Development of Trimethoprim Drug and Innovation of Sulfazane-Trimethoprim Derivatives as Anticancer Agents

Nagham Mahmood Aljamali¹* and Imad Kareem Alwan Alsabri²

¹Synthetic Chemistry Field, Department of Chemistry, Iraq.
²Master in Oncology, College of Medicine, Kufa University, Iraq.
*Corresponding Author E-mail: dr.nagham_mj@yahoo.com

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Cancer tumors cause not few deaths among the total deaths in the world, despite the great progress in the treatment of cancer, so it has become necessary to understand the molecular explanations that contribute to the development and progress of cancer and to search for more effective and less toxic treatments to treat this disease. The work included the innovation and development of the drug trimethoprim by linking it with the innovative compound sulfazane and creation of several derivatives of sulfazane- trimethoprim for the first time then studying the behavior and impact of innovative compounds on vital activities as anti-cancer tumors by following an innovative procedures of preparation for sulfazane compounds, then linking them with several chemical reactions with the drug trimethoprim to develop its effectiveness and efficiency. Several spectral techniques were used to diagnose and demonstrate the formation of innovative derivatives (sulfazane - trimethoprem) thereof (FT.IR - Spectra, H.NMR – Spectra, HMBC- Spectrum, Mass Spectra), chemical properties, Flowing through TLC, in addition to laboratory study of cancerous tumors. The results indicated to formation of these drug derivatives by appearance of new bands and disappearance of bands in starting compounds, besides to conclusions from our paper that gave good data for inhibition efficiency for these drug derivatives against cancer cells.

Keywords: Azo; Coupling; Cancer; Innovation; Invented Compounds; New Reaction of Diazonium; Sulfide-Azo; Sulfazane; Tumor; Toxicity; Trimethoprim; (-S-N=N-).

The newly innovative sulfazane compounds that were invented and prepared for the first time by the researcher (Dr. Nagham Aljamali in year 2019 in the first research work¹ The researcher laid the first foundations for the innovative sulfazane compounds in terms of (methods of preparation, their properties, chemical and physical characteristics, their colors, stability, some of their vital applications and behavior towards some types of bacteria and fungi).¹² Now in this research work continued to prepare other drug derivatives by linking sulfazane to the drug trimethoprim via (-S-N=N-) sulfazane group to study its effect on cancerous tumors. Trimethoprim contains the active ingredient trimethoprim, an antibiotic used to treat infections with bacteria. Bacterial cells in order to grow and multiply need a genetic material (DNA) to produce DNA and need folic acid (folate).³⁻¹⁰ Methoprim works by preventing bacteria from producing folic acid
and without it bacteria cannot produce DNA, and thus become unable to reproduce\textsuperscript{11-22} and increase numbers, so methoprim stops the spread of infection, and kills the remaining bacteria by the immune system\textsuperscript{23-34}.

Trimethoprim (C\textsubscript{14}H\textsubscript{18}N\textsubscript{4}O\textsubscript{3}) : 5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine., its names (Proloprim, Monotrim, Triprim, others). M.Wt : 290.32 g/mol. It is used to treat bacterial infections in the urinary tract and also to prevent frequent bacterial infections in the urinary tract,\textsuperscript{35-44} as well as to treat bacterial infections in the lungs and bronchi (respiratory system), such as acute bronchitis, chronic bronchitis or pneumonia.\textsuperscript{44-56}

The cancer is a type of non-infectious disease that is characterized by uncontrolled growth of cells, there are more than (100) different types of cancer, which were classified according to the type of cell that was affected at the beginning, and cancer causes a death rate of more than 20\% of the total deaths in the world on according to the World Health Organization, where lung cancer is the most prevalent\textsuperscript{57-59} followed by breast, colon and prostate cancer, despite significant progress in cancer treatment, the death rate has not decreased, so it has become necessary to understand the molecular explanations that contribute to the development and progress of cancer and search for more treatments. Efficacy and least toxic for a processor with this disease,\textsuperscript{60-63} there are many methods for treating this disease, including tumor lift, multiple chemical treatments, and radiological treatments.

**Experimental Part**

All chemicals compounds supplied from Sigma –chemicals company and fluka –chemicals company.

An innovative method\textsuperscript{1, 2} was used to develop the trimethoprim drug by linking it to an innovative sulfazane compounds through several steps, chemical reactions and reaction conditions that differ for each of the innovative derivatives prepared in this research. The chemical composition of the innovative derivatives prepared in this research was proven by several investigation techniques (FT-IR spectra (FT-IR 8300 Shimadzu) with the range (400-4000)cm\textsuperscript{-1} using discs of KBr, 1H.NMR–Spectra in solvent (d-DMSO) Fourier transformation broker spectrometer, operating at (400MHz), HMBC- Spectrum, Mass spectra for some of them) in Kashan university, and the preparation was followed by (TLC), then a laboratory study of cancer cells line to know the effectiveness and efficiency of the innovative compounds.
**Paths of Synthesis**

**Innovation and Creation of Sulfazane-Trimethoprim {1}**

P-thiol aniline (0.01 mole) with (0.01 mole) ammonium thiocyanate in bromine with glacial acetic acid, after rotation for (2 hrs) at (10 C°) via three reactions, then precipitation filtered, washed, dried, purification by recrystallization, then (0.01 mole) from precipitation dissolved in basic alcoholic solution, and added to acidic diazo-trimethoprim salt in three steps according to invented procedure in papers (1, 2), after (3 days), filtered, washed, dried, recrystallized to yield sulfazane- trimethoprim {1}.

**Innovation and Creation of Sulfazane-Trimethoprim {2}**

Cystein (0.01 mole) in basic alcoholic solution by two steps, then added to acidic diazo- trimethoprim salt in three steps according to invented procedure in papers (1, 2), after (3 days), filtered, washed, dried, recrystallized to yield sulfazane- trimethoprim {2}.

**Innovation and Creation of Sulfazane-Trimethoprim {3}**

Thiol benzoic acid (0.01 mole) in basic alcoholic solution by two steps, then added to acidic diazo- trimethoprim salt in three steps according to invented procedure in papers (1, 2), after (3 days), filtered, washed, dried, recrystallized to yield sulfazane- trimethoprim {3}.

**Scheme. 2. Creation of Sulfazane-Trimethoprim {2}**

**Scheme. 3. Creation of Sulfazane-Trimethoprim {3}**
Scheme 4. Creation of Sulfazane-Trimethoprim {4}

Trimethoprim {4}

2-Thiol imidazole in previously steps was prepared, then (0.01 mole) dissolved in basic alcoholic solution by two steps, then added to acidic diazo-trimethoprim salt in three steps according to invented procedure in papers (1, 2), after (3 days), filtered, washed, dried, recrystallized to yield sulfazane-trimethoprim {4}.

RESULTS

Our research described invented procedure (1, 2) via original process to creation and preparation of Sulfazane compounds linked with trimethoprim drug via designation of reaction conditions, type of used catalyst, mechanism of this reaction, we called it (Name–Sulfazane) as a first and Original preparation to this novel type of trimethoprim-derivatives., then all these drug derivatives investigated via numerous spectral techniques and other chemical with physical studies:

Spectral Evidences
FTIR- Spectra

The infrared spectrum is the first technique that has demonstrated, with certain evidence, the creation of innovative, advanced derivatives in this study through the emergence of effective and functional grouping frequencies in derivatives indicating the reason for the accuracy of their preparation and the correctness of the innovative procedure to prepare them:

Sulfazane-Trimethoprim {1}

Bands at (-OCH3) methoxy group:

Fig. 1. I.R Spectrum of Invented Sulfazane-Trimethoprim {1}
Sulfazane-Trimethoprim [2]

Bands at (-OCH3) methoxy group: 1118 ,(-N=N-S-) Azo-Sulfide: (1363, 1454, 1496) , (S-CH-) Sulfide : 1224 , (C=N) endocycle of pyrimidine: 1664 ,(-NH2) amine group: (3314 ,3379), (CO-O-)carbonyl of carboxyl : 1729.

Sulfazane-Trimethoprim [3]

Bands at (-OCH3) methoxy group: 1134

Sulfazane-Trimethoprim [4]

Bands at (-OCH3) methoxy group: 1130 ,(-N=N-S-) Azo-Sulfide: (1382, 1460, 1502) , (C=N) endocycle of pyrimidine: 1657 , (C=N) endocycle of imidazole: 1632. Other active groups are shown in some selected spectra (1, 2).

1H.NMR Spectra

The nuclear resonance spectrum is the
second technique that has demonstrated, with certain evidence, the creation of innovative, advanced derivatives in this study through the emergence of effective and functional grouping frequencies in derivatives indicating the reason for the accuracy of their preparation and the correctness of the innovative procedure to prepare them by disappearance of amine signal of (-NH2) as a result of formation of (sulfazane)- group(1, 2) in derivatives, all spectra showed signals at (2.50) for solvent (d-DMSO), besides to other signals like:

**Sulfazane-Trimethoprem[1]**

It gave signals at (5.40) due to proton of amine group (NH2) , (6.78–7.74) to protons of aromatic ring , (2.98) for (O-CH3) protons of methoxy groups.

**Sulfazane-Trimethoprem[2]**

It gave signals at (5.11) due to proton of amine group (NH2) , (6.36–7.42) to protons of aromatic ring , (2.94) for (O-CH3) protons of methoxy groups , (COOH) proton of carboxyl group : (13.26) , (S-CH2-CH-N-) : (3.56 , 3.76).

**Sulfazane-Trimethoprem[3]**

It gave signals at (6.77–7.57) to protons of aromatic ring , (2.86) for (O-CH3) protons of methoxy groups , (COOH) proton of carboxyl group : (13.08).

**Sulfazane-Trimethoprem[4]**

It gave signals at (6.69–7.52) to protons of aromatic ring , (2.92) for (O-CH3) protons of methoxy groups :(NH) proton of amine in imidazole ring: (8.14). Other protons of functional groups appeared in some spectra (3 , 4).

**HMBC- Spectrum**

The HMBC- spectrum is the third technique that has demonstrated, with certain evidence, the creation of innovative, advanced derivatives in this study through the emergence of effective and functional grouping frequencies in derivatives indicating the reason for the accuracy of their preparation and the correctness of the innovative procedure to prepare them via appearance of functional groups for invented derivative, Figures (5).

**Mass Spectra of Sulfazane –Trimethoprim Derivatives**

The mass spectrum is the fourth technique that has demonstrated, with certain evidence, the creation of innovative, advanced derivatives in this study through the emergence of effective and functional grouping frequencies in derivatives indicating the reason for the accuracy of their preparation and the correctness of the innovative procedure to prepare them via appearance of fragments of some of invented derivatives, Figures (6, 7):

All chemical with physical properties and information about TLC for invented derivatives in Table(1).
Fig. 5. HMBC-Spectrum of Invented Sulfazane-Trimethoprim {2}

Fig. 6. Mass Spectrum of Invented Sulfazane-Trimethoprim {1}

Table 1. All chemical with physical properties and information of TLC

| Invented Derivatives         | Product % | Color             | M.P(°C) | Rf   | Solvents (TLC)               |
|------------------------------|-----------|-------------------|---------|------|-----------------------------|
| Sulfazane-Trimethoprim {1}   | 74        | Deep Yellow       | 202     | 0.68 | Ethanol : Benzene           |
| Sulfazane-Trimethoprim {2}   | 80        | Yellowish Orange  | 168     | 0.60 | Ethanol : Benzene           |
| Sulfazane-Trimethoprim {3}   | 78        | Yellowish Orange  | 182     | 0.62 | Ethanol : Benzene           |
| Sulfazane-Trimethoprim {4}   | 82        | Pale Orange       | 194     | 0.70 | Ethanol : Benzene           |
Table 2. Mean Percentage (%) for each cell line (Respond to Treatment) for Derivative{1}

| Sulfazane-Trimethoprim{1} | IC_{50} (µg/ml) : (190.1654) | Mean Percentage (%) for each cell line (Respond to treatment) |
|---------------------------|-------------------------------|---------------------------------------------------------------|
|                           | Conc (µg/ml)                   | Killing and inhibition of Carcinoma Cells %                   |
|                           | 500                           | 66 %                                                          |
|                           | 250                           | 58 %                                                          |
|                           | 125                           | 50 %                                                          |
|                           | 62.5                          | 46 %                                                          |
|                           | 31.5                          | 46 %                                                          |
|                           | 15.6                          | 44 %                                                          |

Table 3. Mean Percentage (%) for each cell line (Respond to Treatment) for Derivative{2}

| Sulfazane-Trimethoprim{2} | IC_{50} (µg/ml) : (228.1930) | Mean Percentage (%) for each cell line (Respond to treatment) |
|---------------------------|-------------------------------|---------------------------------------------------------------|
|                           | Conc (µg/ml)                   | Killing and inhibition of Carcinoma Cells %                   |
|                           | 500                           | 56 %                                                          |
|                           | 250                           | 52 %                                                          |
|                           | 125                           | 46 %                                                          |
|                           | 62.5                          | 40 %                                                          |
|                           | 31.5                          | 32 %                                                          |
|                           | 15.6                          | 30 %                                                          |

Fig. 7. Mass Spectrum of Invented Sulfazane-Trimethoprim {2}
DISCUSSION

Innovative Derivatives Test Against Breast Cancer

Initialization of Cancer Cell Line(13)

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(13, 53) at (37 °C) in incubator [(CO2) % 5]. The suspended cells were centrifuged at (250 g) for (10 minutes) and the supernatant was removed, the cells were re-suspended in a freezing medium, then placed at (-70 °C) in beaker for (1-3) days, the beaker was transferred from the standard freezer boxes to the liquid (N2) container.

Processing Method

MTT was used to determine cell viability by chromatic examination(64-70) of two (MCF-7 and WRL cell lines):

- Cell suspension (100 µL) was added to the wells of a small flat plate bottom.
- The solution was prepared by dissolving the crystals of 5 mg MTT in 1 ml of PBS solution (phosphate buffer solution).
- The concentrations of each innovative derivative of the prepared derivatives were used in this research (500, 250, 125, 62.5, 31.5, 15.6 (µg/ml of methanol, which were added to each well (three replicates per concentration).
- A 10 ml 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 °C (the solution became yellow).
- DMSO was added (200 µL 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 (575 nm) using the ELISA reader. The mean absorption was calculated for each group of iterations and the validity ratio of the

Table 4. Mean Percentage (%) for each cell line (Respond to Treatment) for Derivative{3}

| Sulfazane-Trimethoprim{3} | IC<sub>50</sub> (µg/ml) : (258. 5202 ) | Mean Percentage (%) for each cell line (Respond to treatment) | Toxic Effect on Normal Cell Line |
|---------------------------|----------------------------------|-------------------------------------------------------------|-------------------------------|
| Conc (µg/ml)              | Killing and inhibition of Carcinoma Cells % |                                                                               |                               |
| 500                       | 50 %                             | 12 %                                                         |                               |
| 250                       | 38 %                             | 10 %                                                         |                               |
| 125                       | 36 %                             | 10 %                                                         |                               |
| 62.5                      | 34 %                             | 8 %                                                          |                               |
| 31.5                      | 30 %                             | 8 %                                                          |                               |
| 15.6                      | 30 %                             | 4 %                                                          |                               |

Table 5. Mean Percentage (%) for each cell line (Respond to Treatment) for Derivative{4}

| Sulfazane-Trimethoprim{4} | IC<sub>50</sub> (µg/ml) : (210. 3871 ) | Mean Percentage (%) for each cell line (Respond to treatment) | Toxic Effect on Normal Cell Line |
|---------------------------|----------------------------------|-------------------------------------------------------------|-------------------------------|
| Conc (µg/ml)              | Killing and inhibition of Carcinoma Cells % |                                                                               |                               |
| 500                       | 60 %                             | 22 %                                                         |                               |
| 250                       | 50 %                             | 18 %                                                         |                               |
| 125                       | 44 %                             | 18 %                                                         |                               |
| 62.5                      | 40 %                             | 16 %                                                         |                               |
| 31.5                      | 30 %                             | 16 %                                                         |                               |
| 15.6                      | 30 %                             | 16 %                                                         |                               |
cells exposed to different treatments was obtained as follows (13):

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

**CONCLUSIONS**

The results indicated to formation of these drug derivatives by appearance of new bands and disappearance of bands in starting compounds, besides to conclusions from our paper that gave good data for inhibition efficiency for these drug derivatives against cancer cells. Our results appeared good inhibition for carcinoma cells line for all invented derivatives, and gave high response for derivatives \{1 and 4\} more than other invented derivatives, it was \(66\%\) response percentage of derivative \{1\} for inhibition and killing of cancer cells due to sulfazane (1, 2) group (-S-N=N- Drug) that linked with drug which gave it more response against cancer cells also due to thiazole core in this derivative \{1\}.

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