

## SYNTHESIS AND CHARACTERIZATION OF SOME ANTIMICROBIAL MANNICH BASES

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### ABSTRACT

Three Mannich bases: 2-hydroxymethyl-4-morpholinomethyl-1, 5-diphenyl-pent-1,4-dien-3-one(I); 2-acetamidomethyl 1-4-acetamidophenol(II) and 1-phenyl-3-(pyrrolidino-1-yl)propan-1-one(III) were synthesized via a general synthesis strategy. They were identified by spectral data (UV, IR, NMR and MS). Mannich bases I and II were evaluated for their antimicrobial potential and promising activity was observed.

**KEYWORDS:** Mannich bases, Synthesis, antimicrobial activity.

### INTRODUCTION

The Mannich reaction is a three component, usually single pot reaction, giving products known as "Mannich bases". In the Mannich reaction an N-H amine is condensed with an aldehyde and an active hydrogen component. Secondary amines are usually employed since they do not afford multiproducts. Usually formalin is the aldehyde of choice, but successful aminomethylation was achieved by employing benzaldehyde as an aldehyde component.

Mannich bases are known for their biological potential. Some anticonvulsant Mannich bases have been reported.<sup>[1,2]</sup> Others exhibiting analgesic potency were successfully synthesized.<sup>[3]</sup> Mannich bases with potential chemopreventive properties are known.<sup>[4]</sup> Also the cytotoxic activity of some Mannich bases was documented.<sup>[5,6,7]</sup> Stephen *et.al.*<sup>[8]</sup> claimed antimalarial activity for some aminomethylated phenols, while Tomas *et.al.*<sup>[9]</sup> described the antibacterial activity of some fused Mannich ketones. Afaf *et.al.*<sup>[10]</sup> reported some aminomethylated benzimidazoles with promising antimicrobial activity. The anticancer potential of some Mannich bases was outlined.<sup>[11,12]</sup> Mannich bases are considered as versatile intermediates in chemical and polymer chemistry.<sup>[13,14]</sup>

### MATERIALS AND METHODS

#### Materials

Analytical grade reagents(Sigma-Aldrich) were used. The UV spectra were recorded on a Perkin-Elmer

Lambda 2 UV-Visible spectrophotometer. Infra-red spectra were run on a Perkin-Elmer 1310 Infra-red spectrophotometer.<sup>1</sup>HNMR spectra were measured on EM-360 NMR spectrophotometer. Mass spectra were recorded on a Krates MS 80 RF mass spectrophotometer. The target molecules were evaluated for their antimicrobial potency against the following bacterial strains:

**Table 1: Test organisms.**

Microorganism	Type	Source
Escherichia Coli	Gram -ve	TCC*25922
Bacillus subtilis	Gram +ve	CTC* 8236
Staphylococcus aureus	Gram +ve	TCC 25923
Pseudomonas aeruginosa	Gram -ve	NCTC6750
Aspergillus Niger	Fungus	ATCC9736
Candida albicans	Fungus	CTC10716

\*NCTC:- National Collection of type culture , Colindale England.

\*ATCC:- American type culture collection, Rockville, Maryland, USA.

#### Methods

##### Synthesis protocols

**Synthesis of the Mannich base: 2-hydroxymethyl-4-morpholinomethyl-1,5-diphenyl-pent-1,4-dien-3-one(I)**

The Mannich base I was synthesized by adding formalin dropwise to a mixture of dibenzylideneacetone and

morpholine in absolute ethanol at 0°C. The mixture was then stirred at 0°C for four hours, and left overnight. The solvent was removed under reduced pressure to give the product.

#### Synthesis of the Mannich base: 2-acetamidomethyl-4-acetamidophenol (II)

Mannich base II was prepared by adding formalin dropwise to a mixture of p-acetamidophenol and acetamide in dioxane at 0°C. The mixture was then stirred at 0°C for six hours and left overnight. The solvent was removed *in vacuo* to give the product.

#### Synthesis of the Mannich base: 1-phenyl-3-(pyrrolidino-1-yl) propan-1-one (III)

Mannich base III was prepared by adding formalin dropwise to a mixture of pyrrolidine and acetophenone in dioxane at 0°C. The mixture was then stirred at 0°C for six hours, and left overnight. The solvent was removed under reduced pressure to give the product.

#### Antimicrobial activity

##### Preparation of microbial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10<sup>8</sup> -10<sup>9</sup> colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique<sup>15</sup>. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for 2 hours at room temperature for the drops to dry, and then incubated at 37°C for 24 hours. After incubation the number of developed colonies in each plate was counted. The average number of colonies per drop (0.02ml) was multiplied by 50 to give the viable count of the stock suspension expressed as the number of colony forming units per ml of suspension (C.F.U. /ml). Each time a fresh stock suspension was prepared, all of the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed off with sterile normal saline, and the suspension was stored in the refrigerator until used.

#### *In vitro* testing of synthesized compounds for antimicrobial activity

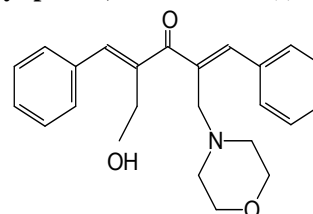
The cup-plate agar diffusion method<sup>[15]</sup> was adopted with some minor modifications. Six (ml) of the standardized

bacterial stock suspension (10<sup>8</sup> -10<sup>9</sup> colony forming units/ml) were homogeneously mixed with 600 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. Twenty (ml) aliquots of the inoculated nutrient agar were distributed into sterile Petri dishes and agar was left to settle. Each of these plates was divided into two halves. Two cups in each half (10mm in diameter) were cut using sterile cork borer (No.4). Each half was designed for a test solution. Separate Petri dishes were designed for standard antimicrobial chemotherapeutics. The agar discs were removed and alternate cups were filled with (0.1ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. The above procedure was repeated for different concentrations of the Mannich bases and the standard antimicrobial chemotherapeutics.

#### RESULTS AND DISCUSSION

Three Mannich bases were synthesized via a general synthesis protocol involving the condensation of an active hydrogen compound with N-H molecules in presence of formalin. The target molecules were evaluated for antimicrobial activity against six standard human pathogens.

#### Mannich base: 2-hydroxymethyl-4-morpholinomethyl-1, 5-diphenyl-pent-1, 4-dien-3-one (I)



The Mannich base I was synthesized by adding formalin dropwise to a mixture of dibenzylideneacetone and morpholine in absolute ethanol at 0°C.

The UV spectrum of compound I (Fig.1) showed  $\lambda_{\max}$  (MeOH) 230,325nm which is a characteristic absorption of an enone chromophore auxochromed by two phenyl moieties.

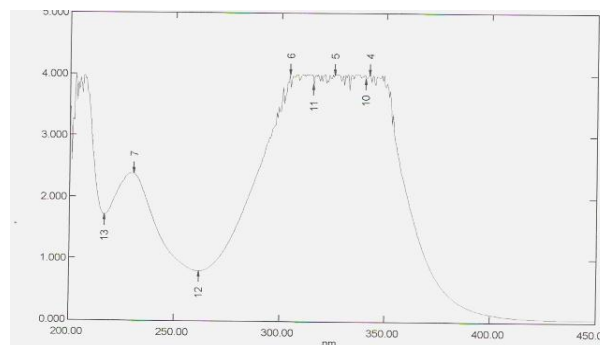


Fig.1: UV spectrum of compound I.

The  $^1\text{H}$  NMR spectrum (Fig.2) revealed the following signals

$\delta$ 2.49	singlet	8H
$\delta$ 3.30	singlet	4H
$\delta$ 7.32	doublet	4H
$\delta$ 7.47	singlet	4H
$\delta$ 7.80	singlet	2H

The signal at  $\delta$  2.49(8H) accounts for four methylenes ( $-\text{N}-$  and  $-\text{CH}_2\text{O}$ ), while the resonance at  $\delta$  3.30(4H) is characteristic of two methylenes attached to oxygen of the morpholine moiety. The aromatic protons resonate at  $\delta$  7.32, 7.47 and 7.80 ppm (residual protons of the solvent (DMSO) usually appear at  $\delta$  2.50 ppm, while DMSO water appears around  $\delta$  3.30 ppm).

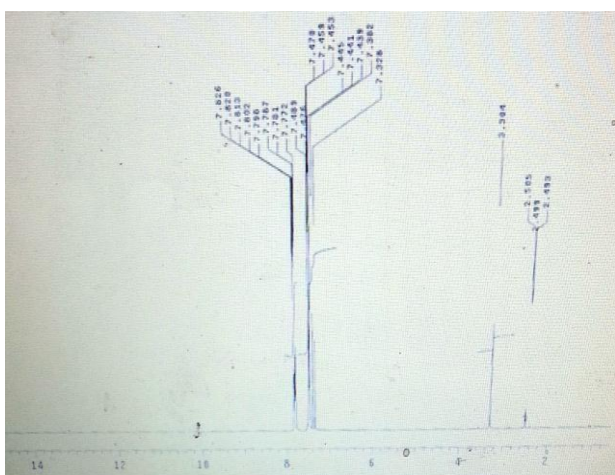


Fig. 2:  $^1\text{H}$  NMR spectrum of compound I.

The Mass spectrum (Fig.3) gave  $m/z$  363 for the molecular ion.

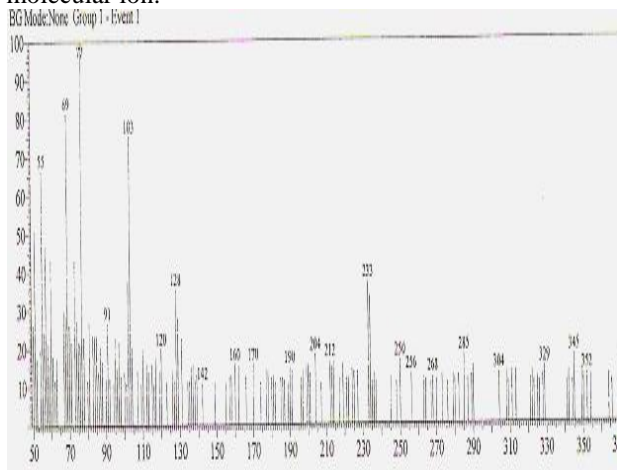
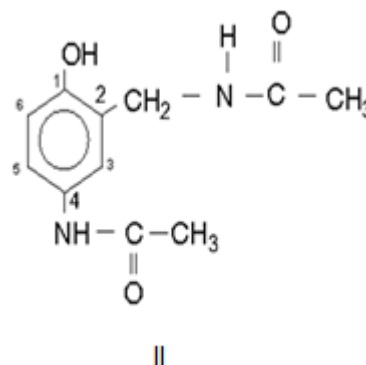


Fig 3. Mass spectrum of compound I.

On the basis of the above spectral data structure I above was assigned for this Mannich base.

Mannich base: 2-acetamidomethyl-4-acetamidophenol (II).



The Mannich base II was synthesized by adding formalin dropwise to a mixture of P-acetamidophenol and acetamide in dioxane at  $0^\circ\text{C}$ .

The UV spectrum (Fig.4) showed  $\lambda_{\text{max}}$  (MeOH) 248nm which is characteristic absorption of a  $\text{C}=\text{O}$  function as extended chromophore.

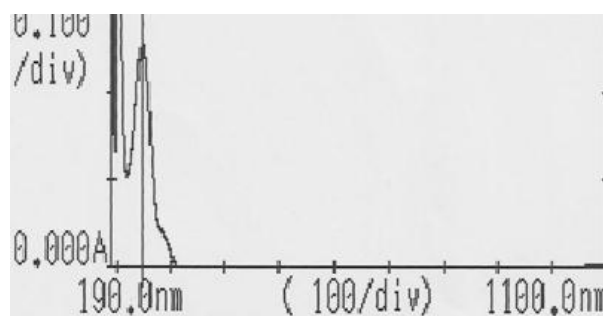


Fig. 4: UV spectrum of compound II.

The IR spectrum (Fig.5) showed  $\nu$  (KBr): 682, 802, 835 ( $\text{C}-\text{H}_{\text{Ar}}$ , bending), 1224(CN), 1440, 1508, 1564 ( $\text{C}=\text{C}$ , Ar), 1654 ( $\text{C}=\text{O}$ ), 3161(NH) and  $3325\text{cm}^{-1}$ (OH).

The Mass spectrum (fig.6) gave  $m/z$  223 for  $\text{M}^+ + 1$ .

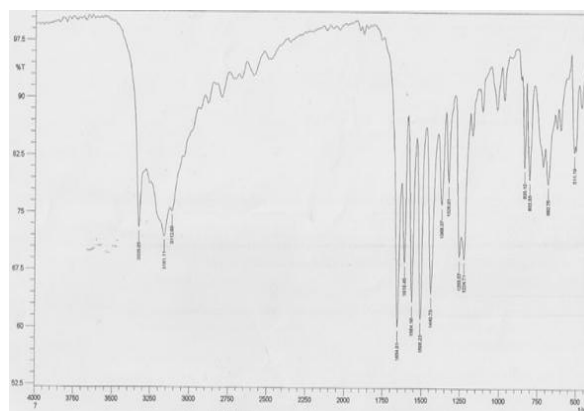


Fig. 5: IR spectrum of compound II.

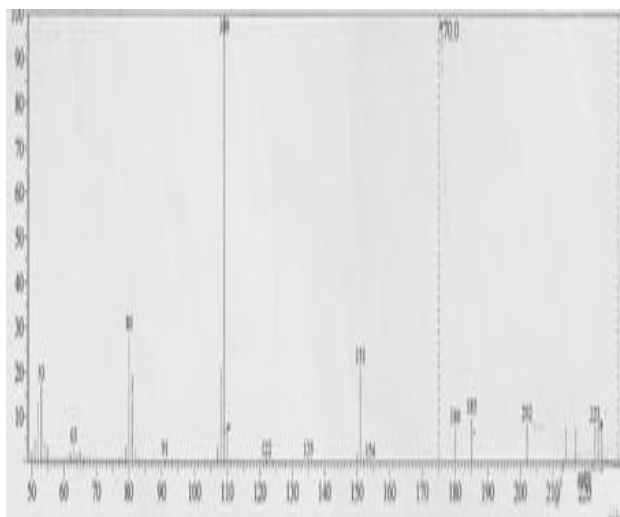


Fig. 6: Mass spectrum of compound II.

The  $^1\text{H}$ NMR spectrum (Fig.7) revealed the following signals

$\delta$ 1.97	singlet	6H
$\delta$ 3.42	singlet	2H
$\delta$ 7.32	doublet	3H
$\delta$ 9.66	singlet	2H

The signal at  $\delta$  1.97 (6H) was assigned for the two methyl groups in  $-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$ , while the singlet at  $\delta$  3.42 (2H) corresponds to the methylene group of the Mannich base being shifted downfield by the electron-withdrawal effect of the neighboring nitrogen. The aromatic protons appear as a doublet at  $\delta$  7.32 (3H), while the singlet at  $\delta$  9.66 (2H) corresponds to two  $-\text{NH}-$  groups shifted downfield by the electron-withdrawal influence of the neighboring carbonyl function.

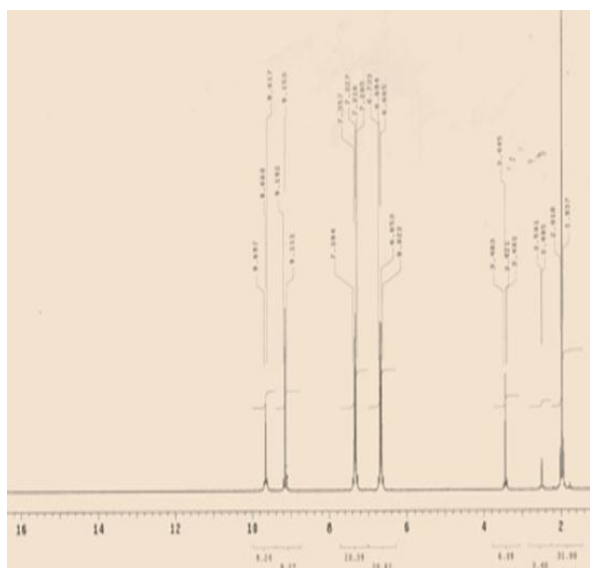
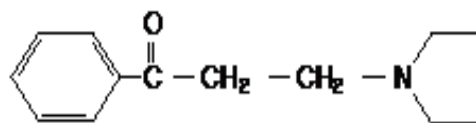


Fig.7: The  $^1\text{H}$  NMR spectrum of compound II

On the basis of its spectral data compound II was assigned structure II above.

Mannich base: 1-phenyl-3-(pyrrolidin-1-yl)propan-1-one(III)



The Mannich base III was synthesized by adding formalin dropwise to a mixture of acetophenone and pyrrolidine in dioxane at  $0^\circ\text{C}$ . The Mass spectrum (fig.8) gave  $m/z$  203 for  $\text{M}^+$ .

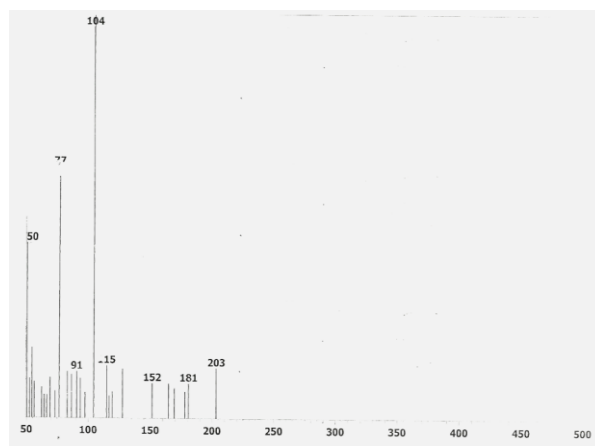


Fig. 8: Mass spectrum of compound III.

The  $^1\text{H}$  NMR spectrum (Fig.9) revealed the following signals.

$\delta$ 1.10-1.29	multiplet	4H
$\delta$ 2.14-2.34	multiplet	6H
$\delta$ 4.19	triplet	2H
$\delta$ 7.263	doublet	3H
$\delta$ 7.552	doublet	2H

The multiplet centered at  $\delta$  2.30 (6H) corresponds to three methylenes directly attached to nitrogen, while the other methylenes of the pyrrolidine moiety appear as multiplet centered at  $\delta$  1.14 (4H). The triplet centered at  $\delta$  4.19 (2H) is due to a methylene directly attached to the carbonyl function. The aromatic protons appeared at  $\delta$  7.263 (3H) and  $\delta$  7.552 (2H). (residual protons of the solvent (DMSO) usually appear at  $\delta$  2.50 ppm, while DMSO water appears around  $\delta$  3.30 ppm).

On the basis of the above argument, structure III above was assigned for this Mannich base.



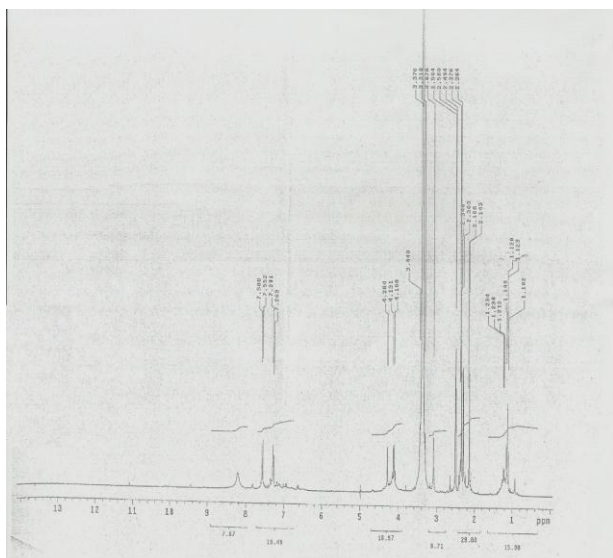


Fig. 9: <sup>1</sup>H NMR spectrum of compound III

#### Antimicrobial assay

The Mannich bases I and II were evaluated for their antimicrobial activity against six standard human pathogens, namely, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans*.

The cup-plate agar diffusion method was adopted with some minor modifications to assess the antimicrobial activity of the synthesized molecules. The diameters of inhibition zones are depicted in Table 2. The results were interpreted in commonly used terms (<9mm: inactive; 9-12mm: Partially active; 13-18mm: active; >18mm: very active).

Significant antifungal activity against *Aspergillus niger* was exhibited by compound I. It also showed moderate antimicrobial activity against all test organisms. Compounds II showed excellent antifungal activity and moderate antibacterial activity. The antibacterial and antifungal activity of standard chemotherapeutic agents is displayed in Tables (3) and (4) respectively.

Table 2: Antibacterial activity of Mannich bases I and II.

Organism	Conc. mg/ml	Comp. I	Comp. II
Bacillus subtilis	100	14	--
Escherichia coli	100	15	15
Pseudomonas aeruginosa	100	16	13
Staphylococcus aureus	100	15	15
Candida albicans	100	15	17
Aspergillus Niger	100	17	20

Table 3: Antibacterial activity of standard drug.

Drug	Conc(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycine	40	25	19	22	21
	20	22	18	18	15
	10	17	15	15	12

Table 4: Antifungal activity of standard drug.

Drug	Conc. (mg/ml)	An	Ca
Clotramizole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*  
 Ec.: *Escherichia coli*  
 Pa.: *Pseudomonas aeruginosa*  
 An.: *Aspergillus niger*  
 Ca.: *Candida albicans*  
 Bs.: *Bacillus subtilis*

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