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# ANTIMICROBIAL ACTIVITIES OF MANNICH BASES DERIVED FROM HETERO ALDEHYDES

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**Abstract:** A series of Mannich bases have been synthesized using secondary amines, amide moieties as active hydrogen compounds and hetero aldehydes. The synthesized compounds are characterized by physical methods such as TLC, melting point determination and elemental analysis. The structures of newly synthesized compounds are established by IR, $^{1}$H-NMR and $^{13}$C-NMR spectral studies. Further, the compounds have been tested for antimicrobial studies. Antimicrobial studies of the compounds have revealed that their inferior potency when compared to the standard employed. The antimicrobial activity of the ligand and its complexes have been extensively studied in microorganisms such as bacteria *E. coli* and *Staphylococcus aureus* and fungi *Candida* albicans and *Aspergillus niger* by agar-well diffusion technique . Antibacterial studies are carried out by observing the changes in minimum inhibitory concentration (MIC) of the complexes and compared with the parent ligands and two antibiotics (Amplicillin and Amoxicillin).

**Keywords:** Antimicrobial, Mannich bases, Ligand, Aldehydes

***PAPER-QR CODE***

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## INTRODUCTION

The use of antimicrobial agents has increased dramatically in the past 50 years. They are used in the treatment and prevention of infectious diseases and non-infectious applications. Antimicrobial agents are used extensively in the agricultural production of cattle for treatment and prevention of diseases and in the promotion of growth. In addition, bacterial diseases in plants and other food producers like honeybees and fish are treated with antimicrobial agents. As existing antimicrobial agents decline in effectiveness, infections are becoming very difficult and expensive to treat; and epidemics have become harder to control. The environmental consequences of the widespread use of antimicrobial agents are still little understood. The medical community, Governments, the World Health Organization, and other nongovernmental international agencies have begun to institute policies to address the problem of antimicrobial resistance.

However, there remains a lack of systematic and coordinated action. Surveillance of the collection, analysis, and reporting of data is crucial. Different types of surveillance data are needed for each component of the biosphere, and that data must be made available for treatment decisions and new drug development. Further scientific research is required to determine the effects of antimicrobials on the environment in order to assess the consequences of resistance to human health and ecology, and develop ways to detoxify antimicrobials. The possibilities for new diagnostic procedures, improved therapies, reintroduction of susceptible microorganisms, and reversal of resistance should be explored. Special efforts and innovative methods must be employed to investigate the global impact of antimicrobial resistance.

Advancement in Chemistry plays an essential role in the synthesis of various antimicrobial agents. A three component reaction named Mannich reaction is grabbing the attention of synthetic chemists for their wide gamut of biological activities. Plenty of reports are available on the Mannich bases of heterocyclic compounds as active **antimicrobials (Mazaahir Kidwai et al., 2005; Chhajed and Padwal, 2006; Saraswathi et al., 2010; H. Kumar et al., 2010)** . Metal complexes of Mannich bases have been studied extensively in recent years due to the selectivity and sensitivity of ligands toward various metal ions **( Tramontini and Angliolini, 1990)**. Complexes of pyridin-2-carbaldehyde [heterocyclic] are reported to possess anti viral properties **( Rapheal et al., 2007; Xiao Dong Jia et al., 2012).** Diethyl amines are a precursor for various pharmaceutical compounds. Xylocaine and Procaine, the derivatives of Diethyl amine are used as Anesthetics **(Nils Lofgren, 2008)**. Diethyl amines are used in the manufacture of Diclofenac ethyl salt. Morpholine derivatives are used as an intermediate in rubber industry and as crop protection agents and dyes. They are also used in toiletry and cosmetic products as a surfactant and emulsifier. Rifampicin and trifluroperazine are antibiotic and antipsychotic

drugs respectively, are prepared from the derivatives of N-methyl piperazine **( Katzung and Trevor's, 2005)**. These derivatives are also used in the manufacture of antianginal and urological drugs. N-methyl piperazine derivatives are used in manufacture of plastic, industrial materials and pesticides. Compounds of urea are used in tropical dermatological products to promote rehydration of the skin. Isotopes of urea are used to detect bacteria in stomach and increase the pH of the stomach environment around the bacteria. Urea plays an essential role in metabolism. It is majorly used in agriculture and also in manufacture of plastics, resins and adhesives **( Kurzer, et al., 2011)**. Semicarbazide, the derivative of Urea containing the moiety has a strong ability to form metal complexes **( Kumar et al., 2008)**. Benzohydrazide compounds are believed to behave as ambient nucleophiles in several reactions. Derivatives of benzohydrazide are used as herbicides. Anthracycline is a derivative of benzohydrazide used in cancer chemotherapy **(Billy Clen et al., 2011 2005)**. Diarrhea is the condition of having three or more loose or liquid bowel movements per day. It is a common cause of death in developing countries and the second most common cause of infant death worldwide. Nifuroxadize, derivative of benzohydrazide, is an oral nitro furan drug used as an antibiotic for Diarrhea. In view of these interesting considerations, we have synthesized a new series of biologically important Mannich base using the above compounds as starting materials.

## 2. MATERIALS AND METHODS

The chemicals used are of Merck products. Purity of the compounds is checked by TLC using silica gel G coated glass plates and iodine vapor as visualizing agent. Melting points are determined in an open capillary tube and are uncorrected. The IR spectra are recorded in KBr on a FT-IR SHIMADZU spectrophotometer. The $^{1}$H-NMR and $^{13}$C-NMR spectra are recorded on Bruker AMX 300MHz NMR spectrophotometer using DMSO-d6 as internal standard and chemical shifts are expressed in ppm. The elemental analyses are performed on a Perkin-Elmer series C, H, N and O analyser-2000.

### 2.1. Materials and reagents

All the chemicals are commercially available. All the solvents are dried before use by the literature methods and moisture is excluded from the glass apparatus using $CaCl_2$ drying tubes. Mannich base (2-((diethyl amino) (pyridine-2-yl)methyl) hydrazine carboxamide, Benzoic acid N'-(furan-2-yl-morpholin-4-yl-methyl)-hydrazide, 2-((4-methyl piperazin-1-yl pyridine-2-yl)methyl) hydrazine carboxamide, Benzoic acid N'-(diethylamino-furan-2-yl-methyl)-hydrazide, Benzoic acid N'-[furan-2-yl-(4-methyl-piperazin-1-yl)-methyl]-hydrazide is synthesized according to literature method.

### 2.1.1. Instruments

All the reagents used for the preparation of ligand and the complexes are of Merck products. Melting points are determined on a open capillary melting point apparatus and are uncorrected. NMR is recorded on 300MHz Shimadzu spectrometer using dmso-$d^6$ with TMS as an internal standard. IR spectra are recorded in Shimadzu spectrometer. The homogeneity of the compounds is monitored by Thin Layer Chromatography (TLC) Silica-G coated glass plate visualized by iodine vapor.

### 2.1.2. Apparatus

Melting points were uncorrected and measured on electric melting point apparatus SMP1. The infrared spectra (vmax in cm-1) was taken in KBr discs using a Perkin Elmer spectrophotometer 57928 RXIFT-IR system. The absorption in the UV-Vis region was recorded by a Perkin Elmer Lambda 35 Spectrophotometer using DMF as solvent. IR and UV/Vis spectral analysis were carried out at Faculty of Science, JAMAL MOHAMED COLLEGE, TRICHY. The NMR study was carried out at the SASCRA UNIVERSITY,THANJAVUR,INDIA. The Magnetic study was carried out at the CLRI Institute ,Chennai, INDIA. Elemental analyses of C, H, and N, study were carried out at the COCHIN UNIVERSITY, KERALA INDIA.

### 2.1.3. Antimicrobial properties:

Antimicrobial study was carried out at CLINICAL PATHOLOGY LABORATORY, AARTHI DIAGNOSTIC CENTRE, CHENNAI, INDIA. The standardized disc–agar diffusion method was followed to determine the activity of the synthesized compounds against the tested microorganisms. Cultures of the microorganisms were used in the following tests: *Gram-positive bacteria: Staphylococcus aureus (ATCC 25923) and Micrococcus luteus (ATCC 6635), Gram – negative bacteria: Pseudomonas fluorescens (S 97) and Salmonella typhimurium (ATCC 14028), Yeast: Candida albicans (ATCC 10231) and Fungus: Aspergillus fumigatus.*

## 3. Synthesis of Ligands

### 3.1 Synthesis of *(2-((diethyl amino) (pyridine-2-yl)methyl) hydrazine carboxamide(1a)*

In this reaction, Diethyl amine (2.6 mL, 0.025 M) was added to the alcoholic solution of Semicarbazide (2.78 g, 0.025 M) followed by pyridine-2-carboldehyde (2.4 mL, 0.025 M). The reaction mixture was taken in a round bottom flask which was kept over a magnetic stirrer with hot plate and stirred at hot condition. The pH of the reaction mixture was adjusted from 8 to 10 by using NaOH. After 4 hrs, the reaction mixture became viscous and solidified. The product was separated out as a solid was washed, filtered, dried over vacuum and recrystalised from absolute ethanol. The above procedure was employed for the compound **1b (Scheme 1).**

### 3.2. Synthesis of *Benzoic acid N'-(furan-2-yl-morpholin-4-yl-methyl)-hydrazide (2a)*

In this reaction, Morpholine (2.2 mL, 0.025 M) was added to the alcoholic solution of Benzohydrazide (3.4 g, 0.025 M) followed by Furan-2-aldehyde (2.1 mL, 0.025 M). The reaction mixture was taken in a round bottom flask which was kept over a magnetic stirrer and stirred at a cold condition. The pH of the reaction mixture was adjusted to 8 to 10 by using NaOH. After 3 hrs, the reaction mixture became viscous and solidified. The product separated out as a solid was washed, filtered, dried over vacuum and recrystalised from absolute ethanol. The above procedure was employed for the remaining compounds **2b & 2c (Scheme 2)**.

### 3.3. *(2-(diethyl amino) (pyridine-2-yl) methyl) hydrazine carboxamide*

IR (KBr, $cm^{-1}$) 3386, 3231, 2929, 1692, 1584, 1421, 1234, 1084.$^1$H NMR (300 MHz, DMSO-$d_6$, δ ppm) 10.52 (s, 1H, CONH), 8.5-7.2 (m, 4H, pyridine ring), 5.0 (d, 1H, CHNH), 2.25 (m, 4H, $CH_2$-N-$CH_2$), 1.9 (s, 1H, NH), 1.12 (m, 6H, $2CH_3$).$^{13}$C NMR (300 MHz, DMSO-$d_6$, δ ppm) 165.2 (CO), 147.2, 145.3, 135, 125, 122, (pyridine ring), 75 (CH), 45 ($CH_2$-N-$CH_2$), 15.2 ($2CH_3$).

**3.4.** ***2-((4-methyl piperazin-1-yl pyridine-2-yl)methyl) hydrazine carboxamide*** IR (KBr, $cm^{-1}$) 3368, 3246, 2950, 2931, 1657, 1522, 1466, 1395, 1223, 1072.$^1$H NMR (300 MHz, DMSO-$d_6$, δ ppm) 10.52 (s, 1H, CONH), 8.51-7.29 (m, 4H, pyridine ring), 5.2 (d, 1H, CHNH) 2.5 (m, 8H, $4CH_2$, piperazine ring) 2.2 (s, $2CH_3$), 1.92 (d, 1H, NH).$^{13}$C NMR (300 MHz, DMSO-$d_6$, δ ppm) 164.1 (CONH), 148, 146, 135, 125, 121.7 (pyridine ring), 77.6 (CH-NH), 54.7 (N-$CH_3$), 47.5 ($CH_2$-N-$CH_2$), 45.6(N-$CH_3$).

### 3.5. *Benzoic acid N'-(furan-2-yl-morpholin-4-yl-methyl)-hydrazide*

IR (KBr, $cm^{-1}$) 3249, 3064, 2945, 1964, 1782, 1642, 1544, 1339, 1150, 1013, 936.$^1$H NMR (300 MHz, DMSO-$d_6$, δ ppm) 11.6 (s, 1H, CONH), 7.9-7.5 (m, 5H, Phenyl ring), 6.9 (d, 2H, in furyl ring), 6.5 (s, 1H, furyl ring), 4.5 (d, 1H, CHNH), 3.56 (t, 4H, $CH_2OCH_2$), 2.5 (t, 4H, $CH_2NCH_2$), 2.0 (s, 1H, NH). $^{13}$C NMR (300 MHz, DMSO-$d_6$, δ ppm) 170.2(CO), 155, 141, 112, 108, (furyl ring), 135, 131, 129, 126(phenyl ring), 79 (CH), 71, 48.2(Morpholine ring).

### 3.6. *Benzoic acid N'-(diethylamino-furan-2-yl-methyl)-hydrazide*

IR (KBr, $cm^{-1}$) 3250, 3062, 2952, 1964, 1783, 1642, 1564, 1339, 1287, 1013, 936.$^1$H NMR (300 MHz, DMSO-$d_6$, δ ppm) 11.7 (s, 1H, CONH), 7.9-7.5 (m, 5H, Phenyl ring), 7.28-6.3 (d, 2H, in furyl ring), 6.1 (s, 1H, furyl ring), 4.81 (d, 1H, CHNH), 3.58 (m, 4H, $CH_2OCH_2$, Morpholine), 2.47 (t, 4H, $CH_2NCH_2$, Morpholine), 2.1 (s, 1H, NH), 1.3 (m, 6H, $2CH_3$). $^{13}$C NMR (300 MHz, DMSO-$d_6$, δ ppm) 170.2(CONH), 155, 141, 113, 106, (furyl ring), 135, 131, 129, 126(phenyl ring), 80 (CH), 71, 48.2(Morpholine ring).

### 3.7. *Benzoic acid N'-[furan-2-yl-(4-methyl-piperazin-1-yl)-methyl]-hydrazide*

IR (KBr, $cm^{-1}$) 3245, 3059, 2949, 1964, 1821, 1964, 1821, 1650, 1623, 1540, 1339, 1287, 1013, 936.$^{1}$H NMR (300 MHz, DMSO-$d_6$, δ ppm) 11.6 (s, 1H, CONH), 7.9-7.4 (m, 5H, Phenyl ring), 6.81-6.20 (d, 2H, in furyl ring), 6.09 (s, 1H, furyl ring), 4.74 (d, 1H, CHNH), 2.5 (m, 8H, $4CH_2$, piperazine ring) 2.2 (s, 3H, $CH_3$). 1.9 (s, 1H, NH), $^{13}$C NMR (300 MHz, DMSO-$d_6$, δ ppm) 170 (CONH), 153, 142, 110, 107(furyl ring), 135, 132, 128, 127(phenyl ring), 78 (CH), 57, 46, 37(N-methylpiperazine ring).

## 4. RESULTS AND DISCUSSION

Molecular weights of the synthesized compounds were determined by Rast Micro method. Melting points were determined in an open capillary tube method. The elemental analyses were carried out using Perkin-Elmer series C, H, N and O analyser-2000. The values of molecular weight, melting point and elemental analysis were shown in table.1. In general, IR spectra of all compounds (2-(diethyl amino) (pyridine-2-yl)methyl) hydrazine carboxamide(1a & 1b)- *Benzoic acid N'-[furan-2-yl-(4-methyl-piperazin-1-yl)-methyl]-hydrazide and Benzoic acid N'-(furan-2-yl-morpholin-4-yl-methyl)-hydrazide(2a & 2c)* showed absorption at around 3386-3245, 2952-2929, 1692-1642, and 1084-1032 regions conforming the presence of $NH_2$, CH, CO and C-N respectively.

The $^{1}$H NMR spectra, the signals of the compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. $^{1}$H NMR spectra of most compounds have characteristic CONH proton of at δ 11.7-10.52 ppm, 4H proton of pyridine ring were at around δ 8.51-7.2 ppm, characteristic protons of phenyl ring at δ 7.9-7.4 ppm, 3H proton of furyl ring at δ 7.28-6.1ppm, 1H proton of CH proton at δ 5.2-4.5 and aliphatic and NH protons showed at δ 3.58-1.12 ppm. $^{13}$CNMR spectra of all compounds have characteristic CO signals appeared around at δ 170.2-164.1 ppm, pyridine ring at δ 148-121.7 ppm, furyl ring at δ 155-106 ppm, phenyl ring at δ 135-126 ppm, CH at 80-75 ppm and other aliphatic regions at δ 71-15.2.

### 4.1. Antimicrobial screening

*4.1.1. Antibacterial activity*

The synthesized compounds were tested for their antibacterial activity by disc diffusion method. The agar solution spread over the disc and ***Gentamycin*** was employed as the standard drug against the bacterial strains. The compounds were tested in various concentrations (25, 50, 100 and 200 mg/ml). The exhibited values of antibacterial activity and graphical plots of the compounds are shown in **table 2** and **Figure 1** respectively.

*4.1.2. Antifungal activity*

The synthesized compounds were tested for their antifungal activity by disc diffusion method. ***Amphotracin–B*** used as standard drug against the pathogenic strains in various concentrations (25, 50, 100 and 200 mg/ml). The observed values and graphical plots of anti fungal activities are given in **Table 3** and **Figure 2** respectively.

## 5. CONCLUSION

Five new Mannich bases have been synthesized by using various secondary amines (Morpholine, Diethyl amine and N-Methyl piperazine), an active hydrogen compound (Benzohydrazide) and different aldehydes namely, Pyridine-2-carboldehyde and Furan-2-aldehydes. The newly synthesized compounds were characterized by elemental analysis, analytical techniques and spectroscopic studies. Further, the synthesized compounds were screened for their antimicrobial activity. All the new compounds possess higher zone of inhibition when compared to the standard drugs. Compounds of pyridine-2-carbaldehyde (1a & 1b) showed higher antimicrobial activity than the compounds of furan-2-aldehyde (2a-2c). Many effective antimicrobial agents show a heterocyclic moiety within their structure **(Anandarajagopal et al., 2010).** The derivatives of heterocyclic compounds have their own importance due to the good biological activities. Among the wide variety of heterocyclic, the compounds bearing nitrogen atom as hetero atom have played an important role in medicinal chemistry **(Anandarajagopal et al., 2010).** It has been reported that the pyridine compounds found to possess higher antimicrobial activity against *Staphylococcus aureus.* This is explained on the basis of QSAR studies. The very reason for their activity is Quantum, constitutional and geometrical parameters. For the antimicrobial activity against *Candida Albicans* is due to Geometrical, functional group and topological parameters **(Sabet et al., 2007).**

## ACKNOWLEDGEMENT

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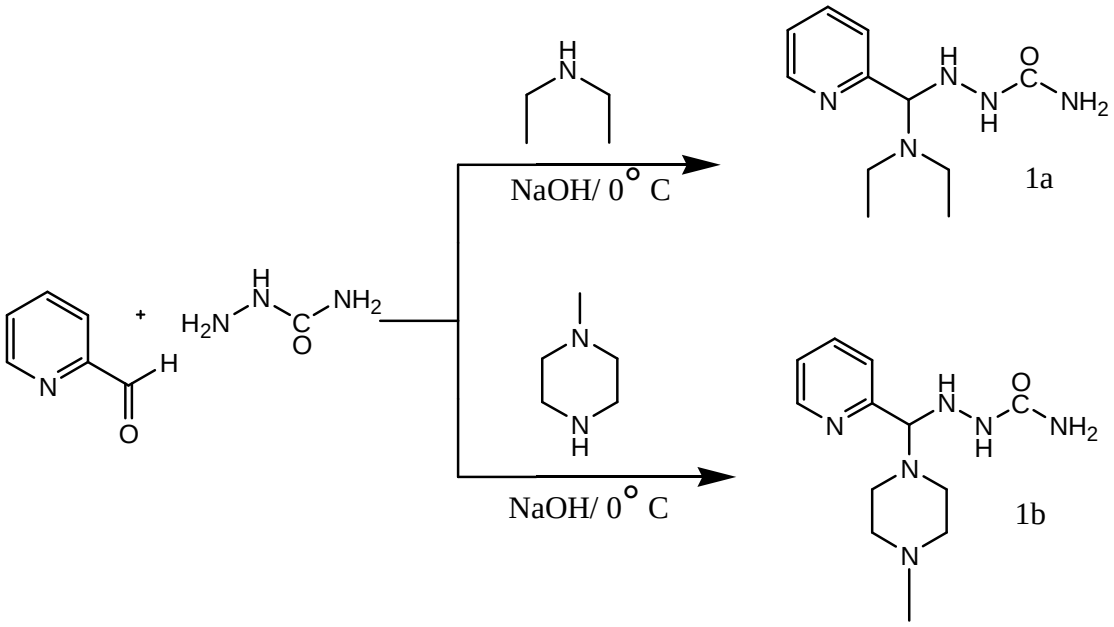
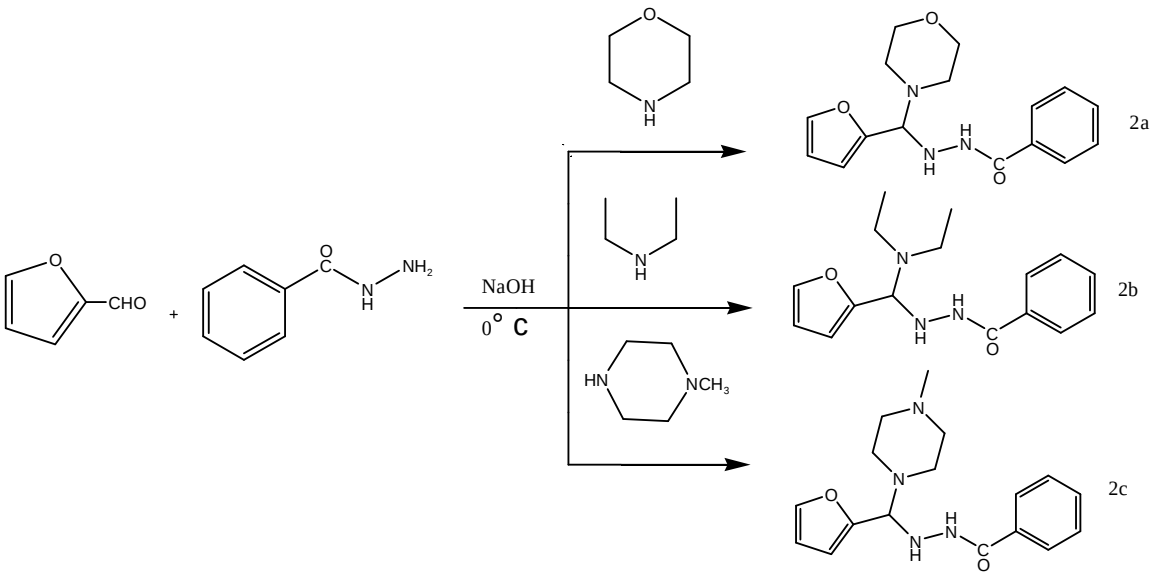



**Scheme 1. Synthesis of compound 1a and 1b.**




**Scheme 2. Synthesis of compound 2a-2c**

**Table.1.**

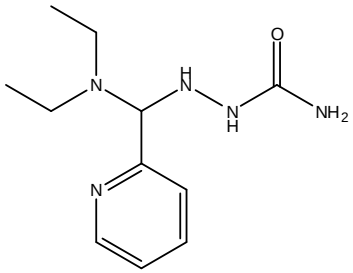
| S. No. | M.F. | m.w. Md. | Rast °C | m.p. % | Yield C | Elemental analysis, [Found% (Calcd.%)] H | N | O | |
|---|---|---|---|---|---|---|---|---|---|
| 1a | $C_{11}H_{19}N_5O$ | 237 | 242 | 190 | 71 | 55.68(55.05) | 8.07(8.12) | 29.51(29.41) | 6.74(6.53) |
| 1b | $C_{12}H_{20}N_6O$ | 264 | 261 | 137 | 73 | 54.53(54.02) | 7.63(7.53) | 31.79(31.87) | 6.05(6.34) |
| 2a | $C_{16}H_{19}N_3O_3$ | 301 | 304 | 168 | 80 | 63.77(63.04) | 6.36(6.44) | 13.94(13.23) | 15.93(15.88) |
| 2b | $C_{16}H_{21}N_3O_2$ | 287 | 286 | 201 | 86 | 66.88(66.32) | 7.37(7.21) | 16.62(16.41) | 11.44(11.12) |
| 2c | $C_{17}H_{22}N_4O_2$ | 314 | 330 | 184 | 83 | 64.95(64.76) | 7.05(6.98) | 17.82(17.44) | 10.18(10.17) |

**TABLE 2**

| S.No | *Candida albicans* | | | | | *Aspergillus niger* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration mg/mL | | | | | Concentration mg/mL | | | | |
| | 25 | 50 | 100 | 200 | Average | 25 | 50 | 100 | 200 | Average |
| 1a | 21.00 | 21.20 | 22.10 | 22.40 | 21.67 | 22.22 | 24.12 | 25.84 | 27.12 | 24.82 |
| 1b | 25.00 | 28.12 | 28.44 | 29.12 | 27.67 | 20.14 | 24.42 | 25.81 | 28.22 | 24.64 |
| 2a | 18.12 | 18.42 | 19.12 | 19.60 | 18.81 | 22.00 | 22.20 | 22.46 | 23.00 | 22.41 |
| 2b | 21.10 | 21.36 | 21.60 | 21.80 | 21.46 | 20.18 | 20.42 | 21.40 | 21.82 | 20.95 |
| 2c | 16.12 | 16.18 | 16.44 | 16.72 | 16.36 | - | - | - | - | - |

**TABLE 3**

| S.No | Gram positive bacteria (*Staphylococcus*) | | | | | Gram negative bacteria (*Escherichia coli*) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration mg/mL | | | | | Concentration mg/mL | | | | |
| | 25 | 50 | 100 | 200 | Average | 25 | 50 | 100 | 200 | Average |
| 1a | 20.00 | 20.40 | 20.65 | 20.80 | 20.46 | 28.20 | 28.30 | 28.41 | 29.11 | 28.52 |
| 1b | 21.20 | 21.30 | 21.40 | 21.62 | 21.38 | 25.30 | 25.40 | 25.61 | 25.84 | 25.64 |
| 2a | 15.12 | 15.22 | 15.34 | 15.44 | 15.28 | - | - | - | - | - |
| 2b | 17.20 | 17.40 | 17.64 | 17.82 | 17.51 | 19.2 | 19.30 | 19.41 | 19.52 | 19.36 |
| 2c | - | - | - | - | - | 23.30 | 23.52 | 23.55 | 23.56 | 23.48 |




2-((diethylamino)(pyridin-2-yl)methyl)hydrazinecarboxamide

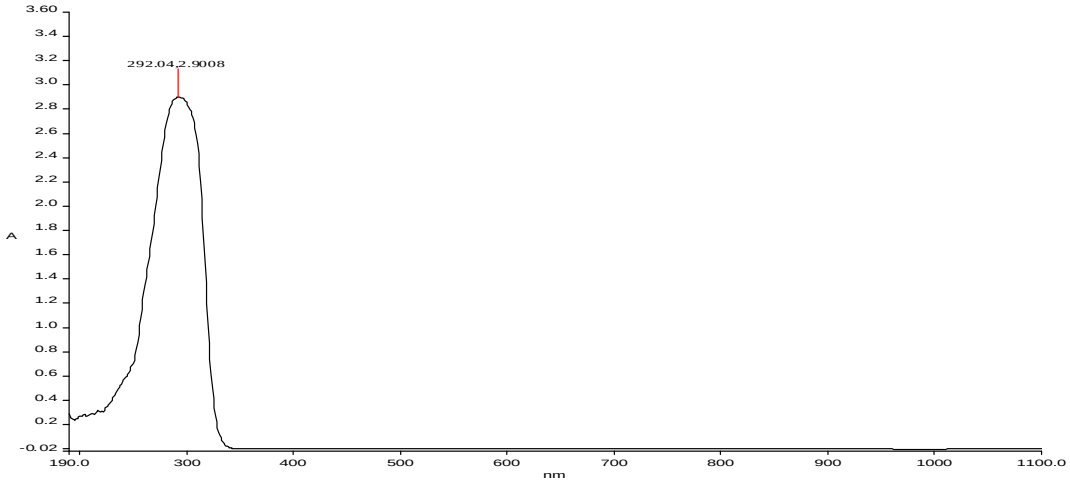



**Fig.1.**

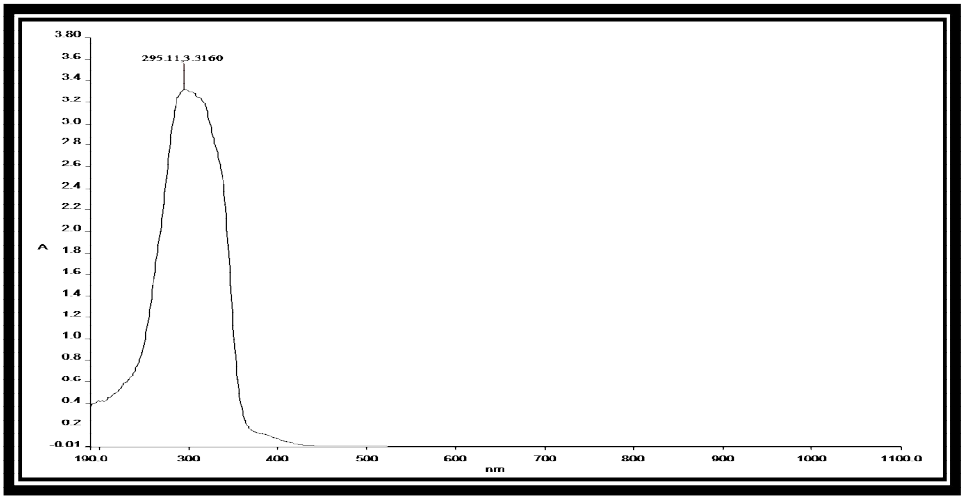
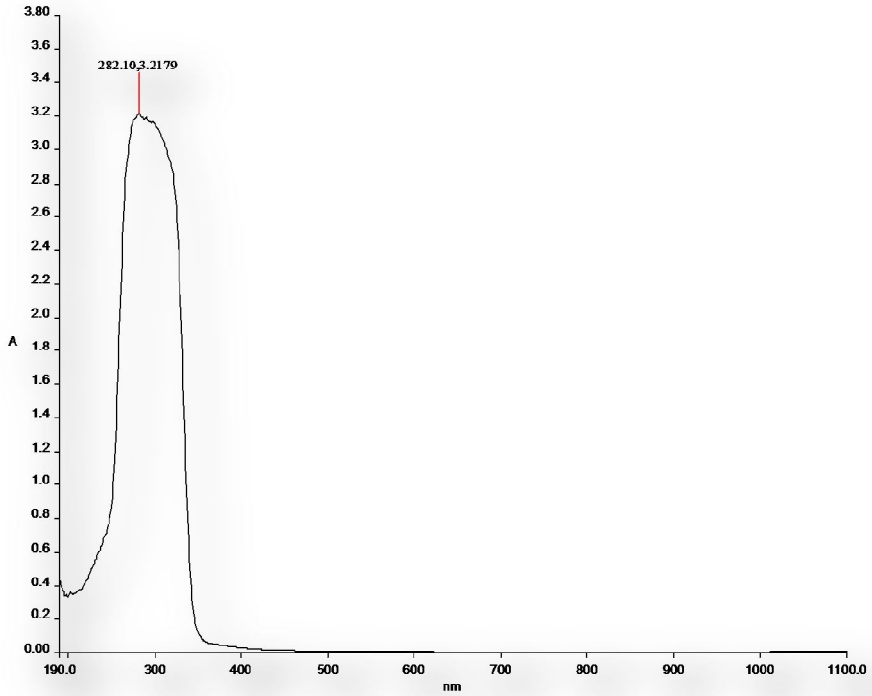



**Figure 2.**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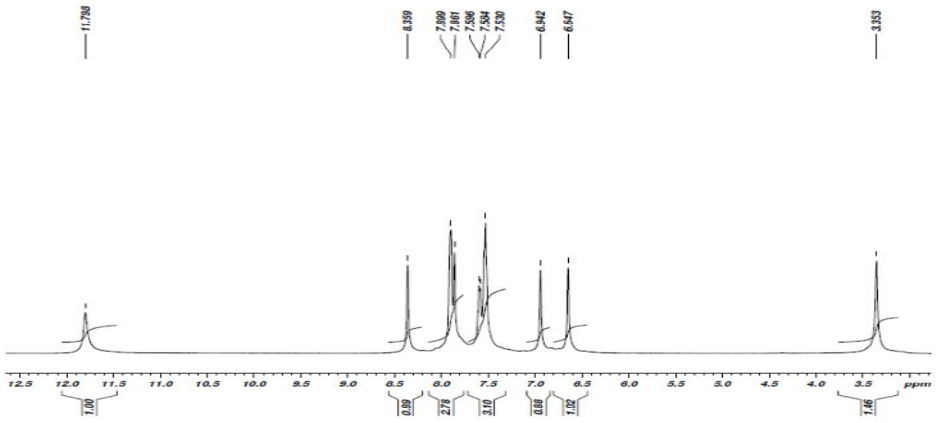
A
nm

**Fig. 3.**


11.798
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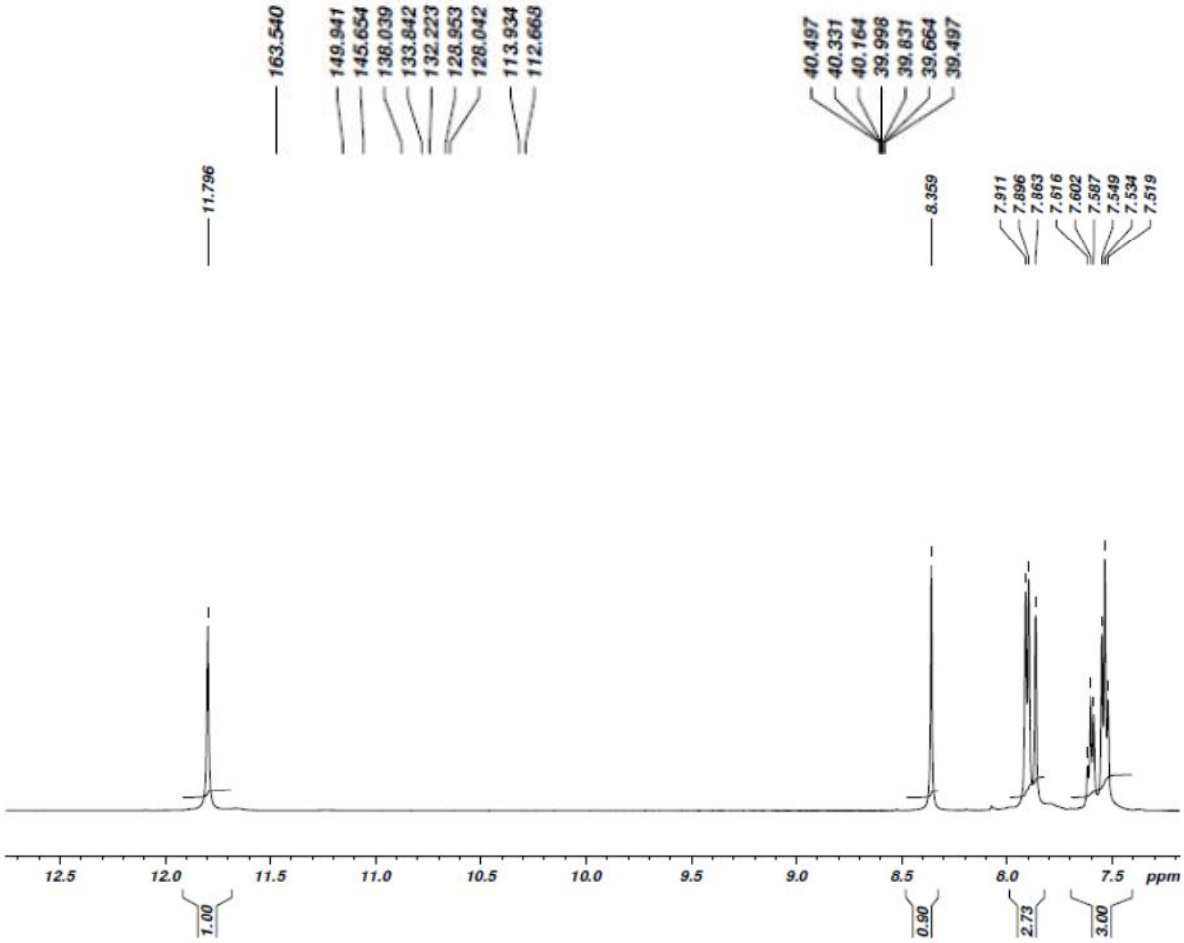
7.530
6.942
6.847
3.353
ppm

**Fig. 4.**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133.842
132.223
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128.042
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39.998
39.831
39.664
39.497
11.796
8.359
7.911
7.896
7.863
7.616
7.602
7.587
7.549
7.534
7.519
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
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ppm
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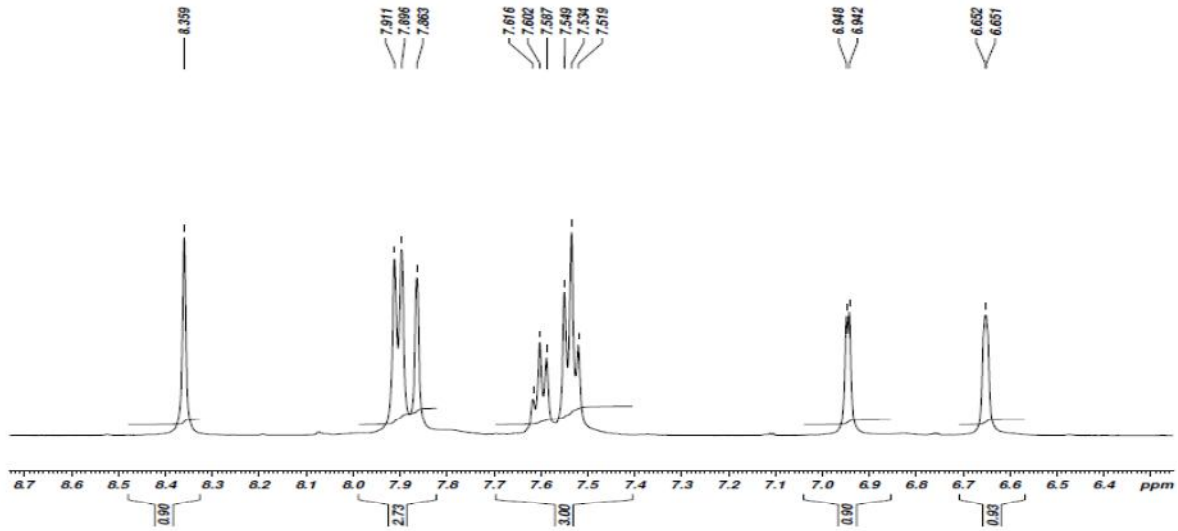
2.73
3.00

**Fig. 5.**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7.616
7.602
7.587
7.549
7.534
7.519
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6.651
8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 ppm
0.90
2.73
3.00
0.90
0.93

**Fig. 6.**

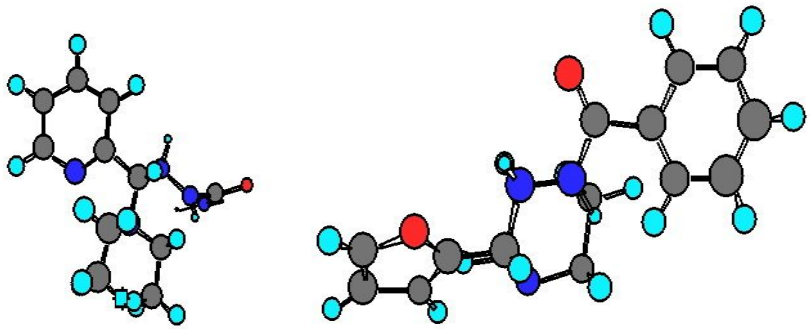

**Fig. 7.**

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