PUBELESE PUBLISHER
ICCER-2017
JOAASR Journal of Advanced Applied Scientific Research
INTERNATIONAL CONFERENCE ON CHEMICAL AND ENVIRONMENTAL RESEARCH

SYNTHESIS, CHARECTERIZATION AND ANTIBIOLOGICAL ACTIVITIES OF N'-(2-HYDROXYPHENYL)-N'- (4-METHYLPIPERAZIN-1-YL) FURAN-2-CARBOHYDRAZIDE

N. ANGAMUTHU *, J. AHAMED SULTHAN and A. SENTHILRAJA.

PG Department of Chemistry, Dr. R.K.S College of arts and science, Kallakurichi, Tamilnadu

ABSTRACT

Present study deals with synthesis of novel Mannich bases from **N'**-(2-HYDROXY PHENYL)-N'-(4-METHYLPIPERAZIN-1-YL) FURAN-2-CARBOHYDRAZIDE instead of formaldehyde, other aliphatic or aromatic aldehydes or ketones can be employed. The amine used may be ammonia or 1^{o} or 2^{o} aliphatic amine. Mostly diethyl amine is preferred to use. The newly synthesized compounds were characterized by elemental analysis for IR, electronic absorption spectra, TLC. All the synthesized compounds were tested for their antibacterial activities against Gram positive and Gram negative bacteria, antimicrobial activities and antifungal activities

Key words: IR, Antimicrobial activities, UV-Visible spectra and TLC.

INTRODUCTION

The Mannich reaction is the aminoalkylation reaction, involving the condensation of an enolizable carbonyl compound (α-CH acidic compound) with a non enolizable aldehyde (like formaldehyde) and ammonia or a primary or a secondary amine to furnish a β-aminocarbonyl compound, also known as Mannich base. The reaction is usually carried out with the hydrochloride salt of amine. This salt exists in equilibrium with the free amine and proton. Hence the acidic conditions are maintained in Mannich reaction. The Eschenmoser's salt, $[(CH_3)_2N=CH_2]^+I^-$ is used as a source of formaldehyde and dimethyl amine for Mannich reactions. Since the β-aminocarbonyl compounds can be conveniently reduced to β-aminoalcohols, which show considerable pharmacological activity, the Mannich reaction plays an important role in pharmaceutical chemistry.

The Mannich reaction of acetophenone with formaldehyde and dimethylaminium chloride in alcohol furnishes the salt of 2-(dimethylamino)-1-phenylethanone, which can be conveniently eliminated to acrylophenone (1-phenylprop-2-en-1-one), an α,β-unsaturated compound.

Dialkylketones, cyclo alkanones as substrates in the formation of Mannich base [1]. The synthesis of Mannich bases using acetone as a substrate to react with formaldehyde and dimethyl ammonium chloride. The product 1-chlorodiethylamino-ethyl methyl-1-one forms in several minutes [2].

2-hydroxy -4-methoxy acetophenone act as a substrate in Mannich base reaction. 2,4-dimethyl naphthol, acetyl furan, acetyl thiophenes, and acetyl pyrrole undergoes the Mannich base reaction corresponding 2-aminoethyl ketones [3]. Benzylidenacetone, anthraquinone derivatives have synthesized by aminomethylation of appropriated alkyl –ketonic anthraquinone [4].

The synthesized Bis-pyrimido imidazole fused ring heterocyclic adduct with urea and urea derivatives of Mannich base for CNS depression. Mannich bases as well as urea derivatives have found to posses potent CNS depression activities [5]. Hydroxyl indoles, hydroxyl quanolines, hydroxybenzofuranes, hydroxy coumarins have been reported as substrate in Mannich base reaction [6].

From the above literature studies it has been clearly understand that no work has been carried out using Benzohydrazide as a substrate for the Mannich base synthesis. Hence, an attempt has been made to synthesis of Mannich base.

Microwave enhanced solventless Mannich condensation of terminal alkynes and secondary amines with para formaldehyde on cuprous iodide doped alumina has been developed [7]. β – aminoalkynes are generated in good yields. A Mannich base, N-(1-morpholinobenzyl)benzamide [8]. (MBB) has been prepared by the condensation of morpholine, benzamide and benzaldehyde in 1:1:1 mole ratio. Using MBB. Cobalt (II), nickel(II), copper(II) and zinc(II) complexes have been synthesized and characterized through spectral methods. It is found that the coordination takes place through deprotonated carbonyl oxygen and nitrogen atom of morpholine.

Acetamide forms a light blue complex [9] with copper (II) sulphate having 2:3 stoichiometry [10]. The complex decomposes in the range of 210-240°C resulting in the formation of a 2:1 complex and the acetamide moiety is lost at 245-260°C. Light green anhydrous copper(II) complex with composition $[Cu(CH_3CONH_2)_2Cl_2]$ in alcoholic solution has been reported by saleeva et al [11]. The infrared spectrum of the complex indicates that coordination is through acetamide oxygen. Coper(II) bromide under similar condition forms a complex with stoichiometry $[CuBr_2.4L.2H_2O]$ [12].

Complex of N-methylurea with chromium(II), manganesh(II), cobalt(II), nickel(II), copper(II), nickel(II) and iron(II) have been isolated and characterized as M-O bonded moieties [13-14].

Several copper (II) complexes with bis(thiosemicarbazone) have been studied to obtain a superoxide dismutase (SOD) like drug that is able to cross the cell membrane and reach intracellular superoxide generating cell [15-17]. It has been reported that salicyladehydesemicarbazone and thiosemicarbazone ligands [18] are advantageous in designing active anti-cancer agent whose antiproliferative activities can be modulated following their copper(II) / copper(I) redox couple.

A thorough literature survey over the synthesis of Mannich bases reveals different aldehydes have been employed as reactant. Among the aldehydes studied so far, formaldehyde, benzaldehyde, substituted bensaldehyde are noteworthy.

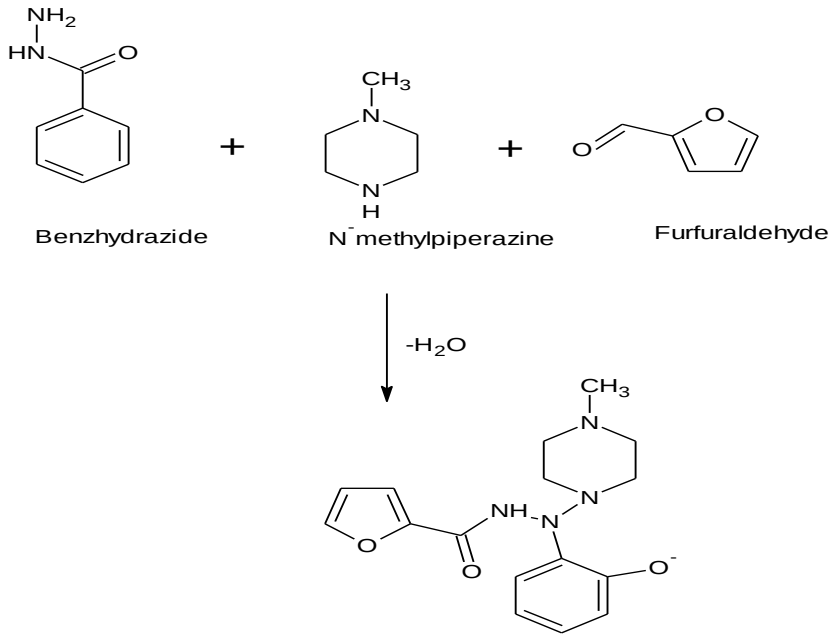
A few reports are available on the synthesis of Mannich bases using hetero aldehyde, such as furan-2-aldehyde, thiophene-2-aldehyde. From the above statement, it is cleared that no work has been carried out using anisaldehyde.

Hence in the present study an attempt has been made to synthesis of Mannich bases by reacting furfuraldehyde, n-methylpiperazine and benzhydrazide.

EXPERIMENTAL METHODS

This provides a brief account of the analytical and physical methods employed for the characterization of ***N*'-(2-HYDROXYPHENYL)-*N*'-(4-METHYLPIPERAZIN-1-YL) FURAN-2-CARBOHYDRAZIDE** and its Cu (II) complex.

Furfuraldehyde, N-methyl pipeprazine and Benzhydrazide were taken in 1:1:1 ratio. 1.7 ml of N-Methyl pipeprazine was taken in a round bottom flask and 5 ml of ethanol was added. To this solution 3.4 g of benzhydrazide was added and stirred well for half an hour by keeping the reaction mixture on magnetic stirrer. Ethanolic solution, of Benzhydrazide was added to the above mixture and stirring was continued under ice cold condition. The compound was formed and recrystallized by using ethanol.



N'-(2-hydroxyphenyl)-*N*'-(4-methylpiperazin-1-yl)furan-2-carbohydrazide

DETECTION of NITROGEN

Lassigne's test was performed to detect the presence of nitrogen. A small piece of sodium metal is fused in a fusion tube and a few crystal of sample are added. The tube is heated to red hot quickly plunged in to 5 ml of water. The solution is boiled and filtered. 1 ml of this solution is mixed with few ml of freshly prepared $FeSO_4$ solution and then acidified with dilute sulphuric acid. The appearance to blue colour indicates the presence of nitrogen in the compound.

3). MELTING POINT DETERMINATION

Melting point is determined in an open capillary tube using melting point apparatus. The synthesized compound was powdered and packed in a fusion capillary tube. Then the capillary tube was inserted in a Toshnival Melting point apparatus. A 360° C sensitive thermometer was inserted. The point at which substance melted was immediately noted. The Melting Point was found to be 160°-162°C.

4). THIN LAYER CHROMATOGRAPHY

TLC is a simple, quick and inexpensive procedure that gives the chemist a quick answer to confirm the formation of product. TLC is also used to analyze the purity of the compound. To prepare the TLC plate, slurry of silica gel prepared by adding chloroform and methanol in the ratio 1:9 to silica powder is uniformly coated on a thin glass plate.

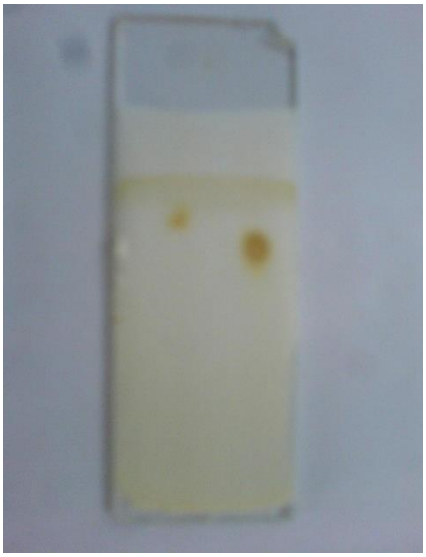
About 100 g of silica powder was taken in a beaker. 50 ml of chloroform was added, followed by 5 ml of methanol and then the mixture was stirred well. The slurry thus prepared was used for coating the plate. Before this, the plate were washed well and kept in an oven and dried. The clean plate was dipped into the slurry and taken out and then it was dried. The dried plates were used for the experiments. A single spot on TLC silica gel glass plates with a methanol conformed the purity of the synthesized sample.

$$R_f = \frac{Distance\ travelled\ by\ Sample}{Distance\ travelled\ by\ solvent}$$

$$R_f = \frac{2.0}{3.7}$$

$$= 0.540\ cm^{-1}$$

TLC PLATE OF *N*'-(2-HYDROXYPHENYL)-*N*'-(4-METHYLPIPERAZIN-1-YL)FURAN-2-CARBOHYDRAZIDE



MOLECULAR WEIGHT DETERMINATION

The molecular mass of the compound is determined by Rast method using naphthalene as the solvent. When a non volatile, solute is dissolved in a non Electrolyte solvent, decrease in freezing point of the solvent is observed. The depression in freezing point is directly related to molality of the solution.

Melting Point of Solvent (Naphthalene) $T_1 = 80°C$

Weight of the Solute (Compound) $W_2 = 0.002$ g

Weight of the Solvent (Naphthalene) $W_1 = 0.02$ g

Freezing Point of Mixture $T_2 = 77.8°C$

$$\Delta T_f = T_1 - T_2$$

$$= 80° C - 77.8°C$$

$$= 2.2° C$$

$$\Delta T_f = \frac{K_f \, X \, W_2 \, X \, 1000}{M_2 \, X \, W_1}$$

$$M_2 = \frac{K_f \, X \, W_2 \, X \, 1000}{\Delta T_f X \, W_1}$$

$$M_2 = \frac{1000 \, X \, 7.0 X \, 0.002}{2.2° X \, O.O2}$$

$$= 318.18 \text{g / mole}$$

The molecular mass of the compound determined by Rast method using naphthalene as the solvent. The molecular weight determination using naphthalene as solvent gave the value of 318.18g / mole, which is very close to expected value of 315 g / mole.

IR SPECTROSCOPY

IR Spectrum of ***N*'-(2-HYDROXYPHENYL)-*N*'-(4-METHYLPIPERAZIN-1-YL) FURAN-2-CARBOHYDRAZIDE** was recorded to find out the functional groups present and to propose a structure. The IR spectrum of the compound shows.

1. The broad band appears at 3245cm^{-1} is assigned to stretching of amide NH.

2. The broad band in the region 2853 cm^{-1} is due to aromatic C-H stretching.

3. The band appearing at 1650 cm^{-1} is assigned to (C=O) stretching.

4. The absorption band in the region 1390 $cm^{-1}$is assigned to (C-N).

5. The C-N-C stretching frequency of N-methyl piperazine is appeares at 1222 cm^{-1} .

Table: 1 IR SPECTRAL DATA

FUNCTIONAL GROUP	FREQUENCY cm^{-1}
N - H (Stretching)	3245
Aromatic C – H bending	2853
C =N	1650
C-N	1390
C-N-C	1222

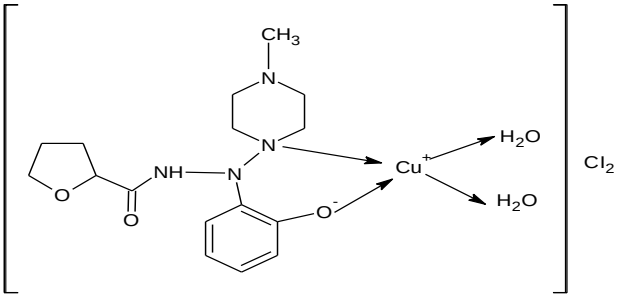
INTERPRETATION OF IR SPECTRUM

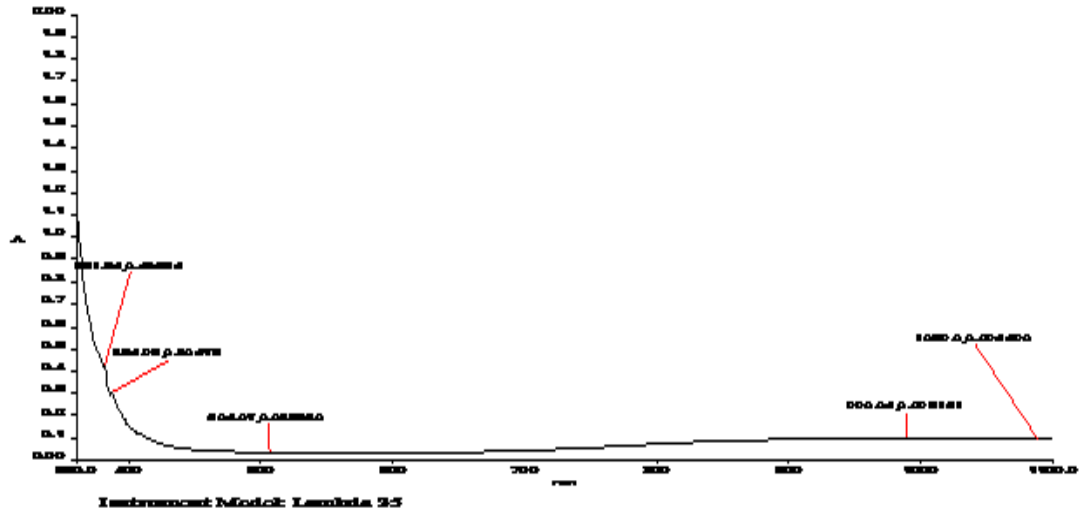
In order to study the binding mode of ligand to the metal ion in the complex, the IR spectrum of the free ligand was compared with the spectrum of complex. The band due to γC=O at 1643 cm^{-1} in the spectrum of the ligand observed has been found shifted to 1598 cm^{-1} in the spectrum of the complex indicating the co-ordination of oxygen atom of C=O with metal ion. The band at 3203 cm^{-1} assigned toγ (OH) in the spectrum of the ligand is shifted to 3428 cm^{-1} in the spectrum of the complex indicating the co-ordination of deprotonated OH and the NH with the metal ion. These results indicate the ligand acts as a negative bidentate donor.

ELECTRONIC ABSORPTION SPECTRUM

The electronic spectra of copper (II) complex in DMSO solution is recorded and is shown in figure. Copper (II) complex shows the absorption band at 19762 cm^{-1}. Which is assigned to $^2B_{1g} \rightarrow {}^2B_{2g}$, $^2A_{1g}$ & ^2E_g transitions. These are attributed to square plannar geometry.Based on the above analytical and spectral studies; the structure of the complex is established as shown below.

UV SPECTRUM OF *N*'-(2-HYDROXYPHENYL)-*N*'-(4-METHYLPIPERAZIN-1-YL) FURAN-2-CARBOHYDRAZIDE COPPER (II) COMPLEX





SYNTHESIS AND CHARACTERISATION

SYNTHESIS OF COPPER II COMPLEX

To a methanolic solution of $Cu(II)Cl_2$ was taken in round bottom flask and methanolic solution of ligand was added. It was stirred well using magnetic stirrer for an hour. The yellow crystalline solid complex was formed and filtered with distilled water and dried.

ESTIMATION OF COPPER

The amount of Copper presence may be estimated by using colorimetric technique. About 0.764 g of copper complex was mixed with 3 ml of concentrated nitric acid in a china dish and roasted for evaporation. The procedure was repeated till the complete decomposition of organic part of the complex. The residue obtained was made up to 100 ml in a standard flask using distilled water. 5 ml of this solution pipetted out into a boiling tube. 2 ml of 5% $K_4[Fe(CN)_6]$ solution was added. The total volume was made up to 20 ml by adding water and the solution was stirred well. The optical density of this solution was measured and the corresponding concentration was determined from the calibration curve.

Weight of the Copper Chloride = 0.759 g

170.48 g of Copper Chloride contains 63.54 g of Copper

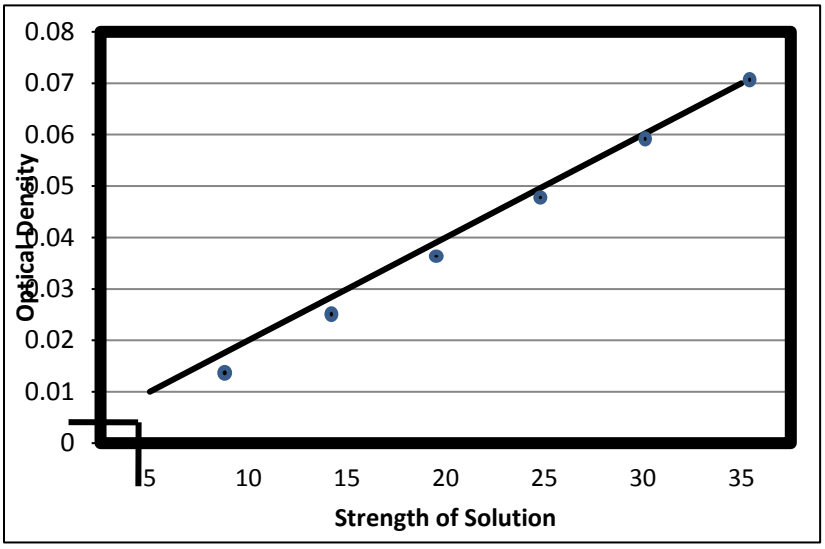
Weight of Cu Present in 0.759 g of $CuCl_2$ $= \frac{63.54}{170.48}$ X 100

Contains in 100 Ml **= 0.3g**

Table: 2

S.No	Volume of the Solution (ml)	Strength of the Solution(ppm)	Optical density
1.	2	0.00199	0.02
2.	3	0.00299	0.03
3.	4	0.00398	0.04
4.	5	0.00498	0.05
5.	6	0.00598	0.06
6.	7	0.00677	0.07

GRAPH



DETERMINATION OF WEIGHT OF Cu^{2+} IN THE COPPER COMPLEX SOLUTION

Weight of the copper complex = 0.76 g

5 ml of Copper Complex Solution Contains

= 36 ppm of Cu

$$= \frac{10\ X\ 6}{20} \times 10$$

30ppm = 30mg

REPORT

The amount of Copper present in the whole of the Copper Complex Solution = 0.3g

MOLAR CONDUCTANCE MEASURMENT

Complex Molecular Weight = 485.48g.

$$M = \frac{W}{M.wt} \text{ x } \frac{1000}{V\ in\ mL}$$

M = 0.048g.

0.01 N (Concentration)

0.02 Specific Conductance (K) = Cell constant x Conductance

$$= 0.108 \text{ X } 1.00$$

$$= 0.108$$

$$\lambda = \frac{1000\ X\ K}{concentration}$$

$$= \frac{1000\ X\ 0.108}{0.001}$$

$$= 108 \text{ X } 10^{-3}$$

The molar conductance of 10^{-3} M solution of the complex in DMSO was measured. The calculated molar conductance of 108 ohm^{-1} cm^2 mol^{-1} indicates the electrolytic behaviour of the complex.

BIOLOGICAL ANALYSIS:

ANTIMICROBIAL ACTIVITY

The synthesis and characterization of biologically activity compound and complexes getting much attention during recent years among chemists. Search through the literatures reveals that compounds of Furfuraldehyde, N-methyl piperazine and Benzhydrazide have great activity against micro organisms. Among the complexes of transition metals, Cu complexes show more activity. In the present work the biological activity for the ***N*'-(2-hydroxyphenyl)-*n*'-(4-methylpiperazin-1-yl)furan-2-carbohydrazide** and its Cu (II) complex were carried out by disk diffusion technique. The test micro organisms of gram positive *Staphylococcus* gram negative *Escherichia coli* and fungi *Candida algilcans* and *Aspergillus niger* were obtained from Eumic analytical lab and research institute, trichy and maintained by periodical sub culturing on nutrient agar and Sabourand dextrose medium for bacteria and fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control.

ANTIBACTERIAL ACTIVITY

To study the antimicrobial activity nutrient agar is used as a medium. This is prepared by dissolving 5 g of yeast extract, 10 g meat extract, 5 g of peptone, 5 g of NaCl and 20 g of agar in 100 ml of distilled water in a clean conical flask and the pH is maintained at 7. The solution is boiled to dissolve the medium completely and sterilized by autoclaving at 7 kg pressure (121°C) for 15 minutes, after sterilization 20 ml media poured into the sterilized petri plates. These petri plates are kept at room temperature for some time after a few minutes, the medium solidifies in plate. Then this is inoculated for 12 hr. After the incubation, this is inoculated with microorganisms, using simple swabs. All these manipulation are carried out with atmospheric air under aseptic condition

ANTIFUNGAL ACTIVITY

The potato dextrose agar PDA is used as a medium for antifungal activity, the nutrient agar is prepared by dissolving 20 g of potato extract 20g of agar and 20 g of dextrose in one liter of dissolving distilled water in a clean conical flask. The solution is boiled to dissolved the media completely and sterilized by autoclaving with 7 kg pressure (121°C)for the 30 minutes after the sterilization 20 ml media poured in to the sterilized petri plates. These petri plates are kept at room temperature for some time. After a few minutes, the medium get solidifies in plate. DMSO (0.5 µL) is used as solvent and clotrimazole (10 µg\disk) as control in a typical procedure, a well made on the agar medium is inoculated with microorganism and it is filled with test solution using a micro pipette (50 µL) and the plate is incubated at 35°C for 72 hr. During this period the test solution diffuses and affects the growth of the inoculated microorganisms. A zone is developed on the plate and the inhibition zone are measured by measuring the diameter of inhibited zone in mm. the zone of inhibition value are given in the table.

ANTIMICROBIAL DATA

The sample and their efficiency of inhibiting effect has been assessed using the disc diffusion method by measuring the diameter by inhibition zones. The result were depicted in the table 3.

Table: 3 ANTIMICROBIAL DATA FOR SYNTHESIZED SAMPLE

S.No	Name of the Micro organism	Antibiotics	Control	DMSO	Sample	Recrystal
1	*E.Coli*	**Gentamycin**	**-**	**-**	**17**	**-**
2	*Staphylococcus*	**Gentamycin**	**-**	**-**	**14**	**-**
3	*Candida*	**Amphotracin-B**	**-**	**-**	**27**	**-**
4	*Aspergillus nigar.*	**Amphotracin-B**	**-**	**-**	**-**	**-**

Antibacterial Screening (gram positive) of N'-(2-hydroxyphenyl)N'-(4-methylpiperazin-1-yl)furan-2-carbohydrazide and its Cu (II) Complex-against

E-coli **Candid** **Staphylococcus** **Aspergillusniger**

CONCLUSION

The first chapter of this dissertation describes the summary of Mannich reaction mechanism and applications. The literature survey reveals that in many of the compound and metal complex of Mannich base reaction.the aim and scope of the present work. In this chapter importance of the synthesis of ***N*'-(2-HYDROXYPHENYL)-*N*'-(4-METHYLPIPERAZIN-1-YL) FURAN-2-CARBOHYDRAZIDE** and its Cu (II) complexes.another one is experimental techniques employed for the characterization of the synthezised compound. The molecular weight and melting point of the compound are determined.The synthesized and characterization of the compound and its Cu (II) complex. The methods, IR,^{1}H NMR studies have been used to elucidate the structure of ***N*'-(2-HYDROXYPHENYL)-*N*'-(4-METHYLPIPERAZIN-1-YL)FURAN-2-CARBOHYDRAZIDE**.The analytical data reveals 1:1 (metal : ligand) ratio in

the Cu (II) complex. The electrolytic conductivity data of the complex reveals its electrolytic nature. The antimicrobial activities of the compound and its Cu (II) complex by disc diffusion method. The biological activity for the synthesized compound and its Cu (II) complex were reported. The results show that sample has inhibition against microbes.

REFERENCE

1. Chrysselies, M.C., Rekka, E.A. Kourounakis, P.N., J.Med Chem. 43 (2000) 609-612.
2. Rekka, E; Kourounkkis, Eur.J. Med. Chem. 23 (1989) 179-184.
3. V.M.Belikov. Y.N.Belokon,M.M.Dolgaya, N.S.Martin-kova, J.Org. Chem. 26 (1970) 1199.
4. J.A. Gautier,M.Micoque,D.Q.Quan, C.R. J. Acad. Sci,258 (1964) 3731.
5. R. Brandes, H. J. Roth, J. Arch. Pharm. 300 (1967) 1005.
6. Avani. H. Sheth. Parimal. M. Prajapati. Yarti R. Shah, J.Pharm.sci. 45 (2009-2010) 468.
7. Kakul Husain, Mohamed Abid and Amir Azam, J.Pharm. 69 (2007) 5463.
8. J.Blass, Bull. J.soc.chim.France. 89 (1966) 3120.
9. A.Burcherle, F.Ducluzeau; F.Haimovicj, J.soc.chim. 410 (1967) 675 C.A.69 (1968) 35644.
10. J.Blass, Bull. J.soc.chim.France. 134 (1966) 3120.
11. J.H.Schauble. E.Hertz. J. Org. Chem. 35 (1970) 2529.
12. Reactions &Reagents in organic chemistry – O.P. Agarwal, O. D. Sharma – Goel publishing house, Meerut sixth edition.
13. Swaminathan, S. & Narisimhan, K., J. chem. Ber., 99 (1996) 889.
14. Reactions &Reagents in organic chemistry – O.P. Agarwal, O. D. Sharma – Goel publishing house, Meerut fourth edition (1973-75).
15. .N. Karali and A. Gursoy, Synthesis and anticonvulsant activity of some new
16. thiosemicarbazone and 4-thiazolidine derivatives bearing an isatin moiety, Farmaco 49(1994) pp 819–822.
17. 2 B. Iddon, J.Org. Chem. 23 (1985) 417.
18. 3 E.S. Raper, J. Coord. Chem. 61 (1985) 115.