Origination of Macrocyclic Formazan with Macrocyclic Sulfazan and Triazan as Innovated Compounds and Compared Their efficiency Against Breast Cancer

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ABSTRACT

Macrocyclic Sulfazan and Macrocyclic Formazan were created and developed globally for the first time by researcher Dr. Nagham Aljamali in April 2021, while same researcher created and innovated Sulfazan compounds as the first time in 2019. Therefore, these compounds considered one of the developed and modern compounds which have a lack of references or scarcity of books and researches. Therefore, the researcher, Dr. Nagham prepared and created a chain of these innovated compounds and studied by numerous applications, containing biological, pharmaceutical studies, also as antifungal, antibacterial, anticancer agents, and here in this research, also cyclic sulfazan and cyclic formazan were studied a several applications. A numeral of practical spectroscopic studies has been used to exhibit their chemical structures which provided strong evidence of their chemical structures through various technical devices like (FT IR-Spectra, 1H NMR-Spectra, Mass-Spectra), Melting points, other studies represented by evolution them as bio-compounds by scanning them as anticancer compounds.

KEYWORDS: Cyclic sulfazan; Cyclic formazan; Formazan; Imine; Schiff base; Azo; Aldamine; Anil; Anticancer

INTRODUCTION

Sulfazan compounds are one of the latest organic chemistry compounds that were invented and developed by researcher Dr. Nagham Aljamali in 2019. The researcher has developed all the methods [1,2] of its preparation, its properties and interactions, and studied its applications in several applied researches this is due to the lack of papers and references on it, the researcher Dr. Nagham carried out many researches [3-9] and studies to provide references on the methods of preparing and applying these newly innovative compounds, so the researcher Dr. Nagham worked on numerous studies of applications for these compounds [3-7], including microscopic scanning to prove that they are nano-compounds that act as drug carriers, anti-fungal, anti-bacterial, anti-tumors, and studies have been conducted for chromatographic separation of a series of them to study the effect of the active groups in these compounds [10-15].

Triazan compounds are one of the newest organic chemistry compounds that were innovated and developed by researcher Dr. Nagham Aljamali in 2019. The researcher has developed the methods [1-4] of its preparation, its properties and reactions, and studied its applications in several applied researches this is due to the lack of papers and references on it, the researcher Dr. Nagham carried out many researches [1,2,8] and studies to provide references on the methods of preparing and applying these newly invented compounds, so the researcher Dr. Nagham worked on series studies of applications for these compounds like studies in fungi assay, bacteria assay, cancer and tumors studies [1,8] to...
supply information about the effect of the active groups in these compounds in various applications [16-25].

Cyclic Sulfazan and Cyclic Formazan are Original-Innovative compounds in the field of organic chemistry and are considered a new innovation by Dr. Nagham Aljamali in April 2021 when these compounds were prepared for the first time globally [1,2], and because their references and papers are few in this field for this reason the researcher Dr. Nagham Aljamali developed various compounds from Macrocyclic Sulfazan and Macrocycle Formazan by using various conditions and different basic medium [3-7] like (Pyridine, Pipridine, 5% Sodium hydroxide, Triethyl amine,...) [1,2], and linked them with heterocyclic compounds and other compounds [26-30] with more than two hetero atoms to increase their effectiveness [31-36], biological [37-41] and industrial applications [42-45].

Cyclic Formazan has cyclic structure of (-N=N=C-N- in cyclic structure ) or (-N=N-C-N-NH- in cyclic structure) according to type of amine or sulfide compound.

Cyclic Sulfazan has cyclic structure of (-N=N-S- in cyclic structure) according to type of amine or sulfide compound.

Cyclic Triazan has cyclic structure of (-N=N-N- in cyclic structure) according to type of amine derivative or hydrazine derivative.

**Instruments and Experimental Part**

All melting points were uncorrected and dignified on an electro-thermal apparatus (Switzerland) in an open capillary tube. FTIR spectra were detailed on Fourier transform infrared spectrometer (FT-IR) in (FT-IR- 3600) infrared spectrometer via employing KBr Pellet technique., 1H.NMR spectra were recorded in DMSO-d6 as solvent using (TMS) as internal standard and chemical shifts are expressed as (δppm), also Mass- Spectra for some of them other studies represented by evolution them as bio-compounds by scanning them as anticancer compounds.

**EXPERIMENTAL METHODS**

**Preparation of Invented Macrocyclic Sulfazan Compounds (1,2)**

Cysteine (0.01 mole) was reacted with thiosemicarbazide(0.01 mole) in (30ml) absolute ethanol (5ml) of (6% NaOH) with refluxing for (28hrs), then cyclization step according to procedures [4-7], the product filtered, dried, recrystallized to yield derivative of Triazole thiol Compound [1], which reacted (0.01 mole) with (0.01 mole) from diazo salt of m-phenyl diamine in pyridine through three steps in basic medium to formation Invented Macrocyclic Sulfazan after (10hrs), the product filtered, dried, washed by distilled water, recrystallized to yield Invented Macrocyclic Sulfazan Compound [2] by following literatures (Scheme 1); [2,8].

**Preparation of Invented Macrocyclic Formazan Compounds (3,4,5)**

Cysteine (0.01 mole) was reacted with thiosemicarbazide(0.01 mole) in (30ml) absolute ethanol (5ml) of (sulfuric acid) with refluxing for (22hrs), then cyclization step according to procedures [4-7], the product filtered, dried, recrystallized to yield derivative of Thiadiazole amine Compound [3], which (0.01 mole) reacted with (0.02 mole) of p-methylbenzaldehyde with (3 drops) of glacial acetic acid and absolute ethanol (30ml), the product filtered, dried, recrystallized to yield derivative of Anil Compound [4], which reacted (0.01 mole) with (0.01 mole) from diazo salt of m-phenyl diamine in (6% NaOH) through three steps in basic medium to formation Invented Macrocyclic Formazan after (20hrs), the product filtered, dried, washed by distilled water, recrystallized to yield Invented Macrocyclic Formazan Compound [5] by following literatures (Scheme 2); [1,2].

**Creation of Inventive Macrocyclic Sulfazan- Formazan and Sulfazan –Triazan Compounds (6,7)**

Compound [1] refluxed (0.01 mole with (0.01 mole) of p-methylbenzaldehyde in presence of (2-3 drops) of glacial acetic
acid for (3hrs) in absolute ethanol according to procedure [3-7], the product filtered, dried, recrystallized to yield Imine - Compound [6] that (0.01 mole) was reacted in presence [1,2] of (Pipyridine) with (0.02 mole) of diazo salt of p-phenyl diamine via many steps in basic medium to formation Invented Macro cyclic Sulfazan-Formazan and Macrocyclic Sulfazan-Triazan after (10hrs), the product filtered, dried, washed by distilled water, recrystallized to yield Invented Macro cyclic Sulfazan-Formazan and Macrocyclic Triazan [7] by following literatures (Scheme 3); [2,8].

**Scheme 2:** Creation of invented macrocyclic Formazan compounds {3,4,5}.

**Scheme 3:** Creation of invented macrocyclic Sulfazan-Formazan and Triazan Compounds {6,7}.

**RESULTS AND DISCUSSION**

In newly scientific paper, a number of Invented Macro cyclic Sulfazan with Formazan and Triazan Compounds have been created in same procedure that followed and invented [1,2] by Dr. Nagham in April 2021 for Macro cyclic Formazan and in 2019 for Sulfazan and Triazan compounds, then several studies were carried out to improve these innovative compounds by the using of spectral identification like: 1H.NMR spectra, FTIR-Spectra, Mass-Spectra., other studies represented by (Melting points, other studies acted with evolution them as anticancer compounds., all the results are shown in Tables and Figures.
Spectral Evidence

FTIR- spectral indication of invented macrocyclic Sulfazan with Formazan and Triazan compounds: The first characterization of invented compounds by shifting of frequencies of Imine group (CH=N) in starting compounds -Imine compounds [4,6] that were about at (1617,1622) cm⁻¹ respectively in all starting materials of imine compounds that were shifted to (1637,1634) cm⁻¹ for (-C=N-) due to formation of Macrocyclic Formazan in compounds [5,7] respectively, while disappearance of band of thiol group (SH) and bands of amine group (NH₂) in Compounds [1,6] and appearance new bands in compounds [2,7] at (1292,1297) cm⁻¹ due to sulfide in Sulfazan group (-S-N-N-), also appearance three bands due to partitions of azo group of Formazan and Sulfazan also in Triazan in Macrocyde (-N=N-) are three bands to every compound like (1433,1468, 1491) cm⁻¹ for Azo group (-N=N-C-) in compound (5), also appearance band at (1300) cm⁻¹ due to (-N-N=N-) in Triazan compound [7], and other compound like this., all frequencies clarified according to reference [33].

H.NMR- spectral indication of invented macrocyclic Sulfazan with Formazan and Triazan Compounds: The second characterization of innovative compounds by disappearance of peak for Anil group (CH=N) in starting compound (Imine compound) that were at δ(8.25, 8.61) in Compounds (4,6) respectively in [starting compound] due to formation of (N=C=N=N) Formazan and (S-N=N-) Sulfazan group in innovated compounds [5-7], also in compound [7] disappeared peak at δ(5.11) due to proton of removing of proton of amine in Triazole ring due to formation (-N-N=N- in cycle structure) of Triazan compound [7], while disappeared peak at δ(4.98) as proton of Thiol group (SH) in compound [1] due to formation of Sulfazan compound [2], all peaks explained according to references [1,33].

Mass- spectral indication of invented macrocyclic Sulfazan with Formazan and Triazan Compounds: The third characterization of innovated compounds by partition of innovative cyclic compounds via appearance of fragments in spectra in Figure 1-3.

Figure 1: Mass-spectrum of invented macrocyclic Sulfazan compound (2).

Figure 2: Mass-spectrum of invented macrocyclic formazan compound (5).
Other Characterization

All Invented Macrocyclic Sulfazan with Macrocyclic Formazan and Triazan derivatives were studied to collect all the chemical and physical properties, in Table 1:

<table>
<thead>
<tr>
<th>Innovated Comps.</th>
<th>P%</th>
<th>Color</th>
<th>MPC°</th>
<th>RF</th>
<th>Solvents (TLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innovated Comp. (1)</td>
<td>74</td>
<td>Bill Yellow</td>
<td>158</td>
<td>0.56</td>
<td>Ethanol: Benzene</td>
</tr>
<tr>
<td>Innovated Comp. (2)</td>
<td>82</td>
<td>Yellowish Orange</td>
<td>200</td>
<td>0.62</td>
<td>Ethanol: Benzene</td>
</tr>
<tr>
<td>Innovated Comp. (3)</td>
<td>70</td>
<td>Bill Yellow</td>
<td>166</td>
<td>0.54</td>
<td>Ethanol: Benzene</td>
</tr>
<tr>
<td>Innovated Comp. (4)</td>
<td>84</td>
<td>Orange</td>
<td>178</td>
<td>0.6</td>
<td>Ethanol: Benzene</td>
</tr>
<tr>
<td>Innovated Comp. (5)</td>
<td>82</td>
<td>Reddish Orange</td>
<td>212</td>
<td>0.62</td>
<td>Ethanol: Benzene</td>
</tr>
<tr>
<td>Innovated Comp. (6)</td>
<td>84</td>
<td>Yellowish Orange</td>
<td>204</td>
<td>0.6</td>
<td>Ethanol: Benzene</td>
</tr>
<tr>
<td>Innovated Comp. (7)</td>
<td>74</td>
<td>Orange</td>
<td>222</td>
<td>0.64</td>
<td>Ethanol: Benzene</td>
</tr>
</tbody>
</table>

Effect of invented macrocyclic Sulfazan and Macrocyclic Formazan compounds against breast cancer cells [1,8,44]: MTT was used to determine cell viability by chromatic examination (64-70) of two (MCF-7 and WRL cell lines):

- Cell suspension (100µL) was added to the wells of a small flat plate bottom.
- The solution was prepared by dissolving the crystals of 5mg MTT in 1ml of PBS solution (phosphate buffer solution).
- The concentrations of each innovative derivative of the prepared derivatives were used in this research (400, 200, 100, 50, 25, 12.5, 6.5 (µg/ml of methanol, which were added to each well (three replicates per concentration).
- A 10ml MTT solution was added to each well of a plate containing 96 wells and then incubated for 4 hours with a test sample at 37 ºC (the solution became yellow).
- DMSO was added (200µL) (to each hole and stirred for 5 minutes (to become a purple DMSO solution).
- After the complete dissolution of the dye, the absorption of the colored solution from the living cells was read at (575nm) using the ELISA reader. 7- The mean absorption was calculated for each group of iterations and the validity ratio of the cells exposed to different treatments was obtained as follows:

\[
\text{Cell Vitality } \% = \left( \frac{\text{Absorption from the treated sample}}{\text{Absorption from the untreated sample}} \right) \times 100
\]

Initialization of cancer cell line for invented macrocyclic formazan compound [5] and macrocyclic Sulfazan compound [2]: Line processing and implantation of breast cancer cells and live cell line were carried out at Biotechnology Center - the Nahrain (MCF-7 cell line) and (WRL cell line grew in 95% of RPMI–1640) supplemented with (10% FBS), cell suspension and incubation at (37 ºC) in incubator (CO\textsubscript{2} 5%). The suspended cells were centrifuged at (250g) for (10 minutes) and the supernatant was removed, the cells were re-suspended in a freezing medium, then placed at (-70 ºC) in beaker for (1-3) days, the beaker was transferred from the standard freezer boxes to the liquid (N\textsubscript{2}) container according to studies [1,8,28], all data in Figure 4 & 5 and Table 2 & 3.

The results improved that the Invented Macrocyclic Sulfazan and Macrocyclic Formazan compounds have good results as inhibitor of cancer of breast cells and the invented macrocyclic Sulfazan compound [2] has more activity than invented macrocyclic formazan compound [5] due to structure of compound [2] involved sulfdie group (-N=C-N=N-) involved, while compound [5] has less inhibition activity due to its structure of Formazan (-N=N-C=N-).
Figure 4: Percentage of remaining cells versus concentration of macrocyclic Sulfazan Compound [2].

Figure 5: Percentage of remaining cells versus concentration of macrocyclic formazan compound [5].

Table 2: Effect of different concentrations on the cancer cell line (MCF-7) and its toxic effect on the live cell line (WRL) of invented macrocyclic Sulfazan Compound [2].

<table>
<thead>
<tr>
<th>Concentration of Invented Macro cyclic Sulfazan Compound [2] (µg/Ml^-1)</th>
<th>MCF-7</th>
<th>WRL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>400</td>
<td>35.81</td>
<td>5</td>
</tr>
<tr>
<td>200</td>
<td>49.72</td>
<td>3.96</td>
</tr>
<tr>
<td>100</td>
<td>58.34</td>
<td>3.09</td>
</tr>
<tr>
<td>50</td>
<td>58.95</td>
<td>3.97</td>
</tr>
<tr>
<td>25</td>
<td>78.45</td>
<td>2.18</td>
</tr>
<tr>
<td>12.5</td>
<td>86.73</td>
<td>1.4</td>
</tr>
<tr>
<td>6.25</td>
<td>90.88</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Table 3: Effect of different concentrations on the cancer cell line (MCF-7) and its toxic effect on the live cell line (WRL) of invented macrocyclic formazan compound [5].

<table>
<thead>
<tr>
<th>Concentration of Invented Macro cyclic Formazan Compound [5] (µg/Ml-1)</th>
<th>MCF-7</th>
<th>WRL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>400</td>
<td>37.44</td>
<td>4.05</td>
</tr>
<tr>
<td>200</td>
<td>39.89</td>
<td>3.84</td>
</tr>
<tr>
<td>100</td>
<td>41.62</td>
<td>3.56</td>
</tr>
<tr>
<td>50</td>
<td>46.2</td>
<td>3.12</td>
</tr>
<tr>
<td>25</td>
<td>59.1</td>
<td>2.63</td>
</tr>
<tr>
<td>12.5</td>
<td>67.02</td>
<td>2.42</td>
</tr>
<tr>
<td>6.25</td>
<td>77.31</td>
<td>2.19</td>
</tr>
</tbody>
</table>
CONCLUSION

All Invented Macroyclic Sulfazan and Formazan also Triazan compounds gave good evidence for their structures via various spectral techniques, also some of them studied as anticancer compounds that gave good data and clear results against selected type of cancer cells.

REFERENCES


