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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF NOVEL MANNICH BASES DERIVED FROM BETA-NAPHTHOL

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ABSTRACT

This present study is concerned with the synthesis of novel β-naphthol Mannich bases with benzimidazole and p-toluidine by condensation reaction with appropriate aldehydes. The compounds 3-(phenyl(p-tolylamino)methyl) naphthalene-2-ol (TNPTB) and 3-((1H-benzo[d]imidazole-1-yl)methyl)naphthalene-2-ol (TNBIF) were synthesized and the chemical structures were characterized using spectral and analytical techniques. Mannich bases were screened for their antibacterial and antifungal activity against opportunistic bacterial and yeast pathogens. The antioxidant activity was tested using established methods. Among the tested ligands, TNBIF showed better antimicrobial and antioxidant activity compared to TNPTB.

KEYWORDS: Mannich base, β-naphthol, p-toluidine, Benzimidazole, Antimicrobial activity, Antioxidant activity.

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INTRODUCTION

Bacterial and fungal infections are increased due to the higher number of immuno-compromised hosts hence emerging necessity to synthesize modern, safer and more effective antimicrobial agents. Various strategies and methods were used to develop antibiotics to improve the activities of new antimicrobial properties. The literature survey clearly indicated the Mannich bases are active in growth-inhibition of pathogens. The heterocyclic compounds have been very good biological activities, specifically triazole, imidazole and piperidine are attractive targets of organic synthesize due to their pharmacological activity. Mannich bases are the potent source to inhibit micro-organisms and could be used an efficient drug with minimum side effects[1]. Mannich bases derived from heterocyclic compounds exhibits unique biological activities like anticancer[2], antitubercular[3], antimicrobial[4], antimalarial[5], anti-inflammatory[6] and antioxidant activities[1]. Mannich bases used in industrial applications as corrosion inhibitors[7] due to the presence of N, O, S atoms and conjugate double bonds[8] in it. Due to the wide range of applications, Mannich bases were fascinating the synthetic and medicinal chemists towards it. This research work intends to synthesize the Mannich bases of β-naphthol compounds by condensation reaction. Mannich reaction is three component condensation reactions with compound containing active hydrogen atom reacts to aldehydes and a primary or secondary amine with liberation of water. In Mannich reaction, C–C and C–N bonds were formed by amino methylation or amino benzylation process causes the reaction more useful and important synthetic applications. Mannich bases have a broad range of application in biochemistry, pharmaceuticals and macromolecular chemistry[9]. The Mannich bases are antimalarial and antiviral[10], anti-inflammatory[11], and antimicrobials[12] activities. The β-naphthol and its derivatives showed an excellent antimicrobial activity due to presence of electron donating substituents[13]. The phenolic and amino derivatives are more active analogs because of the presence of more electrons enriched groups in it[14]. Phenolic compound acts as an effective antioxidant by scavenging radical mechanism through the antioxidant mode of chain breaking pathway[15]. The Benzimidazole and its derivatives were used as antiviral, antimicrobial agents and show's better cytotoxic activity[16].

MATERIALS AND METHODS

All the chemicals and solvents were used in this synthesize were obtained from Aldrich and Merck chemical companies and used without further purification. Precoated silica gel plates (Kieselgel 60 F254, Merck) were monitoring the reactions and visualized using UV lamps. Melting points were determined in an open capillary tube by using Elico instrument and readings were uncorrected. Ultraviolet-visible (UV-Vis) absorption spectrum was recorded in Systronics 2202 spectrophotometer at the wavelength of maximum absorption (λ_{max}). IR spectrum was recorded in Perkin-Elmer FTIR spectrophotometer with KBr disc. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance DPX 300 MHz Ultra-Shield FT-NMR Spectrophotometer using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as internal standard. GC-MS analysis was carried out by agilent-7890A GC instrument coupled with MS-5975 inert MSD with triple axis mass selective ion detector. The micro-elemental analysis was done in Vario-EL instrument.

Synthesis of Mannich bases

3-(phenyl(p-tolylamino)methyl) naphthalene-2-ol (TNPTB)

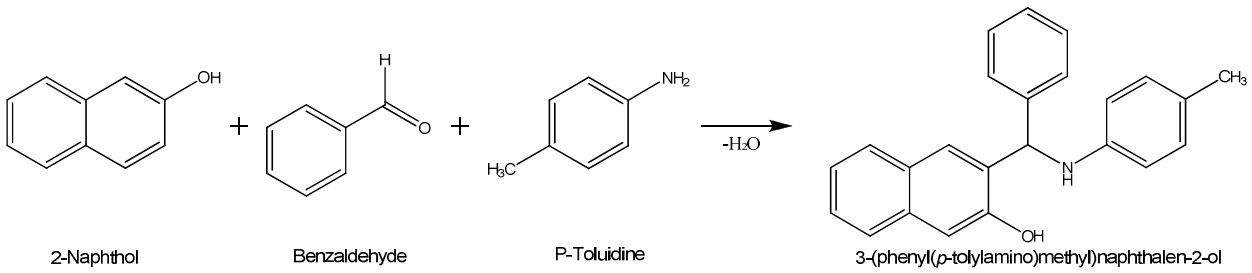
β-naphthol (1.44g, 0. 01M) dissolved in minimum quantity of ethanol and add steadily to saturated ethanolic solution of p-toluidine (1.07g, 0.01M) with constant stirring. Through this mixture 1.00 mL (0. 01M) of benzaldehyde was added slowly with continuous stirring. The mixture was stirred at room temperature for 12 hours, and the reaction monitored by TLC. After one week of ageing light brown colour solid product (Scheme 1) obtained and washed

several times with ethanol then recrystallized with acetone. Mol.F: $C_{24}H_{21}NO$, Mol.wt: 339.43, Yield: 83.33%, M.p: 148-150°C. FT-IR (KBr, υ in cm^{-1}): 3350 (sharp, NH stretching), 3022 (broad, OH stretching), 2889 (CH aromatic stretching), 1509 (NH bending), 1453 (C-N stretching), 1228 (C-O stretching), 1153 (C-N-C stretching), 748 (di substituted aromatic ring). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.77 (s, weak, 1H, OH), 6.80-7.79 (m, 15H, Ar-H), 6.14 (s, 1H, NH), 4.07 (s, 1H, CH), 2.23 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 156.4 (s, 1C, C-OH), 144.0 (d, 1C), 142.9 (s, 1C), 113.4-133.2 (m, 18C-aromatic), 63.1 (s, 1C), 20.6 (s, 1C). EI-MS (positive mode) *m/z*: 341 ($C_{24}H_{21}NO^{+\cdot}$), 261 ($C_{18}H_{15}NO^{+\cdot}$), 233.1 ($C_{17}H_{13}O^{+\cdot}$), 216.1 ($C_{17}H_{12}^{+\cdot}$), 126 ($C_{10}H6^{+\cdot}$). Elemental analysis for $C_{24}H_{21}NO$ %: C 84.92, H 6.24, N 4.13, O 4.71. Found %: C 85.05, H 6.18, N 4.69.

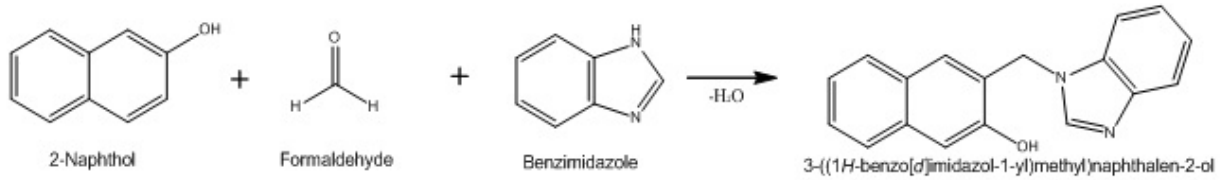
3-((1H-benzo[d]imidazole-1-yl)methyl)naphthalene-2-ol (TNBIF)

Equimolar ratio of β-naphthol (1.44g, 0.01 M) and benzimidazole (1.71g, 0. 01 M) were dissolved in minimum quantity of ethanol with constant stirring. The same molar formaldehyde (1 mL, 0.01 M) added very slowly to this mixture with continuous stirring. The mixture was stirred at room temperature for 12 hrs, and it monitored with TLC. Reddish-brown precipitate obtained and ageing given for 48 hrs to complete the reaction. The creamy-white product (Scheme 2) appeared and washed several times with ether then recrystallized by acetone. Mol.F: $C_{18}H_{14}N_2O$, Mol.wt: 274.32, Yield: 97.0%, M.p: 245-247°C. FT-IR (KBr, υ in cm^{-1}): 3043 (broad, OH stretching), 2942 (CH aromatic stretching), 1443 (C-N stretching) 1278 (C-O stretching), 1194 (C-N-C stretching), 739 (di substituted aromatic ring). ^{1}H NMR (DMSO, 300 MHz): δ 10.48 (s, weak, 1H, OH), 8.24 (s, 1H, CH), 7.13-8.09 (m, 10H, Ar-H), 5.82 (s, 1H, CH_2). ^{13}C NMR (DMSO, 300 MHz): δ 154.0 (s, 1C), 143.9 (s, 1C), 143.3 (s, 1C), 133.8 (s, 1C), 110.7-130.4 (m, 13C), 40.1 (s, 1C). EI-MS (positive mode) *m/z*: 272 ($C_{18}H_{14}N_2O^{+\cdot}$), 257 ($C_{18}H_{13}N_2^{+\cdot}$), 231 ($C_{16}H_{11}N_2^{+\cdot}$), 205 ($C_{14}H_9N_2^{+\cdot}$). Elemental analysis for $C_{18}H_{14}N_2O$ %: C 78.81, H 5.14, N 10.21, O 5.83. Found %: C 78.75, H 5.18, N 10.25.

Scheme 1
The reaction scheme of TNPTB



Scheme 2
The reaction scheme of TNBIF



Biological activity

In vitro antimicrobial activity

The antimicrobial potentialities of synthesized ligands were evaluated by agar well diffusion method using Mueller Hinton agar (bacteria) and Sabouraud dextrose agar (fungus) medium. The *Mycobacterium smegmatis* (NIRT), *Staphylococcus aureus* (NCIM 5021), *Pseudomonas aeruginosa* (NCIM 5029), *Candida albicans* (NCIM 3471), *Candida tropicalis* (NCIM 3118) and *Candida glabrata* (NCIM 3236) used for this assay. The pathogens were activated by inoculating a loopful of strain in the broth (20 mL) in a 100 mL Erlenmeyer flask and incubated at 37°C on a rotary shaker for 24 hrs. Fresh inoculum of 0.1 mL was spread on the surface of disinfected agar plates using a sterilized glass spreader. Wells were made on the seeded plates with the help of a sterilized cork-borer (6 mm Hi-Media). The ligands were further dissolved in dimethyl sulfoxide (DMSO, 4%, v/v). The compounds (100 µg/mL concentration) were dispended into the well, and the plates were incubated aerobically at 37°C for pathogens. In the same way negative control well was made with only 4% DMSO separately streptomycin (25 µg disc) and ketoconazole (50 µg disc) used as a positive control. 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 and free radical scavenging activity of the compounds were measured using three different *in vitro* antioxidant assays. Hydrogen peroxide scavenging activity method used to analyze the ability to scavenge the radicals[17]. Different concentration of the compounds and the standard ascorbic acid solution were added to the 2 ml of hydrogen peroxide solution. Which one was prepared by using buffer solution at pH 7.4. The absorbance of different concentration of compounds with hydrogen peroxide at 230 nm was determined after 20 min against blank solution. 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 1 ml of 0.1 mM methanolic solution of DPPH was added to the various concentrations of compounds. The absorbance at 517nm was noted against DPPH blank after 30 min storage in dark place. The reducing power of Mannich bases were determined by using the standard method[19]. The different concentration of compounds mixed with 2.5 ml of 0.2 M phosphate buffer with pH-6.6 and 2.5 ml of 1 % potassium ferricyanide and then the mixture was incubated at 50^{o}C for 30 min. Afterwards 2.5 ml of 10% trichloro acetic acid added to this mixture. From this mixture 2.5 ml of solution was taken along with same quantity of distilled water and then 0.5 ml of 0.1% ferric chloride added to note down the absorbance at 700 nm against method blank.

RESULTS AND DISCUSSION

The Mannich bases were synthesized by condensation method and its structures were elucidated by analytical and spectroscopic methods. The structural assignments are based upon the spectroscopic data of ligands 3-(phenyl(p-tolylamino)methyl)naphthalene-2-ol (TNPTB) and 3-((1H-benzo[d]imidazole-1-yl)methyl)naphthalene-2-ol (TNBIF) are good agreement with the proposed structure. In synthesized compounds, the IR transmittance at 2889 & 2942 cm^{-1} with broad peak shows the presence of OH streching, 1228 and 1278 cm^{-1} confirm the presence of C-O streching and 1153, 1194 cm^{-1} represents C-N-C streching of TNPTB and TNBIF respectively. In TNPTB the sharp peak at 3350 cm^{-1} confirms the presence of NH streching. In NMR spectroscopy of TNPTB and TNBIF shows, the weak signal at δ 11.77 and δ 10.48 due to the OH group in 2-naphthol ring and the strong signal at δ 4.07 & δ 5.82 due to the CH_2, CH protons respectively. The signals at δ 6.5-7.5 confirms the presence of aromatic protons in both compounds. The structural assignments are based upon the spectroscopic data of ligands 3-(phenyl(p-tolylamino)methyl)naphthalene-2-ol (TNPTB) and 3-((1H-benzo[d]imidazole-1-yl) methyl)

naphthalene-2-ol (TNBIF) are good agreement with the proposed structure. The molecular formulae confirmed with elemental analysis. Both the ligands gave satisfactory IR, ^{1}H NMR, ^{13}C NMR and Mass spectra was consistent with the assigned structures. The detailed spectral and analytical data of both ligands are listed in the materials and methods.

The synthesized TNPTB and TNBIF ligands were subjected to the antimicrobial assay. The TNPTB showed activity only in *M. smegmatis* (10 mm) and other tested pathogens were resistance to this compound, while the TNBIF showed higher antimicrobial activity (> 9 mm) against *M. smegmatis, S. aureus, P. aeruginosa, C. albicans and C. tropicalis,* but this ligand failed to control *C. glabrata* given in the Tables 1 & 2. For control experiment, streptomycin and ketoconazole showed activity in the zone of inhibition (> 8 mm for bacteria and > 12 mm for fungus) against tested pathogens. This study confirms TNPTB and TNBIF ligands having the good antimicrobial property. The Mannich base compounds exhibited significant antimicrobial activities due to the presence of electron donating substituent OH group present in naphthol[13]. The compounds with aromatic, cyclic and heterocyclic structural features showed better activity against Gram – ve bacteria [20]. Both the compounds are active because of more electrons enriched amino and phenolic group in it[14]. In TNPTB, the substituent present in *p*-positions is as well as enriched the antimicrobial activity. Furthermore, presence of heterocyclic ring and hetero atoms may be a potent reason for the significant activity.

Table 1
In vitro antibacterial activity of synthesized Mannich bases

Bacteria	Zone of inhibition (mm)			
	TNPTB	TNBIF	DMSO (4%)	Streptomycin (25µg)
	µg			
Mycobacterium smegmatis	10	16	-	21
Staphylococcus aureus	-	15	-	23
Pseudomonas aeruginosa	-	17	-	8

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

Fungus	Zone of inhibition (mm)			
	TNPTB	TNBIF	DMSO (4%)	Ketoconazole (50µg)
	µg			
Candida albicans	-	12	-	12
Candida tropicalis	-	9	-	14
Candida glabrata	-	-	-	16

The synthesized naphthols derivatives analysed for their inhibition efficiency with the standard methods. Hydrogen peroxide scavenging activity of the compounds decreases in the following order: L ascorbic acid > TNBIF > TNPTB at concentration of 80 µg/mL, 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 compounds increases with increasing the concentration (Fig 4). TNBIF possessed the efficient free radical scavenging activity compared to TNPTB. The reducing power of the compounds increased in following order: L ascorbic acid > TNBIF > TNPTB with concentration respectively (Fig 5). The presence of electron donating group on benzene ring in naphthol is the profound antioxidant activity of both compounds[21]. Moreover, the phenolic compounds were effective antioxidants by scavenging radicals through the mode of a chain breaking path. The TNBIF had two hetero atoms may also increase the antioxidant activity then TNPTB.

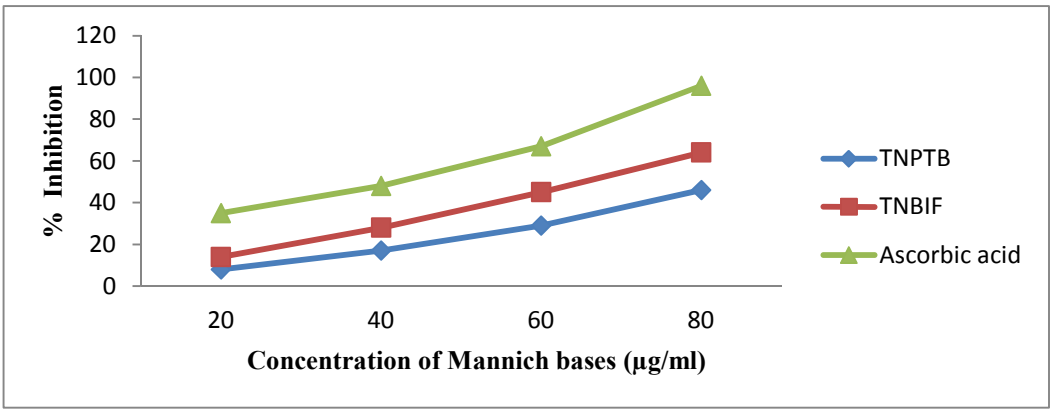


Figure 3
Hydrogen peroxide scavenging activity of synthesized compounds

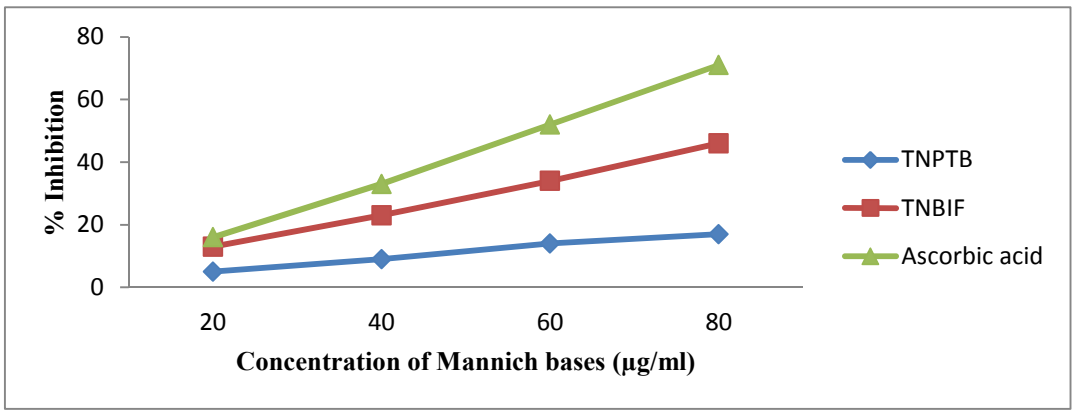


Figure 4
Free radical scavenging activity of Mannich base by DPPH method

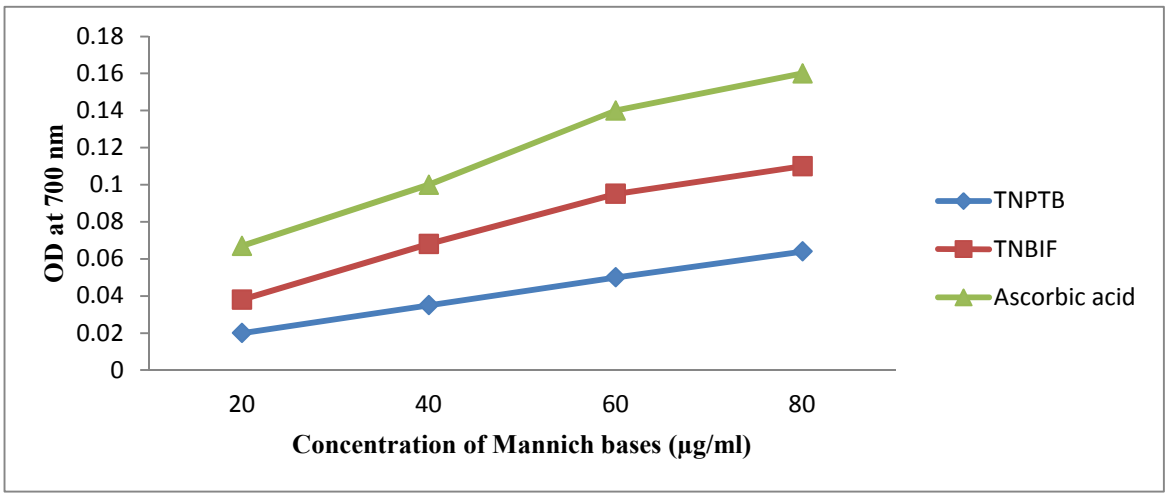


Figure 5
Reducing power activity of synthesized compounds

CONCLUSION

The novel Mannich bases derived from β-naphthol by condensation reactions were characterized on the basis of analytical and spectroscopic methods. The elemental analysis results were coincided with analytically calculated results. The NMR spectrum shows the position and structural details of the both compounds and the IR spectroscopy results confirm the presence of functional groups. The compounds were screened for biological evaluation like antimicrobial and antioxidant activities. Both the synthesized compounds show moderate antimicrobial and anti-oxidant activities. The antimicrobial study specifically focussed the skin infectional microbes and got the good results. The results of anti-bacterial, anti-fungal and anti-oxidant assay concludes the TNBIF shows the good and significant activities compared to the TNPTB. Findings shows the biological activity of TNBIF may be due to the presence of two hetero atoms in the Benzimidazole. The antioxidant activities of derived Mannich bases due to presence of electron releasing OH group. For further *in vivo* investigation in these findings can have good effect on Mannich base compounds can be used as a potential drug for anti-skin inflectional drug.

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