Research Article

Evaluation of Anti-Microbial, Anti-Cancer, and Anti-Oxidant Activity of Novel 1-((1H-Indol-3YL)(Phenyl)Methyl)Pyrrolidine-2,5-Dione Mannich Base

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ABSTRACT

Mannich bases of indole are predominantly popular in metal-mediated and ligand-accelerated catalysis of enantioselective carbon-carbon bond formation. Since these compounds have multiple centres for chelation with metal ions, they are likely to be potent inhibitors of metallo-enzymes. A number of pharmaceutical and agricultural agents have indole in framework. Our present study focuses on the synthesis of Mannich base derived from the condensation of indole, benzaldehyde and succinimde and its biological activities.

Keywords: Mannich base, Spectral studies, Antimicrobial activity, Anti-cancer activity.

INTRODUCTION

The ground of coordination chemistry has grown from a readily defined and limited area into the most active research field particularly in drug designing due to their applications in pharmaceutical chemistry. Mannich bases have been studied extensively in recent times, due to the selectivity of ligands towards various metal ions [1-3]. Mannich bases when form complexes with transition metals can exhibit enhanced microbiological activities due to the presence of metal atoms or other variations of structural factors. Evolution metal ions play important role in metabolic activities of living organisms[4].

Brass chelates of Mannich bases having both sulphur and nitrogen as potential donors have been increased much interest in biochemistry because of their multipurpose use as antibacterial[5], anticancer[6], analgesic, anti-inflammatory[7], anticonvulsant[8], antimalarial[9], antiviral[10], antioxidant [11] and CNS depressant activities[12].

The widespread use of Mannich base metal complexes in biological field lies in the fact that the synthesis of Mannich base ligand introduces the basic function which can provide a molecule soluble in aqueous solvents and they can easily be transformed into a number of compounds. Many research articles are available in the literature for the synthesis of Mannich bases using indole[13,14], benzaldehyde and substituted benzaldehydes[15]. These informations have given a thrust for the synthesis of a new Mannich base from indole, benzaldehyde and succinimde using Mannich reaction.

MATERIALS AND METHODS

All the reagents and solvents used for the synthesis of ligand and its metal complexes were of the highest available purity and used as such received. The IR spectra were recorded as KBrpellets on Perkin-Elmer 1000 unit instrument. Absorbance in UV-Visible region was recorded in DMF solution using UV-Visible spectrometer. The ^{1}H&^{13}C NMR of the ligand was recorded on a Bruker instrument employing TMS as internal reference and DMSO – DMF as solvent. The mass spectral study of the ligand was carried out using LC mass spectrometer.

RESULTS AND DISCUSSION

Synthesis of the Mannich base

The ligand 1-((1H-indol-3-yl)(phenyl)methyl)pyrrolidine-2,5-dione was synthesized by Mannich condensation reaction between indole, benzaldehyde and succinimde in 1:1:1 molar ratio. indole (5g, 0.1mmol), was mixed with benzaldehyde (3.68g, 0.1mmol) followed by adding succinimde (2.5g, 0.1mmol) in DMF solution at room temperature with constant stirring for 48hrs.

After 2 weeks, a light brown colored solid mass was obtained and then washed and dried at 60°C in an air oven and recrystallized from ethanol.

The yield of the compound was obtained as 93%. (Figure 1).

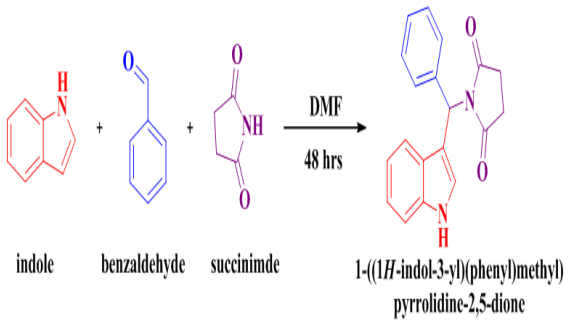
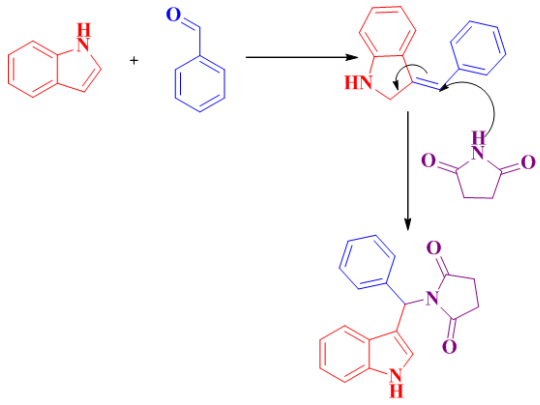


Figure 1: Synthesis of Mannich base

Mechanism

The reaction route for the synthesis of Mannich base involves the condensation reaction of benzaldehyde with indole to form the imine product. This electron deficient

imine then attacked by imide to give the ligand1-((1H-indol-3-yl) (phenyl) methyl) pyrrolidine-2,5-dione.



1-((1*H*-indol-3-yl)(phenyl)methyl) pyrrolidine-2,5-dione

FT-IR ANALYSIS

The coordination mode or bonding sites of the ligand were investigated with the characteristic absorption bands of the free ligand. The IR spectrum of the ligand 1-((1H-indol-3-yl)(phenyl)methyl)pyrrolidine-2,5-dione show a broad band in the region of 3377 & 3275cm^{-1} due to ν_{NH} stretching and aromatic C-H stretching vibrations. Aromatic C-C and C-H bending were observed as sharp bands at 1462 & 813 cm^{-1}. The characteristic C=Cstretching frequency of succinimide for the ligand was appeared in the region of 733 cm^{-1}. In the spectra of the metal complexes, the ν_{NH} stretching frequency was found to be decreased thus showing the coordination of nitrogen atom of succinimide with the carbonyl ion. At the same time, the C=Cstretching frequency of succinimide is almost same in all confirming atom of succinimide.

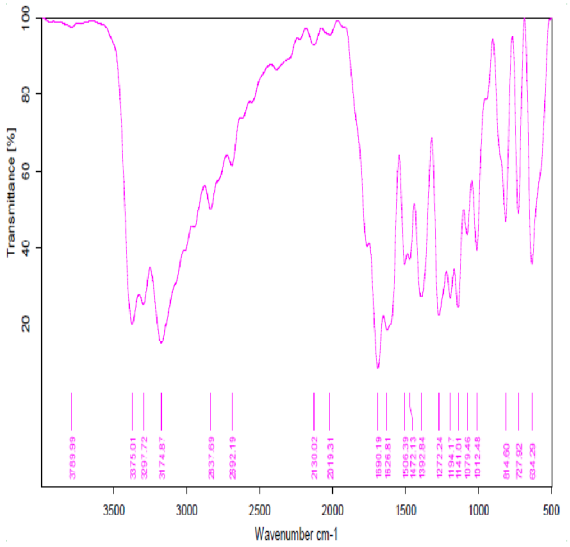


Figure 2: FT-IR Spectrum

^{1}H NMR Data (DMSO/TMS, 500.3MHz)

The ^{1}H NMR spectra of the ligand shows a singlet at 3.383δ due to CH proton of aldehyde. The multiplet between 7.079-7.772 δ corresponds to aromatic protons. The singlet for one proton at 9.716 δ is assigned to amide –NH.

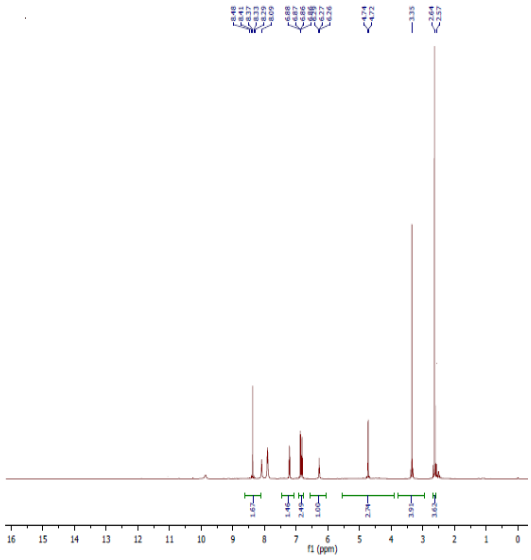


Figure 3: ^{1}H-NMR Spectrum

^{13}C NMR Data (DMSO/TMS, 125.7 MHz)

The number of signals of sharp peaks represents the number of carbons of the ligand which are not chemically equivalent. 134.56-108.61 (aromatic carbon atoms), 155.24(bridge head carbon), 183.85(carbonyl carbon).

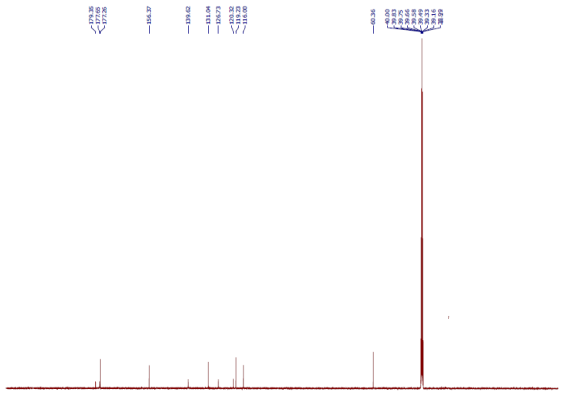


Figure 4: ^{13}C-NMR Spectrum

LC Mass Data: Calculated for $C_{19}H_{16}N_2O_2S$*m/z*=304.40; Found 306.15 (M+2).

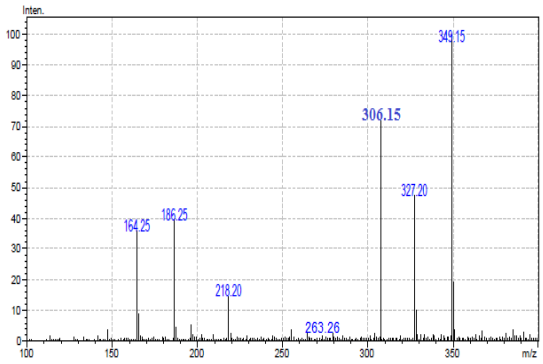


Figure 5: LC-Mass Spectrum

Anti-bacterial activity of Mannich base

The minimal inhibitory concentration of ligand 1-((1H-indol-3-yl)(phenyl)methyl)pyrrolidine-2,5-dione was found to be 300µg for *E.coli* and *B.subtilis.* This is well marked with the reduction A_{600} with the increase in concentration of drug in the medium. The activity was higher rate at high concentration, at low concentrations survival of bacteria was observed. The inhibitory effect was proved with well-diffusion method and cleared zone of inhibition was observed with Mannich base shown in Table 1. The effect of ligand as anti-bacterial agents has been discussed in the literature[16].

Table 1: Antibacterial and antifungal activities (mm) of the synthesized compound

Tested microorganisms	Compound
E. coli	16
S. aureus	20
K. pneumonia	20
M. smegmatis	15
P. aeruginosa	15
E. cloacae	15
B. megaterium	16
M. luteus	17
K. fragilis	14
R. rubra	10
S. cerevisiae	17

Cytotoxicity of Mannich base

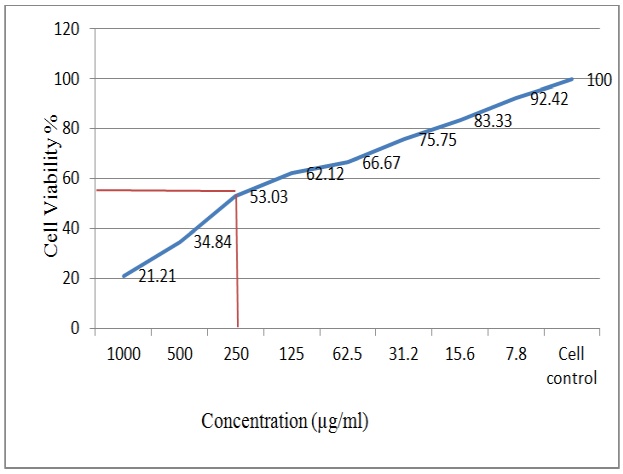


Figure 6: The effect of the ligand BNBTU in inhibition of the growth of cancer cells.

The effect of Mannich base ligand against cancer cells was analyzed by the MTT assay. The drug was able to reduce the viability of HeLa cells in a dose-dependent manner, as shown in Figure 4. The IC_{50} value of the enzyme was found to be 250 µg/ml when the cells were treated with the drug for 24 hrs.

These results proved that the cytotoxic nature of the Mannich base against HeLa cells was effective. IC_{50}concentration was used anti-oxidant assays. The result was concurred that Mannich base unveil the dose dependent toxicity against cancer cells.

When the concentration of the ligand goes beyond 250 µg/ml, more than 50 % of the cells shown ruined structure. Based on the studies, scientists agreed that Mannich bases having the potential to inhibit the proliferation of cancer cells[17]. The ligand had shown greater effect against cancer cells than normal cells (Figure 6).

Effect on iron reduction as anti-oxidant

The ligand exhibited anti-oxidant activity as, measured by DPPH method. These assays prove that ligand compound have the ability to scavenge free radicals generated *in vitro* by donating hydrogen atom[18]. The ligand compound at a concentration of 250 µg/ml demonstrated equal or higher activity than the standard anti-oxidants analyzed as illustrated in Table 2. Observing the outcomes from DPPH assay, act as anti-oxidant agents.1-((1H-indol-3-yl)(phenyl)methyl)pyrrolidine-2,5-dione had shown greater anti-oxidant effect of the compound.

Table 2: Anti-oxidant activity of selected metal complexes Anti-oxidant Activity

Concentrations (%)	Anti-oxidant Activity
0.05%	+
0.15%	+
0.20%	+
0.25%	+
0.50%	+

Anti-fungal activity

The results from well-diffusion assay confirmed that the ligand have the potential of inhibiting fungal growth. Samples were shown the inhibition against fungal growth. The inhibition zones were measured and compared with controls. At the concentration of 400µg/ml the metal complexes potentially increase the clear zone against the growth of the fungus. This demonstrates that Mannich base compound have the anti-fungal activity (Table 1). The antifungal activity of the compound was compared with standard drug Flucanozole. Among screened compounds, ligand emerged as active against fungal strains. Mannich bases are physiologically active because of the molecule solubility in aqueous phase. Compared with other compounds the ligand 1-((1H-indol-3-yl)(phenyl)methyl)pyrrolidine-2,5-dione show cases its potential in reducing the growth of fungus[19].

CONCLUSION

This paper describes the summary of Mannich reaction, its important properties and also discussed about and their biological importance. Based on the spectral data, the ligand behaves as bidentate through the oxygen atom of indole and nitrogen atom of succinimide. The biological activity of the synthesized compound shows marked

activity against the selected microorganisms. The cytotoxic effect of the newly synthesized ligand 1-((1H-indol-3-yl)(phenyl)methyl)pyrrolidine-2,5-dione have been found good inhibition activity against the cancer cell line.

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