



# MICROWAVE INDUCED SYNTHESIS AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF A NEW MANNICH BASE, N-(1-MORPHOLINOSALICYLYL) BENZAMIDE

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## ABSTRACT

The synthesis of a new Mannich base, N-(1-morpholinosalicylyl) benzamide, has been carried out under the microwave condition. The structure of the compound was established by spectral analysis and tested for antimicrobial activity against Gram positive bacteria, i.e. *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria, i.e. *Proteus vulgaris* and antifungal activity against *Candida albicans*. Tetracycline was used as standard for antibacterial activity and amphotericin for antifungal activity. The compound has shown maximum activity against both Gram positive and Gram negative bacteria.

**Key words:** Mannich base, Antimicrobial, Microwave induced synthesis.

## INTRODUCTION

In recent years, there has been much interest in the use of microwave radiation in organic synthesis with improved yield and less reaction time<sup>1-4</sup>. The application of microwave heating under solvent free condition<sup>5</sup> is a promising field of non-polluting reaction and has been a topic of current interest. It is well known from literature that compounds containing amide moiety have a strong ability to form metal complexes and exhibit a wide range of biological activities<sup>6-15</sup>. Keeping the above facts in mind, we report the synthesis of N-(1-morpholinosalicylyl) benzamide, a new Mannich base (MSB) under microwave radiation in this paper.

The demand for new and better antibacterial compounds is driven by (a) the problem of bacterial resistance and (b) the rate at which bacterial resistance develops and spreads. The most alarming aspect of this acceleration is the speed with which the resistance spreads

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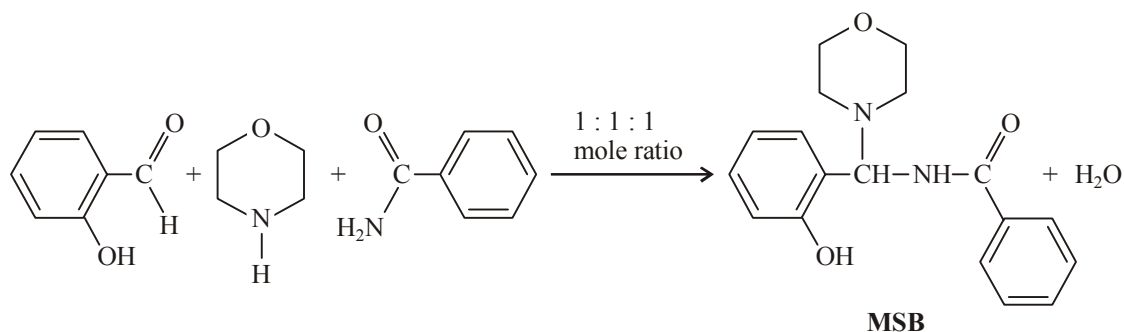
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among Gram positive organisms (*pneumococci*, *enterococci* and *staphylococci*). Many of them release relatively large amounts of  $\beta$ -lactamase into the surrounding medium, and they can destroy the  $\beta$ -lactamic antibiotics by hydrolysis of the  $\beta$ -lactam ring, this being the most prevalent mechanism of resistance<sup>16-18</sup>. Many metal complexes possess toxicological and pharmacological properties but the problem is that some lose their activity *in vivo* upon exposure to proteins and appear to have better affinities than the ligands studied for metal ions, which are deactivated once; they are embedded in the proteins<sup>19</sup>. Many drugs possess modified toxicological and pharmacological properties in the form of metal complexes, which have proved beneficial to many diseases such as tuberculosis, gastric ulcers, rheumatoid arthritis and cancers.

## EXPERIMENTAL

### Synthesis of Mannich base (MSB)

All the chemicals used were of AR grade. Salicylaldehyde, morpholine and benzamide were taken in 1 : 1 : 1 mole ratio. Morpholine 1 mL (10 mM), benzamide 1.2 g (10 mM) and 1 mL of salicylaldehyde (10 mM) were mixed and kept under microwave radiation at 130°C for 1 min and the product obtained was a yellow solid. It was recrystallised from methanol. Thin layer chromatography was used to check the purity of the compound. Yield : 71%., m.p.161°C.



*In vitro* antimicrobial activities of MSB were tested using the *well diffusion* method. The test bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris* and *Candida albicans* cultures were obtained from Microbial type culture collection (MTCC), Punjab, India. The micro-organisms were grown overnight at 37°C in Mueller-Hinton Broth at pH 7.4. Muller Hinton Broth was used for preparing basal media for the bio-assay of the organisms. The turbidity of the culture suspension was adjusted with broth or a sterile saline

solution (0.85 – 0.9%). The density of this culture was adjusted with 0.5 McFarland standard and finally inoculum size approximately of  $5 \times 10^5$  CFU/ mL.

The well diffusion test was performed using Mueller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA). The medium was prepared and autoclaved at 121°C for 15 minutes and immediately cooled in a 50-55°C water bath. The cooled medium was poured into sterile petri dishes to a uniform depth of 4 mm; this is equivalent to approximately 25 mL in a 90 mm plate. Once the medium had solidified; then the culture was inoculated on the medium. Within 15 minutes of adjusting the density of the inoculum, a sterile cotton swab was dipped into the bacterial and yeast (*Candida*) suspension or inoculated with 1 mL of the organism suspension. The sterile swab was used to streak on the surface of the MHA and SDA medium. The plate was left undisturbed for 3 to 5 minutes to absorb excess moisture. Sterilized 9 mm cork borer was used to make agar wells. Solution of the synthesized compound was diluted with 100% dimethyl sulfoxide and immediately dispensed into each agar wells of culture inoculated MHA plate using the sterilized micropipette. The plate was incubated at 35-37°C for 24 hours. Standard tetracycline (for bacteria) and amphotericin (for *Candida albicans*) were used as references. Each experiment was repeated three times to minimize the error.

## RESULTS AND DISCUSSION

The elemental analyses values were consistent with the stoichiometry of the Mannich base and the data are given in Table 1.

**Table 1: Analytical data of Mannich base**

Compound	Found (Calculated)		
	% C	% H	% N
<b>MSB</b> <b>(Mol. Wt. 312.37)</b>	69.38 (69.21)	6.15 (6.45)	8.49 (8.96)

IR spectra was recorded on the Perkin-Elmer 783 spectrometer by using KBr pellet. The bands observed at 3420, 1610 and 1115  $\text{cm}^{-1}$  have been assigned to  $\nu_{\text{N-H}}$ , amide  $\nu_{\text{C=O}}$  and  $\nu_{\text{C-N-C}}$  of morpholine group, respectively.  $^1\text{H}$  NMR spectrum was recorded on a JEOL FX-90X instrument using  $\text{CDCl}_3$  as solvent and TMS as internal standard (chemical shifts in  $\delta$ , ppm). The  $^1\text{H}$  NMR spectrum of MSB displayed the following signals: a multiplet at 7-7.5  $\delta$  (Ar-H), 6.7  $\delta$  (d, 1 CH), 6.05  $\delta$  (d, 1H, NH), 2.75  $\delta$  (morpholine N- $\text{CH}_2$ ) and 3.77  $\delta$  (morpholine O $\text{CH}_2$ ). Thus,  $^1\text{H}$  NMR and IR results confirm the structure of MSB ligand.

UV-Visible spectra were recorded on Perkin-Elmer Lambda EZ201 spectrophotometer and the data of Mannich base are given in Table 2.

**Table 2: Electronic absorption spectral data of Mannich base**

Compound	$\lambda_{\max}$	Absorbance	$\epsilon_{\max}$
MSB	303	1.832	183.2

The susceptibility of certain stains of bacterium towards MSB (a ligand) was judged by measuring the size of the inhibition diameter. As assessed by color, the compound remains intact during biological testing. The results shown in Table 3 indicate that the MSB (ligand) shows good antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus vulgaris*. Further, the antifungal activity of the compound was found to be significant against *Candida albicans*. It was also noticed that the compound was more active as antifungal than as antibacterial.

**Table 3: Antimicrobial activity of MSB**

Compound	<i>Bacillus subtilis</i>	<i>Staph. aureus</i>	<i>Proteus vulgaris</i>	<i>Candida albicans</i>
MSB	11	16	10	14

(All doses were 10  $\mu\text{g}/\text{well}$ )

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