Zainab RAMZI

SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF NEW MULTIFUNCTIONAL HETERO MACROMOLECULAR COMPOUNDS

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Zainab RAMZI

A thesis submitted to

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Master of Science

in

Chemistry

June 2015
Istanbul, Turkey

APPROVAL PAGE

This is to certify that I have read this thesis written by Zainab RAMZI and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree Master of Science in Chemistry.

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June 2015

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Zainab RAMZI

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June 2015

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ABSTRACT

Some macromolecular dialdehyde and di-imine compounds have been synthesized. All the synthesized compounds have been purified and their melting points have been measured. The compounds have been characterized in terms of the recorded FT-IR and NMR spectra. The antimicrobial activities of the di-mines have been estimated by using the diffusion and the minimal inhibitory concentration dilution methods in dimethyl sulfoxide against $Gram^+$ and $Gram^-$ bacteria. Their antifungal activities have been measured on several yeast cultures. Some of the presently synthesized compounds demonstrate higher antimicrobial and antifungal activities than the standard reagents.

Keywords: Dialdehyde, Di-imine, FT-IR, NMR, Antimicrobial and antifungal activities.

YENİ ÇOK FONKSİYONLU HETERO MAKROMOLEKÜLLERİN SENTEZİ, SPEKTROSKOPİK KARAKTERİZASYONU VE ANTİMİKROBİYAL AKTİVİTELERİ

Zainab RAMZI

Yüksek Lisans Tezi – Kimya
Haziran 2015

Tez Danışmanı: Prof.Dr.Naz Mohammed ATABAY

ÖZ

Bazı makromoleküler dialdehid ve di-imin bileşikleri sentezlendi. Sentezlenen bütün bileşikler arıtıldı ve erime noktaları tespit edildi. FT-IR ve NMR spektrumları ile bileşiklerin karakterizasyonu yapıldı. Di-iminlerin antimikrobial aktiviteleri difüzyon ve minimum yasaklayıcı konsentrasyon seyreltme metodları ile dimetil sulfoksit içinde $Gram^{+}$, $Gram^{-}$ bakterilerine karşı tespit edildi. Antifungal aktiviteler birçok maya kültürü üzerinde ölçüldü. Sentezlenen bileşiklerden bazılarının mevcut ilaçlardan daha yüksek antimikrobial ve antifungal aktiviteye sahip oldukları gözlendi.

Anahtar Kelimeler: Dialdehid, Di-imine, FT-IR, NMR, Antimikrobial ve antifungal aktiviteler.

To my family

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOL/ABBREVIATION

^{13}C NMR	Carbon (13) NMR Spectroscopy
^{1}H NMR	Proton NMR Spectroscopy
AM	Ampicillin
$CDCl_3$	Deuterated Chloroform
CL	Clotrimazole
CT	Cefotaxime
d	Doublet
dd	Doublet of doublet
DMSO-d_6	Deuterated Dimethylsulphoxide
FT-IR	Fourier Transform Infrared Spectroscopy
GE	Gentamycin
J	coupling constant
KE	Ketoconazole
m	Multiplet
MP	Melting Point
NMR	Nuclear Magnetic Resonance Spectroscopy
NY	Nystatin
OF	Ofloxacin
PE	Penicillin
PPM	Parts per million
s	Singlet
t	Triplet
TE	tetracycline
VA	Vancomycin
δ	Bending vibration

δC	Carbon(13) chemical shift
δH	Proton chemical shift
υ	Stretching

CHAPTER 1

INTRODUCTION

Nowadays there has been an increasing demand for searching for new novel therapeutic agents because of the highly increasing rate of resistance to the current antimicrobial therapeutic agents, the search for new selective and nontoxic antimicrobial agents has become an influential area of investigation in medicinal chemistry [1,2].

Heterocyclic chemistry is widely expanding because a lot of research works have been done in this area. Heterocyclics have more flexibility and better ability to respond to many demands of biochemical systems. The activity of heterocyclic compounds in biological system is essential in natural and pharmaceutical products. Heterocyclic are present in most vitamins, many natural products, biomolecules, and biological active compounds including: antitumor, antibiotics, anti-inflammatory, antidepressants, antimalarial, anti-HIV, antibacterial, antifungal, antiviral [3,4].

Compounds having within an azomethine group (CH=N) are distinguished as Schiff bases .They can be obtained from the reaction of primary amines and carbonyl compounds; these compounds were discovered by Hugo Schiff. Schiff bases can be found in different natural, semi-synthetic, and synthetic compounds and it has proved to have an important function in the organic, biological, analytical chemistry researches due to their simple synthesis [5-10].

Many influential antibacterial antifungal compounds prepared by the condensation of aldehydes with heterocyclic compounds. Synthesis,characterization and structural activity relationship of Schiff bases have been studied vastly as it has proved that (C=N) linkage in Schiff bases is a fundamental feature for bioactivity.

The various substituents in the phenyl rings of aromatic Schiff bases are responsible for antifungal activity, which can be modified depending upon the type of of the substituent present on the aromatic rings [5-10].

In this thesis, synthesis of heterocyclic compounds containing dialdehydes and di-imines functional groups have been carried out. Such of hetero macromolecular compounds containing dialdehydes and di-imines; have been proved a wide flexibility and easy synthesis pathways. Many researchers prepared many heterocyclic compounds containing;dialdehydes,di-imines,Schiff base ligands and their metal complexes (for example: Co (II), Ni (II), Cu (II) and Zn (II) and more) and dendritic macromolecules; as it has been proved they have a promising range of bioactivity and various uses in organic chemistry, biochemistry and pharmaceutics [11-28].

In this project, all compounds that have been synthesized were purified,the melting point for each compound has been measured and the structural features of the macromolecules have been characterized by FT-IR, ^{1}H and ^{13}C NMR spectroscopies.

Figure 1.1 Hogue Schiff in his lab on the right side [29]

Bacterial infection caused by $Gram^{+}$ or $Gram^{-}$ bacteria,which might cause either a local infection or might spread in the whole body causing a systemic infection. Antimicrobial agents can be classified in to bacteriostatic that inhibits the cell growth of the bacteria or bactericidal that induces cell death of the bacteria. In general antimicrobial agents act by following mechanisms:

1) Inhibition of cell wall synthesis (e.g.Vancomycin).
2) Inhibition of DNA synthesis (e.g. Ciprofloxacin).
3) Inhibition of RNA synthesis (e.g.Rifampin).
4) Inhibition of protein synthesis (e.g. Gentamicin) [30].

Figure 1.2 which shows mechanism of some antibiotics. While fungal infections could happen by invading of a microorganism in to an epithelial tissue. The infection can be superficial in case when it infects the skin (Dermophytic infection) for example: Tinea capis it is a fungal infection that infects the scalp caused by (MicrosPorum canis and Trichophyton mentagrophytes).While the systemic fungal infection may happened when spores of fungi inhaled causing fungal pneumonia (e.g.Histoplsmosis caused by Histoplasma capsulatum. In addition to that there is an opportunistic infection can be happened (e.g.mucormycosis caused by murcor sp. That infects; sinuses, eyes, blood and brain). Antifungal agents can work by several mechanisms of action:

1) Inhibition of cell wall synthesis (e.g. Caspofungin acetate).
2) Disruption of Cell membrane (e.g. Amphotericin B).
3) Inhibition cell division through inhibiting microtubule polymerization inhibiting mitotic spindle formation (e.g. Griseofulvin) . Figure 1.3 shows cell division.
4) Inhibiting of DNA transcription or Defecting the RNA of the fungi (e.g. Flucytocin) [31]. Figure 1.4 shows DNA and RNA synthesis.

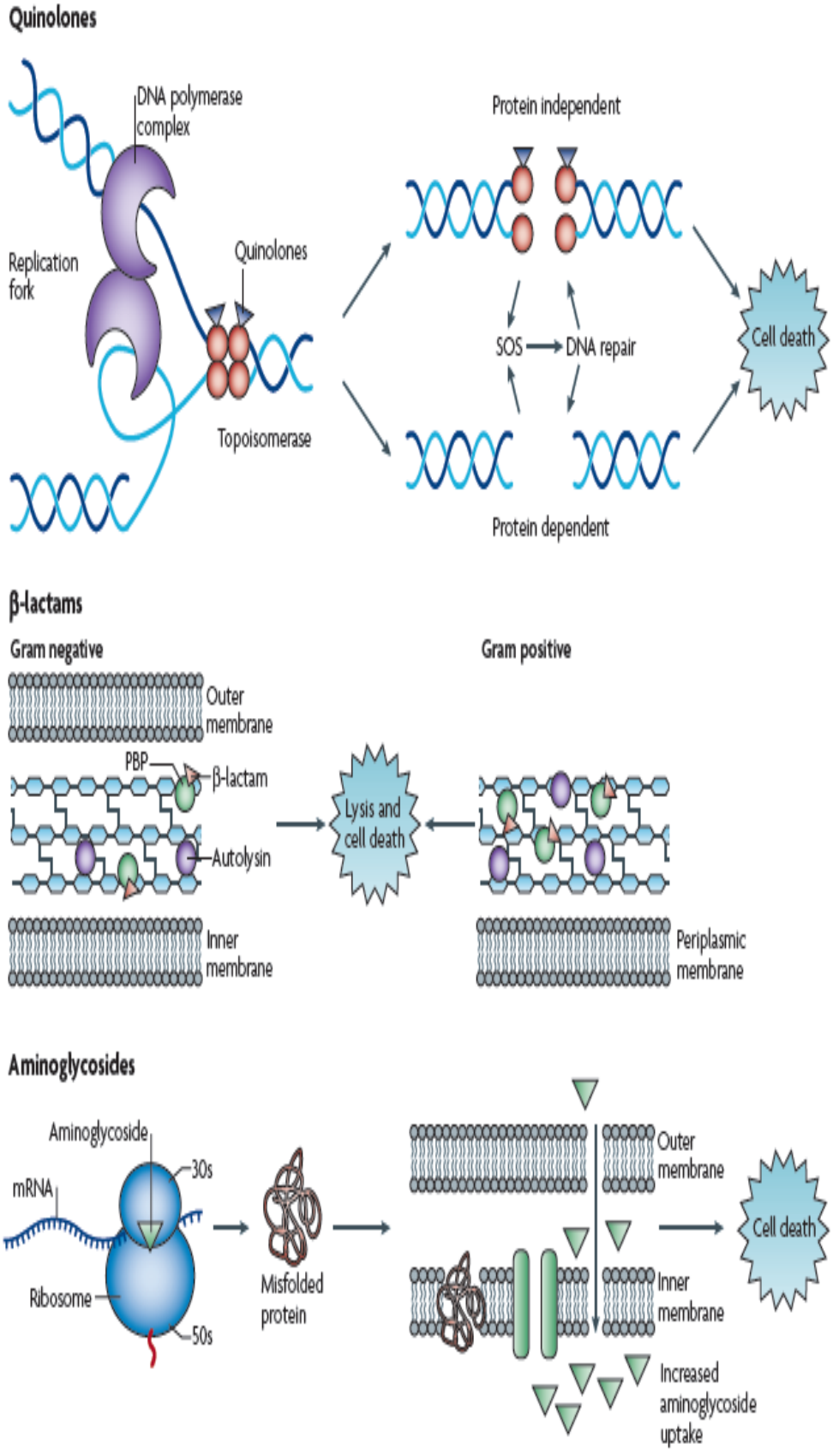

Figure 1.2 Antimicrobial activities [30]

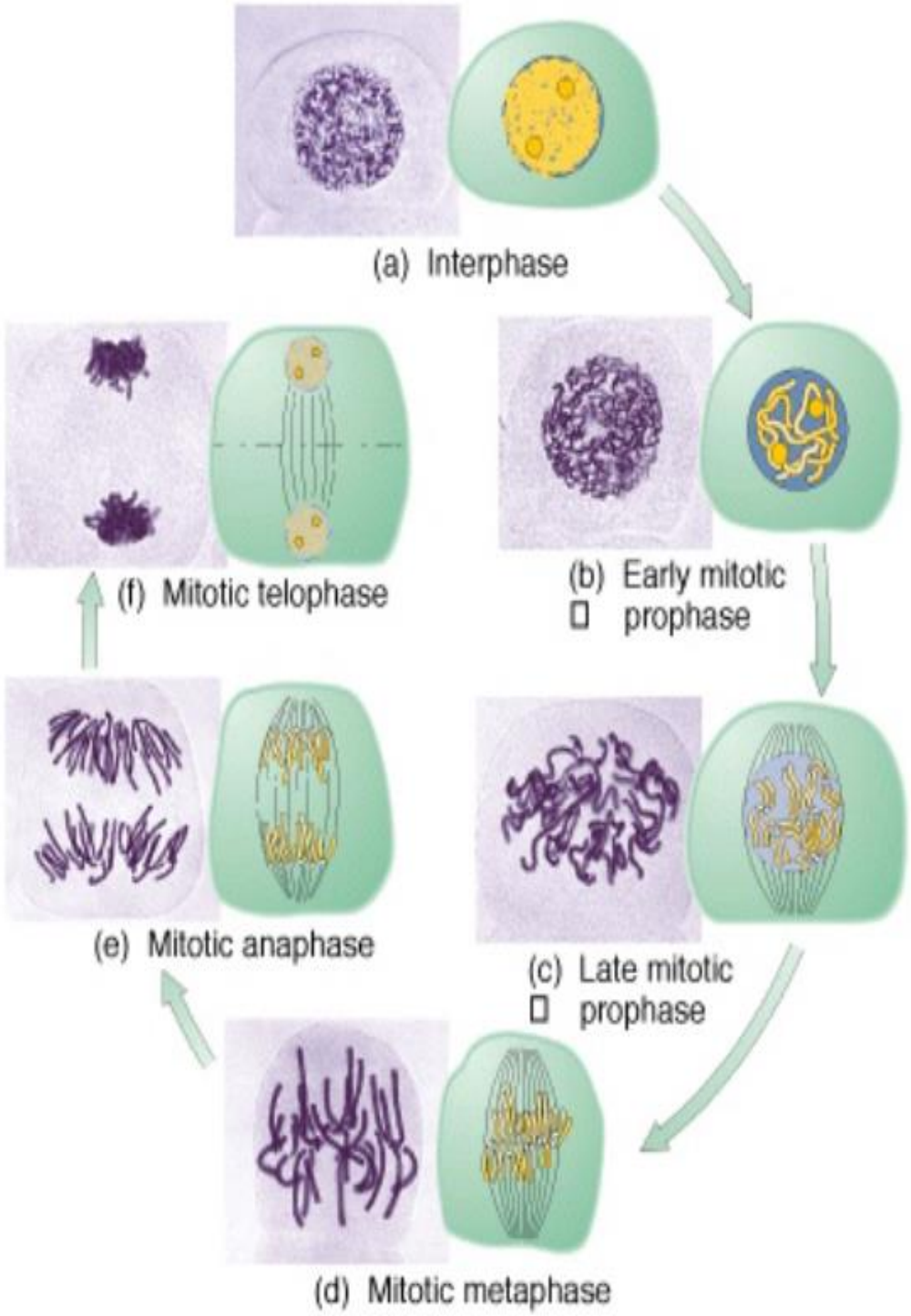

Figure 1.3 cell division [31]

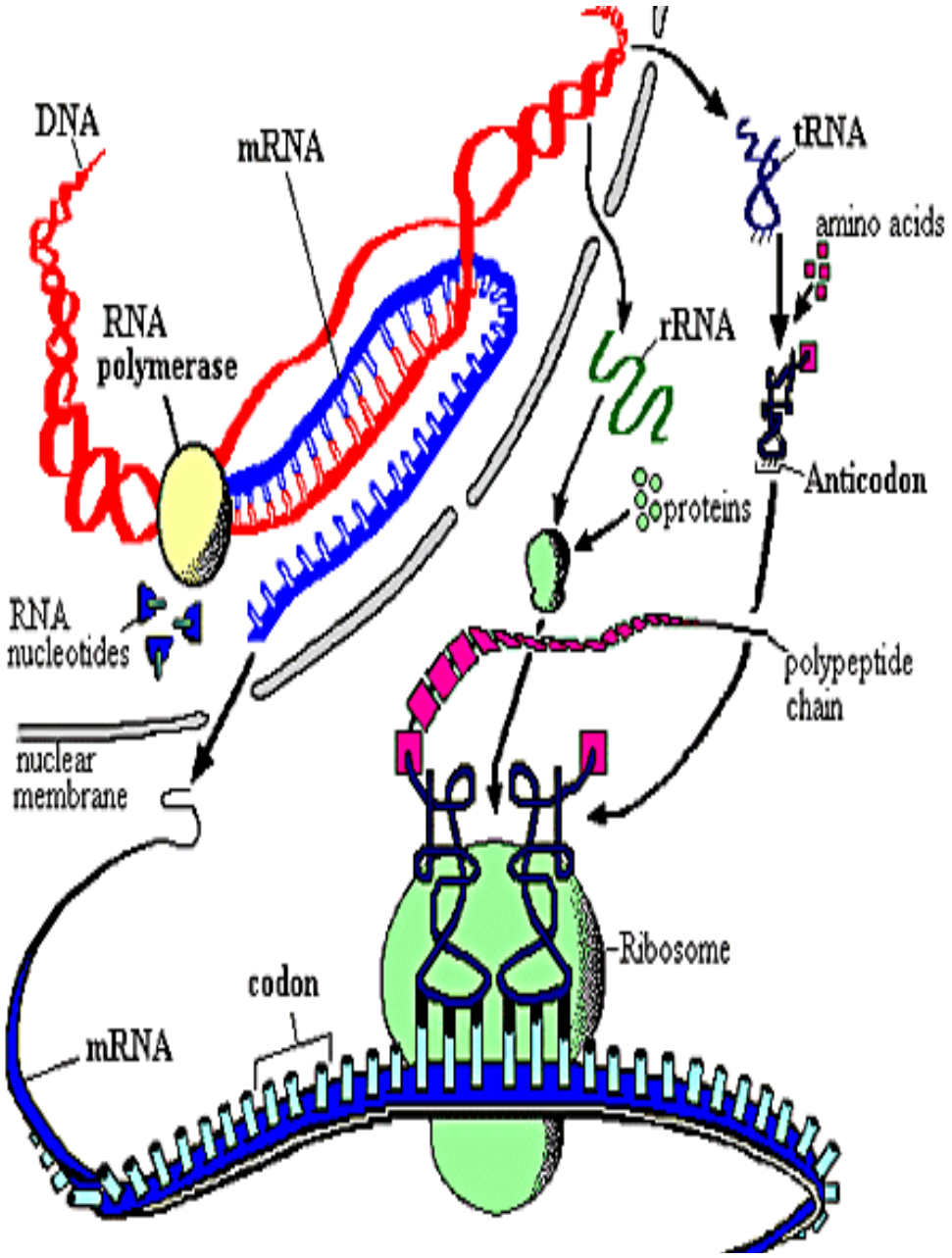

Figure 1.4 DNA and RNA synthesis [31]

In this thesis the antimicrobial activities of the di-mines estimated by utilizing the diffusion method and the minimal inhibitory concentration dilution method in dimethyl sulfoxide respectively against some bacteria including: Gram+ and Gram$^-$ bacteria. The antifungal activities carried out on several yeast cultures. The results compared with standard reagents using the following antibiotics and antifungals: Penicillin,Ampicillin,Cefotaxime, Vancomycin, Ofloxacin, Tetracycline, Gentamycin, Nystatin, Ketoconazole and Clotrimazole.

CHAPTER 2

EXPERIMENTAL

2.1 CHEMISTRY

Chemicals, reagents and solvents of standard grade were used as bought without further purification. Melting points were detected using Electro-thermal 9100 melting point apparatus. FT-IR spectra were measured using the Burker Alpha-P in the range 3500-400 cm^{-1}. ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were measured by using DMSO-d_6 at room temperature on a Burker Ultra shieled plus 400 MHz apparatus .chemical shift (δ) are expressed in units of parts per million relative to TMS.

2.2 GENERAL SYNTHETIC PATHWAY

Ortho, Meta, Para hydroxybenzaldehyde were reacted with KOH and the resulting potassium salt of the hydroxybenzaldehyde was then reacted with α,α'dichloro-m-xylene. Dialdehydes (A_1-A_3) were then precipitated, purified, collected and reacted with 1,3-Benzodioxol-5-amine to give the subsequent di-imines (B_1-B_2) as follows:

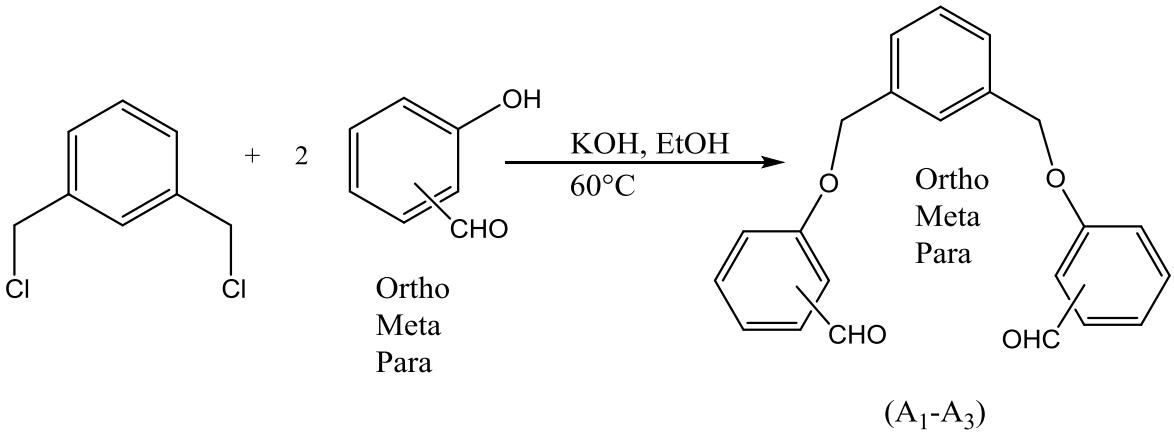

Figure 2.5 dialdehyde synthesis

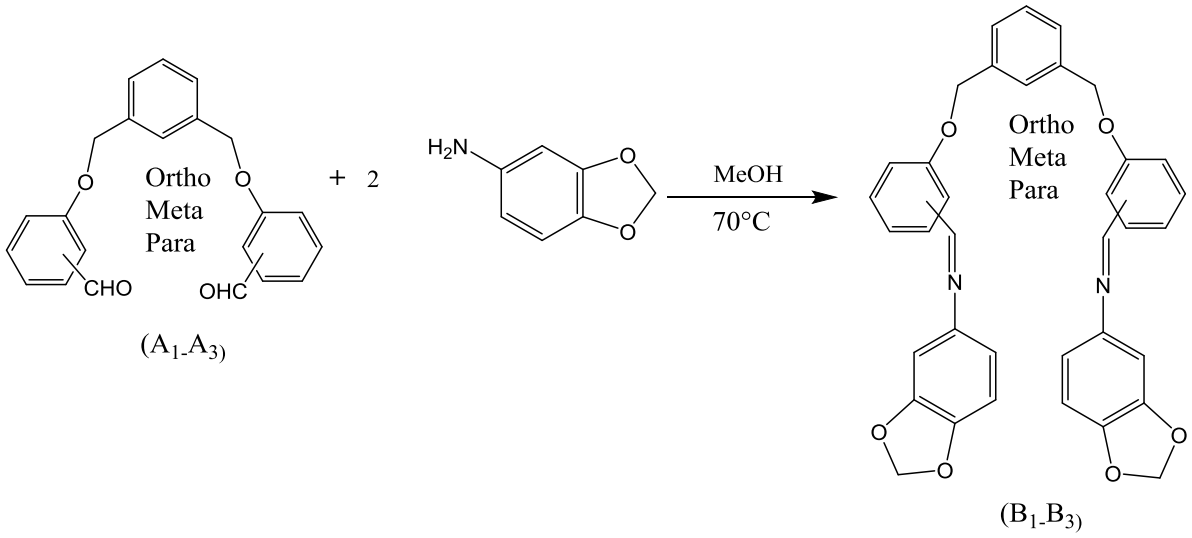

Figure 2.6 di-imines synthesis

2.3 SYNTHESIS OF DIALDEHYDES (A_1-A_3)

2.3.1 1,3bis (2-phenyl methoxy)dibenzaldehyde (A_1)

Orthohydroxybenzaldehyde (1.22 g , 10 mmol) was added to a solution of KOH (0.566 g, 10.1 mmol) in ethanol (25 ml) and stirred for 1 hr. in an external oil bath .

α,α′-dichloro-m-xylene (0.857 g, 5 mmol) was added slowly during a 30 minutes. The mixture was stirred over night; the temperature of the reaction was 60°C.The resulting compound was purified in cold distilled water and ice twice to

remove unreacted starting materials after filtration a light brown solid was obtained. $C_{22}H_{18}O_4$: 1.63 g yield 96% and m.p 112°C. FT-IR (solid cm^{-1}): 3076-3040 υ(C≕C-H), 2878, 2877 υ(CHO), 1670 υ(C=O), 1587 υ(C≕C), 1231 δ(C-O), 718 δ(C≕C-H). ^1H NMR (400 MH_Z, DMSO-d_6), $\delta_H$ppm: 10.44 (s, 2H, CHO), 7, 7.1 (m, 4H), 7.3(d, J= 8 H_Z, 2H), 7.4,7.5 (m, 2H), 7.6,7.7 (m, 4H), 5.34 (s, 4H, CH_2).^{13}C NMR (50 MH_Z, $CDCl_3$) $\delta_C$ppm: 71.3(CH_2), 114.9, 125.7, 126.8, 128.3, 130.6, 134.7, 162.5, 190.9 (CHO) [32].

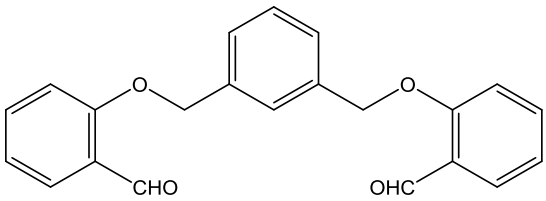

Figure 2.7 dialdehyde structure (A_1)

2.3.2 1,3bis (3-phenyl methoxy) dibenzaldehyde (A_2)

Metahydroxybenzaldehyde (1.22 g, 10 mmol) was added to a solution of KOH (0.566 g, 10.1 mmol) in ethanol (25 ml) and stirred for 1 hr. in an external oil bath α,α′-dichloro-m-xylene (0.857 g, 5 mmol) was added slowly during a 30 minutes. The mixture was stirred over night; the temperature of the reaction was 60°C.The resulting compound was purified in cold distilled water and ice twice to remove unreacted starting materials after filtration a light brown solid was obtained.

$C_{22}H_{18}O_4$: 1.48 g yield 87% and m.p 78°C. FT-IR (solid cm^{-1}): 3061-3035 υ(C≕C-H) , 2884-2730 υ(CHO), 1686 υ(C=O), 1585 υ(C≕C), 1256 δ(C-O), 772 δ(C≕C-H).^1H NMR (400 MH_Z, DMSO-d_6), δ_H ppm: 10.44 (s, 2H, CHO), 7.36-7.38 (m, 2H), 7.43-7.45 (m, 4H), 7.44-7.46 (m, 4H), 7.53-7.56 (m, 2H), 5.24 (s, 4H, CH_2).

^{13}C NMR (50 MH_Z, $CDCl_3$), $\delta_C$ppm:(CH_2) 70.2, 115.3, 126.6, 127.5, 129.3, 130.4, 132.2, 136.8, 163.7, 190.9 (CHO) [32].

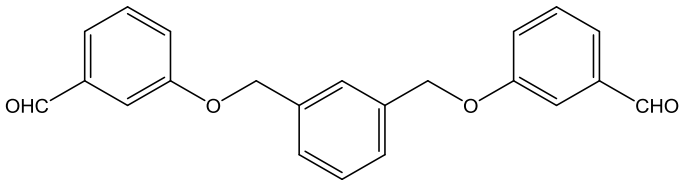

Figure 2.8 dialdehyde structure (A_2)

2.3.3 1,3bis (4-phenyl methoxy)dibenzaldehyde (A_3)

Parahydroxybenzaldehyde (1.22 g, 10 mmol) was added to a solution of KOH (0.566 g, 10.1 mmol) in ethanol (25 ml) and stirred for 1 hr. in an external oil bath α,α′dichloro-m-xylene (0.857g, 5mmol) was added slowly during a 30 minutes. The mixture was stirred over night; the temperature of the reaction was 60°C. The resulting compound was purified in cold distilled water and ice twice to remove unreacted starting materials after filtration a white solid was obtained. $C_{22}H_{18}O_4$: 1.63 g yield 96% and m.p 77°C.

FT-IR (solid cm^{-1}): 3069-3037 υ (C=C-H), 2873-2738 υ(CHO), 1686 υ(C=O), 1597 υ (C=C), 1249 δ(C-O), 781 δ(C=C-H).^{1}H NMR (400 MH_Z, DMSO-d_6), $\delta_H$ppm: 9.9 (s, 2H, CHO), 7.21-7.24 (m, 4H), 7.47 (d, J= 1.3 H_Z, 2H), 7.4-7.6 (m, 2H), 7.8-7.9 (m, 4H), 5.27 (m, 4H, CH_2),^{13}C NMR (50 MH_Z, $CDCl_3$) $\delta_C$ppm,:(CH_2) 71.8, 115.1, 123.7, 126.3, 129.1, 132.4, 161.4, 190.9 (CHO) [32].

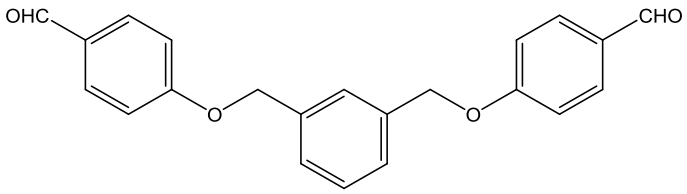

Figure 2.9 dialdehyde structure (A_3)

2.4 SYNTHESIS OF DI-IMINES (B_1-B_3)

2.4.1 1,3bis(2-phenyl methoxy)diphenyl bis(methanylylidene benzo[1,3]dioxol-5-amine)(B_1)

To the first dialdehyde (A_1) (0.346 g, 1mmol) a (0.174 g , 2 mmol) of 1,3 Benzodioxol-5-amine was added in methanol (15 ml) and stirred overnight in an external over night at temperature 70°C. The resulting product was purified in cold distilled water and ice twice to remove unreacted materials; after filtration an ocher product was obtained. $C_{36}H_{28}N_2O_6$: 0.527 g 90% yield and m.p 62°C. FT-IR (solid cm^{-1}): 3073-3035 υ(C=C-H), 1601 υ(CHN), 1555 υ(C=C), 1220 δ(C-O), 751.6 δ(C=C-H).^{1}H NMR (400MH_Z, DMSO-d_6), δ_H ppm: 8.85 (s, 2H, CHN), 6.1 (s, 4H, CH_2), 6.6-6.7 (m, 2H), 6.85-6.87 (m, 4H), 7 (t, J= 7.4 H_Z, 2H), 7.2 (d, J= 7.8 H_Z, 2H), 7.44-7.47 (m, 4H), 7.68-7.70 (m, 2H), 7.97-7.99 (m, 2H), 5.28 (s,4H, CH_2), ^{13}C NMR (100 MH_Z, DMSO-d_6), δ_C ppm: 70.26 (CH_2), 101.76, 102, 108.76, 114.2, 115.22, 121.46, 124.97, 127.39, 127.60, 129.27, 133.17, 137.66, 146, 146.99, 148.45, 154.36, 158.63 (CHN).

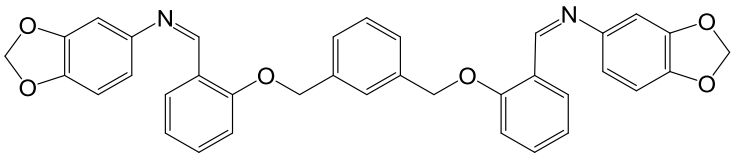

Figure 2.10 di-imine structure (B_1)

2.4.2 1,3bis(3-phenyl methoxy)diphenyl bis (methanylylidene benzo[1,3]dioxol-5-amine) (B_2)

To the second dialdehyde (A_2) (0.346 g, 1mmol) a (0.174 g, 2 mmol) of 1,3 Benzodioxol-5-amine was added in methanol (15 ml) and stirred overnight in an external over night at temperature 70°C. The resulting product was purified in cold distilled water and ice twice to remove unreacted materials and then after filtration an ocher product was obtained. $C_{36}H_{28}N_2O_6$: 0.538 g 92% yield and m.p 101°C. FT-IR (solid cm^{-1}): 3068-3005 υ(C=C-H), 1680 υ(CHN), 1562 υ (C=C), 1232 δ(C-O), 716 δ

(C=C-H).^{1}H NMR (400 MH, DMSO-d_6), δH ppm: 8.61 (s, 2H, CHN), 6.1 (s, 4H, CH_2), 6.83 (dd, J= 8.2,2.1 H_Z, 2H), 6.96(d, J= 8.3 H_Z, 2H), 7(d, J= 2.3 H_Z, 2H), 7.17-7.18 (m, 4H), 7.45-7.47 (m, 4H), 7.49-7.51(m, 4H), 7.58-7.63 (m, 4H), 5.22 (s, 4H, CH_2).^{13}C NMR (100 MH_Z, DMSO-d_6) δ_C ppm: 69.17 (CH_2), 101.39, 108.30, 113.50, 115.78, 118, 121.67, 127.26, 128.62, 129.91, 137.17, 137.62, 145.46, 145.87, 148, 158.59 (CHN).

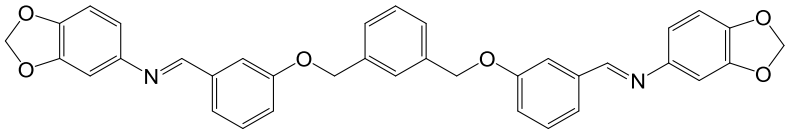

Figure 2.11 di-imine structure (B2)

2.4.3 1,3bis(4-phenyl methoxy)diphenyl bis (methanylylidene benzo[1,3]dioxol-5-amine) (B_3)

To the third dialdehyde (A_3) (0.346 g, 1mmol) a (0.174 g, 2 mmol) of 1,3 Benzodioxol-5-amine was added in methanol (15 ml) and stirred overnight in an external over night at temperature 70°C.The resulting product was purified in cold distilled water and ice twice to remove unreacted materials and then after filtration an ocher product was obtained. $C_{36}H_{28}N_2O_6$: 0.125 g 91% yield, m.p 186°C. FT-IR (solid cm^{-1}): 3066-3014 υ(C=C-H), 1601 υ(CHN), 1572 υ(C=C-H), 1220 δ(C-O), 719 δ (C=C-H).^{1}H NMR(400 MH_Z, DMSO-d_6),δ_H ppm: 8.55 (s, 2H, CHN), 6.77(d, J= 2 H_Z, 1H),6.93-6.96 (m, 4H), 7.14-7.16 (m, 4H), 7.23 (d, J = 8.9 H_Z, 1H), 7.46-7.47 (m, 2H), 7.6 (s, 2H), 7.85-7.87 (m, 4H),6.1 (s, 4H, CH_2),5.23 (s, 4H, CH_2).^{13}C NMR(100 MH_Z, DMSO-d_6),δ_C ppm: 69.25 (CH2), 101.25, 101.39, 108.26, 115, 115.28, 127, 127.39, 128.67, 129.24, 130.20, 136.97, 145.44, 145.99, 147.95, 158, 160.70 (CHN).

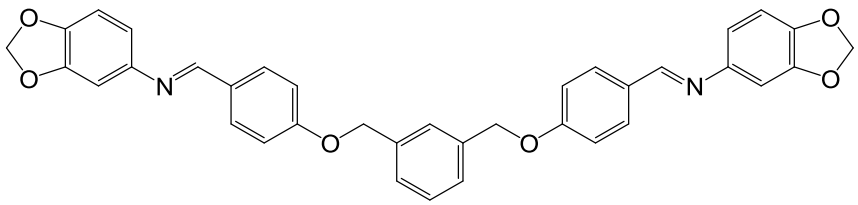

Figure2.12 di-imine structure (B_3)

2.5 Biological activity

The antimicrobial activities of the di-mines (B_1-B_3) estimated by utilizing the diffusion method and the minimal inhibitory concentration dilution method in dimethyl sulfoxide respectively against some bacteria including: Gram+ and Gram$^-$ bacteria. The antifungal activities carried out on several yeast cultures. The results compared with standard reagents using the following antibiotics and antifungals: Penicillin, Ampicillin, Cefotaxime, Vancomycin, Ofloxacin, Tetracycline, Gentamycin, Nystatin, Ketoconazole and Clotrimazole.

2.5.1 Disk diffusion method

In this method a standard way were used for preparation of samples according to standard methods in microbiology tests. Compounds were dissolved in dimethyl sulfoxide (2 mg ml^{-1}). A sterilized diffusion disk (6 mm) were utilized. All bacteria incubated at 30 ± 0.1 ° C for 24 hours in a Nutrient Broth. The yeasts incubated for 48 hours in Malt Extract Broth. Disks injected with the prepared solutions. They placed on agar injected with different microorganisms. A group of samples incubated in case of injecting agar with bacteria for 24 hours at 35°C . Other samples in case of injecting the agar with yeast incubated for 72 hours at 25°C . In each case a triplicate tests have been done and the average was taken as the final reading [33,34].

2.5.2 Minimal inhibitory concentration dilution method (MIC)

In this method a standard way were used for preparation of samples according to a manual in clinical microbiology. All bacteria incubated at 30 ± 0.1 ° C for 24 hours

in a Nutrient Broth. The yeasts incubated for 48 hours in Malt Extract Broth. All compounds dissolved in dimethyl sulfoxide (2 mg ml^{-1}). Then they were diluted carefully adjusted Mueller Hinton Broth. Two fold serial used to determine the minimal inhibitory concentration (MIC) and the lowest concentration that cause complete inhibition considered as (MIC). In each case a triplicate tests have been done and the average was taken as the final reading [35].

CHAPTER 3

RESULTS AND DISCUSSION

3.1 VIBRATIONAL SPECTROSCOPIC STUDIES OF DIALDEHYDES

FT-IR for dialdehydes (A_1-A_3) has been investigated in the range (3500-400) cm^{-1} and it revealed a clear and nice peak in the following ranges: (1670-1686) cm^{-1} which suggests presence of aldehyde functional group,(1587-1590) cm^{-1} indicating the presence of (C=C-H),(1231-1256) cm^{-1} in which (C-O)can be observed, (772-781) cm^{-1} indicating the presence of (C=C-H) as shown in Figures (313.,3.14,3.15).

3.2 VIBRATIONAL SPECTROSCOPIC STUDIES OF DI-IMINES

FT-IR for di-imines (B_1-B_3) has been investigated in the range (3500-400) cm^{-1}. and revealed a clear and nice peaks in the following ranges within range: (1601-1608) cm^{-1} which suggests presence of (CHN),(1555-1572) cm^{-1} indicating the presence of (C=C-H),(1220-1232)cm^{-1} in which(C-O) can be observed, (716-751.6) cm^{-1} indicating the presence of (C=C-H) as shown in Figures (3.16,3.17,3.18).

3.3 NUCLEAR MAGNETIC RESONANCE (NMR) STUDIES FOR DIALDEHYDES

The ^1H NMR (400 MH_Z, DMSO-d_6) δ_H ppm for dialdehydes (A_1-A_3) has been investigated and it showed a clear signals for H protons in following ranges:(9.9-10.44) ppm for 2H protons belong to (CHO) functional group, (7.1-7.9) ppm for 12 H protons

belong to the aromatic functional group and (5.24-5.34) ppm for 4H protons belong to the methoxy functional group.as shown in Figures (3.19,3.20,3.21).

^{13}C NMR (50 MH_Z, $CDCl_3$), δ_C ppm have been investigated and it showed the exact numbers of carbons in the chemical structures of dialdehydes(A_1-A_3) at the following ranges: (70.2-71.8) ppm for 2 carbons belong to (CH_2) group, (114.9-163.7) ppm for 18 carbons belong to the aromatic region and 2 carbons at 190.9 ppm which suggests presence of (CHO) functional group [32].

3.4 NUCLEARMAGNETIC RESONANCE (NMR) STUDIES FOR DI-IMINES

The^{1}H NMR (400 MH_Z, DMSO-d_6) δ_H ppm for di-imines(B_1-B_3) has been investigated and it showed a clear signals for H protons in following ranges: (8.55-5.85) ppm for 2 H protons belong to (CHN) functional group, (6.7-7.9) ppm 18 H protons for the aromatic functional group, (5.23-5.28) ppm for 4 H protons belong to the methoxy functional group, (6,6.1) ppm for 4H protons which belongs to dioxol functional group as shown in Figures (3.22,3.23,3.24).^{13}C NMR (100 MH_Z, DMSO-d_6) δ_C ppm have been investigated and it showed the exact numbers of carbons in the chemical structure of di-imines (B1-B3) at the following ranges:(69.17-70.26) ppm for 4 carbons of (CH_2) group belong to methoxy and dioxol functional groups, (101.25-148.45) ppm for 30 carbons belong to the aromatic region and 2 carbons at (158.5-160.7) ppm which suggests presence of (CHN) functional group and disappearance of (CHO) functional groupas shown in Figures(3.18.,3.19.,3.20).

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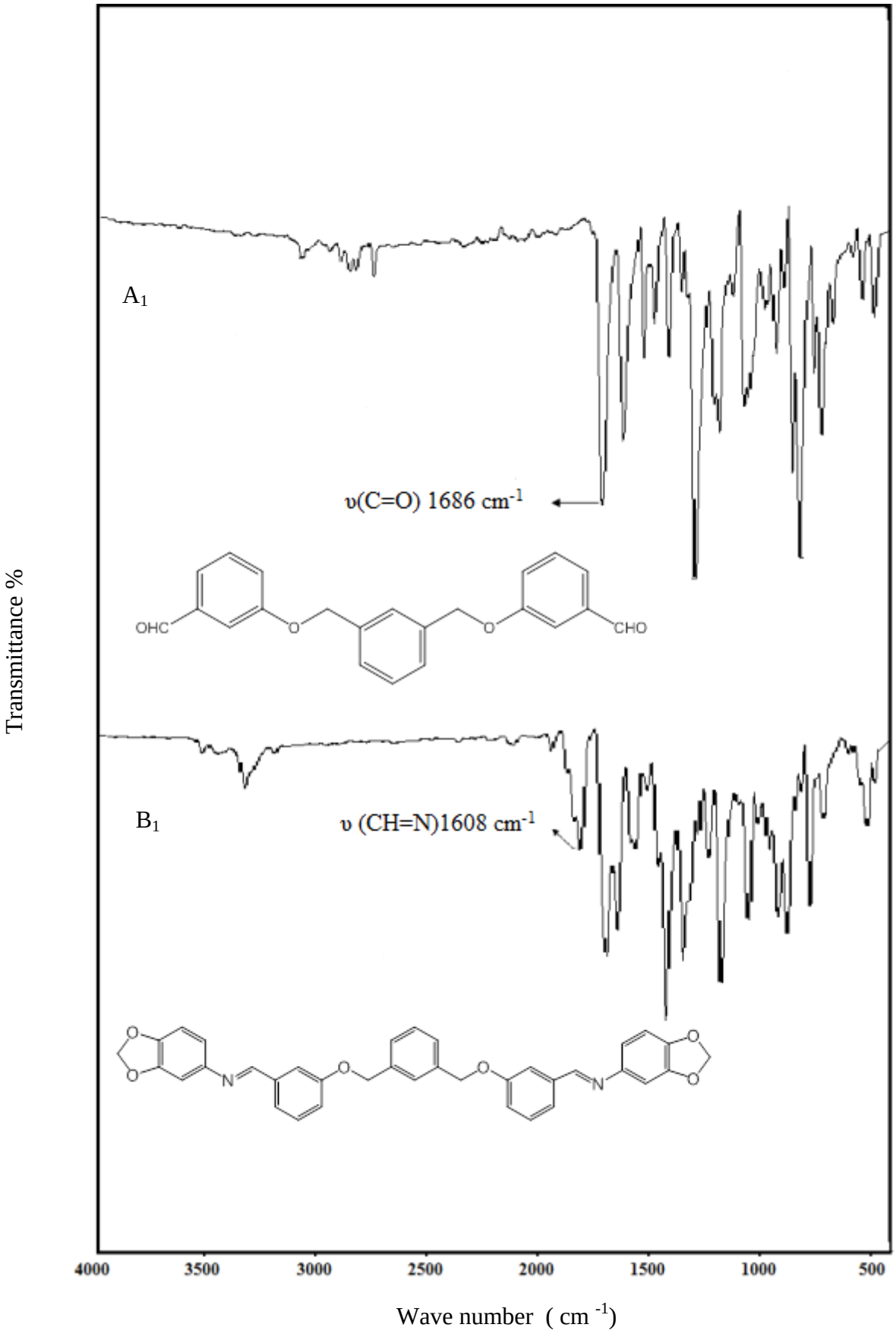


Figure3.13 Comparative FT-IR spectra of dialdehyde (A_1) and di-imine (B_1)

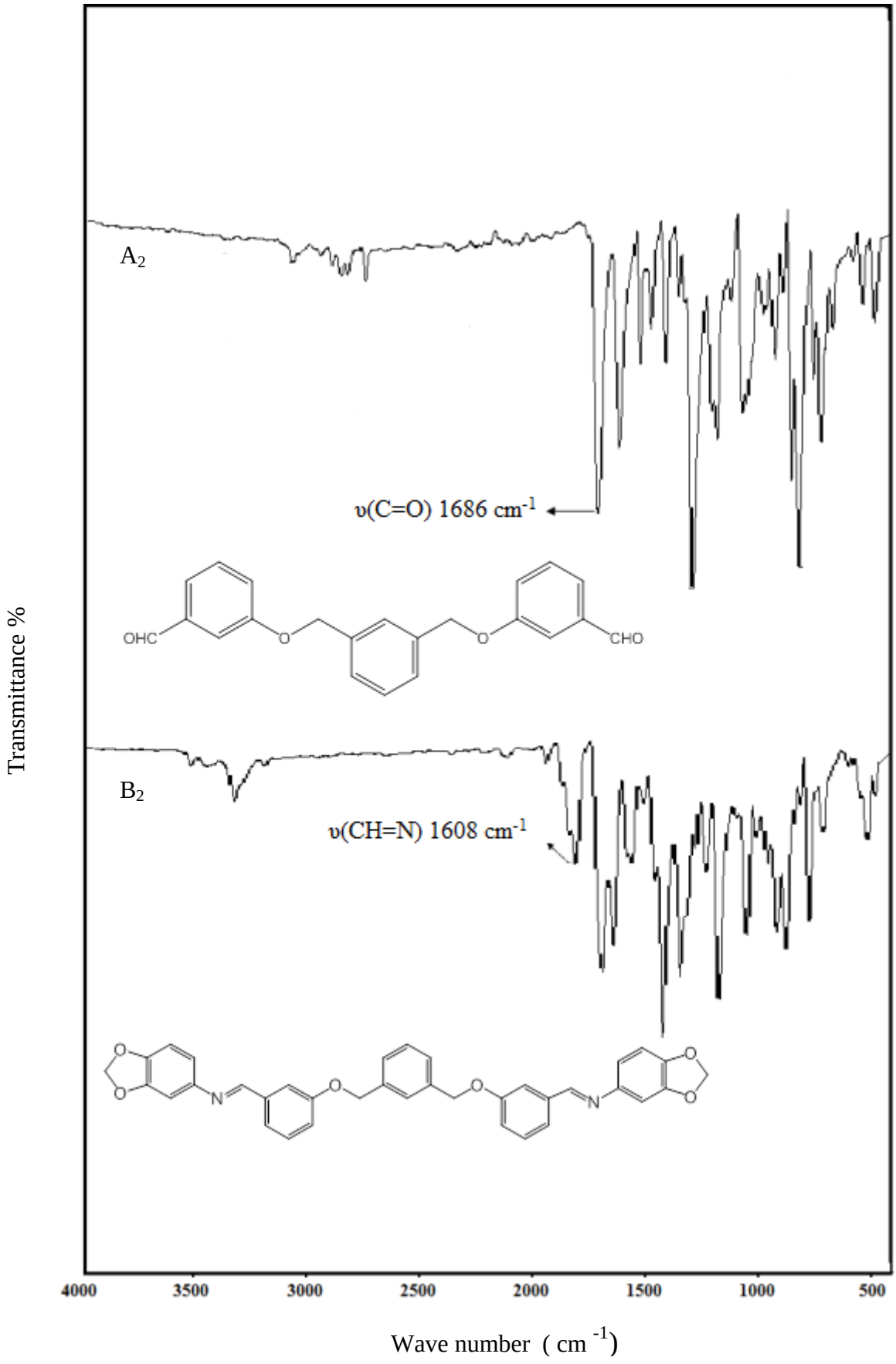


Figure3. 14 Comparative FT-IR spectra of dialdehyde (A_2) and di-imine (B_2)

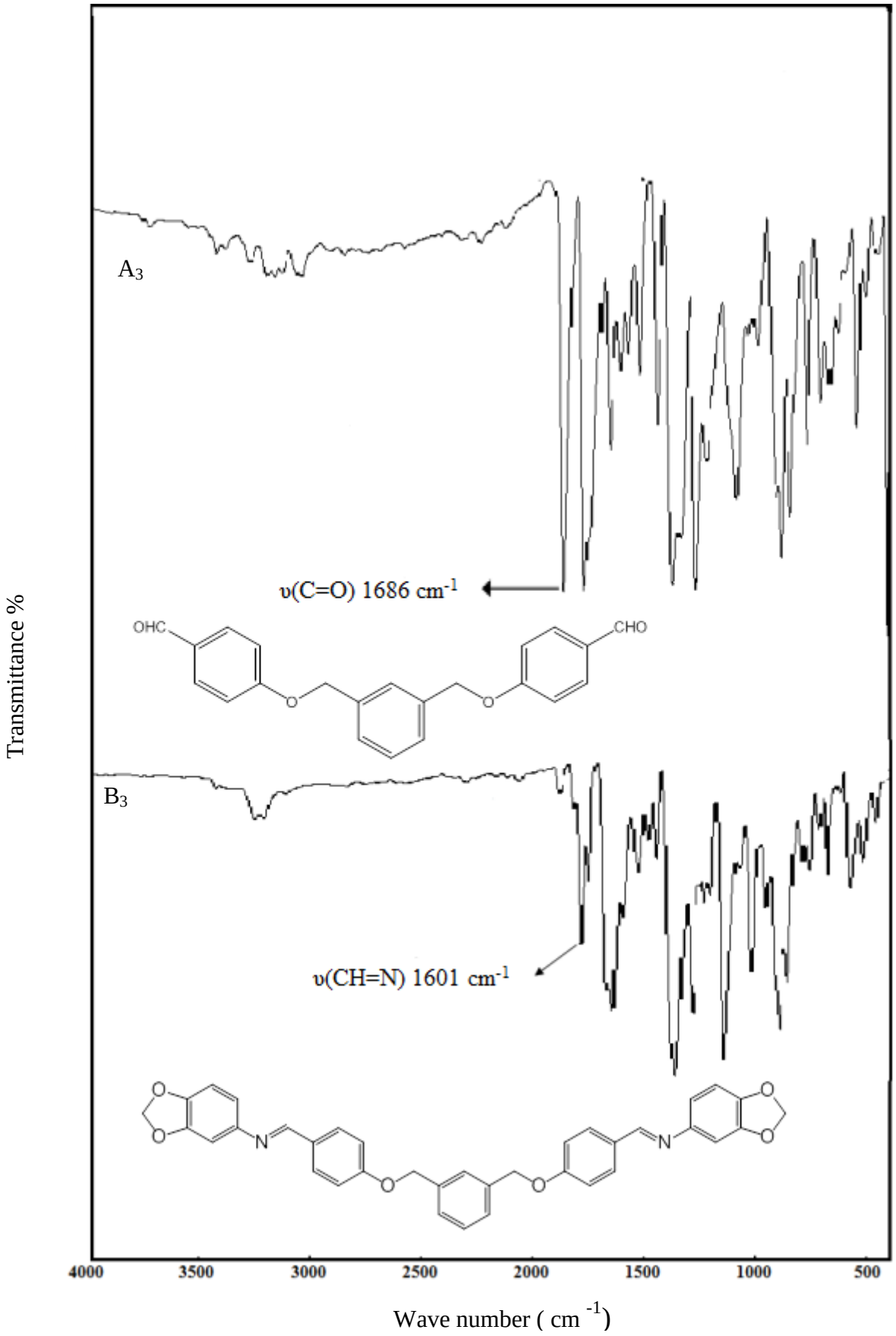


Figure3.15 Comparative FT-IR spectra of dialdehyde (A_3) and di-imine (B_3)

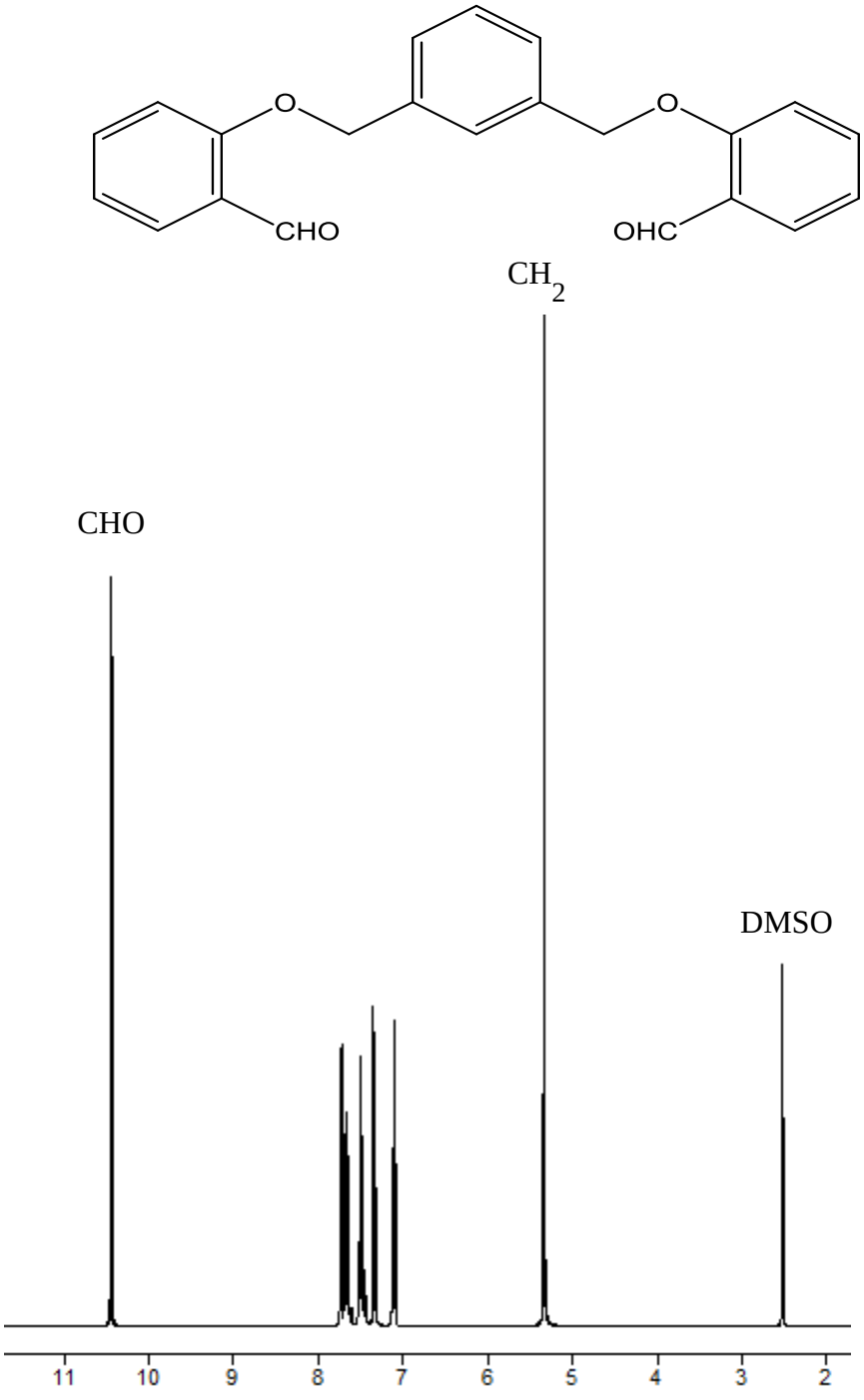


Figure 3.16 ^{1}H NMR spectra for dialdehyde (A_1)

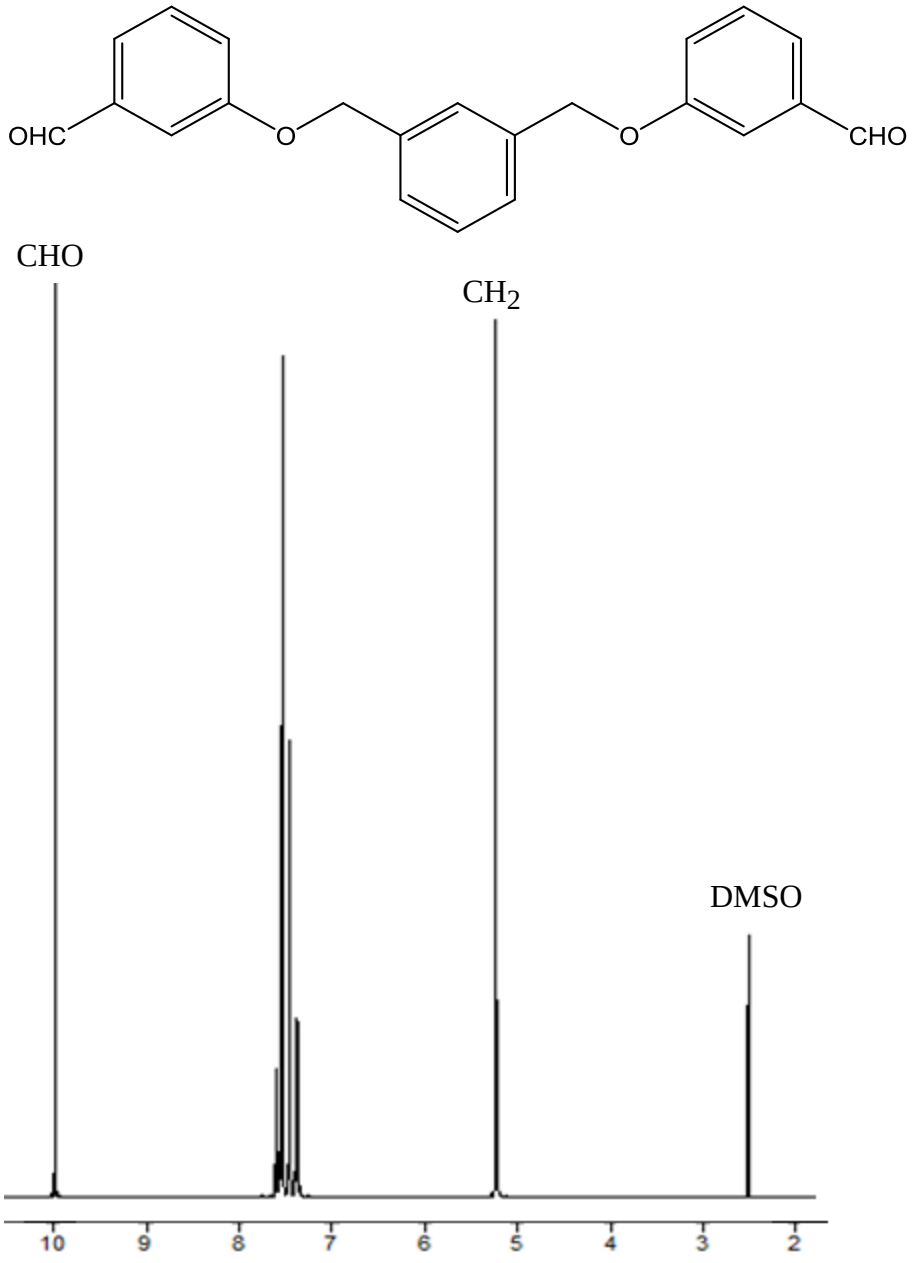
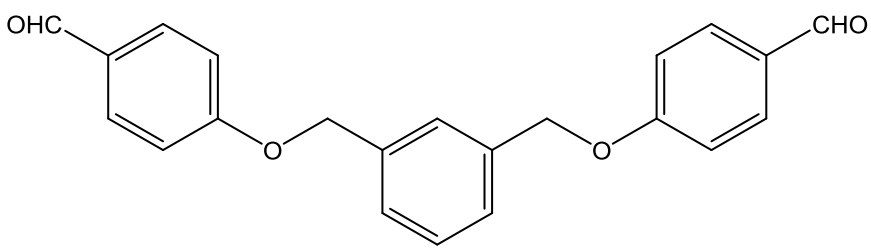


Figure 3.17 ^{1}H NMR spectra for dialdehyde (A_2)



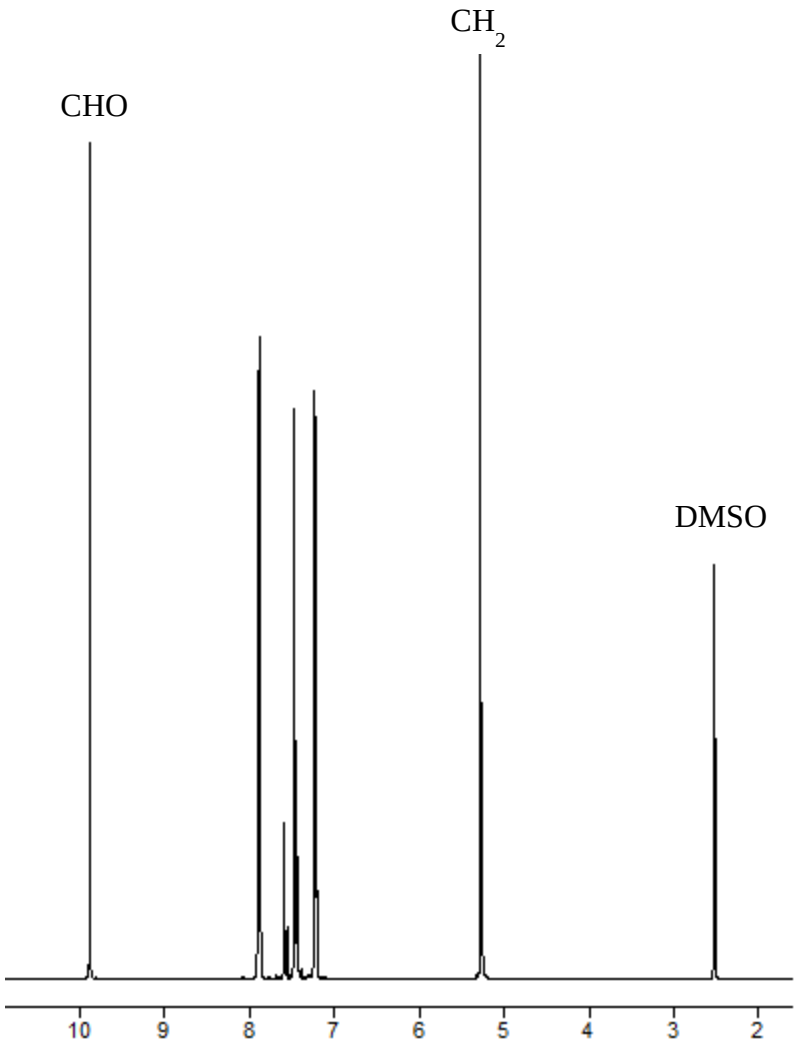


Figure 3.18 ^{1}H NMR spectra for dialdehyde (A_3)

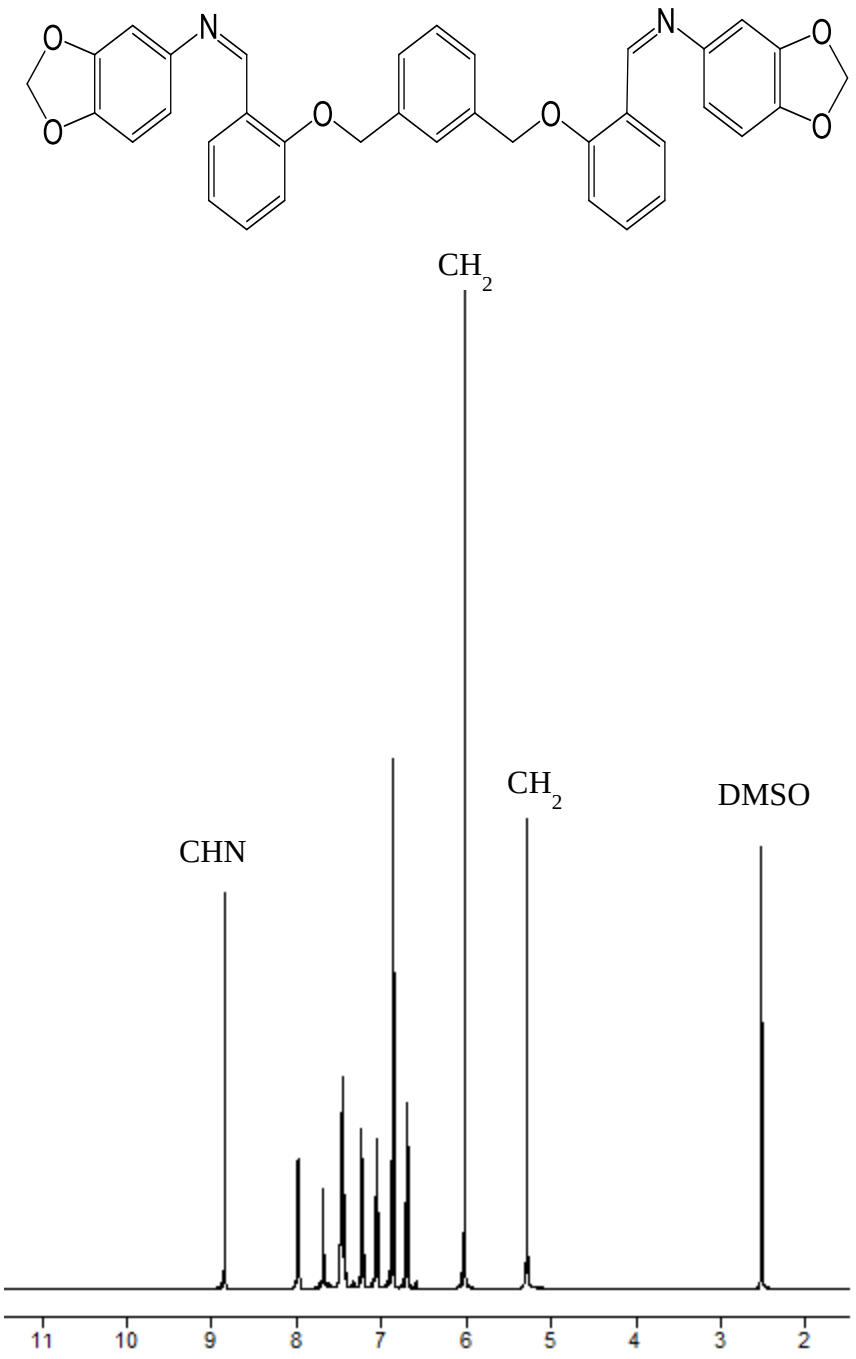
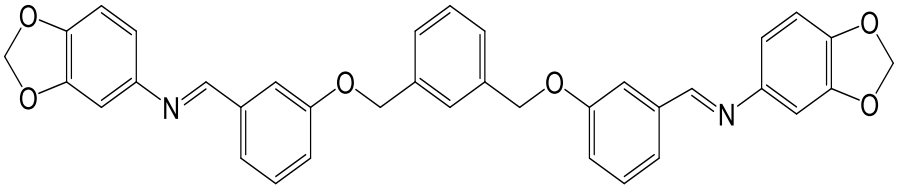


Figure 3.19 ^{1}H NMR spectra for di-imine (B_1)



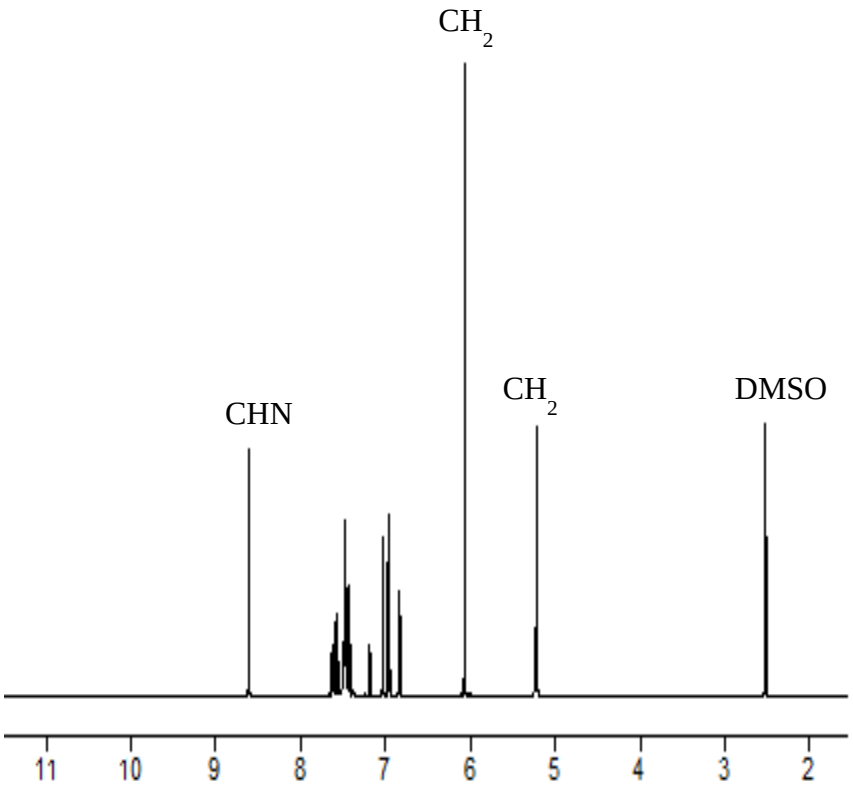


Figure 3.20 ^{1}H NMR spectra for di-imine (B_2)

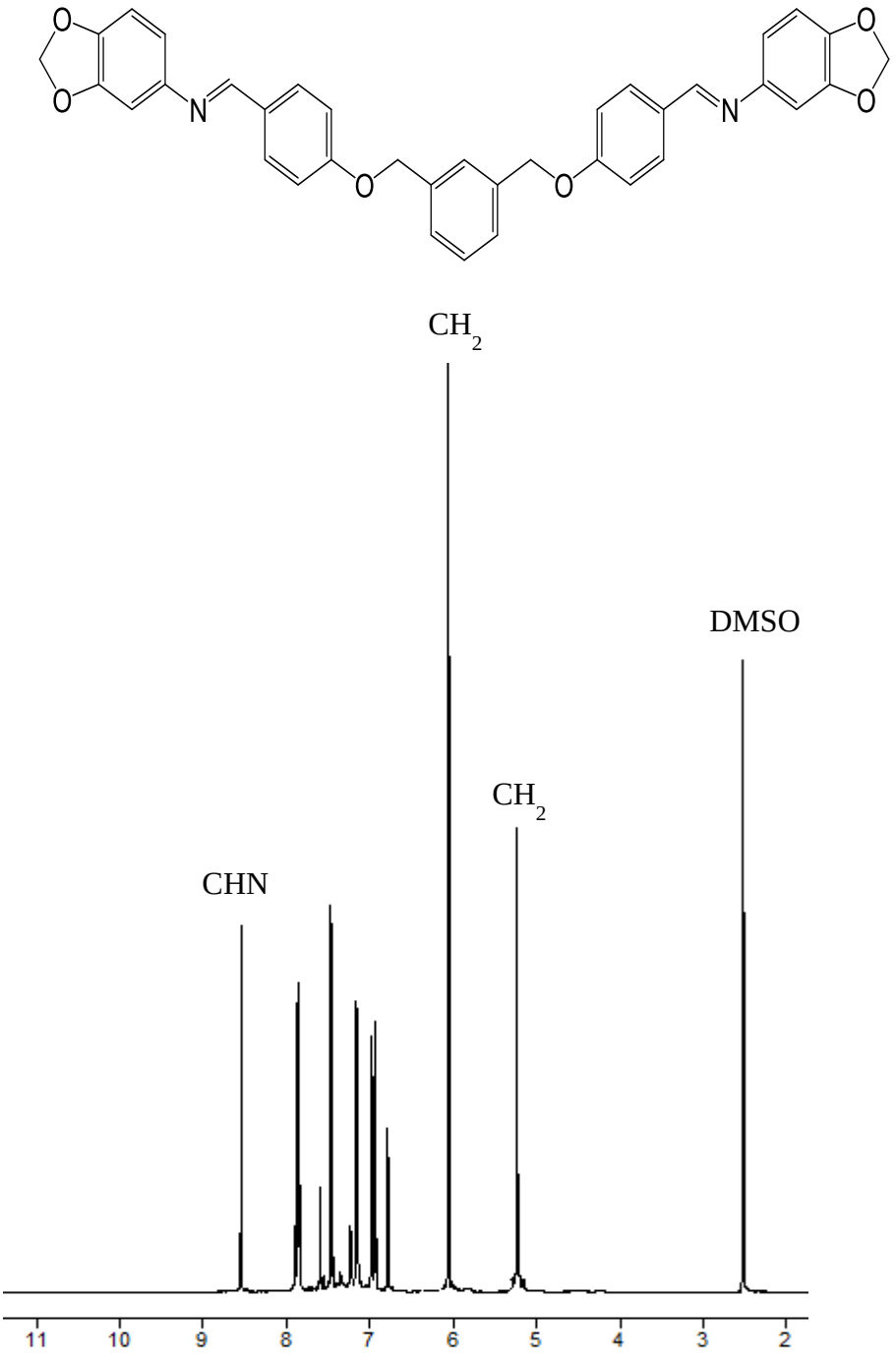


Figure 3.21 ^{1}H NMR for di-imine (B_3)

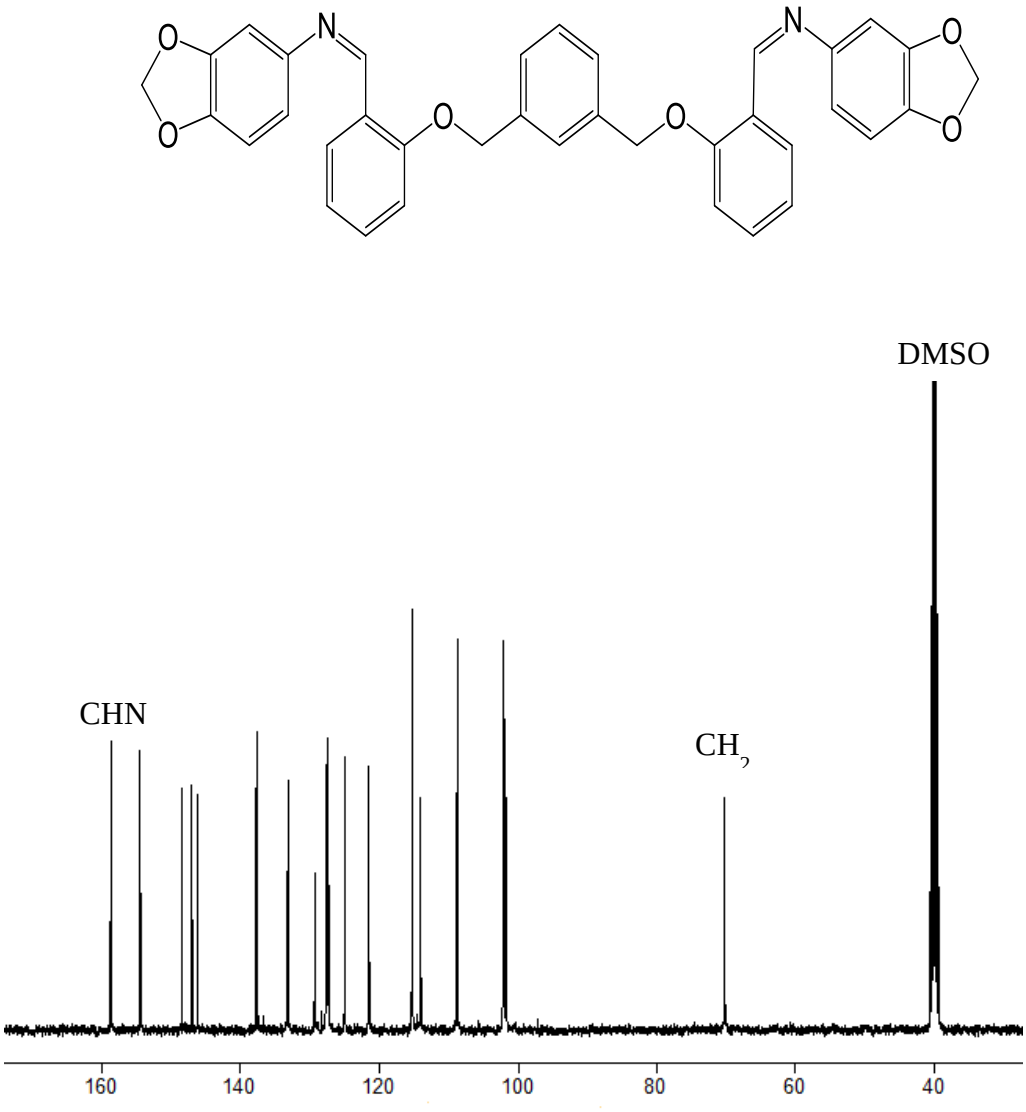


Figure 3.22 ^{13}C NMR spectra for di-imine (B_1)

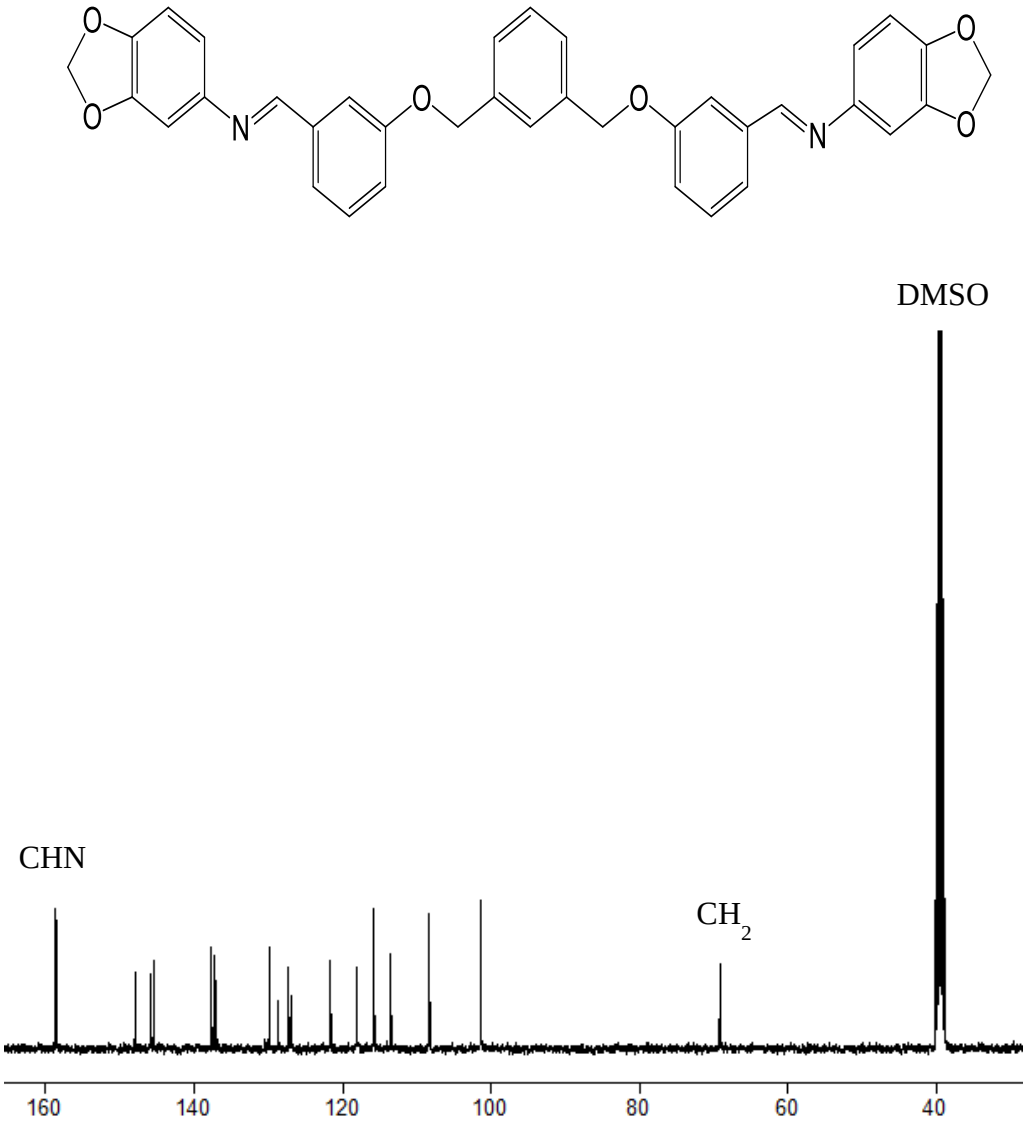


Figure 3.23 ^{13}C NMR spectra for di-imine (B_2)

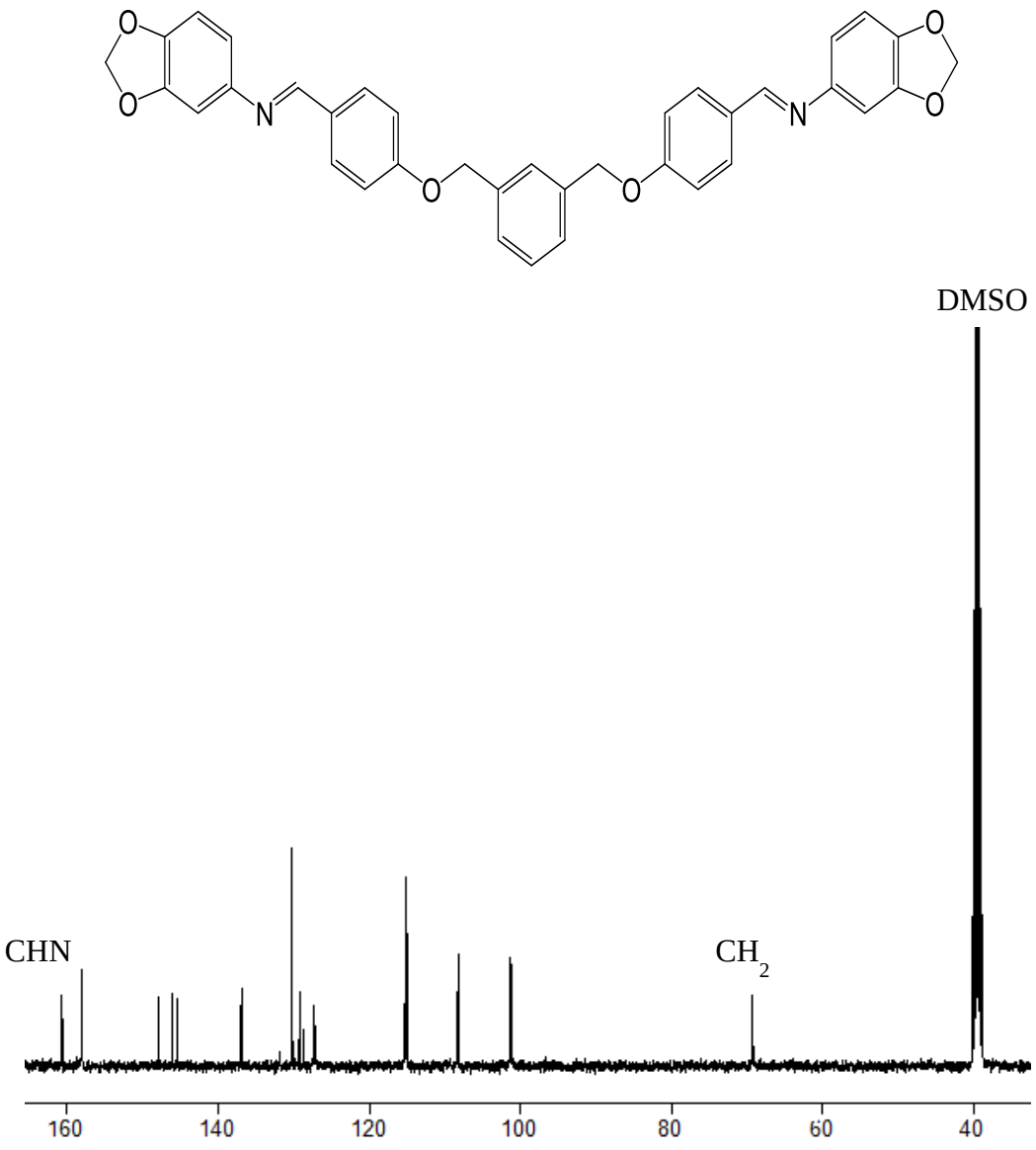


Figure 3.24 ^{13}C NMR spectra for di-imine (B_3)

3.5 ANTI MICROBIAL STUDIES FOR DI-IMINES

The antimicrobial activities of the di-mines (B_1-B_3) estimated by utilizing the disk diffusion method and the minimal inhibitory concentration dilution method in dimethyl sulfoxide respectively against some bacteria including: Gram+ and Gram$^-$ bacteria. The antifungal activities carried out on several yeast cultures. The results compared with standard reagents using the following antibiotics and antifungals: Penicillin, Ampicillin, Cefotaxime, Vancomycin, Ofloxacin,Tetracycline,Gentamycin, Nystatin, Ketoconazole, and Clotrimazole. Some of the presently synthesized compounds demonstrate higher antimicrobial and antifungal activities than the standard reagents.

In vitro antimicrobial activity for (B_1- B_3), using the disk diffusion method showed that Some of them having higher antimicrobial and antifungal activities than the standard reagents.

B_1 showed the highest antimicrobial activity against Bacillus cereus$^+$, Escherichia coli$^-$ and Klebsiella pneumoniae$^-$. B_2 showed the highest antimicrobial activity against Bacillus cereus$^+$, Micrococcus luteus$^+$, Mycobacterium smegmatis$^+$, Listeria monocytogenes, Escherichia coli$^-$, and Klebsiella pneumoniae$^-$. B_3 showed the highest antimicrobial activity against Bacillus cereus$^+$, Escherichia coli$^-$, Klebsiella pneumoniae$^-$ and Pseudomonas aeruginosa$^-$ as shown in the Table 3.1.

In vitro antimicrobial activity for (B_1- B_3) using the disk diffusion showed that Some of them having higher antifungal activities than the standard reagents. B_1 showed the highest antifungal activity in comparison with the standards as shown in the Table 3.1.

In vitro antimicrobial activity for (B_1-B_3) utilizing dilution method also showed that Some of them having higher antimicrobial and antifungal activities than the standard reagents.

B_2 showed the highest antimicrobial activity against Pseudomonas aeruginosa$^-$. B_3 showed the highest antifungal activity Kluyveromyces fragilis, Rhodotorula rubra, Candida albicans, Hanseniaspora guilliermondii and Debaryomyces hansenii standards as shown in the Table 3.2.

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Table 3.1 In vitro antimicrobial activity (mm zone) of (B_1- B_3) and standard reagents.

Microorganisms\compounds	B_1	B_2	B_3	PE	AM	CT	VA	OF	TE	NY	KE	CL
Staphylococcus aureus $^{+}$	18	12	15	13	16	12	13	24	26		-	-
Bacillus cereus^{+}	22	24	26	14	12	14	18	30	25		-	-
Micrococcus luteus^{+}	18	20	13	36	32	32	34	28	22		-	-
Mycobacterium smegmatis^{+}	18	22	16	15	21	11	20	32	24		-	-
Listeria monocytogenes	16	22	16	10	12	16	26	30	28		-	-
Escherichia coli^{-}	26	28	22	18	12	10	22	30	28		-	-
Proteus vulgaris $^{-}$	18	16	18	10	16	18	20	28	26		-	-
Klebsiella pneumoniae^{-}	28	22	26	18	14	13	22	28	30		-	-
Pseudomonas aeruginosa^{-}	16	18	24	8	10	54	10	44	34		-	-
Kluyveromyces fragilis	20	22	18	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	22	16	18	-	-	-	-	-	-	18	22	16
Candida albicans	20	18	20	-	-	-	-	-	-	20	21	15
Hanseniaspora guilliermondii	18	16	22	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	22	16	18	-	-	-	-	-	-	16	14	18

Table 3.2 In vitro antimicrobial activity (MIC, µg ml $^{-1}$) of (B_1- B_3) and standard reagents.

Microorganisms\compounds	B_1	B_2	B_3	GEN	NY
Staphylococcus aureus $^{+}$	12.5	12.5	12.5	25	-
Bacillus cereus^{+}	6.25	12.5	12.5	6.25	-
Micrococcus luteus^{+}	12.5	25	25	25	-
Mycobacterium smegmatis^{+}	12.5	12.5	12.5	12.5	-
Listeria monocytogenes	6.25	25	12.5	12.5	-
Escherichia coli^{-}	12.5	12.5	6.25	6.25	-
Proteus vulgaris $^{-}$	6.25	25	12.5	6.25	-
Klebsiella pneumoniae^{-}	6.25	6.25	12.5	6.25	-
Pseudomonas aeruginosa^{-}	12.5	50	25	6.25	-
Kluyveromyces fragilis	6.25	12.5	25	-	6.25
Rhodotorula rubra	12.5	12.5	12.5	-	6.25
Candida albicans	6.25	12.5	12.5	-	3.125
Hanseniaspora guilliermondii	6.25	12.5	6.26	-	3.125
Debaryomyces hansenii	6.25	12.5	25	-	12.5

CHAPTER 4

CONCLUSION

Finally, in this thesis, three of dialdehyde compounds and three di-imine compounds have been synthesized. All compounds were purified and their melting points have been measured. FT- IR and NMR spectroscopies have been taken. FT-IR data revealed all the functional groups for each compound. Both ^{1}H NMR and ^{13}C NMR gave the exact number of hydrogen atoms and carbon atoms subsequently. In addition, they gave signal ranges for each functional group in the normal range. Other researchers have been synthesized dialdehyde compounds which have been synthesized in this project[32,36-47].However the three di-imine compounds that have been synthesized are new.

The antimicrobial activities of the di-mines (B_1-B_3) estimated by utilizing the disk diffusion method and the minimal inhibitory concentration dilution method in dimethyl sulfoxide respectively against some bacteria including: Gram+ and Gram$^-$ bacteria. The antifungal activities carried out on several yeast cultures. The results compared with standard reagents using the following antibiotics and antifungals: Penicillin, Ampicillin, Cefotaxime, Vancomycin, Ofloxacin, Tetracycline, Gentamycin, Nystatin, Ketoconazole, and Clotrimazole. Some of the presently synthesized compounds demonstrate higher antimicrobial and antifungal activities than the standard reagents.

Heterocyclic compounds have a lot of applications in medicinal chemistry therefore my recommendations are:

1) There is need for more investigations about compounds containing Schiff base for possibility of using them as an anticancer agents.

2) To do more researches and finding new ways to use Schiff base as prodrug as they are flexible and easy to be modified.

The importance of prodrug is to increase; permeability, solubility, absorption, distribution and targeting of drugs especially for cancer treatment. At the same time to decrease side effect of drugs and duration of the therapy.

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