Article

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Synthesis, Identification and Study of Anti-Tumor Activity for Macrocyclic Formazan-Triazan Compounds

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Abstract

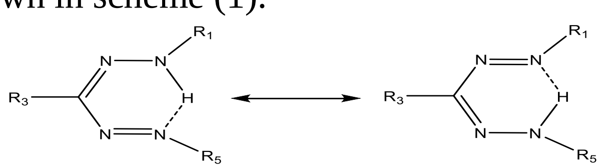
Formazan derivatives composites were equipped concluded condensation reactions and using altered solvents and mediums. They were derived from Mefenamic acid and were converted to a primary amine by reacting it with semicarbazide and going through an esterification step, where this amine was converted to Anil derivative for coupling reaction. Preparation of Macro-Cyclic-Formazan and Macro-Cyclic-Formazan-Triazan, which was synthesized for the first time as a bioorganic compounds via cyclization step with coupling reaction. The physical and chemical properties of the synthesized compounds are provided. The compounds were identified by FTIR, ^{1}H.NMR and ^{13}C.NMR spectroscopy, and evidence of the formation of these compounds was obtained. The second part of the research included a study of its behaviour against breast cancer as a type of cancer, where the results showed the emergence of strong inhibition of tumour cells at a concentration of (600 ppm) for Macro-Cyclic-Formazan-Triazan indicating the effect of heteroatoms on the effectiveness of the compound.

Keywords: Mefenamic acid, Macrocyclic Formazan, Triazan, anticancer, schiff base.

1. Introduction

Mefenamic acid, or (2-[(2,3-dimethylphenyl)-amino] benzoic acid, contains carboxylate and amino groups. It has brand names Mefenamic[(1)], Ponstel. Mefenamic acid is a non-steroidal anti-inflammatory drug, with antipyretic, and analgesic properties. Mefenamic acid is an Anthranilic acid derivative. especially, this medication is connected to 3-hydroxyanthranilic acid, a metabolite that occurs naturally of tryptophan[2]. ,Molecular formula is $C_{15}H_{15}NO_2$, Conventionally it is available as tablets, capsules and suspensions, It is commonly used to treat mild to moderate pain, such as headaches, tooth pain, dysmenorrhea, rheumatoid arthritis[(3)], osteoarthritis, and other joint disorders. However, it might cause gastrointestinal problems such as gastrointestinal bleeding and distress in the stomach[(4)]. Several researchers have reported the crystal structures of various Mefenamic acid-metal complexes[(5,6)]. The results showed that the activity of Mefenamic is enhanced by coordination with metal ions[(7,8)]. As a result, metal complexes have been employed to treat a variety of ailments, as well as to synthesize drugs[(9)]. Scientists prefer nitrogen-based ligands because they exhibit significant metabolic activity when completed with metal ions[(10-12)].

Formazan: Formazans have garnered major attention in the chemical and biological worlds lately because of their increasing biological, technological, and other unique applications[(13)]. Because of the conjugated double bonds in formazan's (-NH–N=C–N=N–) backbone, these compounds are colored. Thus, a great deal of these molecules have been synthesized recently[(13،14)]. The conjugated double bonds within the formazan skeleton produce colourful molecules. The tetrazolium/formazan combination acts as a proton acceptor or oxidant in a unique redox system. It is widely used in biological sciences such as medicine, pharmacology, immunology, and botany, especially in biochemistry and histo chemistry[(15)]. showed that some Formazans could be changed from red to yellow forms upon exposure to visible light. A formazan molecule allows the existence of four possible structures due to geometrical isomerism about the two double bonds (C = N, syn – anti and N = N, cis – trans), the possibilities of tautomerism being ignored for the present. The stereoisomers can be indicated[(14)], as shown in scheme (1).



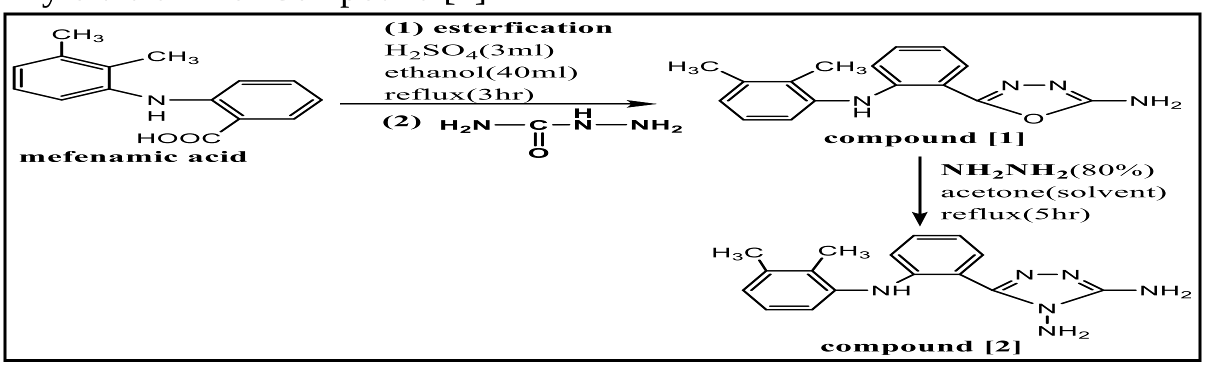
Scheme- 1 Mesomeric forms of formazans

Triazan: In 2019, researcher(13, 14) synthesized and produced Sulfazan , Triazan and Macrocyclic Formazan chemicals for the first time in his initial experiments(13, 14). It includes these compounds in its composition (Ar-N=N-N-R)(13). Triazan Ingredients were invented and discovered for the first time in year 2019 that synthesized these compounds via many steps of conjugated process of Azo group with Nitrogen of Amine group in basic medium and special condition to format (N-N=N-), Formation of Triazan compound acts one of Diazo-salts reactions. The Triazan was created with basic solution from (Pipyridine) medium followed studies(13 ,14) , Linear Triazan Compound has higher efficiency towards Cancer cells and high inhibition on cancer cells than Macrocyclic Triazan compound(13, 14).

2. Experimental Methods

2.1.Preparation of compound[1,2]by Insertion Reaction[13, 14] :

By dissolving (0.01mole) of Mefenamic acid in (30ml) of ethanol, adding (2ml) of (H_2SO_4), and (0.01mole) of Semicarbazide were added with refluxing for (15hrs), the product filtered ,dried ,recrystallized to yield amine Derivative Compound [1], which reacted (0.01mole) in (30 ml) absolute acetone with (0.01M) (0.36gm) of (hydrazine) with refluxing for (17hrs), the product filtered, dried , recrystallized to yield diamine -Compound [2]



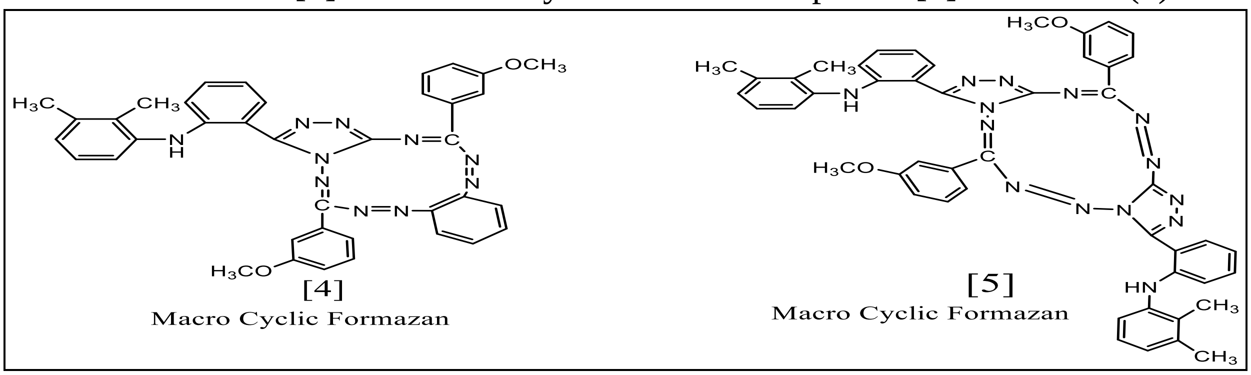
Scheme- 2 Synthesis of compound [1, 2]

2.2. Preparation of imine compound [3]:

(0.01mole) of compound [2] was reacted with (30ml) of absolute ethanol, (0.02mole) of compound (3-methoxybenzaldehyde) was added to the reaction flask and refluxing for (23hrs) in presence of (3drops of glacial acetic acid) to it, the product filtered ,dried ,recrystallized to yield Imine derivative [3].

2.3. Preparation Of Macrocyclic Formazan Compound [4,5](13, 14):

(0.01mol) Various aromatic amines was dissolved in (3ml) of hydrochloric acid and (5ml) distilled water with stirring, The mixture was cooled in at (0-5C^o), then adding solution of (0.3 gm) sodium nitrite ($NaNO_2$) drop wise with stirring at (0–5C^o) in an freeze bath via two steps, (0.01ml) from Imine compounds were dissolved in (20ml) in absolute ethanol with basic medium of solution (10ml) a dilute of (10% Sodium hydroxide) with cooling and stirring. Then followed by mixing the prepared solution of diazonium salt(in first step) to the solution Imine compound (of second step) gradually, while maintaining the temperature at (0-5C^o), then leaving (from 20 to 25 hrs), then filtered, dried, and the crystallized in ethanol, to get a product of pure Mefenamic-formazan derivative [4] and Macrocyclic Triazan compound [5] in scheme (2).



Scheme- 2 synthesis of compound [4,5]

3. Results and Discussion

In recently study, various of Invented Macrocyclic Sulfazan- Formazan Compounds and Macrocyclic Formazan have been created in same procedure that followed and invented[13] in year 2019 that got a patent to invention of Macrocyclic Formazan and Triazan-Formazan compounds by researcher Prof. Dr. Nagham Aljamali, then several studies were carried out to improve these innovative compounds by the using of spectral identification like : ^{1}H.NMR spectra, FT.IR- Spectra,C^{13}NMR ,other studies represented by (Melting points, evaluation against type of cancer cells)., all results are revealed in Tables and figures.

3.1. Spectral Analysis

FT.IR- Spectral Evidence of Invented Macrocyclic Triazan-Formazan Compounds and Macrocyclic Formazan Compounds: The first characterization of innovative compounds spectrum show appearance at (3425) cm^{-1} (N-H str.), (3307, 3255) cm^{-1} (NH_2), (1182) cm^{-1} (C-O-C oxadiazol) in **compound [1]**, (3423) cm^{-1} for (N-H), 1658 (C=N endocycle) , 3309, 3255 (NH_2), 1217 (C-N Triazol) in **compound [2],** 3468 (N-H), 1651

(C=N endocycle) , 1616 (CH=N imine), 1068 (-OCH_3 ether), 1236 (C-N Triazol) to **compound [3],** 1654 (C=N endocycle) , 1638 (-C=N- Formazan), (1467, 1502) cm^{-1} (-N=N-C=N- Formazan), (1153) cm^{-1} (-OCH_3 ether), (1238) cm^{-1} (C-N Triazol) **compound [4],** (1635) cm^{-1} (-C=N- Formazan), (1450, 1500) cm^{-1} (-N=N-C=N- Formazan)) ,while band for (-N=N-N-) of Triazan group appeared at (1320 , 1342) cm^{-1} and (1022) cm^{-1} (-OCH_3 ether), in **compound [5].**

^{1}H.NMR- Spectral Evidence of Invented Macrocyclic Formazan Compounds and Macrocyclic Triazan- Formazan Compounds showed a signal at (4.20 ppm) to the proton of the amine group (NH), and signals appeared at (6.62-7.68 ppm) dating back to Protons of the aromatic ring, as well as the appearance of a strong signal at (3.78 ppm) to the signal protons of the primary amine (NH_2), (1.10,1.28 ppm) of ($2CH_3$) **in compound [1]**, a signal due to the amine group (NH) at (4.23 ppm), and signals appeared at (6.53-7.83 ppm) dating back to Protons of the aromatic ring, as well as the appearance of a strong signal at (3.79, 4.23 ppm) to the signal protons of the primary amine (NH_2) **in compound [2],** signal at (4.42 ppm) to the proton of the amine group (NH), and signals appeared at (6.64-7.69 ppm) dating back to Protons of the aromatic ring, as well as the appearance of a strong signal at (8.86 ppm) to the signal protons of the Imine group (CH=N) **in compound [3]**, signal at (4.08 ppm) to the proton of the amine group (NH), and signals appeared at (6.71-7.94 ppm) dating back to Protons of the aromatic ring, showed signal at (1.04,1.00 ppm) of ($2CH_3$), (2.55 ppm) to (-OCH_3) in compound [4], signal at (4.20 ppm) to the proton of the amine group (NH), and signals appeared at (6.51-7.85 ppm) dating back to Protons of the aromatic ring, showed signal at (1.10,1.32 ppm) of ($2CH_3$) **in compound [5]**.

C^{13}NMR spectrum appear a signal appeared at (10.0, 12.0 ppm) belonging to the methyl group (-CH_3), a signal at (120-141 ppm) belonged to the aromatic carbon atoms **in compound [1].** The **compound [2]** appears a signal at (13.0, 14.0ppm) belonging to the methyl group (-CH_3), where a signal appeared at (115-141 ppm) belonging to the aromatic carbon atoms. C^{13}NMR spectrum appear a signal appeared at (10.0, 15.5 ppm) belonging to the methyl group (-CH3), multiple signals belong to the carbon atoms in the aromatic rings at (115-146ppm), a signal appeared at (155.5 ppm) related to the imine carbon (HC=N) in the compound as a result of the formation of the Imine in **compound [3]**. a signal appeared at (13.5, 16.4 ppm) belonging to the methyl group (-CH_3), multiple signals belong to the carbon atoms in the aromatic rings at (119-133 ppm), a signal appeared at (95.0 , 91.4 ppm) related to the carbon of Forman (-N=C-N=N) in the compound as a result of the formation of the Formazan derivative, where a signal appeared at (64.0) belonging to the methoxy group of ether (-OCH3) in

compound [4]. appear a signal appeared at (10.0, 12.0 ppm) belonging to the methyl group ($-CH_3$), multiple signals belong to the carbon atoms in the aromatic rings at (114-129 ppm), a signal appeared at (99.8 ppm) related to the carbon of Forman (-N=C-N=N) in the compound as a result of the formation of the Formazan derivative, where a signal appeared at (61.95) belonging to the methoxy group of ether ($-OCH_3$), and at (15.0 ppm) due to ($-CH_3$) in **compound [5].**

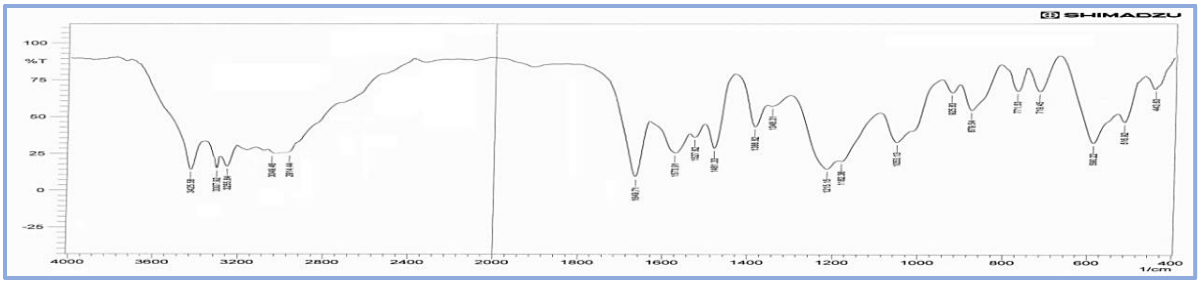


Fig- 1 FT-IR spectrum of Compound [1]

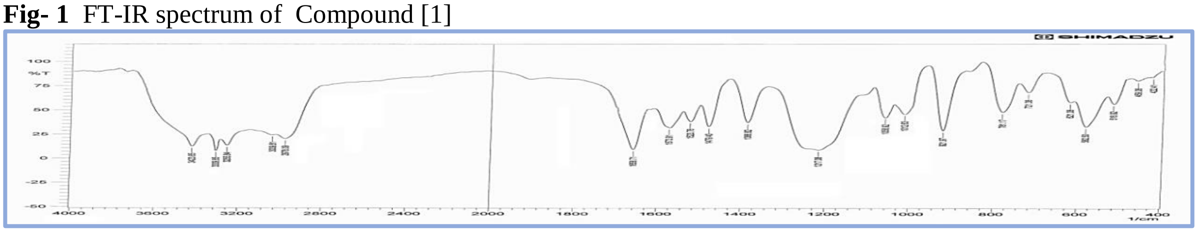


Fig- 2 FT-IR spectrum of Compound [2]

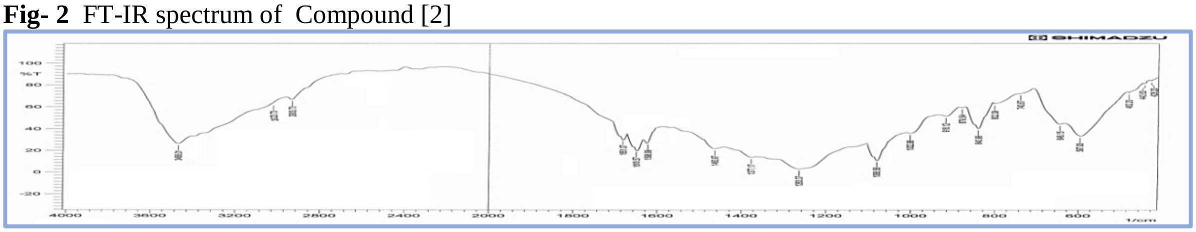


Fig- 3 FT-IR spectrum of Compound [3]

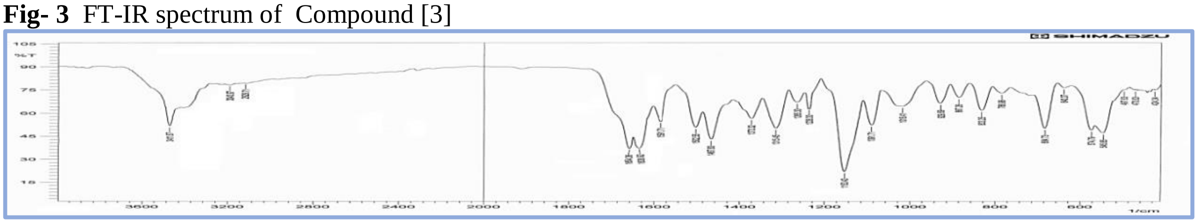


Fig- 4 **F**T-IR spectrum of Compound [4]

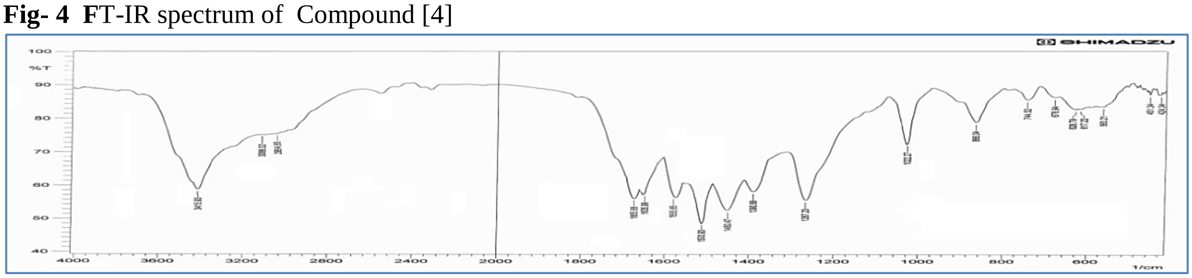


Fig- 5 FT-IR spectrum of Compound [5]

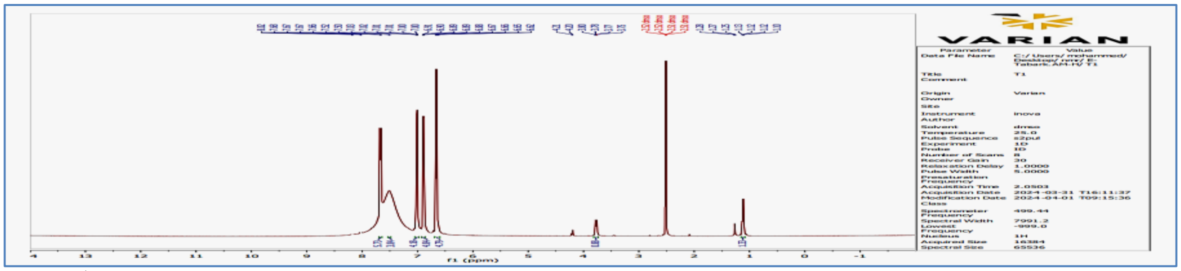


Fig- 6 [1]H.NMR spectrum of Compound [1]

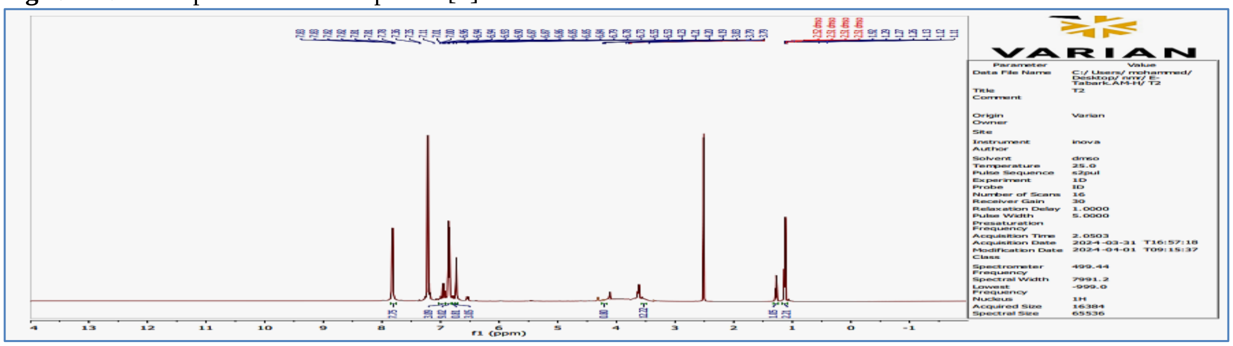


Fig- 7 [1]H.NMR spectrum of Compound [2]

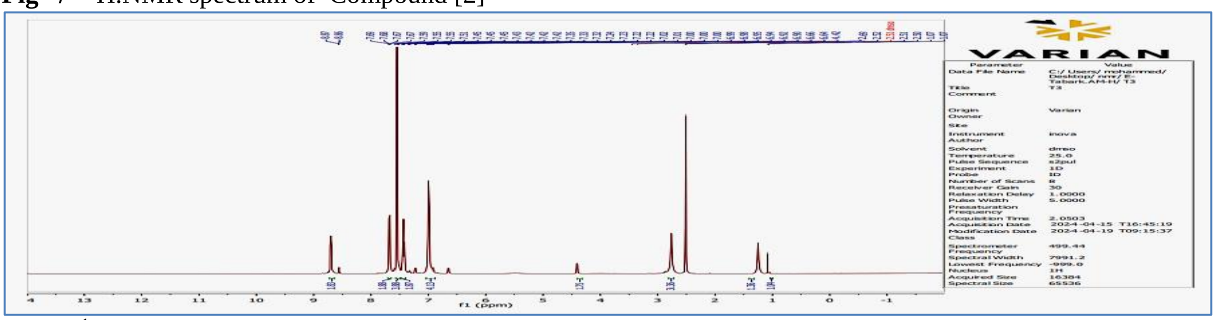


Fig- 8 [1]H.NMR spectrum of Compound [3]

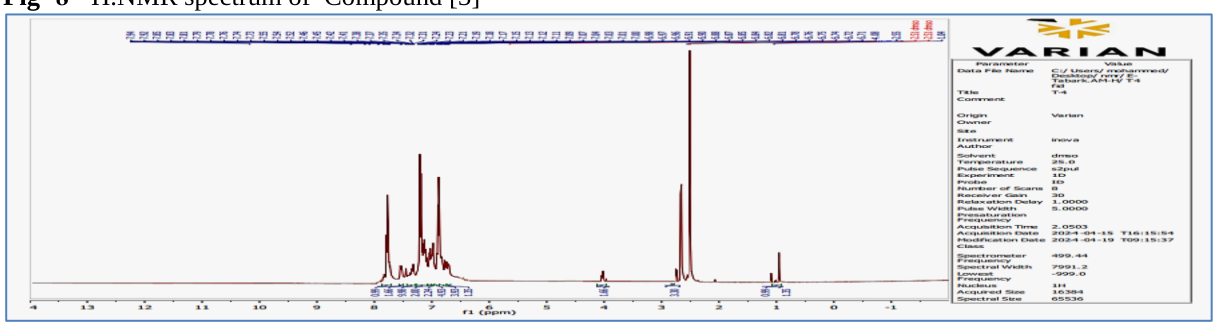


Fig- 9 [1]H.NMR spectrum of Compound [4]

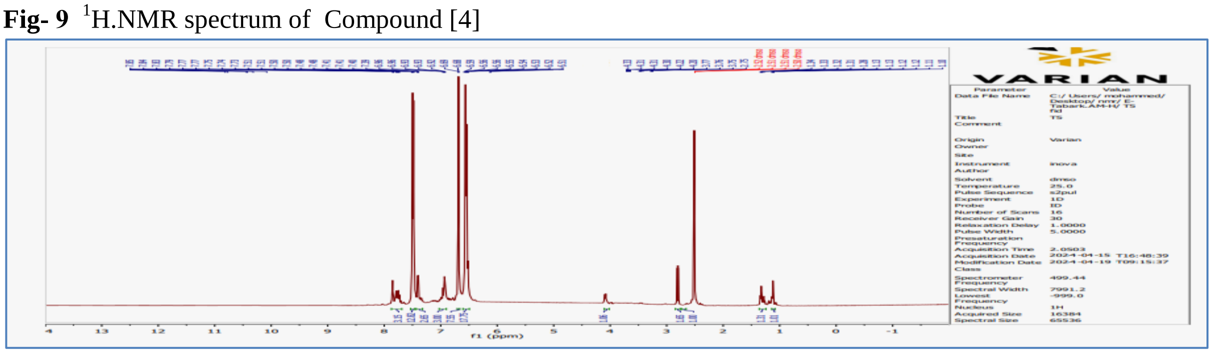


Fig- 10 [1]H.NMR spectrum of Compound [5]

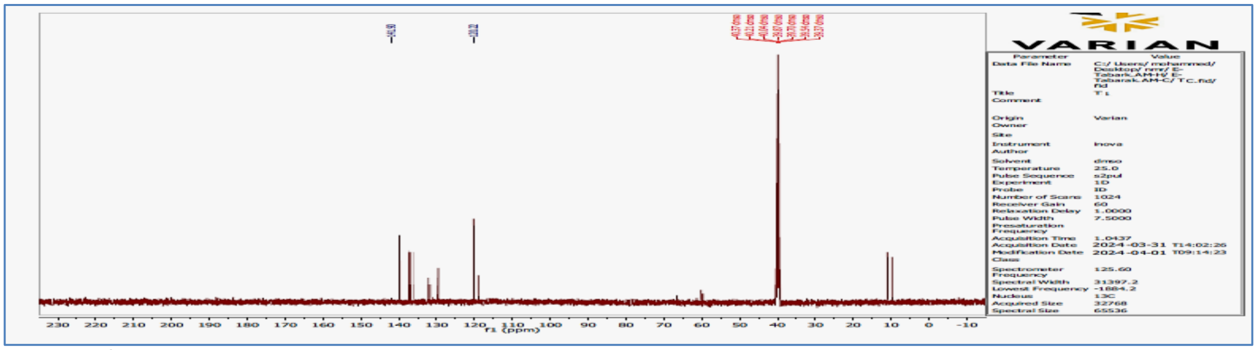


Fig-11 C^{13}NMR of compound [1]

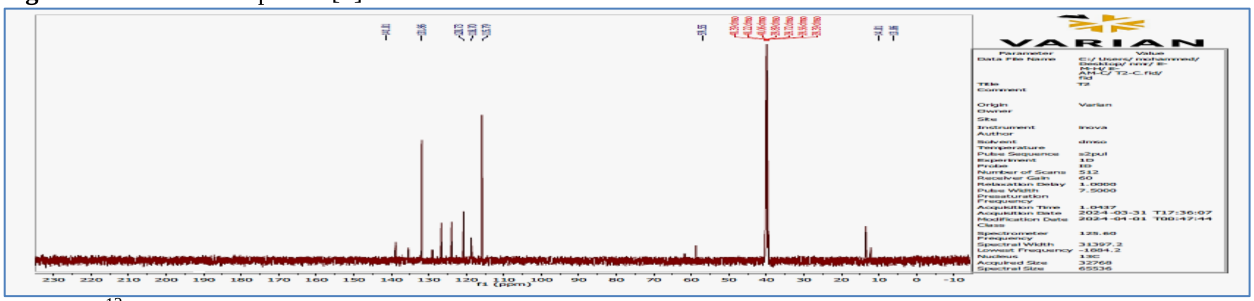


Fig-12 C^{13}NMR of compound [2]

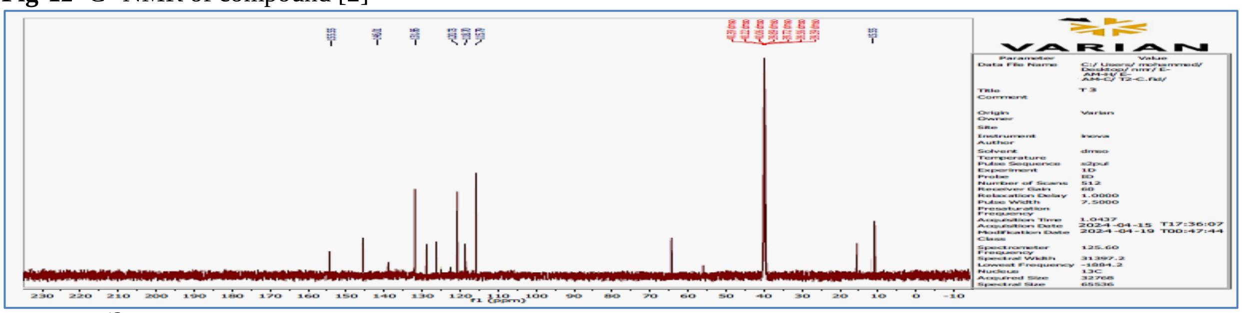


Fig-.13 C^{13}NMR of compound [3]

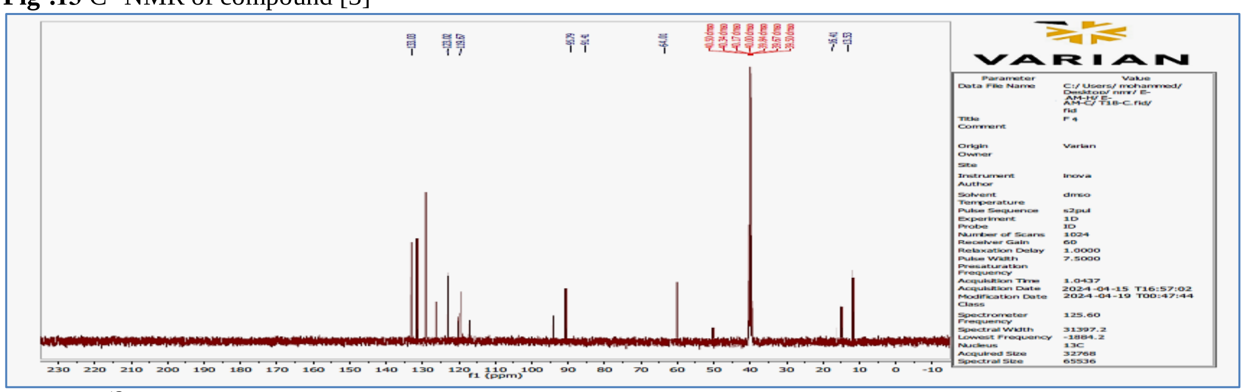


Fig-14 C^{13}NMR of compound [4]

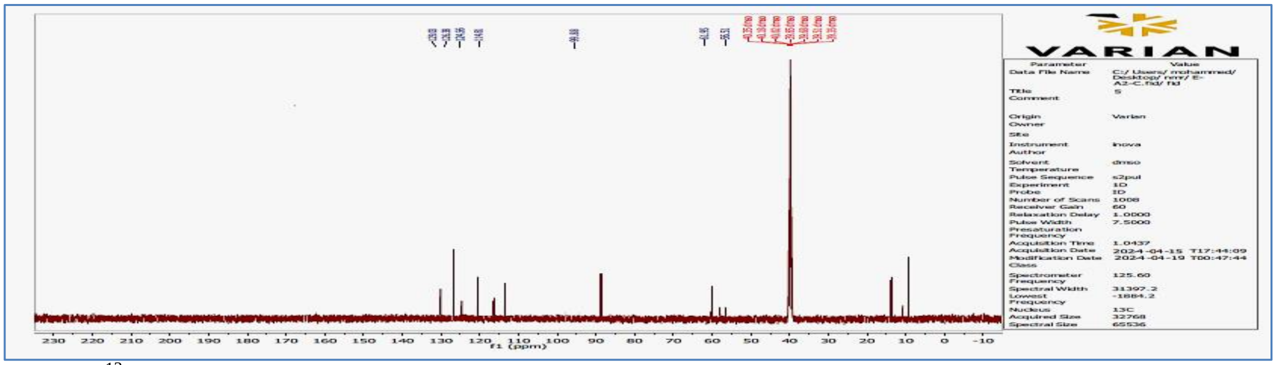


Fig-15 C^{13}NMR of compound [5]

3.2.Bioactivity Study

Anti-cancer study was carried out for Compounds [4, 5] were nominated for anti-malignance appraisal (breast malignance) via MTT-Checking for (MCF-7) as malignance cell besides (MCF- 10 A) as well cell bestowing to study(13) , in photos (1-4), table (1). The results gave good data indicated to high inhibition of tumor cells , Through the studies obtained, it is clear that the cyclic triazine gave an inhibition efficiency linked to the type of successive bonding present in the triazine-formazan structure, which confirmed that the successive system of donor atoms for the doublets gives the highest percentage of inhibition of the size of cancerous tumors., the compounds that we selected for anticancer study are :

Stand. (1): Cytotoxic Activity of Cyclic Compound [4] continuously Breast Melanoma Cells (MCF-7) and Well Cells (MCF-10A) at the equivalent meditation exhausting 24 hrs ., MTT trial 37^{0}c.

Con. (μg.mL^{-1})	Proportion (%) in percentage of every cells			
	MCF-7 , IC_{50} = 50. 18		MCF-10A , IC_{50}= 255. 03	
Cyclic Compound [4]	Melanoma cells (MCF-7)		Well cells (MCF-10A)	
	Cell - Feasibility	Cell -Reserve	Cell - Feasibility	Cell - Reserve
35.63	85.64	11.98	91.51	7.34
75. 00	71.54	24.32	94.00	6.00
150. 00	767.40	28.65	94.92	5.08
300	45.54	50.63	95.50	4.50
600	37.76	64.00	96.78	3.22
Control	100.0	0.00	95.76	6.88

Stand. (2): Cytotoxic Activity of Cyclic Compound [5] continuously Breast Melanoma Cells (MCF-7) and Well Cells (MCF-10A) at the equivalent meditation exhausting 24 hrs ., MTT trial 37^{0}c

Con. (μg.mL^{-1})	Proportion (%) in percentage of every cells			
	MCF-7 , IC_{50} = 42. 84		MCF-10A , IC_{50}= 300. 76	
Cyclic Compound [5]	Melanoma cells (MCF-7)		Well cells (MCF-10A)	
	Cell - Feasibility	Cell - Reserve	Cell - Feasibility	Cell - Reserve
35.67	84.72	12.69	90.33	9.42
75. 00	80.66	17.48	92.00	8.00
150. 00	71.70	24.61	93.50	6.50
300	54.67	49.39	94.42	5.58
600	37.85	58.04	95.93	4.07

Control	100	0.00	93.79	6.21

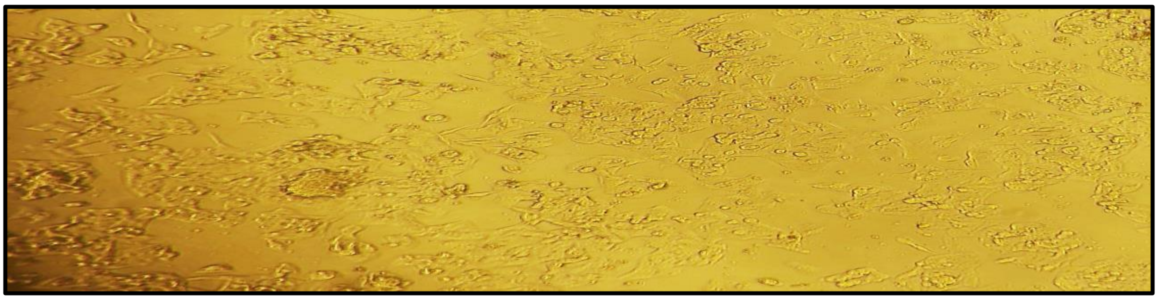


Photo. 1:Anti-cancer commotion of Cyclic {4} on (MCF-7) at 600μg/ml

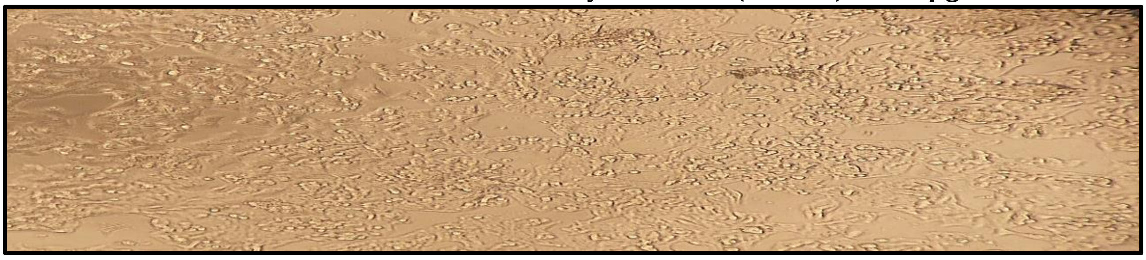


Photo. 2: Anti-malignance commotion of Cyclic {4}on (MCF-A 10) at 600μg/ml

Photo. 3: Anti-cancer commotion of Cyclic {5} on (MCF-7) at 600μg/ml

Photo. 4: Anti- malignance commotion of Cyclic {5}on (MCF-A 10) at 600μg/ml

Conclusions

Through the studies obtained, it is clear that the cyclic triazine gave an inhibition efficiency linked to the type of successive bonding present in the triazine-formazan structure, which confirmed that the successive system of donor atoms for the doublets gives the highest percentage of inhibition of the size of cancerous tumors.

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