Journal of Kufa for Chemical Sciences
ISSN(P) :2077-2351 ISSN( E ): 3006-3280
Journal Homepage:
https://journal.uokufa.edu.iq/index.php/jkcs/index

Article

# Synthesis, Identification and Breast cancer Studies of New Bi-Cyclic Oxazepine and Diazepine Derivatives

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**ABSTRACT**

In this study, new compounds were produced to form several new Schiff bases by combining compounds {1, 4, 5, 6, and 7} with di-carboxyl groups, and by combining compounds {2 with 3} with compounds containing an amino group. Through this condensation, six- and seven-membered rings were obtained, in addition to five-membered rings such as triazoles and oxazoles with seven membered ring like oxazepane and diazepine, which are important and fundamental compounds used in various industrial fields, such as pharmaceuticals, vitamins, dyes, and many industrial and natural products. Triazoles and their derivatives are among the important compounds used in the manufacture of nucleic acids, enzymes, and polymers. The structural formulas of these compounds were diagnosed and determined using spectroscopic studies such as infrared spectroscopy (FT-IR), carbon nuclear magnetic resonance spectroscopy (13C-NMR), and proton nuclear magnetic resonance spectroscopy (1H-NMR). Biological activity was utilized according to the functional groups of these compounds and their appearance in each spectrum. The difference was

also found in the substituted groups during the comparison of the derivatives preparation process.

**Keywords**: Schiff base, biological activity, triazole, oxazole, heterocyclic ,oxazepine, diazepine, five ring.

## Introduction

Heterocyclic compounds are unsaturated organic compounds that have different atoms such as oxygen, nitrogen, and sulfur. These compounds contain two or more of these atoms. An example of these compounds are five-membered rings such as oxadiazole and triazole, with seven membered ring : oxazepane, diazepine and their derivatives [1-4]. These compounds are formed by replacing oxygen and nitrogen atoms with carbon atoms within the backbone of the ring [5,6] These compounds are of chemical importance because they are used in the preparation of many drugs and antibiotics [7-11]. The reason for their effectiveness is that they contain an electron density that gives them high effectiveness among their derivatives and other compounds [12-16],

## 2. Methodology

### 2.1 . Experimental Methodologies:

**Synthesis of Compound{B} :**

Compound {B} was prepared from compound {A} via using (2 mole) of maleic anhydride by sublimation for( 8 hours) using benzene as a solvent, after filtration, drying , crystallization by quit solvent to yield 74% of compound.

**Synthesis of Compound{1} :**

Synthesis of Compound{1} from (0.001 mol ) of Compound{B} refluxed (0.001 mol ) of urea refluxed (6 hrs ) with ethanol and with (2 ml HCl). The linking step and the output formation diazepine ring were completed ring derivative, product filtered ,desiccated ,purified by using (DMF) as a solvent DMF to yield compound {1} according to studies [11, 13].

**Synthesis of Compound{2} :**

The second compound was prepared using compound {B} refluxed (6hrs) with (0.001 mol) of Guanidine with ((2 ml HCl)) with ethanol (50 ml), the product filtered ,desiccated ,purified by using (DMF) as a solvent DMF to yield compound {2} according to studies [11, 13].

**Synthesis of Compound{3} :**

Cyclic compound {3} prepared from reaction of compound {B} with (0.001 mol) of diamine compound refluxed (6 hrs) for cyclization step to formation diazepine ring the creation of cyclic,the product filtered ,desiccated ,purified by using (Eth.OH) as a solvent cyclohexane to yield cyclic compound {3} according to studies [11, 13].

**Synthesis of Compound{4} :**

Cyclic compound {4} prepared from reaction of compound {A} refluxed (6 hrs) with (0.001 mol) of Formaldehyde with $H_2SO_4$ in basic medium (5ml of 5% NaOH), the creation of cyclic compounds for cyclization step to formation Five membered ring derivative, the product filtered ,desiccated ,purified by using (DMF) as a solvent to yield cyclic compound {4} according to studies [11, 13] .

**Synthesis of Compound{5} :**

Cyclic compound{5}prepared from reaction of compound{A}with (0.001 mol)for methyl Benzaldehyde with $H_2SO_4$ refluxed (6 hrs) with ethanol (50 ml), the reaction of cyclic compounds for cyclization step to formation Five membered ring derivative, the product filtered ,desiccated ,purified by using (Eth.OH) as a solvent cyclo hexane to yield cyclic compound {5} according to studies [11, 13].

**Synthesis of Compound{6} :**

Cyclic compound {6} prepared from reaction of compound {A} with (0.001 mol) of diethylmalonate with ethanol (50 ml) for cyclization step to formation seven membered ring derivative, the creation of cyclic compounds ,the product filtered ,desiccated ,purified by using (Eth.OH) as a solvent to yield cyclic compound {6} according to studies [11, 13].

**Synthesis of Compound{7}:**

Cyclic compound{7}prepared from reaction of compound {A} with (0.001 mol) of dicarbonyl compound refluxed (6 hrs) for cyclization step to formation six membered ring the creation of cyclic, the product filtered ,desiccated ,purified by using (Eth.OH) as a solvent to yield cyclic compound {7}according to studies [11, 13].

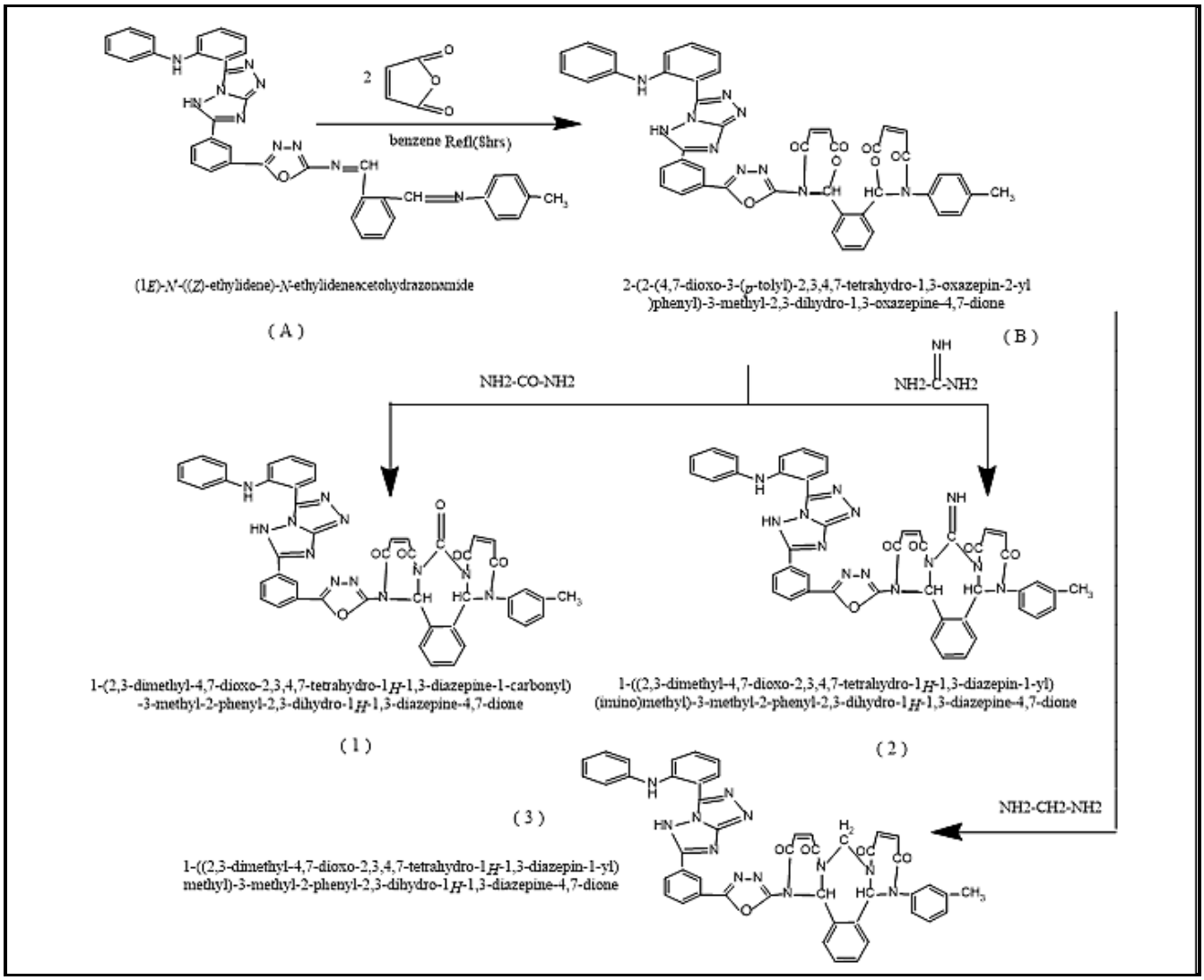
**Cell viability and cytotoxicity assays (MTT)**

The main purpose of the assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)), which is basically a colorimetric test, is to infer and detect the toxicity of the compounds on healthy and infected cells that are being tested. outside the body of the organism and determine the extent of toxicity by calculating the percentage of live cells (cells viability). That is why it was possible to use

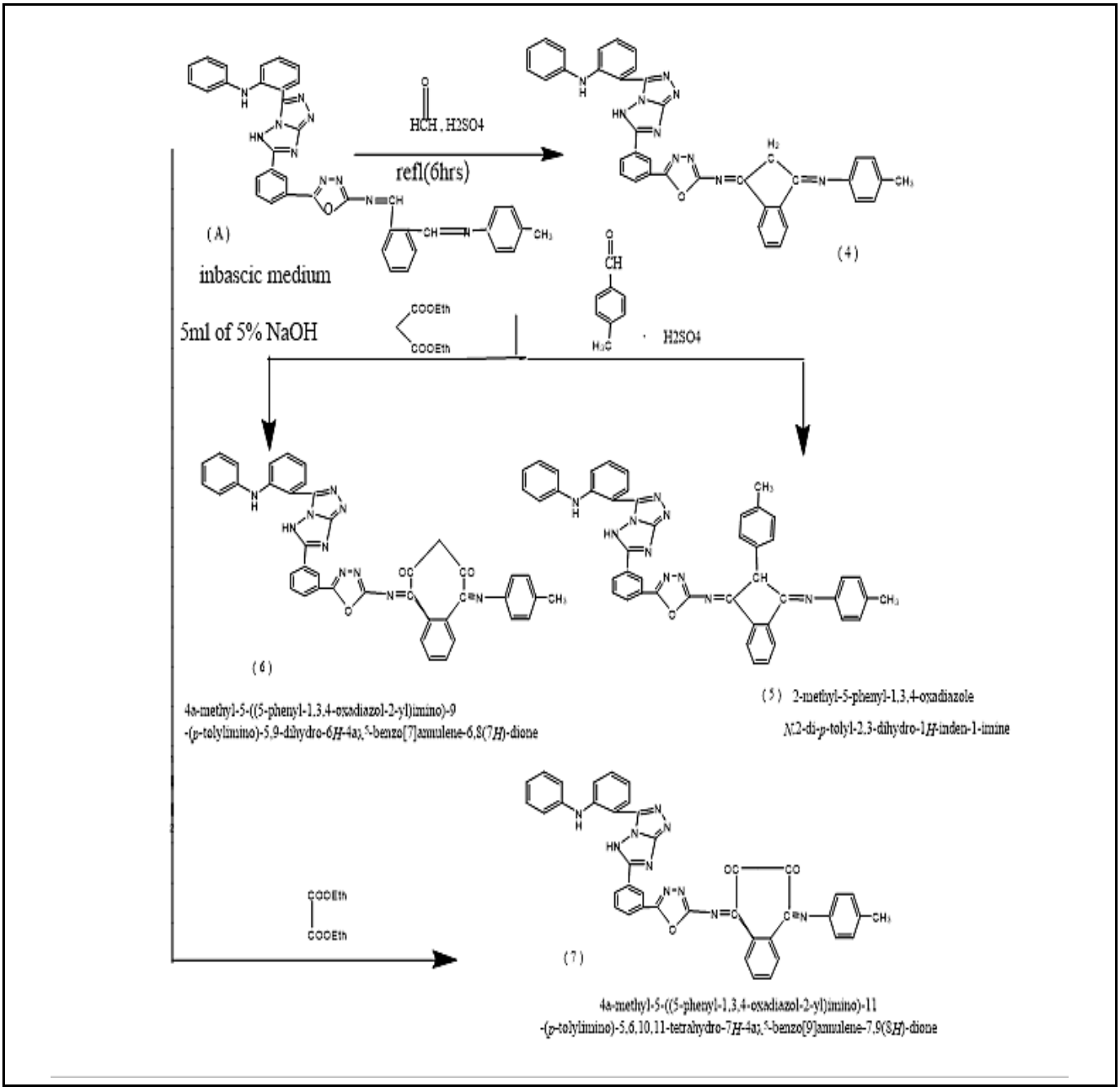
these prepared compounds as a treatment for various types of this disease in the event that healthy cells were not affected and showed toxicity towards these infected cells, as the living cells work to change the color of the (MTT) dye from yellow to the infected cells, As the living cells change the color of the pigment (MTT) from yellow to blue, and the more blue color spreads, this means the increase in the number of living cells

**Initialization of the cancer cell line:**

The cell line (MCF-7) and the cell line (MCF-10A) were grown in (95%) of (RPMI-1640) supplemented with (PBS 10%), the cells were suspended and incubated in (˚C37) in a 5$CO_2$% incubator. The suspended cells were centrifuged at (250gm) for (10min) and the supernatant was removed, the cells were suspended in freezing medium, then placed in a beaker (-70°C) for (1-3) days, the beakers were transferred from the Standard freeze to liquid $N_2$ container (99,100) .




**Pattern.2: : Synthesis of Saturated and Unsaturated Derivatives {A,B,1,2,3}**




**Pattern.2: : Synthesis of Saturated and Unsaturated Derivatives {4,5,6,7}**

## RESULTS AND DISCUSSION

Through the study prepared in this research, unique and new compounds were created. These prepared compounds were studied according to the available spectral studies and

compared between them through the different groups present in them, as the condensation reaction was relied upon in their preparation by focusing on the amine groups (CH=N), present in these compounds and subjecting them to interaction with groups of aromatic aldehydes and intermediate compounds containing dicarboxylic groups. New derivatives of five- and seven-membered rings such as oxadiazole and diazepine were produced. These compounds were compared using spectral studies such as FT-IR spectroscopy. This was done based on the appearance and disappearance of different spectra and frequencies in the prepared compounds, measuring their melting points and determining their molecular weights. They were interpreted based on previous interpretations related to spectral diagnosis techniques, as shown in the calculations in the following tables

**Table.(1): Compounds via Spectral Identification FT.IR- Spectra**

| Com | (CH) aliph (CH) arom | (C=N) endocycle | (NH) or ($NH_2$) | Other bands $cm^{-1}$ |
|---|---|---|---|---|
| 1 | (2924-3010) | (1644) | (3250) (3317) | (CO-N) Lactam:1674,1670 ,(C=CH) alkene(3159) ,(C-O-C)oxadiazol:(1122) |
| 2 | (2950-3014) | (1641) | (3421) (3200) | (CO-N) Lactam:1690,1670 ,(C=CH) alkene (3100),(C-O-C)oxadiazol:( 1147) |
| 3 | (2920-3093) | (1638) | (3444) (3250) | (CO-N) Lactam:1673,1690, (CH=CH) alkene(3194) ,(C-O-C)oxadiazol:(1149) |
| 4 | (2916-3066) | (1678) | (3448) (3299) | (C=N):(1653),(C-O-C)oxadiazol:(1149) |
| 5 | (2950-3000) | (1664) | (3437) (3200) | (C=N):(1641), (C-O-C)oxadiazol:(1149) |
| 6 | (2954-3064) | (1660) | (3437) (3246) | (C=N):(1636), (C-O-C)oxadiazol:( 1147) (C=O) Ketone:(1711) |
| 7 | 2926,3066 | 166 | 3429,3271 | (C=N):(1636), (C-O-C)oxadiazol:( 1147) (C=O) Ketone:(1722) |

**Table.( 2 ): Compounds via Spectral Identification HNMR -Spectra**

| Com | (CH3) methyl | (NH) amine | Aromatic protons | Other peaks |
|---|---|---|---|---|
| 1 | 1.23 ,1.91 | 5.20 | (6.57-7.73) | (N-CH-N) diazepine:2.32 ,(CO-CH=CH-CO) alkene:(6.01,6.15) ,(CH3) :( 0.83) (NH) cycle : (5.89) |

| | | | | |
|---|---|---|---|---|
| 2 | 1.11 , 1.50 | 5.0 | (6.97-7.95) | (N-CH-N) diazepine :2.84 ,(N-C=NH) diazepine :9.79 , (CO-CH=CH-CO) alkene:(6.0,6.05) (CH3) :( 0.90) , (NH) cycle : (5.10) |
| 3 | 1.20, 1.50 | 4.50 | (6.94-8.0) | (N-CH-N) diazepine :2.90 ,(N–CH2-N) diazepine :2.70, (CO-CH=CH-CO) alkene:( 6.0,6.10) (CH3) :( 1.0) ,(NH) cycle : (5.0) |
| 4 | 1.302 , 1.50 | 4.95 | (6.60-7.95) | (=C-CH2-C=) :2.10, (CH3) :(1.11) (NH) cycle : (5.20) |
| 5 | 1.14 , 1.80 | 5.0 | (6.66-7.89) | (CO-CH2-CO) :(2.10) ,(CH3) :(1.0) (NH) cycle : (5.42) |

**Table.( 3 ): Compounds via Spectral Identification $^{13}$C- NMR -Spectra**

| Com | (CH3) carbon of methyl | Aromatic carbon Atoms | Other peaks |
|---|---|---|---|
| 1 | 12.0,18.0 | (113-135) | (CH=CH) alkene : (100 ,104),(CO-N) Lactam :(160 ,164,166),(N–CO-N):(170.0), (CH3) : (0.80) |
| 2 | 31.9 , 34.3 | (116-132) | (CH=CH) alkene : (98.8 ,106), (CO-N) Lactam :( 161.3 ,164.7 ,166.0), (N–C=NH):(79.6), (CH3) : (14.0) |
| 3 | 31.9 , 20.0 | (112-136) | (CH=CH) alkene : (98.7 ,106.2),(CO-N) Lactam :(156 ,161.3 ,164.6),(N–CH2-N):(56.10), (CH3) : (10.0) |
| 4 | 24.0 , 31.9 | (112-134) | (-CH2-) (35.0), (CH3) :( 14.0),(C=N):(150.0,152.0) |
| 5 | 25.0 ,31.0 | (108-135) | (-CH-) (34.0), (CH3) :(15.0 ,18.0),(C=N):(148.0,150.0) |
| 6 | 15.0 , 20.0 | (114-136) | (-CH2) (31.0), (CH3) :(10.0), (C=N):(152.0,154) (CO-) ketone:(185.0 , 186.0) |
| 7 | 32.0 , 20.0 | (114-135) | (CH3):(10.0),(C=N):(150,152)(CO) ketone:(196.7,192.0) |

**Table.(4): Chemical and Physical Properties**

| No. of Compounds | *M .wt* | *M .P C ° ±2* | $R_f$ | Yield % | Color |
|---|---|---|---|---|---|
| Comp. {1} | $C_{59}H7_6N_{12}O_6$ | 242 | 0.72 | 80 | Yellowish Brown |
| Comp. {2} | $C_{59}H_{77}N_{13}O_5$ | 240 | 0.72 | 80 | Deep Orange |
| Comp. {3} | $C_{59}H_{78}N_{12}O_5$ | 238 | 0.70 | 72 | Bill Orange |
| Comp. {4} | $C_{47}H_{56}N_{10}O$ | 200 | 0.62 | 70 | Deep Yellow |
| Comp. {5} | $C_{55}H_{66}N_{10}O$ | 218 | 0.58 | 72 | Yellowish Orange |
| Comp. {6} | $C_{50}H_{62}N_{10}O_3$ | 210 | 0.64 | 70 | Bill Orange |
| Comp. {7} | $C_{51}H_{62}N_{10}O_3$ | 206 | 0.64 | 68 | Yellowish Orange |

**Anticancer Test :**

Compound {2} was selected for anticancer evaluation (breast cancer) as a cyclic compound using MTT assay for two types of cells (MCF-7) as cancer cells and (MCF-10A) as healthy cells according to studies [11-13]. Through the results of cancer studies, we observed that compound {2} has anticancer activity due to its structure containing fused rings [14-17], and also a diazepine ring containing nitrogen atoms [18-20]. All data are presented in Figures (1-3) and Table (5)

**Table (5): Cytotoxic activity of compound {2} on breast cancer cell lines (MCF-7) and healthy cells (MCF-10A) at the same concentration using MTT assay for 24 hours at 37°C.**

| Con. ( µg.mL$^{-1}$) | Mean Percentage (%) for each cell line | | | |
|---|---|---|---|---|
| | MCF-7 / $IC_{50}$ = 22.74 | | MCF-10A / $IC_{50}$= 172.46 | |
| Compound {2} | Cancerous line cells of MCF-7 | | Normal line cells of MCF-10A | |
| | Cell Viability | Cell Inhibition | Cell Viability | Cell Inhibition |
| 31. 25 | 85.76 | 14.24 | 85.83 | 14.17 |
| 62. 5 | 70.91 | 29.08 | 86.64 | 13.36 |
| 125.0 | 62.25 | 37.75 | 87.42 | 12.58 |
| 250 | 54.31 | 45.64 | 88.11 | 11.89 |
| 500 | 46.88 | 53.12 | 88.67 | 11.33 |
| Control | 100 | 0.00 | 85.35 | 14.65 |

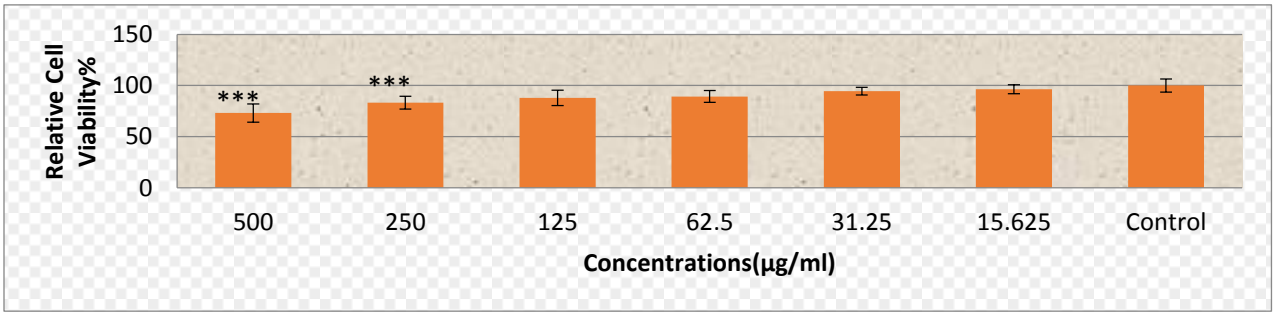



**Fig (1): IC50 for Cancer Cells MCF-7 for Compound{2}**

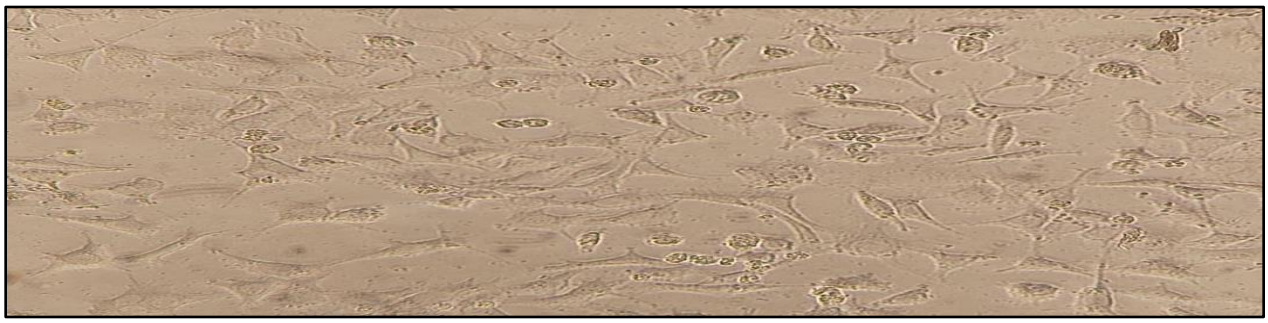


**Fig. 2:Anti-cancer activity of Compound{2}on ( MCF-7) at 500µg/ml**

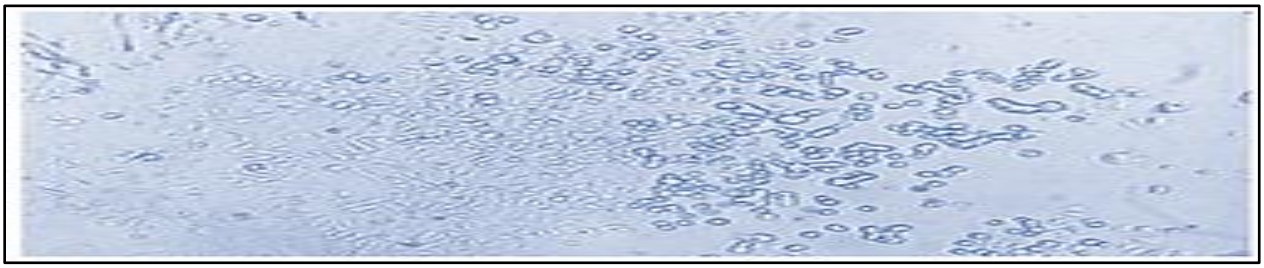


**Fig. 3:Anti-cancer activity of Compound{2}on ( MCF-A 10) at 500µg/ml**

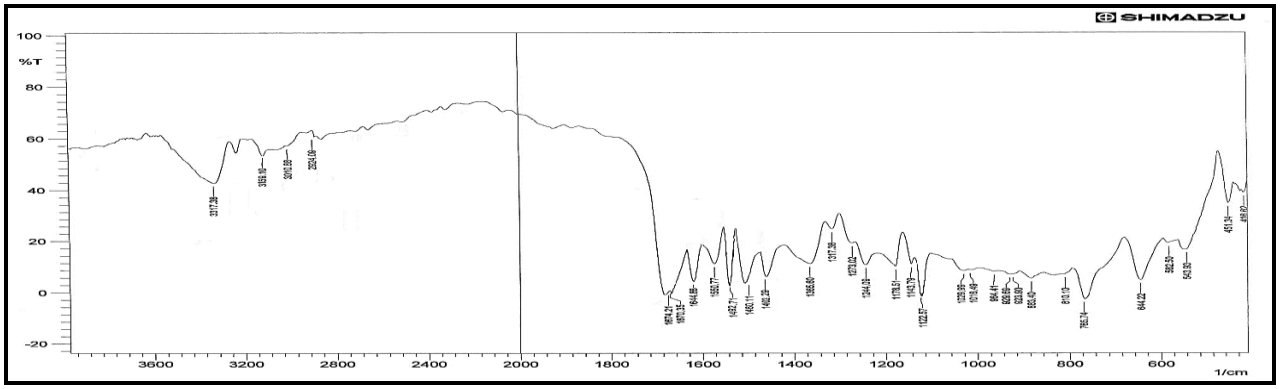



**Fig (4): I.R of Compound [1]**

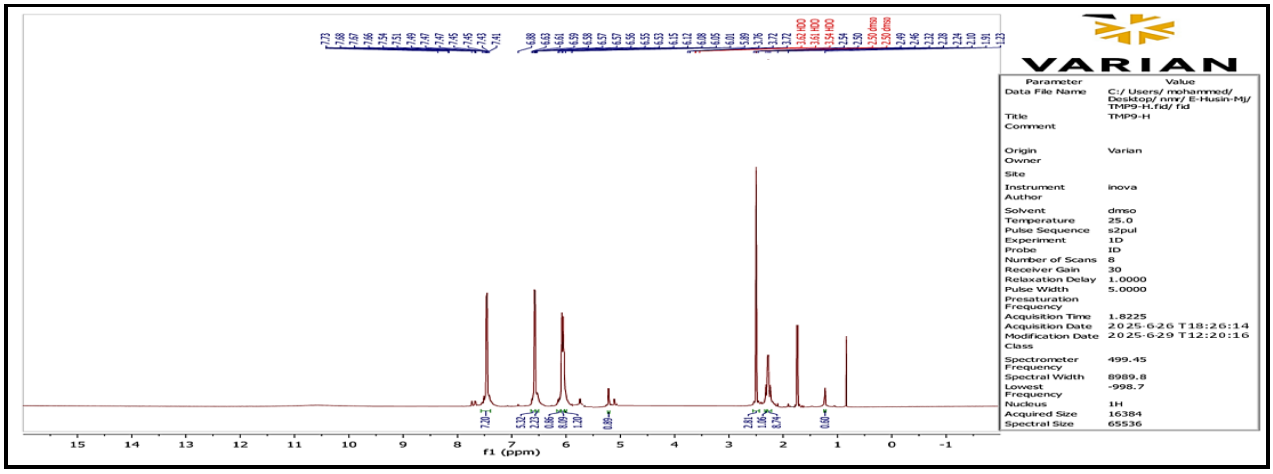



**Fig (5): $^{1}$HNMR Spectrum of Compound [1]**

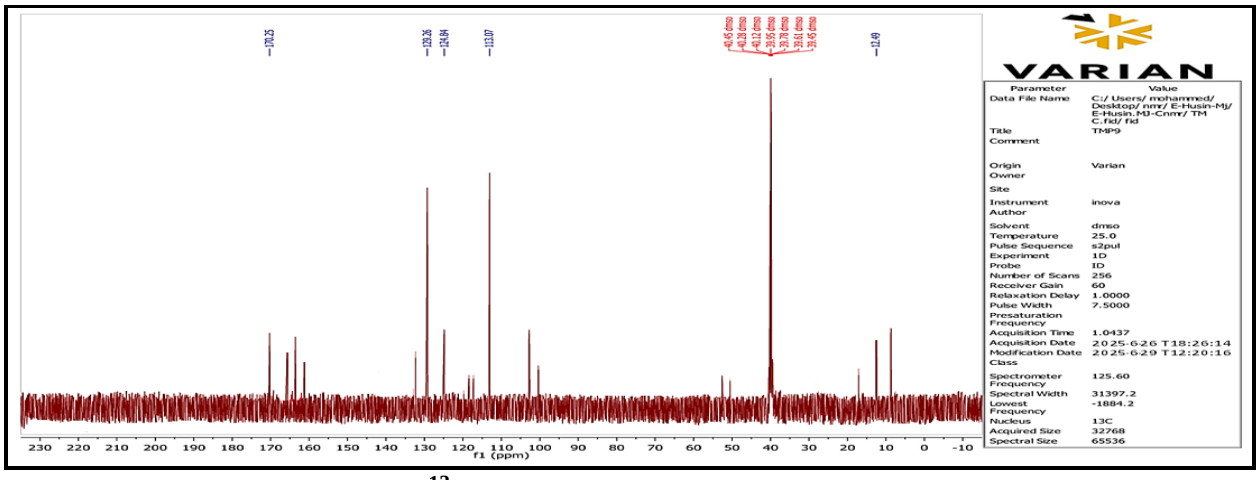



**Fig (6): $^{13}$CNMR Spectrum of Compound [1]**

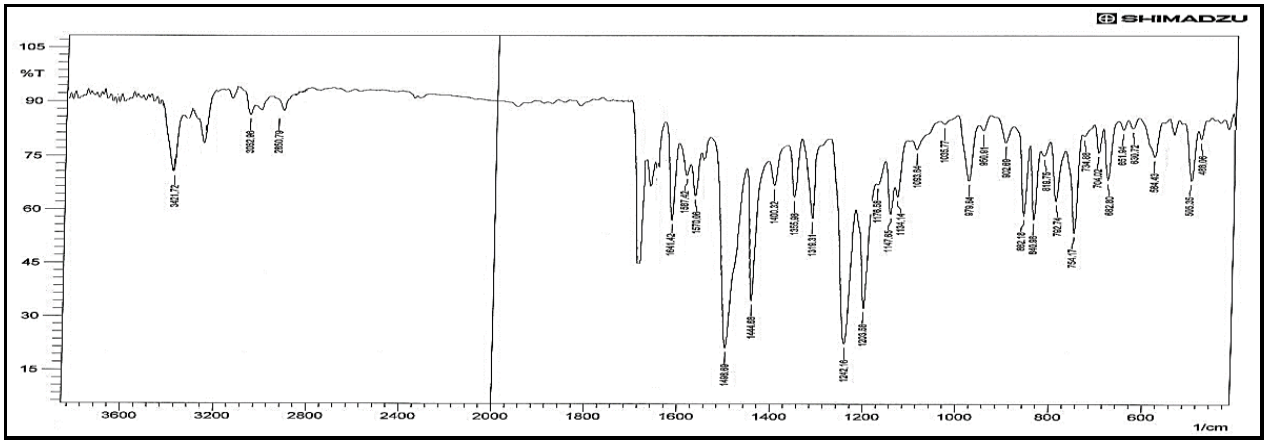



**Fig (7): I.R of Compound [2]**

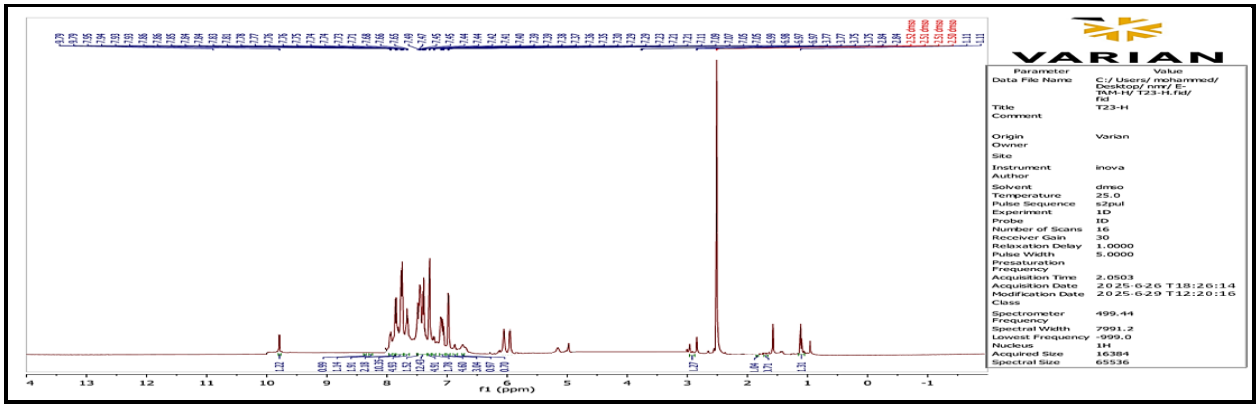



**Fig (9): $^{1}$HNMR Spectrum of Compound [2]**

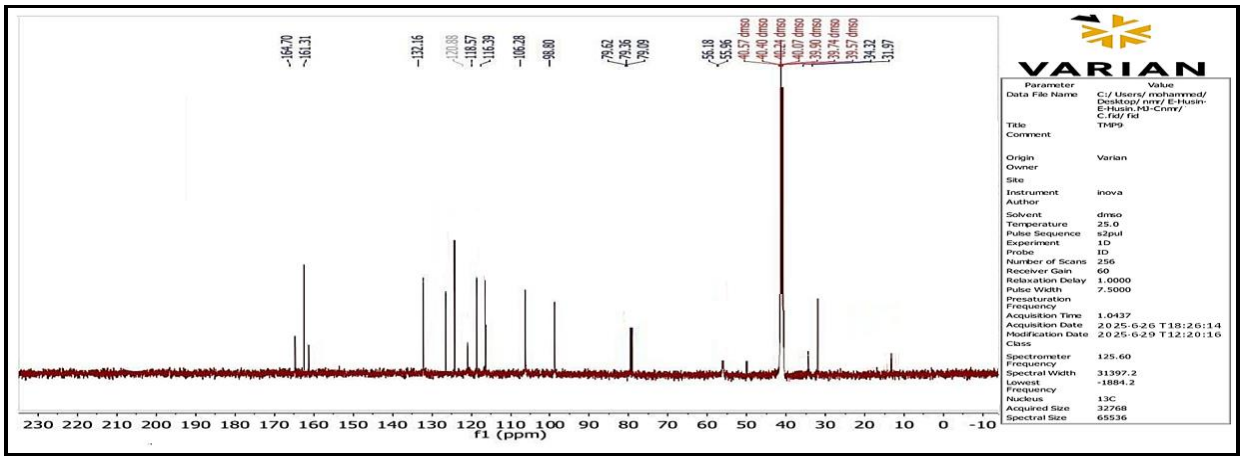



**Fig (10): $^{13}$CNMR Spectrum of Compound [2]**

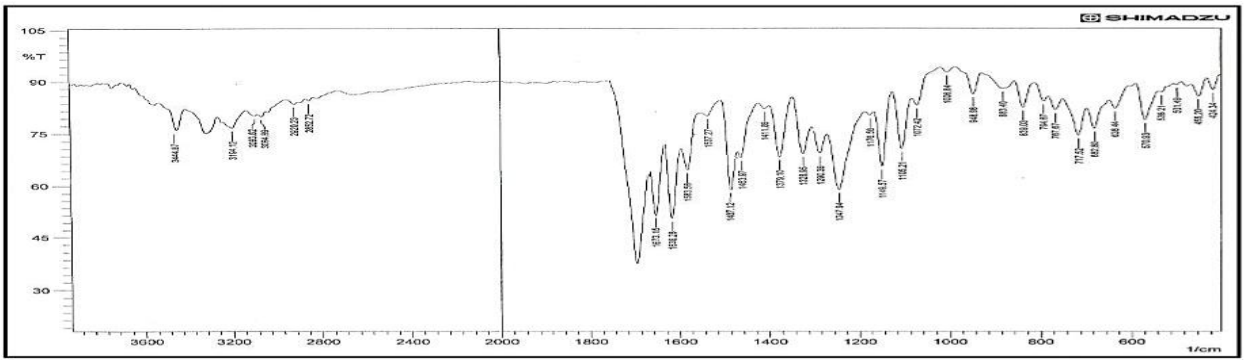



**Fig (11): I.R of Compound [3]**

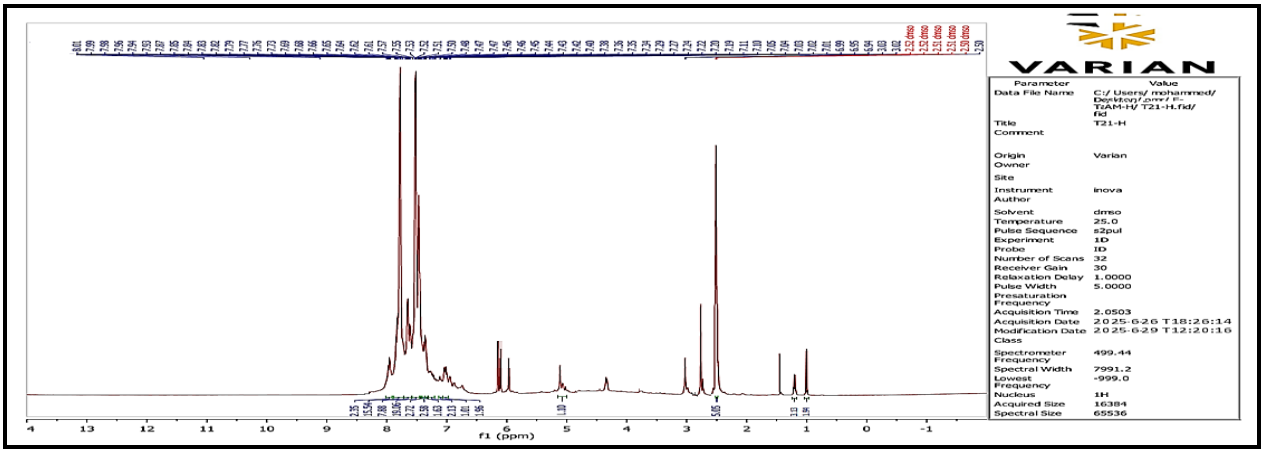



**Fig (12): $^{1}$HNMR Spectrum of Compound [3]**

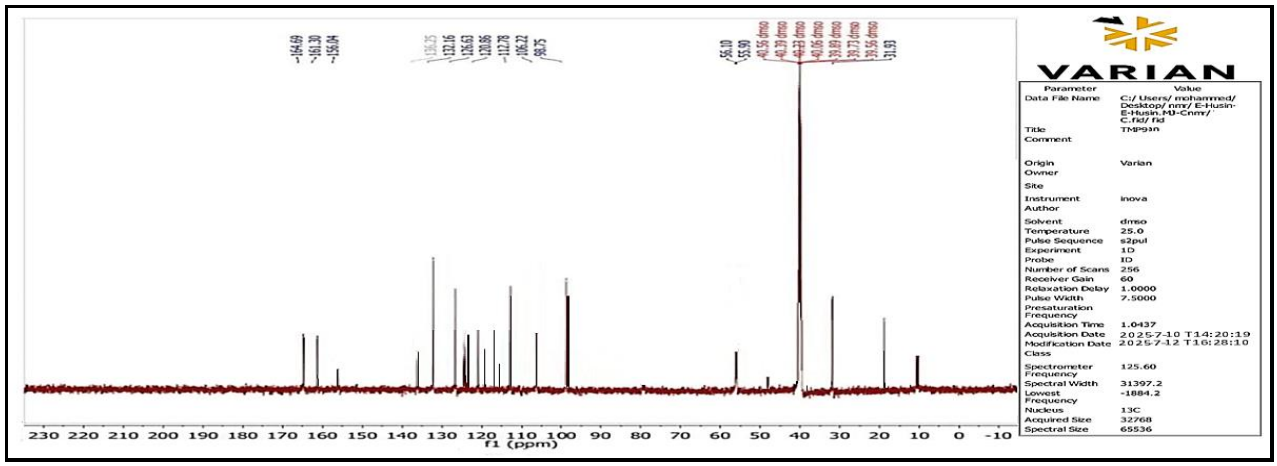



**Fig (13): $^{13}$CNMR Spectrum of Compound [3]**

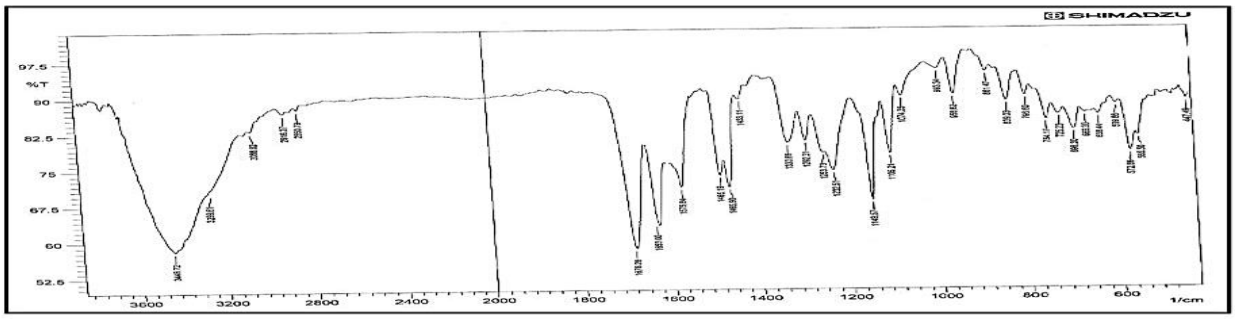



**Fig (14): I.R of Compound [4]**

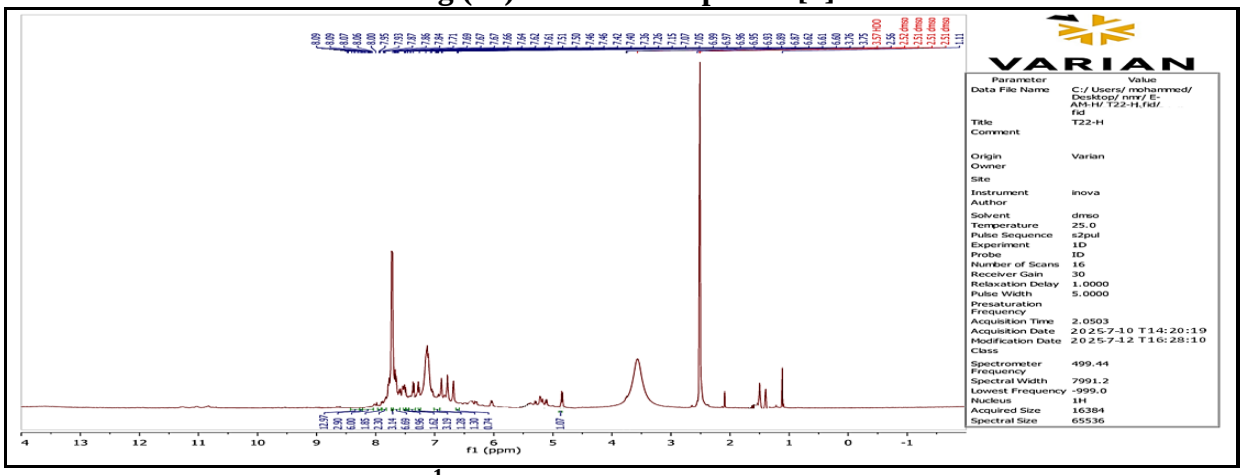



**Fig (15): $^{1}$HNMR Spectrum of Compound [4]**

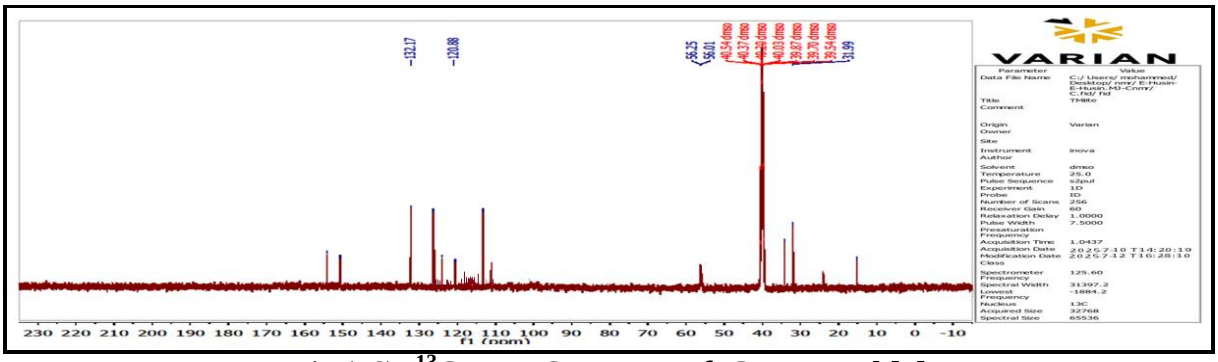



**Fig (16): $^{13}$CNMR Spectrum of Compound [4]**

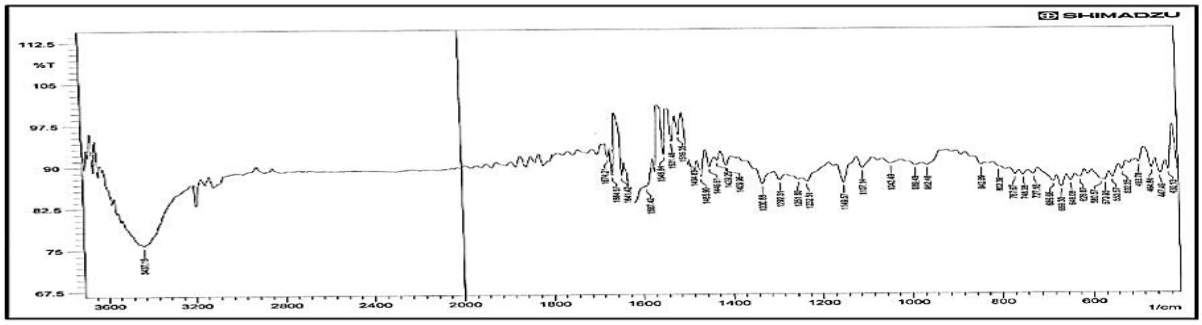



**Fig (17):IR of Compound [5]**

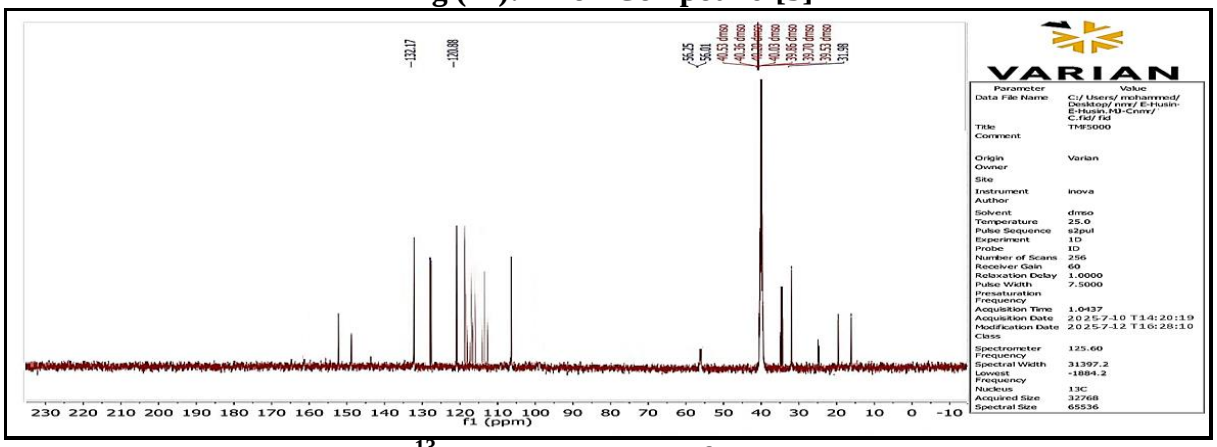



**Fig (18): $^{13}$CNMR Spectrum of Compound [5]**

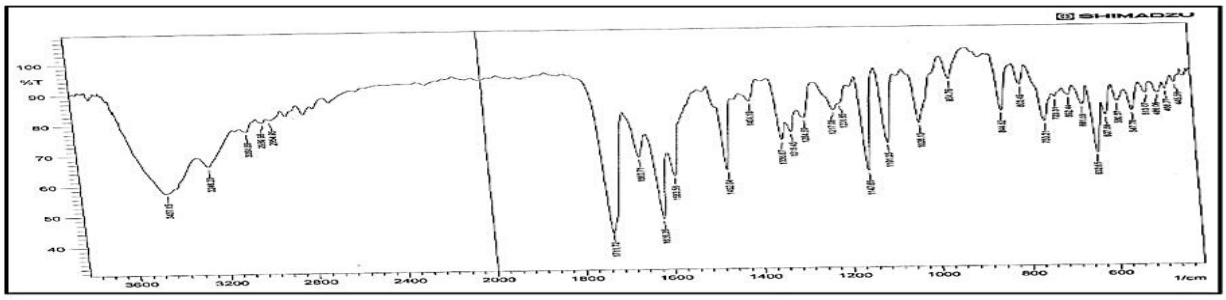



**Fig (19): I.R of Compound [6]**

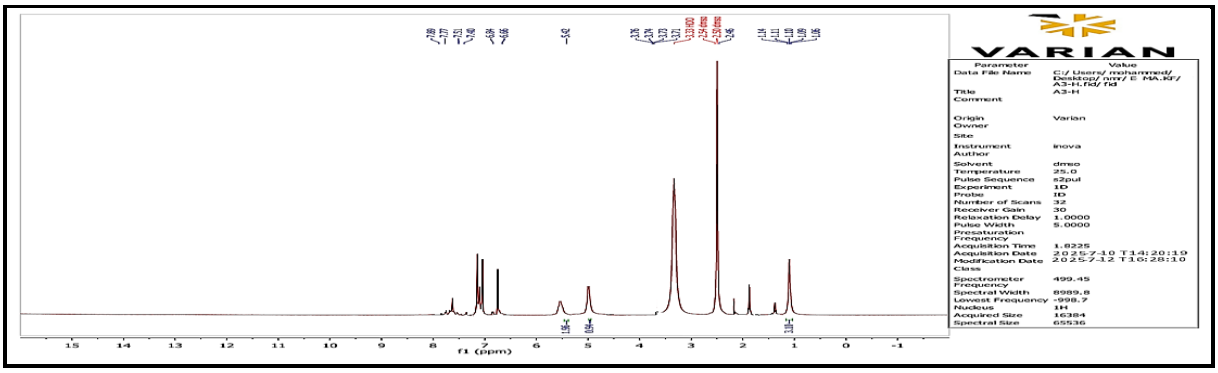



Fig (20): $^{1}$HNMR Spectrum of Compound [6]

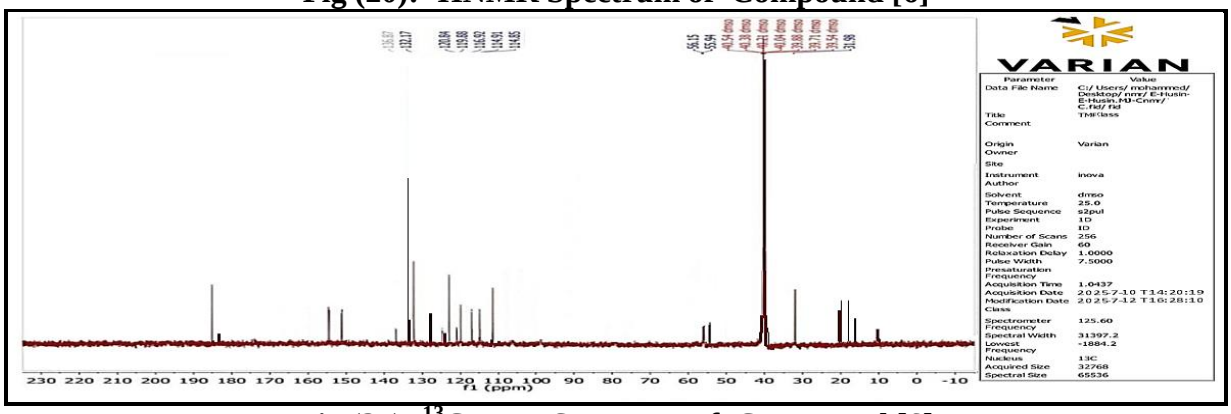



Fig (21): $^{13}$CNMR Spectrum of Compound [6]

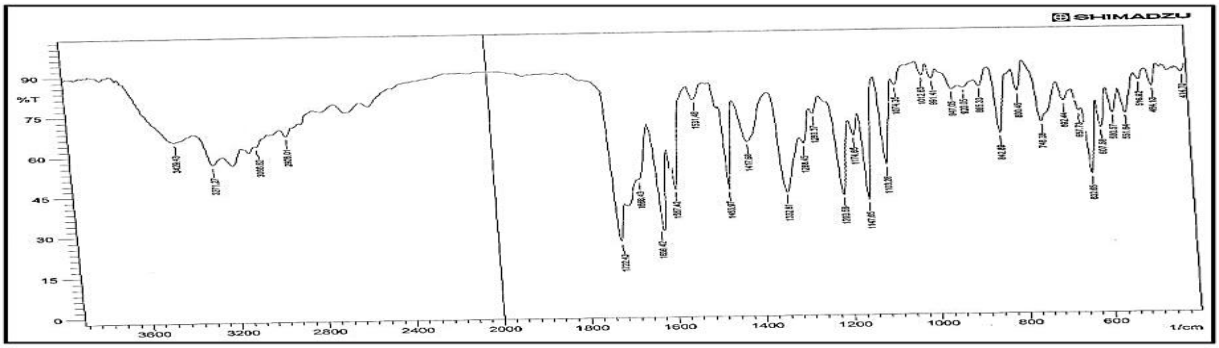



Fig (22): I.R of Compound [7]

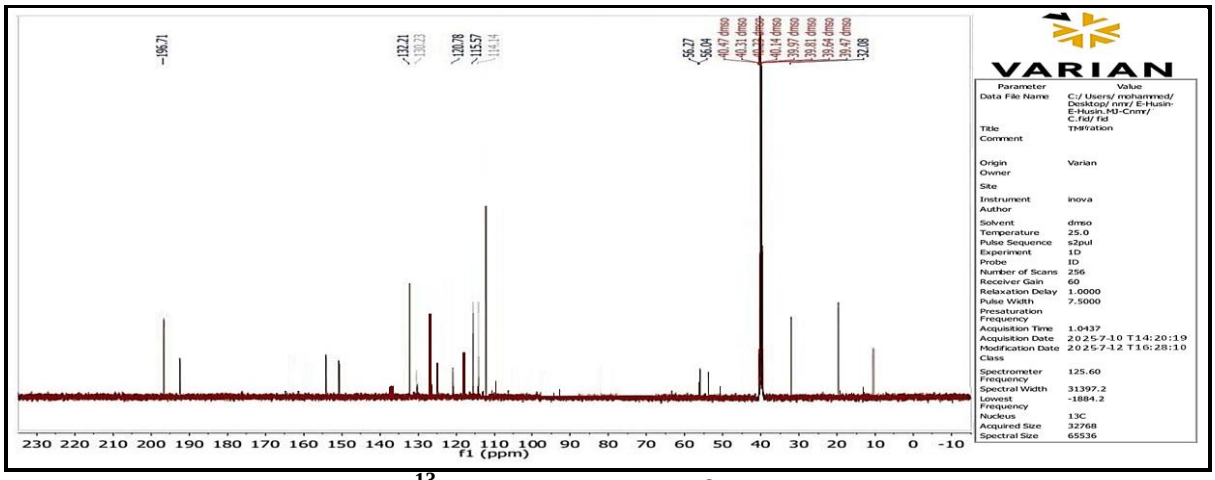



**Fig (23): $^{13}$CNMR Spectrum of Compound [7]**

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