Synthesis, Identification and Anticancer Evaluation of new Heterocyclic Compounds Derived from 2-Benzimidazolylacetonitrile

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Abstract
(E)-2-((3-acetylphenyl)diazenyl)-2-(1H-benzo[d]imidazole-2-ylacetonitrile (I) was prepared via diazotization of 3-aminoacetophenone and coupling of the product with 2-benzimidazolylacetonitrile, then it was reacted with terphathaldehyde to obtain bis azo-chalcone (IA). Then chalcone was reacted with deferent compounds such as urea thiobarbituric acid,acetoacetanilide,acetylacetone, 2-amino phenol,2-amino-5-methyl phenol malonitrile, ethylacetoacetate to yield azo-heterocyclic compounds (IA1-IA7). The structures of prepared compounds were characterized by FT-IR and 1H NMR 13C NMR, Mass spectroscopies. The efficacy of compound [I] was evaluated against MCF-7(human breast adenocarcinoma) cell line.
Keyword: 2-benzimidazolylacetonitrile, bis azo-chalcone, Anticancer

1. 1.Introduction

Benzimidazole are class of heterocyclic aromatic compounds is present as N-ribosyl-dimethylbenzimidazole and it can be found in vitamin B12 [1] Benzimidazole derivatives have many pharmacological activates such as antihypertensive, antiviral [2] anticancer [3] and antihistaminic [4] Azo compounds are one of the important varieties of compounds which have wide applications, especially those prepared from heterocyclic compounds have gained particular attention from several decades as they exhibit antifungal [4-6] anti-convulsant [5] anti-tubercular [6-8] DNA binding and analgesic properties [11-12] Chalcones are well-known precursors for synthesizing various heterocyclic compounds. This is due to the presence of reactive α,β-unsaturated keto group which could be reacted with diazoalkanes [9].
Chalcone derivatives have α,β-unsaturated carbonyl moiety have wide spectrum of biological activity in both medicinal and pharmaceutical, for example antimicrobial [14], anti-inflammatory [10], antitubercular, antioxidant and anticancer.

2. Experimental

2.1. Reagents and Solvents

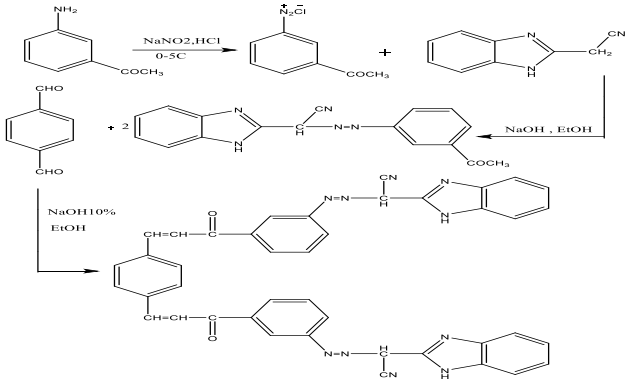
All chemicals, reagents, and solvents obtained from Sigma–Aldrich and Fluka.

2.2. Instrumentation

All FT-IR spectra for the products were recorded using Shimadzu FT-IR spectrophotometer. All 1H NMR, 13CNMR spectra for the products measured with a Bruker Advance (400 MHz) spectrometer in deuterated dimethyl sulfoxide (DMSO) as a solvent. The chemical shifts were measured in parts per million (ppm).
2.3. Synthesis (E)-2-((3-acetylphenyl)diazenyl)-2-(1H-benzo[d]imidazole-2-ylacetonitrile [I] 3-aminoacetophenone (0.01 mole) was dissolved in (5 ml) of concentrated hydrochloric acid and (20 ml) of distilled water. The solution was cold at (0 – 5) $_{o}$C in ice-water bath. The sodium nitrite (0.01 mole) was dissolved in (10 ml) of distilled water and added drop wise to the solution with stirring. 2-benzimidazolylacetonitrile (0.01 mole) was dissolved in (20 ml) of ethanol and (5 ml) of (10 %) sodium hydroxide and cooled to (0 – 5) $_{o}$C, added to the diazonium solution in drope wise and stirring at (0 – 5) $_{o}$C for 2 hours for obtaining the coupling agent. The result compound was precipitated, filtered and washed well with ethanol.
yield 81.94 %, m. p 210-212°C; 1H-NMR (DMSO): δ 2.51 (s, 3H, COCH3), δ 7.23-8.13 (m, H) for aromatic ring, δ 11.91-12.91 (s, 1H, NH), δ 2.68 (s, 1H) for (HC-CN); 13C-NMR (DMSO)
105.12,106.66,111.43,112.90,116.45,119.87,123.39,124.48, 125. 23,130.55,138.45, 142.56, 143.59,145.11 (C) Phenyl ring, 147.75 C(C=N),197.91-199.15 C (C=O), 27.29 C(CH3), 57.19 C(CH-CN), 114.26 C(CN); IR: (N-H) 3452.58cm−1, 3244.27 cm−1,(C-H, aliphatic) 2900cm−1,(C-H, aromatic) 3076.46 cm−1,(N=N) 1498.76-1550.77,(C=N endocyclic) 1645.28 cm−1, (C=O ketone) 1678.07 cm−1, (C=C aromatic) 1593.20-1612.49 cm-1, (CN) 2220.07 cm−1.MS(m/z) 303.1(100%), 260.1(34%), 232.2 (45.6%), 205.2 (17.2%), 156.2(60.8%), 129.1 (25.3%),91.2(27.52%), 43.2 (22.3%).
2.4 Synthesis Chalcone derivative 2,2'-((1E,1E'))-((2E,2E'))-3,3'-(1,4-phenylene)bis(acryloyl))bis(3,1-phenylene))bis(diazene-2,1-diyl))bis(2-(1H-benzo[d]imidazole-2-yl)acetonitrile) [IA]
The prepared of chalcone (0.002 mole) of compound (I) was dissolved in 30ml of absolute ethanol, and added to the solution of an appropriate Terphthaldehyde (0.001 mole) and added (6 ml) of 10% sodium hydroxide. The mixture was stirred at room temperature for (1-7 h) until the formation of chalcone, and then the solid crystals dried and purified by recrystallization from ethanol.
yield 78.7 %, m. p >297°C decomp.; 1H-NMR (DMSO): δ 6.80-8.58 (m,H) for aromatic ring, δ 10.42 (s, 1H, NH), δ 3.46 (s, 1H) for (HC-CN), δ 4.07-4.54(d,2H)for (CH=CHCO)13CNMR(DMSO)116.06,116.24,120.54,120.9

doi.org/10.31838/hiv22.02.327 Received: 07.06.22, Revised: 07.07.22, Accepted: 02.09.22

5,121.16,121.49,128.14,129.20,129.58,129.98(C) Phenyl ring, 138.33 C(C=N),162.47 C (C=O), 57.14 C(CH-CN), 115.17 C(CN); IR: (N-H) 3273.20cm−1,(C-H, alkene) 3070.68cm−1,(C-H, aromatic) 3010.46 cm−1,(N=N) 1420-1440 cm−1, (C=O ketone) 1670.35 cm−1,(C=C alkene) 1630.00 cm−1, (C=C aromatic) 1550.77 cm-1, (CN) 2223.92 cm−1.



Scheme *1*

2.5. Synthesis Compound 2,2'-(3,3'-(1,4-phenylene)bis(1-(4,6-dioxo-2-thioxotetrahydropyrimidin-5(6H)-ylidene)prop-2-ene-3,1-diyl))bis(3,1-phenyl ene))bis(diazene-2,1-diyl)bis(2-(1H-benzo[d]imidazol-2-yl)acetonitrile [IA1]

Chalcone [IA] (0.0003 mole) and Thiobarbituric acid (0.0006 mole) was taken in ethyl alcohol, catalytic amount of AcOH was added and the reaction mixture was refluxed for 15 h.After the completion of reaction, the reaction mass was cooled to room temperature, poured into crushed ice and neutralized with NaHCO3 solution. The product separated out was filtered, dried and recrystallized using ethanol.

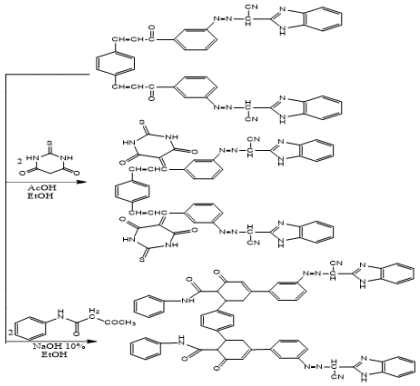
yield 71.63 %, m. p 238-240°C; IR: (N-H) 3176.76cm−1,3352.28 cm-1, 3408.22 cm-1,(C-H, aliphatic) 2974.23cm−1,(C-H, alkene) 3124.68 cm−1,(N=N) 1430.00,(C=C alkene) 1598.99 cm−1, (C=O amide) 1659.00 cm−1, (C=C aromatic) 1548.84 cm-1, (CN) 2223.92 cm-1.

2.6.Synthesis Compound

3,3''''-bis((E)-((1H-benzo[d]imidazol-2-yl)(cyano)methyl)diazenyl)-5',5'''-dioxo-N4',N6'''-diphenyl-1''',2',2''',3',4',5',5''',6'''-octahydro-[1,1':3', 1'':4'',1''':3''',1''''-quinquephenyl]-4',6'''-dicarboxamide. [IA2]

Chalcone [IA] (0.0003 mole) and Acetoacetanilide (0.0006 mole) was taken in ethyl alcohol, catalytic amount (5ml)of NaOH 10% was added and the reaction mixture was refluxed for 20 h.The product separated out was filtered, dried and recrystallized using ethanol.

yield 72.21 %, m. p 249-251°C; 1H-NMR (DMSO): δ 6.73-7.79 (m, H) for aromatic ring, δ 12.61 (s, 1H, NH), δ 10.16 (s, 1H, -CO-NH), δ 3.35 (s, 1H) for (HC-CN), δ 4.10(s,2H)for(CH2),IR: (N-H) 3462.22-3259.70cm−1,(C-H, alkene) 3061.03cm−1,(C=O, amide) 1670.35 cm−1,(N=N) 1400.40-1452.40 cm−1, (C=O ketone) 1708.93 cm−1,(C=C alkene) 1600.92 cm−1, (C=C aromatic) 1544.98 cm-1, (CN) 2360.87 cm−1.



Scheme 2

2.7 Synthesis Compound

2,2'-((E,1'E)-((4',6'''-diacetyl-5,5'''-dioxo-1''',2',2''''3',4',5',5''',6'''-octahydro-[1,1':3',1'':4'',1''':3''',1'''-quinquephenyl]-3,3'''-diyl)bis(diazene-2,1-diyl))bis(2-(1H-benzo[d]imidazol-2-yl)acetonitrile). [IA3]

Chalcone [IA] (0.0003 mole) and Acetylacetone (0.0006 mole) was taken in ethyl alcohol, catalytic amount (5ml) of NaOH 10% was added and the reaction mixture was refluxed for 14 h.The product separated out was filtered, dried and recrystallized using ethanol.

yield 69.98 %, m. p >330°C decomp.; 1H-NMR (DMSO): δ 7.00-8.48 (m,9H) for aromatic ring, δ 11.03 (s, 1H, NH), δ 2.66 (s, 1H) for (HC-CN), δ 1.67(s,2H-CH2),δ2.61(s,3H,COCH3),13CNMR(DMSO)116.26,119.70, 120.37,120.65,121.69,122.54, 127.63, 129.51,129.90,138.25(C) Phenyl ring,162.87 C(C=N),198.81 C (C=O),175.82 C (C=C) 58.84 C(CH-CN), 115.21 C(CN), 25.28 C(CH2), 27.44 C(COCH3); IR: (N-H) 3290.56cm−1, 3180.62 cm−1, (O-H) 3433.29cm−1,(C-H, alkene) 3100.68cm−1,(N=N) 1413.82 cm−1, (C=O ketone) 1697.36 cm−1,(C=C alkene) 1641.42 cm−1, (C=C aromatic) 1558.48 cm-1, (CN) 2229.71 cm−1

2.7 Synthesis Compound 2,2'-((E,1'E)-((1,3-phenylenebis(2,3dihydrobenzo[b] [1,4] oxazepine-2,4-diyl))bis(3,1-phenylene))bis(diazene-2,1-diyl))bis(2-(1H-benzo[d]imidazol-2-yl)acetonitrile [IA4]

Chalcone [IA] (0.0003 mole) and 2-aminophenol (0.0006 mole) was taken in ethyl alcohol, catalytic amount (2ml)of Et3N was added and the reaction mixture was refluxed for 18 h.The product separated out was filtered, dried and recrystallized using ethanol.

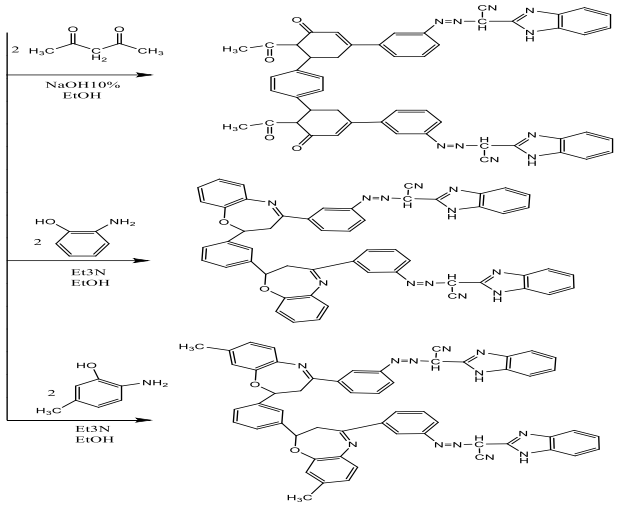
yield 66.5 %, m. p 280-282°C; 1H-NMR (DMSO): δ 7.13-7.89 (m,9H) for aromatic ring, δ 10.88 (s, 1H, NH), δ 3.19 (s, 1H) for (HC-CN), δ 6.37(s,1H)for (CH-O), δ 1.17(t,2H)for(CH2-C=);13CNMR(DMSO)117.37,120.24,121.95,125.36,126.02,127. 62,129.31,129.46 (C) Phenyl ring, 24.46 C(CH2),150.58 C(C=N), 141.87 C (C=C) 58.67 C(CH-CN), 111.45 C(CN); IR: (N-H) 3431.36cm−1, (C-H, alkene) 3061.03cm−1,(N=N) 1436.97-1500.62 cm−1, (C=N endocyclic) 1664.57 cm−1,(C=C alkene) 1595.13 cm−1, (C=C aromatic) 1556.55 cm-1, (CN) 2362.80 cm−1.

2.8 Synthesis Compound 2,2'-((E,1'E)-((8-methyl-1,3phenylenebis(2,3dihydro benzo[b] [1,4]oxazepine-2,4-diyl))bis(3,1-phenylene))bis(diazene-2,1-diyl))bis(2-(1H-benzo[d]imidazol-2-yl)acetonitrile [IA5]

Chalcone [IA] (0.0003 mole) and 2-amino-5-methylphenol

(0.0006 mole) was taken in ethyl alcohol, catalytic amount (2ml)of Et3N was added and the reaction mixture was refluxed for 18 h.The product separated out was filtered, dried and recrystallized using ethanol.

yield 71.43 %, m. p 297-299°C; IR: (N-H) 3390.86cm−1, (C-H, alkene) 3061.03cm−1,(C-H, aliphatic) 2981.95cm−1,(N=N) 1436.97-1517.98 cm−1, (C=N endocyclic) 1658.78 cm−1,(C=C alkene) 1593.20 cm−1, (C=C aromatic) 1558.48 cm-1, (CN) 2362.80 cm−1.



Scheme 3

2.7 Synthesis Compound 4,4'-(1,4-phenylene)bis(6-(3-((E)-((1H-benzo[d]imidazol-2-yl)(cyano)methyl)diazenyl)phenyl)-2-aminonicotinonitrile)

[IA6]

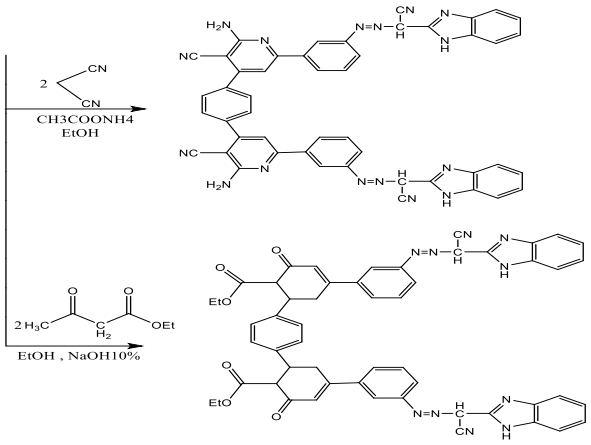
Chalcone [IA] (0.0003 mole) and malononitrile (0.0006 mole) was taken in ethyl alcohol, (0.0006mole)of ammonium acetate was added and the reaction mixture was refluxed for 18 h.The product separated out was filtered, dried and recrystallized using ethanol.

yield 77.90 %, m. p 241-243°C; 1H-NMR (DMSO): δ 7.00-7.90 (m,9H) for aromatic ring, δ 11.47 (s, 1H, NH), δ 3.47 (s, 1H) for (HC-CN), δ 6.95-6.99(s,2H)for (NH2), 13CNMR(DMSO)115.29,115.65,117.04,119.73,120.32 (C) Phenyl ring, 174.55 C(C=N),56.48 C(CH-CN), 108.03 C(CN); IR: (N-H) 3435.22cm−1, (C-H, aromatic) 3070.00cm−1,(N=N) 1417.68 cm−1, (C=N endocyclic) 1629.85 cm−1, (C=C aromatic) 1560.41 cm-1, (CN) 2206.57-2358.94cm−1.

2.8 Synthesis Compound diethyl 3,3''''-bis((*E*)-((1*H*-benzo[d]imidazol-2-yl)(cyano)methyl)diazenyl)-5',5'''-dioxo-1''',2',2''',3',4',5',5''',6'''-octahydro-[1,1':3',1'':4'',1''':3''',1''''-quinquephenyl]-4',6'''-dicarboxylate [IA7]

Chalcone [IA] (0.0003 mole) and Ethylacetoacetate (0.0006 mole) was taken in ethyl alcohol, catalytic amount (5ml)of NaOH 10% was added and the reaction mixture was refluxed for 16 h.The product separated out was filtered, dried and recrystallized using ethanol.

yield 80.3 %, m. p 291-293°C; IR: (N-H) 3441.01cm−1, (C-H, alkene) 3100.03cm−1,(N=N) 1481.33 cm−1, (C=N endocyclic) 1625.00 cm−1,(C=C alkene) 1610 cm−1, (C=C aromatic) 1552.70 cm-1,,(C=O ester) 1724.36 cm−1, (C=O ketone) 1674.21 cm−1 (CN) 2223.92 cm−1.



Scheme 4

2.9 Cell viability And Cytotoxicity assay

2.9.1Cell lines and culture.

MCF7 (a human breast cancer cell line) was purchased from National Cell Bank of Iran (Pasteur Institute, Iran). Cells were grown in RPMI-1640 medium (Gibco) with 10% FBS (Gibco) supplemented with antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cells were maintained at 37 °C under humidified air containing 5% CO2 and were passaged using trypsin/EDTA (Gibco) and phosphate- buffered saline (PBS) solution. Culturing media and conditions used to grow the cells as 3D colonies was the same as monolayer cell culture.

2.9.2MTT cell viability assay in MCF7 Cells.

Cell growth and cell viability were quantified using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide] (Sigma-Aldrich) assay. In brief, for monolayer culture, cells (MCF7) were digested with trypsin, harvested, adjusted to a density of 1.4 × 104 cells/well and seeded to 96-well plates filled with 200 µl fresh medium per well for 24 h. When cells formed a monolayer, they were treated with 100-6.25 µg/ml of the compounds for 24 h at 37 °C in 5% CO2. At the end of the treatment (24 h), while the monolayer culture was left untouched in the original plate, the supernatant was removed and 200 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added and the plate was incubated at 37 °C for an additional 4 h. MTT solution (the supernatant of cells was removed and dimethyl sulfoxide was added (100 µl per well). Cells were incubated on a shaker at 37 °C until crystals were completely dissolved. Cell viability were quantified by measuring absorbance at 570 nm using an ELISA reader (Model wave xs2, BioTek, USA). The concentration of the compounds that resulted in 50% of cell death (IC50) was determined from respective dose-response curves

3. Results and Discussion

Azo Compound [I] is the starting material of this research were synthesized by coupling reaction between 3-aminoacetophenone and 2-benzimidazolylacetonitrile

which are characterized by FT-IR where the azo group (-N=N-) at 1498.76 cm-1 and also amino group (-NH2) at 3369.64-3466.08 cm-1.Chalcone Compound is prepare by cliesen –shemidt condensation which are characterized by FT-IR where appear absorption bands due to stretching vibration of (-C=C-). Compounds[IA1-IA7] are cyclized with thioburbituric acid to produce [IA1]. Acetylacetone, Acetoacetanilide and Ethylacetoacetate respectively in a separated reactions to obtain cyclohexenone derivatives [IA2,IA3,IA7].FT-IR spectrum good evidence to formation these compounds by inspection the changing in the absorption bands the major difference is disappearing of (-C=O) of the [IA] compound and appearing (-C=C-) of the cyclohexenone ring at 1600.92-1641.42 cm-1. Oxazepine derivatives [IA4-IA5] were synthesized by the reaction of compound [IA] with phenol derivatives like [2-amino phenol, 2-amino-5-methyl phenol] in absolute ethanol with triethylamine as a catalyst. The FT-IR is used to detect formation of these compounds by showing the stretching vibration band of (-N=C) endocyclic at 1658.78-1664.57 cm-1 also the stretching vibration of amine group (-NH2) are disappeared. Some extra characteristic bands were mentioned in experimental part. Pyridine derivative compound [IA6] was synthesized by the reaction of chalcone derivative [IA] with malononitrile in ethyl alcohole with ammonium acetate.The FT-IR technique indicate on the disappearing of stretching vibration of (-C=O) group of Chalcone derivative at 1670.35 cm-1 and appearance new absorption bands for (-C=N) group of Pyridine ring at 1629.85 cm-1 also peak at 2206.57 cm-1 due to stretching vibration of (-CN), Also the reaction progress monitored by TLC. H1 NMR,C13NMR and mass spectra were recorded for the prepared compound.

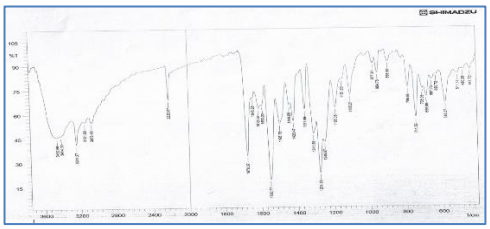


Fig.1:FT-IR Spectra of Comp. I

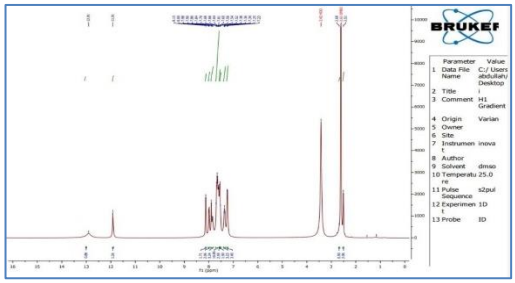


Fig.2: 1H-NMR Spectra of Comp. I

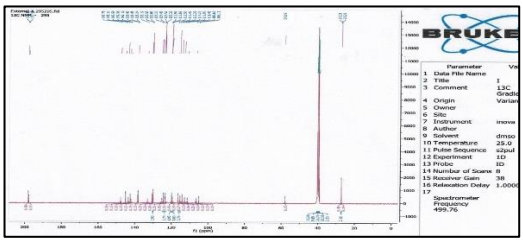


Fig.3: 13C-NMR Spectra of Comp. I

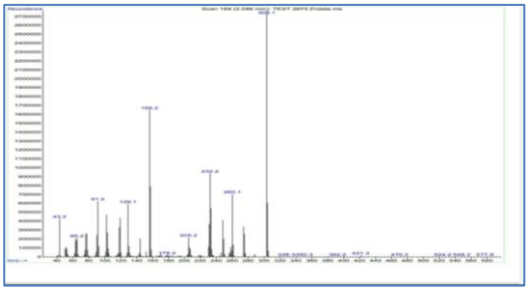


Fig.4: Mass Spectrum of Comp. I

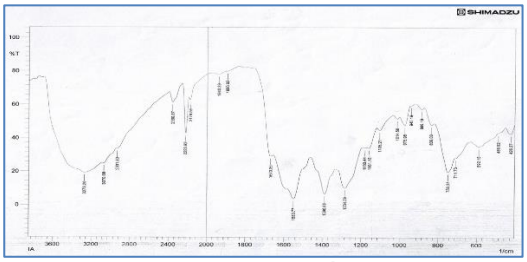


Fig.5: FT-IR Spectra of Comp. IA

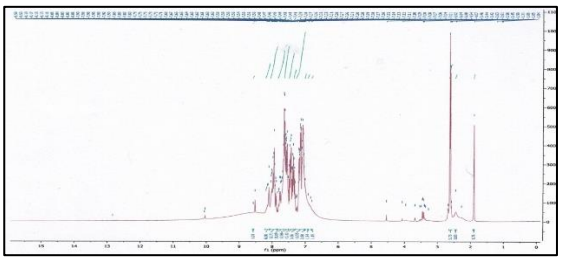


Fig.6: 1H-NMR Spectra of Comp. IA

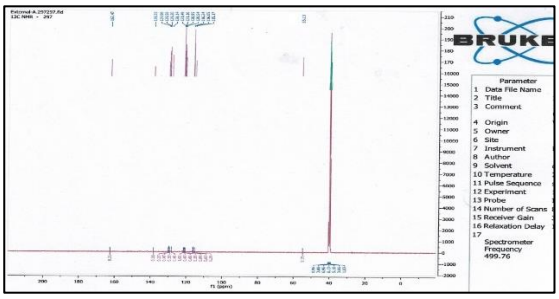


Fig.7: 13C-NMR Spectra of Comp. IA

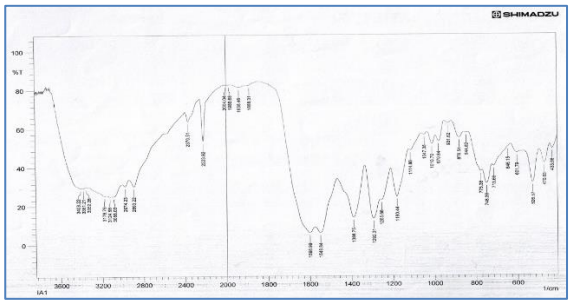


Fig.8: FT-IR Spectra of Comp. IA1

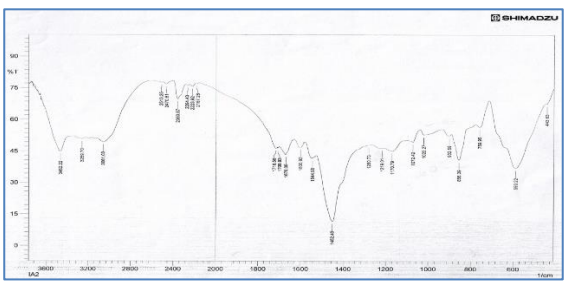


Fig.9: FT-IR Spectra of Comp. IA2

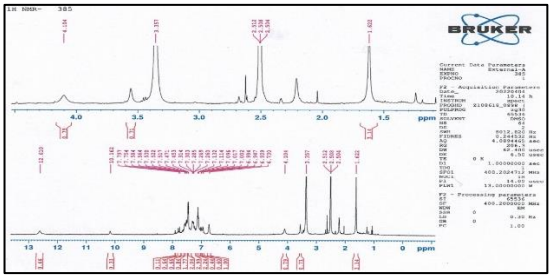


Fig.10: 1H-NMR Spectra of Comp. IA2

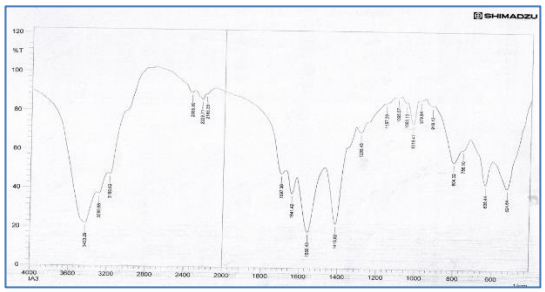


Fig.11: FT-IR Spectra of Comp. IA3

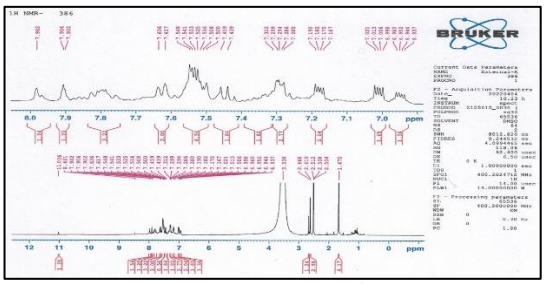


Fig.12: 1H-NMR Spectra of Comp. IA3

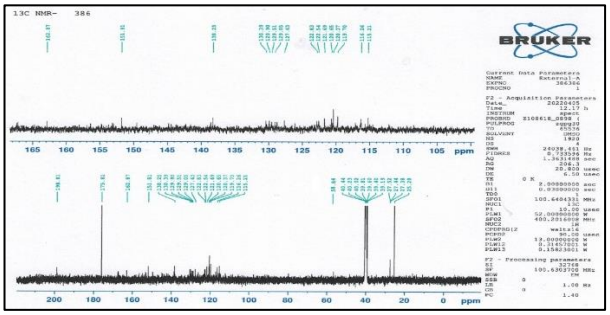


Fig.13: 13C-NMR Spectra of Comp. IA3

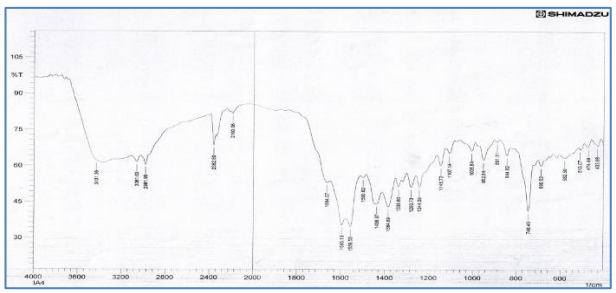


Fig.14: FT-IR Spectra of Comp. IA4

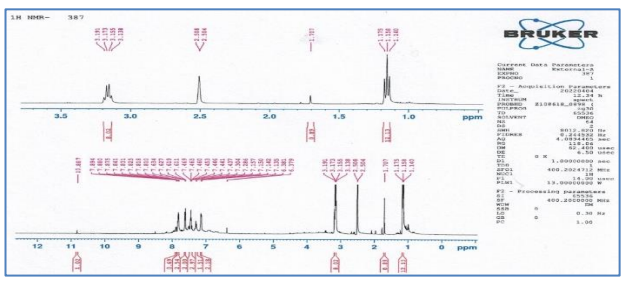


Fig.15: 1H-NMR Spectra of Comp. IA4

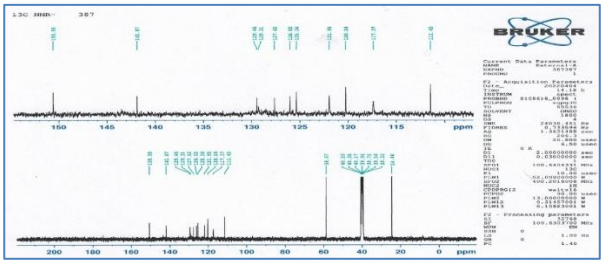


Fig.16: 13C-NMR Spectra of Comp. IA4

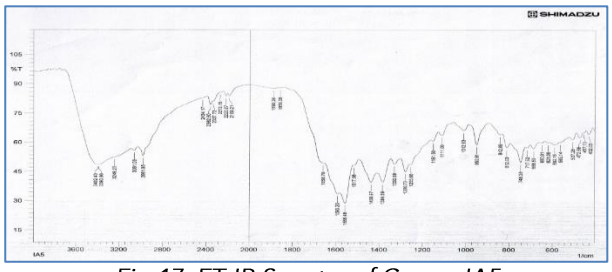


Fig.17: FT-IR Spectra of Comp. IA5

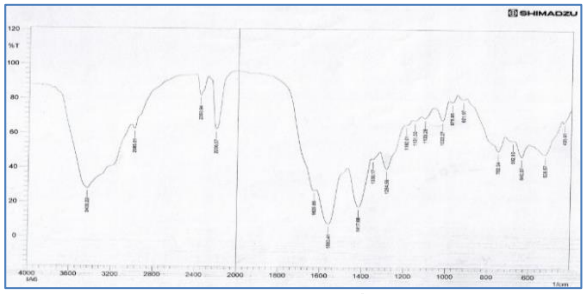


Fig.18: FT-IR Spectra of Comp. IA6

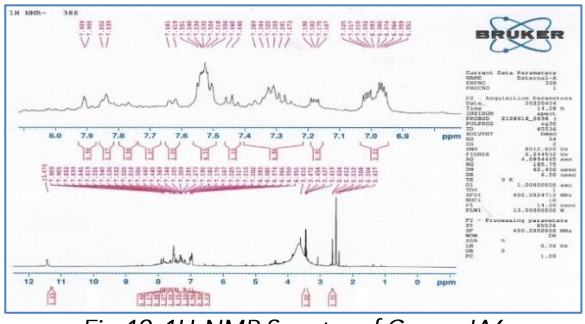


Fig.19: 1H-NMR Spectra of Comp. IA6

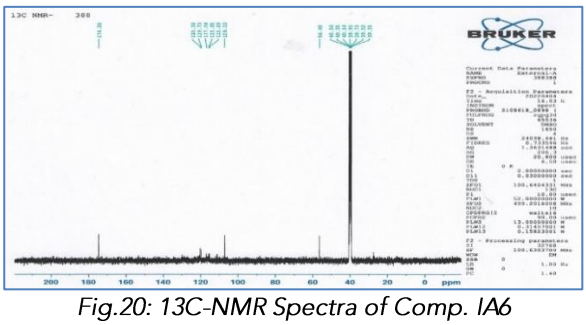


Fig.20: 13C-NMR Spectra of Comp. IA6

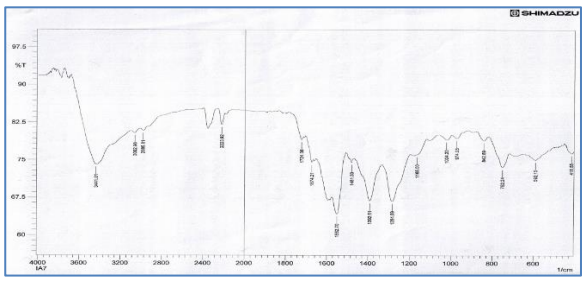


Fig.21: FT-IR Spectra of Comp. IA7

3.2 Cytotoxic Activity of (E)-2-((3-acetylphenyl)diazenyl)-

2-(1H-benzo[d]imidazole-2-ylacetonitrile on Cancerous Cells Line (MCF-7)

This study compared the activity of compound (E)-2-((3-acetylphenyl)diazenyl)-2-(1H-benzo[d]imidazole-2-ylacetonitrile against breast cancer cells and normal breast cells (MCF10A).. The results demonstrated that IC50 = 22.61µg/ml for the compound

[I] on breast cancer cell while IC50 = 118.45 µg/ml for the same compound on normal breast cell, this means that the concentration of this compound to kill half of (MCF-7) is lower than the concentration of the same compound to kill half of normal cells (MCF10A). this results indicated that compound I may use as suitable medicine of breast cancer cells.

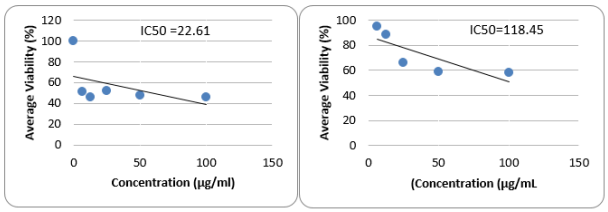


Figure 1: Effect of different concentrations of compound [I] on cell viability of breast cancer cell lines (MCF-7) and normal breast cells (MCF10A).

4. Conclusion

The current search has involved synthesized new heterocyclic compounds derivatives from bis Azo-chalcone, the results of spectra studies of the prepared compounds showed the validity of their chemical structures through the appearance of new bands in prepared compounds represent by heterocyclic compounds, Anti- cancer studying also gave good data by inhibition of tumors level against selected cancer cells.

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