

## SYNTHESIZE, SPECTRAL, ANTIMICROBIAL AND ANTIOXIDANT STUDIES OF DIAMIDE MANNICH BASE DERIVATIVES

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### ABSTRACT

Objectives: Preparation of biologically active Mannich bases by using diamides with  $\beta$ -naphthol and Benzimidazole.

Methods: The synthesized Mannich bases 1-((1H-benzo[d]imidazole-1-yl)methyl)urea (BIUF) and 1-((3-hydroxynaphthalen-2-yl)methyl)thiourea (TNTUF) were characterized by UV-Vis, FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, MASS and Elemental analysis and evaluated to their antimicrobial and anti-oxidant activity.

Results: All the synthesized compounds showed better activity against tested microbial strains and significant anti-oxidant activities.

Conclusion: Synthesized Mannich base compound BIUF shows, better antimicrobial and anti-oxidant activity than TNTUF. More activity in BIUF may due to the presence of two N atoms in the benzimidazole adjoin with amide group.

**Keywords:** Mannich base, Urea,  $\beta$ -Naphthol, Benzimidazole, Antimicrobial activity, Antioxidant activity.

### INTRODUCTION

In the fast growing urban area the life threatening diseases and health disorders caused by new pathogens. Available drugs in the market cannot meet out the threat of newly evolving microbes and also may have some side effects. In this scenario chemists along with life science researchers involving to evolve new drugs with excellent therapeutic activities. On this basis new drugs were prepared by using Mannich reaction. In this reaction a compound containing an active hydrogen atom is reacted with aldehydes and amines with loss of water. In Mannich base reaction the active hydrogen is replaced by amino-methyl group and form carbon-carbon and carbon-nitrogen bonds which make the reaction to synthesis of many heterocyclic compounds. Mannich bases are used in wide range applications of pharmaceuticals and macromolecular chemistry [1]. Mannich bases compounds are used as antimicrobials [2], antimalarial, antiviral [3], anticonvulsive [4], anti-inflammatories [5] and fungicidal [6] agents.

The compounds derived from Mannich base reaction were implicit source to inhibit pathogens with less side effects [7]. Urea and its derivatives were showed better antimicrobial and antioxidant activities [2]. Generally heterocyclic ring with side chain have more activity than aliphatic side chained groups [8]. Design and synthesis of N containing drugs are very essential owing to its individual and broad biological characteristics. The electron withdrawing substituents showed less activity than electron donating substituents against microorganisms [9, 10]. Furthermore the electron releasing groups have more radical scavenging activity than electron withdrawing substituents attached to the phenyl ring [11, 12]. It is also enhancing the anti-inflammatory and analgesic activities [13]. The phenolic compounds are effective free radical scavenging activity [14]. Benzimidazole derivatives have higher cytotoxic activity than the thiazoline derivatives [15].

### MATERIALS AND METHODS

The chemicals and solvents were obtained from Aldrich and Merck chemical companies and used without any purification. For thin layer chromatography (TLC) readily available silica gel plates (Kieselgel 60 F254, Merck) were used to monitor the reactions and the TLC spot visualized using UV lamps. Melting points were identified by using open capillary tubes in Elico instrument and the readings were uncorrected. IR spectra were recorded in Perkin-Elmer FTIR spectrophotometer with KBr pellets. <sup>1</sup>H NMR, [<sup>13</sup>C] NMR spectra were recorded in Bruker Advance DPX 300 MHz Ultra-Shield FT-NMR Spectrophotometer using CDCl<sub>3</sub> as a solvent and

tetramethylsilane (TMS) as an internal standard. Ultraviolet-visible (UV-Vis) absorption spectra were recorded in Systronics 2202 double beam Spectrophotometer. GC-MS analysis carried out by Agilent-7890A GC instrument coupled with MS-5975 inert MSD mass selective ion detector. The micro-elemental analyses were done in Vario-EL instrument.

### EXPERIMENTAL

#### Preparation of Mannich bases

##### 1-((1H-benzo[d]imidazole-1-yl)methyl)urea (BIUF)

Urea (0.60g, 0.01M) dissolved in a minimum quantity of water and mixed with saturated ethanolic solution of benzimidazole (1.18g, 0.01M). To this solution 1 mL of 0.01M formaldehyde added very slowly, the mixture was stirred at room temperature for 24 hours and the excess of ethanol was removed under vacuum. The creamy white colour precipitate (Fig. 1) were obtained after one week of ageing. The product was washed several times with water and acetonitrile. Then recrystallized by using ethanol.

The reaction was monitored by TLC until the reaction was complete. Mol. F: C<sub>9</sub>H<sub>10</sub>N<sub>4</sub>O. Yield: 98.83%, Mol. wt: 190.20, M. P: 110-112°C. FT-IR (KBr,  $\nu$  in cm<sup>-1</sup>): 3417 (N-H, stretching), 3091 (C-H, stretching), 1662 (C=O, stretching), 1157 (C-N-C, stretching). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  8.19 (s, 1H NH), 7.17-7.65 (m, 4H, Ar-H), 5.83 (s, 2H, NH<sub>2</sub>), 5.49 (t, 2H, CH<sub>2</sub>). [<sup>13</sup>C]-NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  157.9 (s, 1C), 144.0 (s, 1C), 111.0-133.0 (m, 5C), 49.53 (s, 1C). EI-MS (positive mode) *m/z*: 190.80 (C<sub>9</sub>H<sub>10</sub>N<sub>4</sub>O<sup>+</sup>), 132.0 (C<sub>8</sub>H<sub>7</sub>N<sub>2</sub><sup>+</sup>), 118 (C<sub>7</sub>H<sub>5</sub>N<sub>2</sub><sup>+</sup>), 91 (C<sub>6</sub>H<sub>4</sub>N<sup>+</sup>). Analytical calculated from C<sub>9</sub>H<sub>10</sub>N<sub>4</sub>O%: C 56.83, H 5.30, N 29.46, O 8.41. Found %: C 56.96, H 5.28, N 29.48.

##### 1-((3-hydroxynaphthalen-2-yl)methyl)thiourea (TNTUF)

Thiourea (0.76g, 0.01M) dissolved in a minimum quantity of water and add saturated ethanolic solution of  $\beta$ -naphthol (1.44g, 0.01M) with constant stirring. Then add formaldehyde (1 mL, 0.01M) very slowly with continuous stirring. The mixture was stirred at room temperature for 24 hours. Excess of ethanol was then removed under vacuum. The mixture was standing for 48 hours to complete the reaction and the creamy white powder (Fig. 2) was obtained. Then washed several times with chloroform and water and recrystallize by using ethanol.

The reaction was monitored by TLC until the reaction was complete. Mol. F: C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>OS. Yield: 98.0%, Mol. wt: 232.30, M. P:

180-183°C. FT-IR (KBr,  $\nu$  in  $\text{cm}^{-1}$ ): 3322 (N-H stretching), 3045 (C-H stretching), 2940 (broad, O-H stretching), 1594 (C=O stretching), 1152 (C-N-C stretching).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  10.15 (s, weak, 1H, OH), 8.23 (d, 1H NH), 7.26-7.78 (m, 6H, Ar-H), 7.04 (d, 2H,  $\text{NH}_2$ ), 4.81 (s, 2H,  $\text{CH}_2$ ).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 300

MHz):  $\delta$  176.2 (s, 1C), 151.6 (s, 1C), 117.2-133.5 (m, 9C), 42.1 (s, 1C). EI-MS (positive mode)  $m/z$ : 233 ( $\text{C}_{12}\text{H}_{12}\text{N}_2\text{OS}^+$ ), 172 ( $\text{C}_{11}\text{H}_{10}\text{NO}^+$ ), 157 ( $\text{C}_{11}\text{H}_9\text{NO}^+$ ), 144 ( $\text{C}_{10}\text{H}_7\text{O}^+$ ). Analytical calculated from  $\text{C}_{12}\text{H}_{12}\text{N}_2\text{OS}$ : C 62.04, H 5.21, N 12.06, O 6.89, S 13.8. Found %: C 62.12, H 5.14, N 12.02.

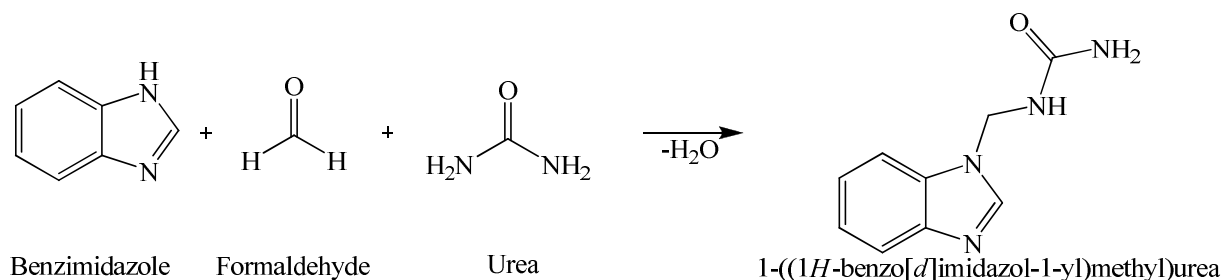


Fig. 1: 1-((1H-benzo[d]imidazol-1-yl)methyl)urea

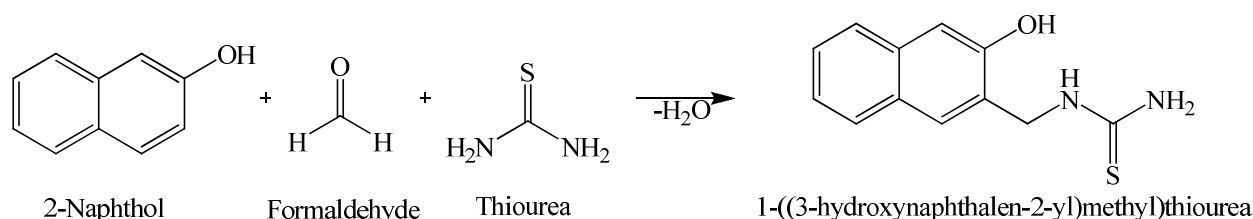


Fig. 2: 1-((3-hydroxynaphthalen-2-yl)methyl)thiourea

## Biological evaluation

### In vitro Antimicrobial activity

The antimicrobial activity of synthesized ligands were evaluated by using agar well diffusion method. The Mueller Hinton agar (bacteria) and Sabouraud dextrose agar (fungus) were used as medium preparation for *in-vitro* antimicrobial activity [16]. The microbial cultures *Staphylococcus aureus*(NCIM 5021), *Mycobacterium smegmatis*(NIRT), *Pseudomonas aeruginosa*(NCIM 5029), *Candida albicans*(NCIM 3471), *Candida tropicalis*(NCIM 3118) and *Candida glabrata* (NCIM 3236) were used for this investigation. Fresh inoculum (0.1 mL) was spread over the surface of newly prepared agar plates. The wells were made on the agar plates with the help of sterilized cork-borer (6 mm Hi-Media). The compounds were dissolved in dimethyl sulfoxide (DMSO, 4%, v/v) and 50  $\mu\text{l}$  of 20mg/mL of samples were dispensed into the agar well. The sample loaded agar plates were incubated aerobically at 37°C. The negative control well also made with 4% DMSO in the same way the above separately. Streptomycin (25  $\mu\text{g}$  disc) and ketoconazole (50  $\mu\text{g}$  disc) were used as a positive control for bacteria and fungus respectively. The entire microbial assay was carried out under strict aseptic conditions. The zones of inhibition (mm) of the compounds were examined after 24 hrs.

### In vitro antioxidant activity

The antioxidant activity of synthesized compounds were measured by three different *in vitro* antioxidant assays. Hydrogen peroxide scavenging activity method used to analyze the ability of compounds to scavenge the radicals [17]. In this method the hydrogen peroxide (20 mM) solution prepared by using phosphate buffer (pH-7.4). Various concentration of the compounds were prepared and added to the hydrogen peroxide (2mL) solution. The absorbance maxima of hydrogen peroxide at 230 nm were noted after 10 min against blank solution (phosphate buffer without hydrogen peroxide). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical, which was widely accepted tool for estimating free radical scavenging activities of antioxidants. DPPH free radical scavenging activity usually

involves hydrogen atom transfer reaction [18]. The 0.1 mM methanolic solution of DPPH (1mL) added to the various concentrations of compounds and kept for 30 min in dark place. The absorbance measured at 517nm against DPPH blank. The reducing power of Mannich bases were determined by using the standard antioxidant method [19]. The different concentration of compounds mixed with 2.5 ml of phosphate buffer (0.2 M, pH-6.6) and 2.5 ml of 1 % potassium ferricyanide then the mixture was incubated at 50°C for 30 min. Then 10% trichloro acetic acid 2.5 ml added to this mixture and 0.1% of ferric chloride 0.5 ml and absorbance maxima measured at 700 nm. For the above studies ascorbic acid was used as reference material.

## RESULTS AND DISCUSSION

The urea derivatives synthesized by Mannich condensation method and its structures were elucidated by analytical and spectroscopic methods. By doing an elemental analysis, we identified the C, H and N percentage of synthesized compounds and it is concurrece with our calculated results. In BIUF and TNTUF, Infrared spectroscopy (IR) transmittance, sharp peak at 3417 & 3322  $\text{cm}^{-1}$  show NH stretching, 1662 and 1594  $\text{cm}^{-1}$  show C=O stretching and 1157, 1152  $\text{cm}^{-1}$  represents C-N-C stretching respectively. In TNTUF the broad peak at 3300 confirms the presence of OH is searching in it. In NMR spectroscopy of BIUF and TNTUF, the signal at  $\delta$  8.19 and 8.20 due to the  $\text{NH}_2$  of urea and thiourea respectively,  $\text{CH}_2$  proton chemical shift at  $\delta$  5.49 & 4.81 respectively. The weak signal at  $\delta$  10.15 of TNTUF is due to the OH group in  $\beta$ -naphthol ring. The gas chromatography with mass spectrum analysis concluded the molecular weight of the synthesized compounds with the proposed structure of both compounds. The structural assignments are based upon the spectroscopic data of ligands 1-((1H-benzod]imidazole-1-yl)methyl)urea (BIUF) and 1-((3-hydroxynaphthalen-2-yl)methyl)thiourea (TNTUF) are good agreement with the proposed structure. The molecular formulae and elemental composition were confirmed by elemental analysis. Both the ligands gave satisfactory IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and Mass spectral results and consistent with the proposed structures. The detailed spectral and analytical data of both ligands are listed in the materials and methods.

Table 1: *In vitro* anti-bacterial activity of synthesized Mannich bases

| Bacteria                       | Zone of Inhibition (mm) |       | DMSO (4%) | Streptomycin (25µg) |
|--------------------------------|-------------------------|-------|-----------|---------------------|
|                                | BIUF                    | TNTUF |           |                     |
| <i>Staphylococcus aureus</i>   | 16                      | 16    | -         | 23                  |
| <i>Pseudomonas aeruginosa</i>  | 16                      | 17    | -         | 8                   |
| <i>Mycobacterium smegmatis</i> | 20                      | 19    | -         | 21                  |

Table 2: *In vitro* anti-fungal activity of synthesized Mannich bases

| Fungus                    | Zone of inhibition (mm) |       | DMSO (4%) | Ketoconazole (50µg) |
|---------------------------|-------------------------|-------|-----------|---------------------|
|                           | BIUF                    | TNTUF |           |                     |
| <i>Candida tropicalis</i> | 24                      | 11    | -         | 14                  |
| <i>Candida glabrata</i>   | 25                      | -     | -         | 16                  |
| <i>Candida albicans</i>   | 14                      | 10    | -         | 12                  |

The synthesized compounds BIUF and TNTUF were subjected to the antimicrobial activity against selected bacteria and fungus. The BIUF showed superior antibacterial activity against tested bacteria *S. aureus*(16mm), *P. aeruginosa*(16mm), *M. smegmatis*(20mm) and fungus *C. tropicalis*(24mm), *C. glabrata*(25mm) and *C. albicans*(14mm). The TNTUF showed good activity against tested bacteria. In fungus *C. glabrata* alone showed resistant to this compound. From this study BIUF and TNTUF have excellent activity against tested microbes given in the Tables 1 & 2. For control experiment, streptomycin (25 mg) and ketoconazole (50 mg) showed the zone of inhibition (> 8 mm for bacteria and > 12 mm for fungus) against tested bacteria and fungus. This study confirms BIUF and TNTUF ligands are the good antimicrobial agents.

The above Mannich base compounds exhibited excellent antimicrobial activities due to the presence of electron donating substituent NH and OH groups present in it [13]. The presence of heterocyclic ring and hetero atoms containing compounds has more biological activity [2]. These compounds are active because of more electrons enriched amino and phenolic group in it [14]. The structural activity relationship explained the activity of BIUF more than TNTUF because off our electron donating N atom is enriched

the antimicrobial activity. Furthermore, the presence of heterocyclic ring and hetero atoms may be a potent reason for the excellent activity.

The synthesized Mannich derivatives tested for their inhibition efficiency by using standard methods. Hydrogen peroxide scavenging activity of the compounds decreases in the following order: L ascorbic acid >BIUF > TNTUF at all concentration respectively. The potential of L-ascorbic acid to scavenge Hydrogen peroxide is directly proportional to the concentration (Fig. 3). The DPPH assay shows free radical scavenging activity of above compounds increases with increasing the concentration (Fig. 4). BIUF possessed the efficient free radical scavenging activity compared to TNTUF. The order of reducing power decreased in following order: L ascorbic acid >BIUF > TNTUF with concentration of compounds respectively (Fig. 5). The presence of electron donating group on the benzene ring in naphtholis the profound antioxidant activity [20] of TNTUF. Moreover, the thioamide moiety, hetero atom and azole compounds were effective antioxidants by scavenging radicals [20]. The BIUF had heteroatoms combined with an amide group may also increase the antioxidant activity than TNTUF.

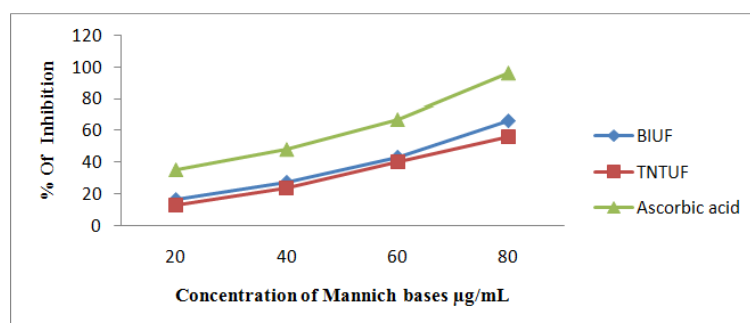


Fig. 3: Hydrogen peroxide scavenging activity of synthesized compounds

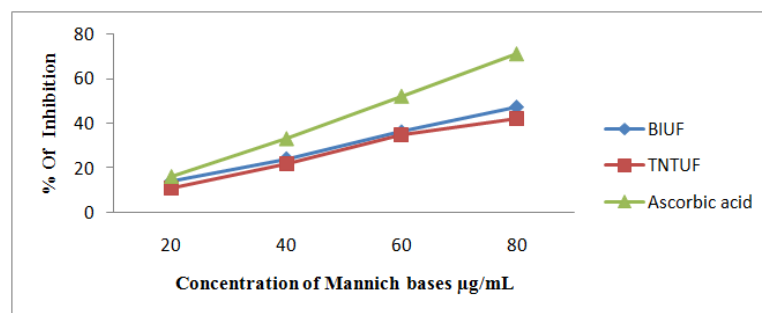


Fig. 4: Free radical scavenging activity of Mannich base by DPPH method

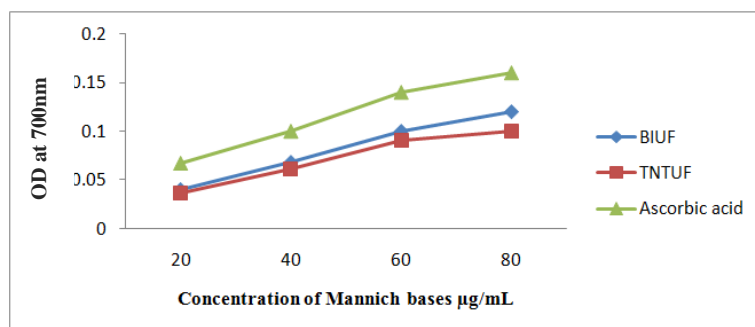


Fig. 5: Reducing power activity of synthesized Mannich base compounds

## CONCLUSION

The novel diamide derivatives obtained by Mannich condensation reactions and were characterized on the basis of analytical and spectroscopic methods. The spectral results were concluded the new compounds BIUF and TNTUF and it is concurrence with our proposed structure. The compounds were screened by biological evaluation shows better antimicrobial and antioxidant activities. The structure and biological activity relationship denotes the more activity in BIUF may due to the presence of two N atoms in the benzimidazole adjoin with amide group. The antioxidant activities of derived Mannich bases due to presence of electron releasing amide group in it. In all the cases, the BIUF shows the better activities compared to the TNTUF.

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