatives of N-alkylated benzotriazole</td>
<td>S Nanjund Swamy et al</td>
<td>Antibacterial activity, Antifungal.</td>
<td>[33]</td>
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<td>2006</td>
<td>Benzotriazole esters</td>
<td>Chung-Yi Wu. et al</td>
<td>Anticoronavirus</td>
<td>[34]</td>
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<td>2006</td>
<td>Derivatives of 1-[3-(4-benzotriazol-1/2-y1-3-fluoro-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-3-substituted-thiourea</td>
<td>Prasad PD and coworkers</td>
<td>Antitubercular activity</td>
<td>[35]</td>
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<tr>
<td>2008</td>
<td>Derivatives of 5-[2-(1,2,3-benztriazole)-1-yl-methyl]-1'-arylidene hydrazine-1,3,4-thiadiazoles and 5-[2-(1,2,3-benztriazole)-1-yl-methyl]-1'(4'-substituted aryl-5'-chloro-2'-oxoazetidine])-amino-1,3,4-thiadiazoles</td>
<td>Shukla DK and Srivastav SD</td>
<td>Antibacterial activity, Antifungal.</td>
<td>[36]</td>
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<td>2008</td>
<td>Derivatives of Benzotriazole esters-1иль-(4-Dimethylaminobenzoyloxy)-Benzotriazole</td>
<td>Koen HG et al</td>
<td>Anticorona virus(SARS) activity</td>
<td>[37]</td>
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<td>2009</td>
<td>2-(substituted)-5-[N-(Benztiazolomethyl)-1,3,4-Thiadiazolyl]-4 Thiazolidiones</td>
<td>KP Namdeo et al</td>
<td>Antifungal</td>
<td>[38]</td>
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<tr>
<td>2009</td>
<td>Derivatives of 1-Trityl-1H-1,2,3-</td>
<td>Rezaei Z et al</td>
<td>Antifungal</td>
<td>[39]</td>
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</table>
CONCLUSION:

The literature study of investigations on synthesis and antimicrobial screening of Benzotriazole derivatives in the past two decades showed antimicrobial activities like antibacterial, antifungal, antiviral, antiprotozoal and anthelminthic action. These reports of investigations suggest the possibility of emerging a lead compound of benzotriazole having a potential antimicrobial activity.

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EVALUATION OF ANTIOXIDANT ACTIVITY FOR SOME BENZO TRIAZOLE 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 Benzo triazole substituted with N-Phenylacetamide (Ia to Vd), acetylcarbamic acid (VI to Vb) Derivatives. The derivatives were synthesized with planned synthetic pathway and screened for antioxidant activity by adopting Gross reaction assay method. Sodium Nitroprusside was used to produce nitric oxide which oxidizes sulphydryl groups present in Griess reagent to form diazotium salt and on further react with N-(naphthalene-1-sulphonylamino) (NED) to chromophore. The nitrogen scavenging activity of the derivatives was measured by absorption of chromophore formed with spectrophotometer at 540nm using acetone acid as standard. Ia, IIa and IVb derivatives showed highest antioxidant activity and rest of the derivatives were having medium activity. The data obtained was expressed statistically.

Keywords: Antioxidants, Scavenging activity, Benzo triazole 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 providing prophylactic agents in pathogenesis [1]. Some food items, vegetables, and fruits act as antioxidants [2-6]. Antioxidant activity reported for indazoles/benzenes[7], Aminothiole[8], thiole derivatives[9], benzene-1,2-dicarboxylates[10], 1-aryl-4-cycloalkyl/arylsulfonylbisamides and 1-acyl-5-benzofuran/ hydroxycarinamido carbamates[11] and herbal extracts [12-13]. 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 Benzo triazole endowed with antiseptic, antifungal activities [24-30]. Benzo triazole also reported for anticancerous and anti-inflamatory [31], antitumor [32] activities and literature study reveals the antioxidant activity[33]. The methods of synthesis of Benzo triazole derivatives with different techniques have been reported[34].

The Benzo triazole derivatives of N-Phenylacetamide (Ia to Vd) and acetylcarbamic acid (VI to Vb) were synthesized (Scheme 1) and antioxidant activity was reported [35-39].

![Scheme 1: Synthetic pathway for Benzo triazole derivatives (Ia to Vb)](image)

Present study was focused on evaluation of 3-(4H-1,2,3-Benzotriazole-1-y1)-phenylacetamide and 3-[1H-benzo triazole-1-y1]-aminocarboxylic 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 produces nitric oxide at physiological pH which interacts with oxygen to produce nitric ion. The synthesized derivatives contain carbonyl and amino functional groups which interact with nitric oxide resulting in reduced production of nitric ion, a strong oxidizing agent. The remaining vacant ion combines the sulfonamide to form diazotium salt which couples with N-(naphthalene-1-sulphonylamino) (NED) to form 4-(1H)-4-(1H)-1,2-benzo triazole-1,2-dicarboxylate - a purple colored complex. Thus formed complex was measured spectrophotometrically at 540nm.

MATERIALS AND METHODS

All the reagents used were analytical grade. DMSO was used as solvent. Griess Reagent: Prepared with 0.3% naphthylethenediamine dihydrochloride and 2% salicylaldehyde in 4% phosphoric acid. Phosphate buffer saline (PBS) and Sodium nitroprusside (SNP) were prepared. All absorbance was measured with Digital Spectrophotometer Model VSI: SPF. Absorption of radical scavenging activity were recorded at 540nm using DMSO as blank and Ascorbic acid as standard or control using formula given below.

% Nitric Oxide Scavenging Activity = \( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \)

Procedure

Test solutions of Benzo-triazole derivatives were prepared in range of 25μg/ml -100μg/ml in DMSO. 1ml of test solution of each concentration transferred to 10ml volumetric flask. 1ml of Phosphate Buffer Saline (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 37°C for 150 minutes. The Griess reagent of 2ml was added to all the solutions. Chromogenic 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 Benzo-triazole derivatives (Ia to Va and Ib to Vb)

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Mol. Formula</th>
<th>Derivative Name</th>
<th>Structure</th>
<th>Mol. Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>C_{12}H_{15}N_{2}O_{2}</td>
<td>2-[[1H-benzo-triazol-1-yl]-N-(naphthalen-1-yl)]acetamide</td>
<td><img src="structure.png" alt="Structure Image" /></td>
<td>302.22</td>
</tr>
<tr>
<td>Ib</td>
<td>C_{12}H_{15}N_{2}O_{2}</td>
<td>2-[[1H-benzo-triazol-1-yl]-N-(4-vanilinyl)phenyl]acetamide</td>
<td><img src="structure.png" alt="Structure Image" /></td>
<td>334.54</td>
</tr>
<tr>
<td>Ic</td>
<td>C_{12}H_{15}N_{2}O_{2}</td>
<td>2-[[1H-benzo-triazol-1-yl]-N-(4-hydroxyphenyl)]acetamide</td>
<td><img src="structure.png" alt="Structure Image" /></td>
<td>268.27</td>
</tr>
<tr>
<td>Id</td>
<td>C_{12}H_{15}N_{2}O_{2}</td>
<td>2-[[1H-benzo-triazol-1-yl]-N-(4-aminophenyl)]acetamide</td>
<td><img src="structure.png" alt="Structure Image" /></td>
<td>297.26</td>
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<tr>
<td>Ie</td>
<td>C_{12}H_{15}N_{2}O_{2}</td>
<td>4-[[1H-benzo-triazol-1-ylacetyl]amino]benzoic acid</td>
<td><img src="structure.png" alt="Structure Image" /></td>
<td>296.20</td>
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Table 2: Antioxidant activity Benzotriazole derivatives (Ia to Va and Ib to Vb)

<table>
<thead>
<tr>
<th>Derivative</th>
<th>% Conc. 25mg/ml</th>
<th>% Conc. 50mg/ml</th>
<th>% Conc. 75mg/ml</th>
<th>% Conc. 100mg/ml</th>
<th>Average</th>
<th>#STDEV</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>40.27726</td>
<td>50.68691</td>
<td>45.31722</td>
<td>52.61769</td>
<td>47.99742</td>
<td>6.28007</td>
<td>3.710095</td>
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<tr>
<td>IIa</td>
<td>48.67168</td>
<td>58.10949</td>
<td>50.37478</td>
<td>62.47264</td>
<td>54.86497</td>
<td>7.947654</td>
<td>5.977822</td>
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<tr>
<td>IIIa</td>
<td>47.05212</td>
<td>50.70754</td>
<td>50.54645</td>
<td>61.49060</td>
<td>53.30596</td>
<td>5.955993</td>
<td>3.757796</td>
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<tr>
<td>IVa</td>
<td>43.67140</td>
<td>50.50562</td>
<td>47.26263</td>
<td>57.31130</td>
<td>50.31467</td>
<td>5.942724</td>
<td>2.557166</td>
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<tr>
<td>Va</td>
<td>42.13446</td>
<td>56.92258</td>
<td>46.29888</td>
<td>66.74443</td>
<td>50.57804</td>
<td>8.937804</td>
<td>2.296676</td>
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<tr>
<td>Ib</td>
<td>44.6514</td>
<td>53.35558</td>
<td>45.31722</td>
<td>52.61769</td>
<td>48.76249</td>
<td>4.377573</td>
<td>2.188786</td>
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<tr>
<td>IIb</td>
<td>42.12649</td>
<td>50.20194</td>
<td>49.1673</td>
<td>61.00629</td>
<td>51.30002</td>
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<td>IIIb</td>
<td>48.69518</td>
<td>51.57448</td>
<td>51.84409</td>
<td>60.07293</td>
<td>51.97292</td>
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<td>IVb</td>
<td>45.12454</td>
<td>50.00344</td>
<td>50</td>
<td>60.20333</td>
<td>49.37783</td>
<td>6.640294</td>
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<tr>
<td>Vb</td>
<td>45.41724</td>
<td>40.17052</td>
<td>50.41096</td>
<td>67.62425</td>
<td>50.62099</td>
<td>5.632387</td>
<td>2.051649</td>
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</table>

* 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.
RESULTS AND DISCUSSION

The percentage of scavenging activity of derivatives was calculated with absorption of different concentrations. The derivatives like IIa, Illa and IIIb showed remarkable scavenging activity when compared to ascorbic acid (Figure 1 & 2). Greater the value 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 III b showed comparable percentage of nitric oxide scavenging activity with standards reference ascorbic acid. The other derivatives show medium activity.

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