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Article

Sulfisoxazole-1,2,3-triazole derivatives synthesis, characterization, and evaluation against breast cancer MCF-7 cell line

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ABSTRACT

The current work describes synthesizing, characterizing and evaluating two new 1,4-disubstituted 1,2,3-triazole-linked sulfisoxazole derivatives against a breast cancer MCF-7 cell line. The newly synthesized compounds 1 and 2 were characterized by 1H-NMR, 13C-NMR, and mass spectra. The synthesized compounds were evaluated against the breast cancer MCF-7 cell line. The results show that the synthesized compounds 1 and 2 have weak activity against the breast cancer MCF-7 cell line with IC50 values of 1873.42 µM, and 3750.02 µM, respectively.

Keywords: Sulfisoxazole, 1,2,3-triazole, cycloaddition reaction, breast cancer, MTT essay.

1. Introduction

Sulfisoxazole belongs to a class of compounds called Sulfonamides. Sulfonamides or sulfonamides have the formula (A-SO_2NHR) where the active group is linked to an aliphatic, aromatic, heterocyclic, or sugar moiety represented by the (A) moiety. As a result, preparing numerous compounds with significant pharmacological properties involves this class of compounds.[1], [2], [3].Among the compounds that can be prepared from sulfonamides are 1,2,3-triazoles via the diazotization reaction of the amine group [4], [5] and preparation of azides, then 1,3-dipolar cycloaddition reactions with unsaturated compounds. [6] One of the most common scaffolds used in pharmaceuticals is 1,2,3-triazoles. These molecules have unique properties that make them useful.[7] For example, they can form hydrogen bonds, are stiff and stable, and have a wide range of biological activities, such as killing bacteria, [8] viruses, [9] cancer, [10] and malaria. [11]

Considering cancer is currently the second-greatest cause of death worldwide, it is a complicated disease. Because they are ineffective and have low selectivity, which increases the risk of negative side effects, the medications that are already on the market that are used to treat cancer are ineffective as well.[12], [13], [14] Drug resistance has also made it more difficult for these medications to be successful in clinical settings. To overcome the present restrictions on chemotherapy treatment, it is imperative to discover novel, safe, efficacious, and selective anticancer drugs.[15]

In this study, 1,2,3-triazole molecules were made using a 1,3-dipolar cycloaddition reaction to help get only 1,4-disubstituted triazole derivatives.

Chemicals and Instruments.

All the chemicals had not been further purified. Sigma-Aldrich was the source of all the chemicals. In the deuterated solvent DMSO-d6, NMR spectra were obtained at the University of Basrah in Iraq using Bruker 400 MHz for 1H-NMR and 100 MHz for 13C-NMR. Internal reference tetramethylsilane (TMS) was employed.

Cell lines and culture.

MCF7, a human breast cancer cell line, was acquired from the National Cell Bank of Iran (Pasteur Institute, Iran). Cells were grown in RPMI-1640 media (Gibco) with 10% FBS (Gibco) and treated with antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cells were cultured at 37 °C in humidified air with 5% CO_2 and were subcultured using trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution.

MTT cell viability assay in MCF7 Cells

Cell proliferation and vitality were assessed with the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium Bromide] test from Sigma-Aldrich. Cells were digested with trypsin, collected, adjusted to a density of 1.4×10^4 cells per well, and seeded onto 96-well plates containing 200 µl of new media per well for 24 hours. Upon achieving a monolayer, the cells were subjected to treatment with chemicals at concentrations ranging from 400 to 25 µg/ml (dissolved in DMSO) for 24 hours at 37 °C in a 5% CO_2 atmosphere. After the treatment (24 hours), the monolayer culture remained undisturbed in the original plate, the supernatant was discarded, and 200 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was introduced, followed by incubation at 37 °C for an additional 4 hours. The MTT solution was prepared by removing the supernatant from the cells and adding 100 µl of dimethyl sulfoxide per well. Cells were incubated on a shaker at 37 °C until the crystals were fully dissolved. Cell viability was evaluated by measuring absorbance at 570 nm using an ELISA reader (Model Wave XS2, BioTek, USA). The IC_{50}, or concentration of chemicals causing 50% cell mortality, was ascertained from the corresponding dose-response curves.

Synthesis of 4-azido-N-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide.

A solution of concentrated hydrochloric acid (2.25 mL) and water (4 mL) was added to 4-amino-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide (2.53 g, 0.01 mole). A drop-wise addition of sodium nitrite solution (0.76 g, 0.011 mole) in water (2.5 mL) was carried out to the above mixture at 0 ºC. After the sodium nitrite solution was completed, it was followed by the drop-wise addition of sodium azide (0.78 g, 0.012 mole) solution in water (2.5 mL) under a continuing stirring for 30 min. The resulting solid was separated using chloroform and re-crystallized.[16]

General method for the synthesis of sulfisoxazole-1,2,3-triazole compounds.

In a round-bottom flask with a stirrer, the following substances were added: *4-azido-N-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide* (0.018 moles), beta-dicarbonyl derivatives (0.018 moles), potassium carbonate (0.057 moles), and absolute ethanol (95%, 45 ml). For 24 hours, the reaction mixture was stirred at 75 °C. TLC was used to track the reaction's development. The solvent was eliminated once the process was finished. After being quenched in the ice-water mixture, the remaining bulk was neutralized using a 10% HCl solution. Diethyl ether was used to extract the product, and anhydrous sodium sulfate was used to dry it. The crude product obtained from the solvent's evaporation was refined via column chromatography with chloroform serving as the eluent, and it was then recrystallized from absolute ethanol.

4-(4-acetyl-5-methyl-1H-1,2,3-triazol-1-yl)-N-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide. (1)

The compound was as a light-cream powder, yield: 66%; mp 162–164, ^{1}**H NMR** (DMSO-d_6) δ/ppm: 2.82 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 7.89(d, J= 8 Hz; 2H, $H_{aromatic}$), 8.9 (d, J= 8 Hz; 2H, $H_{aromatic}$), 11.46 (s, 1H, NH). **^{13}C NMR** (DMSO-d_6) δ/ppm: 10.28 ($C_{1,}$ CH_3), 12.57 (C_2, CH_3), 21.40 (C_3, CH_3), 28.20 (C_4, CH_3), 96.06 (C_5, of isoxazole ring), 126.66 C_6, 128.77 C_7, 138.54 C_8, 138.85 (C_9, of triazole ring), 141.46, 143.50 (C_{11}, of isoxazole ring), 158.14 (C_{12}, of triazole ring), 170.93 (C_{13}, of isoxazole ring), 193.81 (C_{14}, C=O). **MS (EI)** m/z= 375.4 $[M+H]^+$.

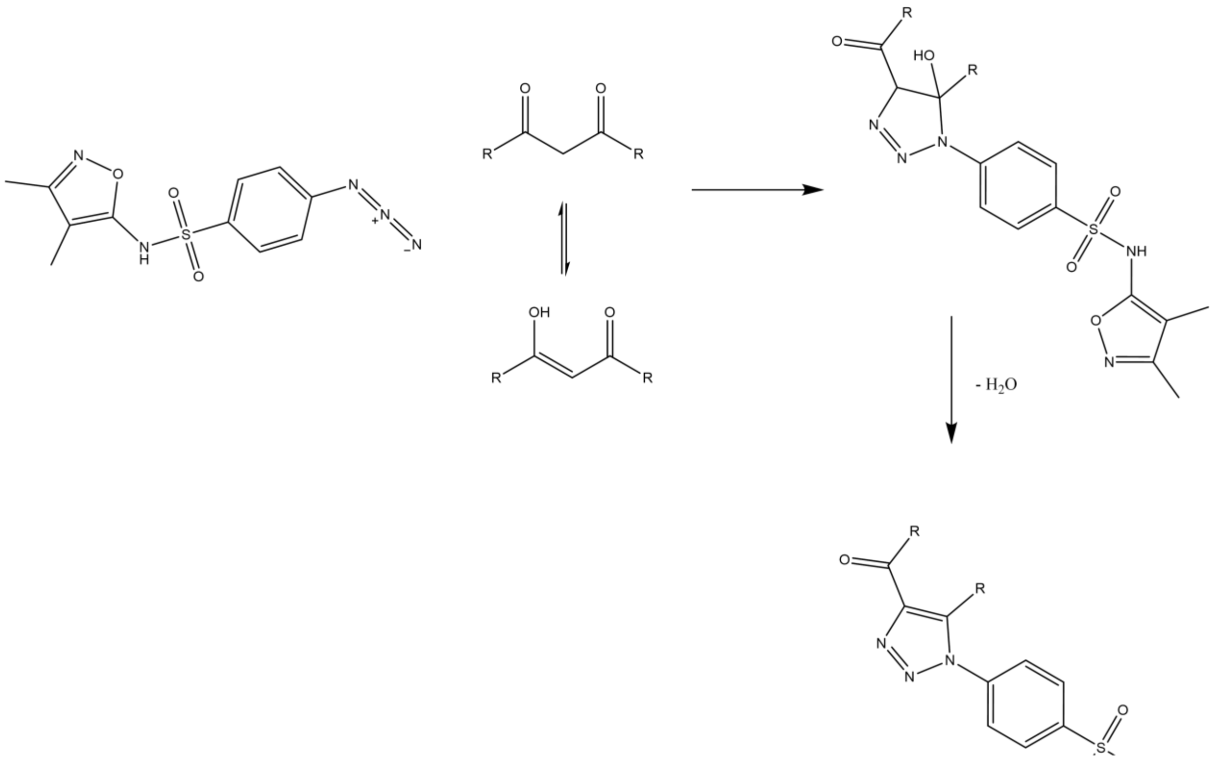
4-(4-benzoyl-5-phenyl-1H-1,2,3-triazol-1-yl)-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide (2)

The compound was isolated as a light-yellow powder, yield: 83%; mp 173–176 °C; **^{1}H NMR** (DMSO-d_6) δ/ppm 1.695 (s, 3H, CH_3), 1.88 (s, 3H, CH_3), 7.27-7.96 (m, 14H, $H_{aromatic}$), 11.50 (s, 1H, NH). **^{13}C NMR** (DMSO-d_6) δ/ppm: 12.71 (C_1, CH_3), 23.51 (C_2, CH_3), 120.39 $C_{3,3'}$, 95. 86 (C_{15}, of isoxazole ring), 129.01 $C_{4,5}$, 131.79 $C_{6,7}$. 132.56 C_8, 133.31 C_9, 135.86 C_{10}, 145.11 (C_{13}, of triazole ring), 157.57 (C_{14}, of triazole ring), 166.71 (C_{15}, of isoxazole ring), 170.84 (C_{18}, of isoxazole ring), 195.76 (C_{17}, C=O). MS **(ESI)** m/z= 499.13 $[M]^+$.

2. Results and discussion

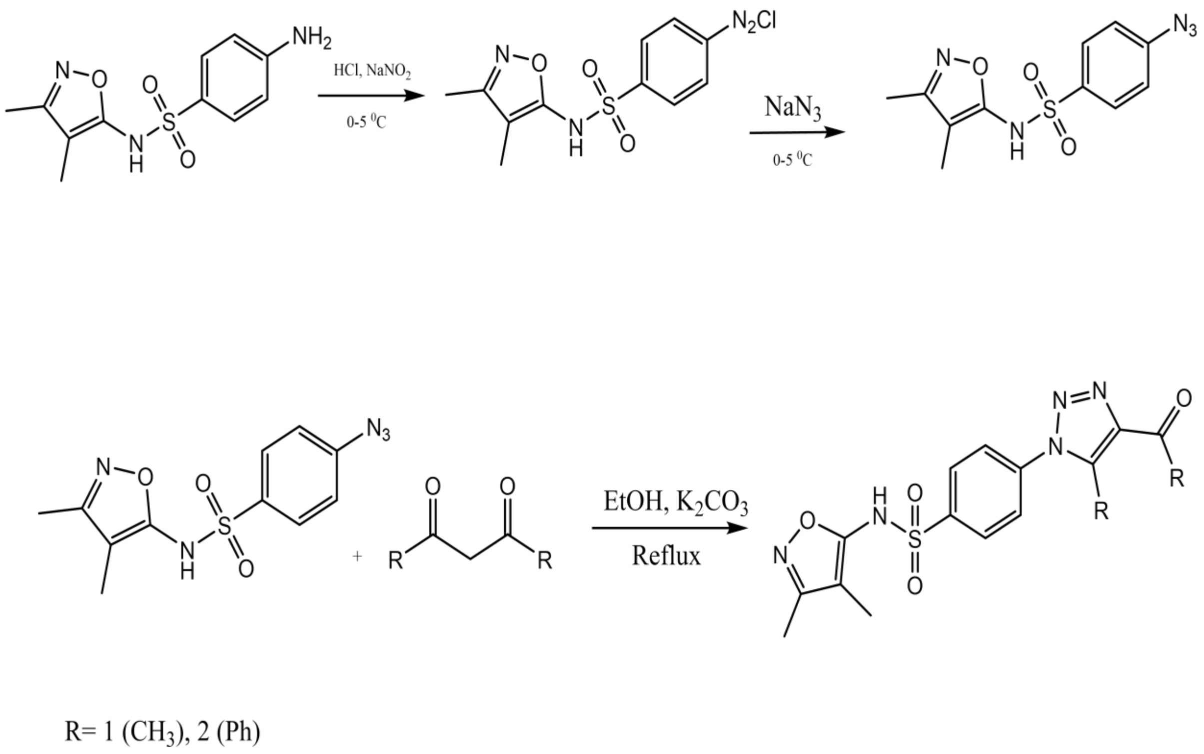
2.1. Synthesis of 1,2,3-triazole compounds (1, and 2)

The thermodynamic reaction between β-dicarbonyl compounds occurs through the symmetric equilibrium between β-dicarbonyl compounds and their enolic forms. They are distracted by the 1,3-dipolar cycloaddition reaction between the enolic form and the azide, and finally, a water molecule is expelled and the triazole is formed.



Scheme 1. Mechanism of thermal reactions of the beta-dicarbonyl compound with the 4-azido-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide.

At first step, 4-azido-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide was synthesized by reacting 4-amino-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide with sodium nitrate in the presence of hydrochloric acid to form diazonium salt, then dropping a solution of sodium azide to produce the target azide. Next step, 4-azido-N-(3,4-dimethylisoxazol-5-yl) benzenesulfonamide was reacted with acetylacetone, and dibenzoyl methane respectively, in the presence of K_2CO_3 to produce 1, and 2 as a final product. As shown in the Scheme 2.



Scheme 2. Synthesis route of sulfisoxazole-1,2,3-triazole compounds

TABLE 1. 1,3-dipolar cycloaddition for the synthesis of new sulfisoxazole-1,2,3-triazole derivatives.

Compound	Molecular formule	Reaction time (hr)	M.p (°C)	Yield (%)
1	$C_{16}H_{17}N_5O_4S$	24	162–164	66
2	$C_{26}H_{21}N_5O_4S$	24	173–176	83

The successful synthesis of novel sulfisoxazole-1,2,3-triazole derivatives 1 and 2 was demonstrated through their analysis using ^{1}H-NMR. Compound 1 exhibited three single signals in the aliphatic region at 1.24, 2.28, and 2.64 ppm with integration for 9 protons belonging to 3 groups of CH_3, as well as integration in the aromatic region of 4 protons at 7.89 and 8.09 ppm. Compound 2 displayed two single signals at 1.695 and 1.895 ppm with integration 6 attributed to 6 protons of 2 CH_3 groups, while singles at 7.27 and 7.97 ppm with integration belong to 14 aromatic protons.

^{13}C-NMR spectra were characterized by the presence of signals at 10-28 which are attributed to methyl groups, in addition to signals in the aromatic region which correspond to the carbon atoms in that region. As for the signals at 193 and 195, they are attributed to the carbonyl carbon.

Moreover, the mass spectra data of 1,2,3-triazole derivatives 1 and 2 were by the anticipated structures. As demonstrated in the experimental section.

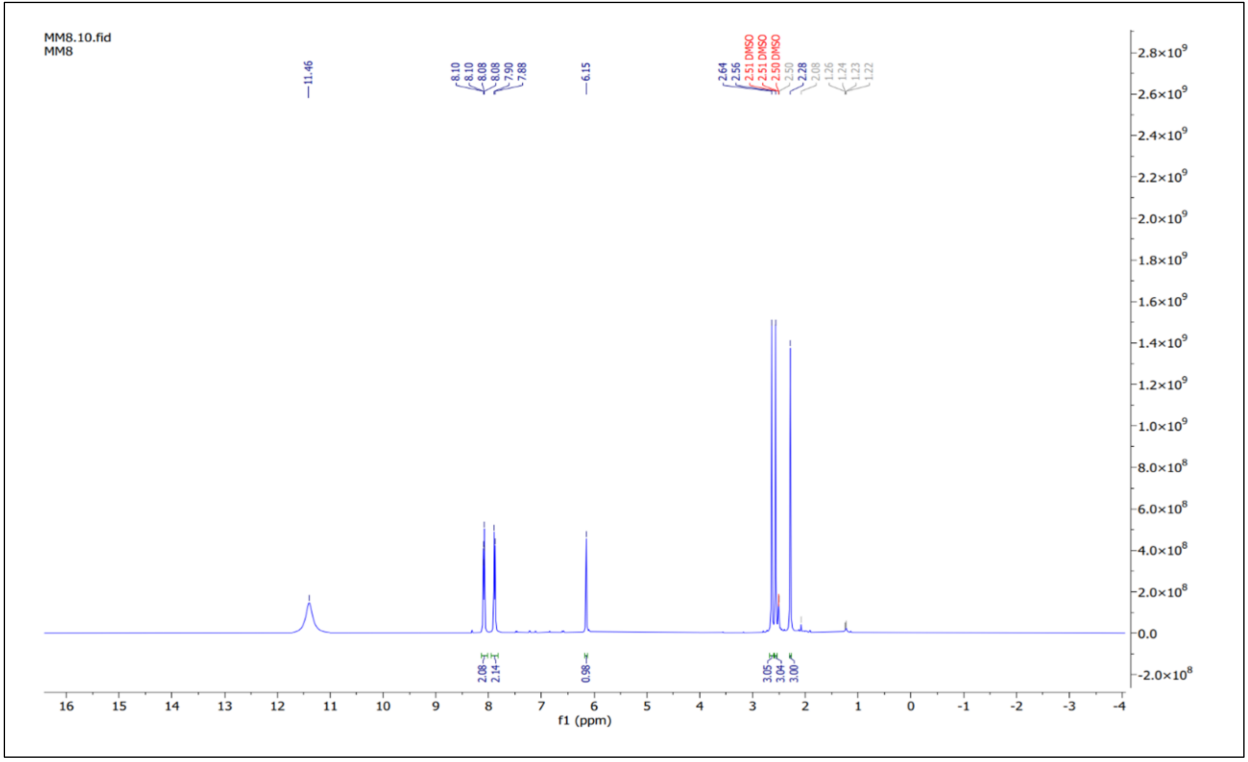


Fig. 1. HNMR spectrum of compound 1

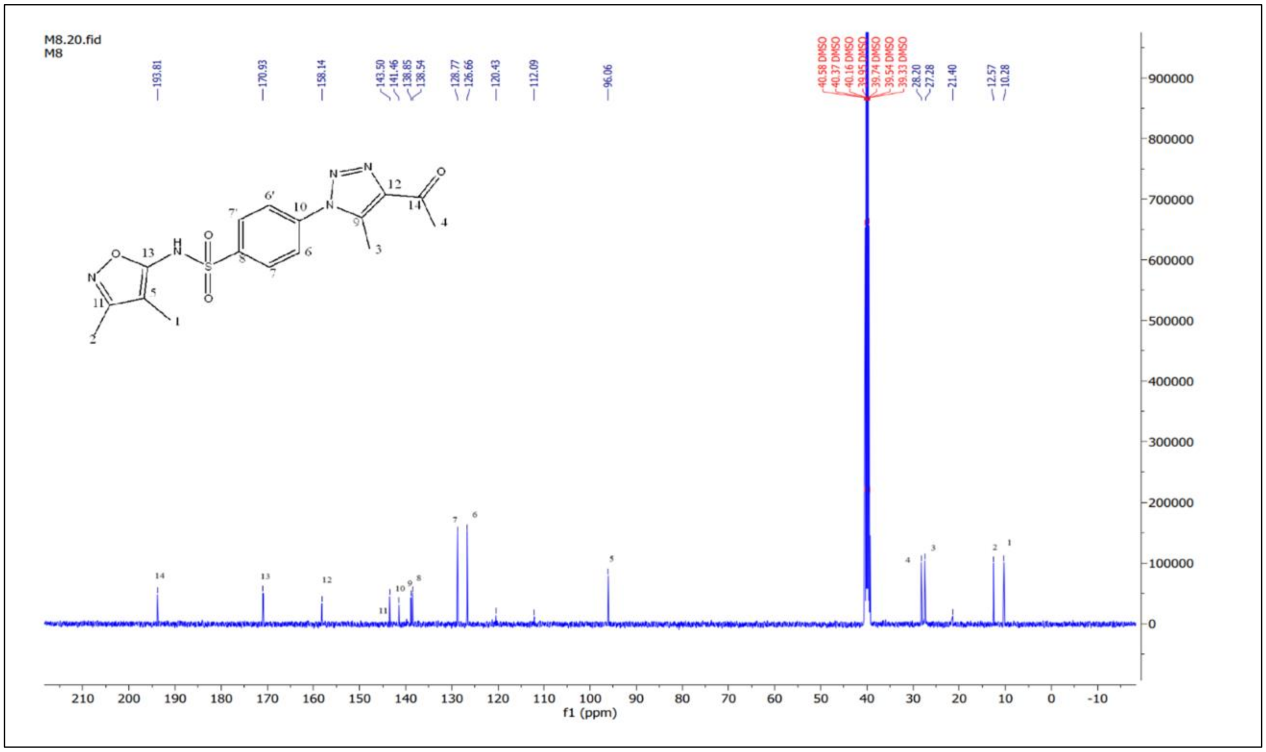


Fig. 2. 13CNMR spectrum of compound 1

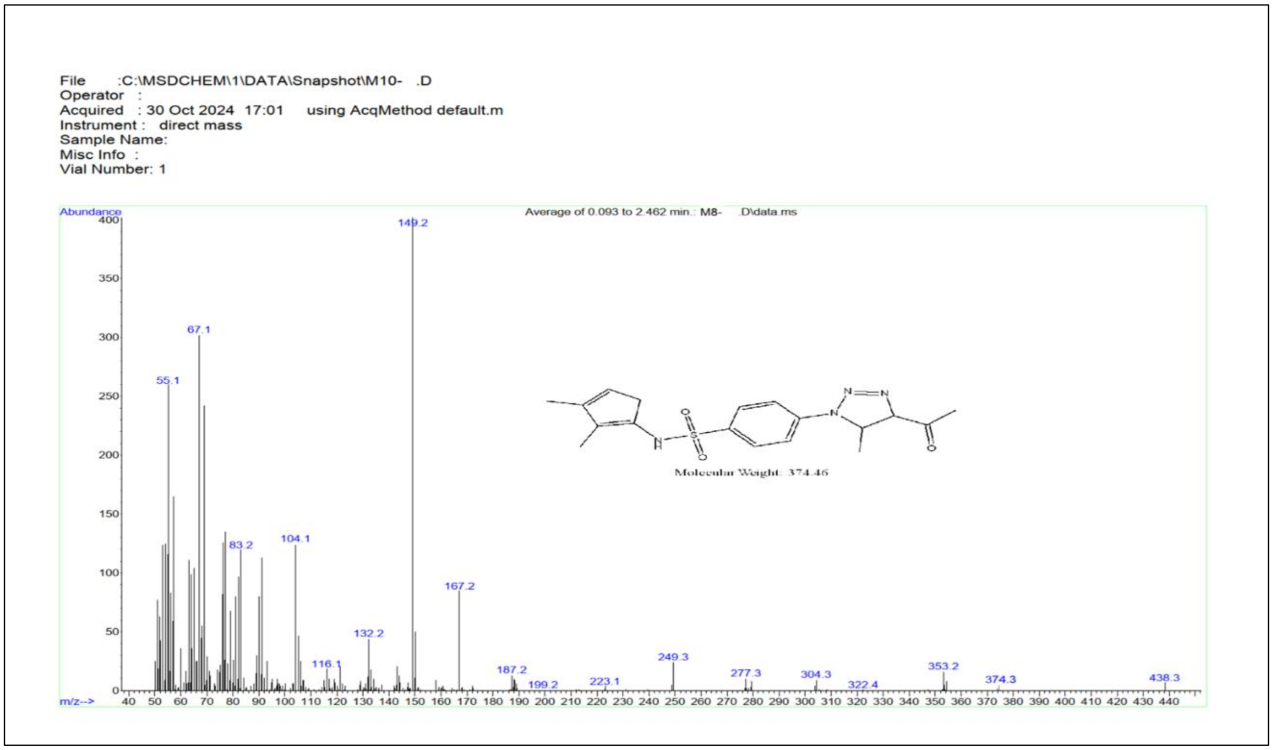

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Fig. 3. Mass spectrum of compound 1

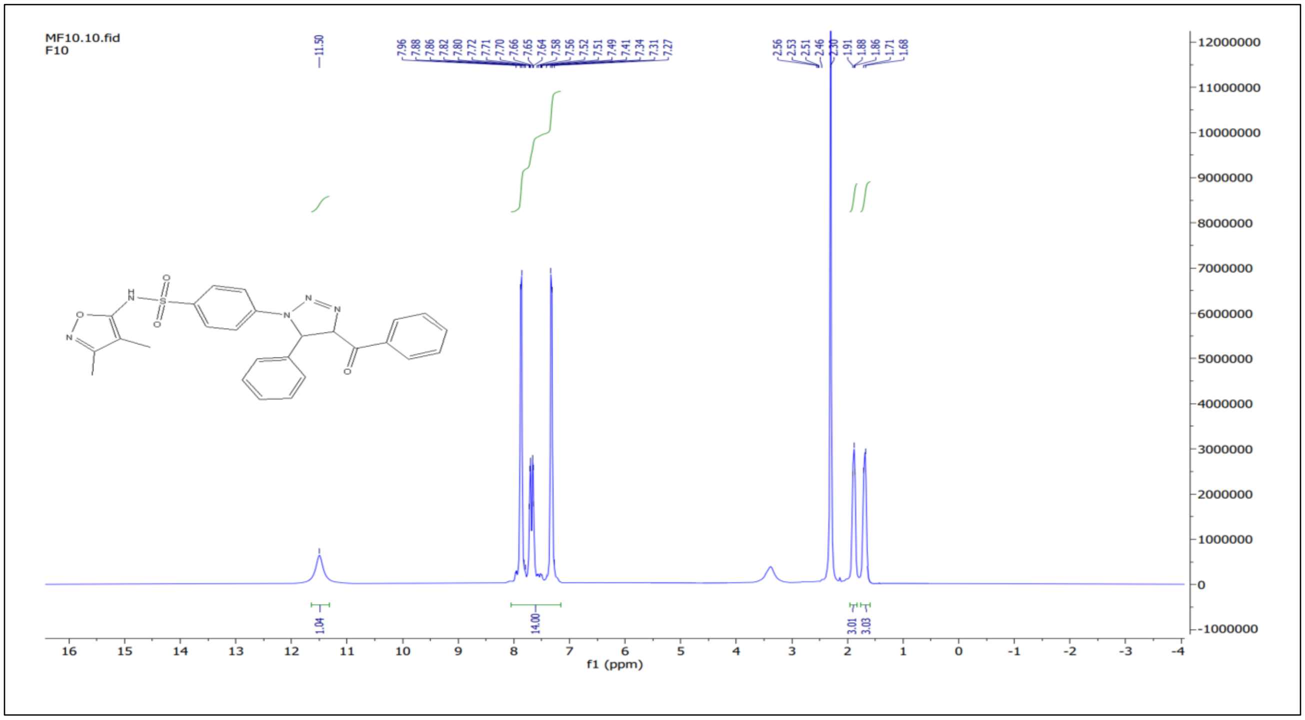

Fig. 4. HNMR spectrum of compound 2

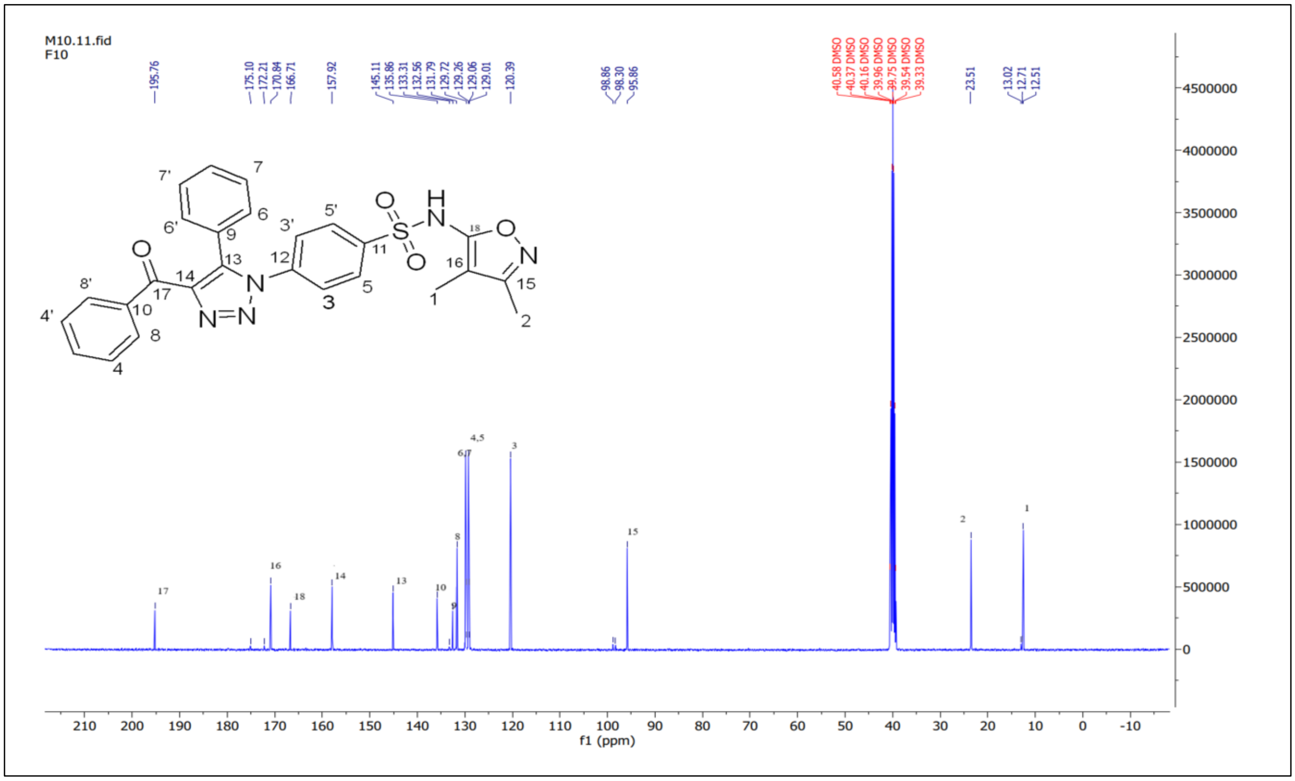

Fig. 5. 13CNMR Spectrum of compound 2

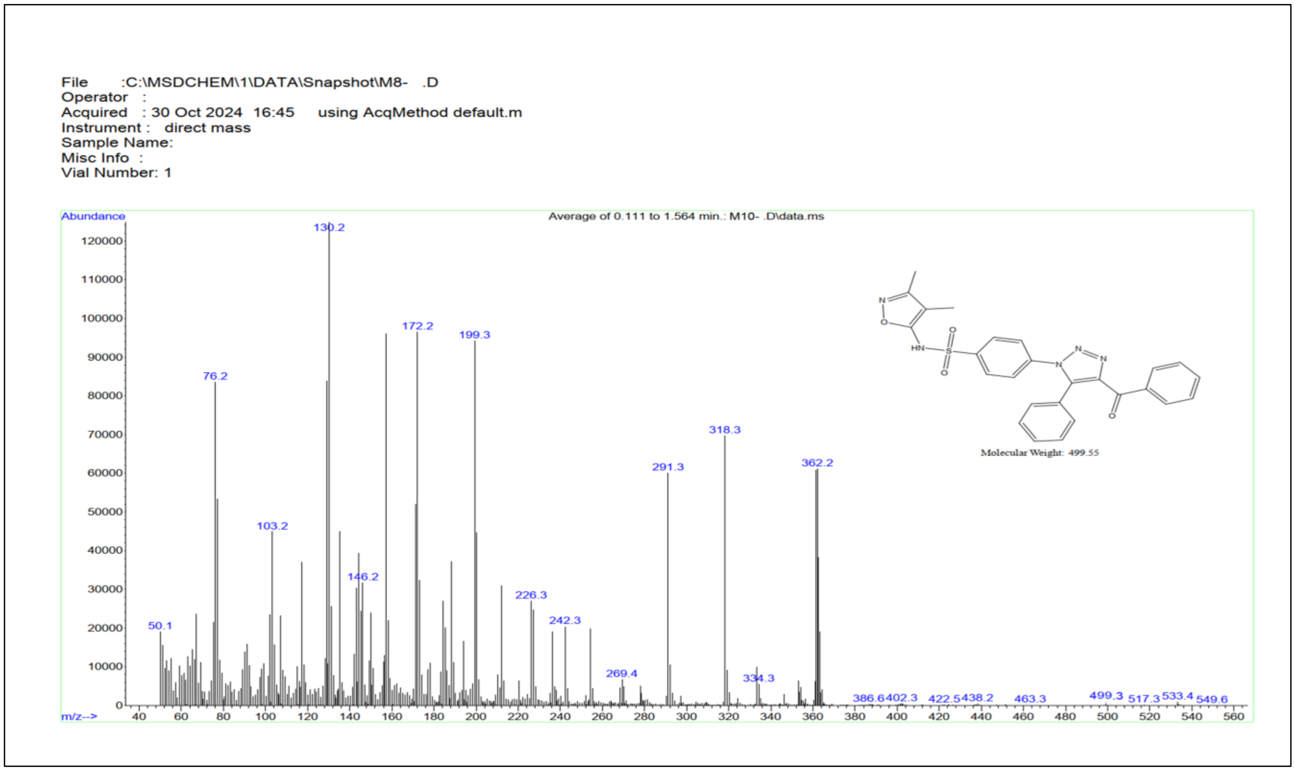

Fig. 8. Mass spectrum of compound 2

Cytotoxicity of the 1,2,3-triazole derivatives

The MTT assay was used to test sulfisoxazole-1,2,3-triazole 1 and 2 anticancer effects on breast cancer MCF-7 cell lines compared to doxorubicin (the positive control). The IC_{50} values demonstrated that compound 1 and 2 had weak activity against the cancer cell line MCF-7, with an IC_{50} of 1873.42, and 3750.02, respectively as shown in table 3. This difference in the effectiveness of the prepared compounds is attributed to the structure of the compounds and functional groups that increase or decrease the effectiveness of the compound. The low toxicity of the prepared compounds is attributed to the nature of the functional groups in these compounds. They contain methyl groups in compound 1 and phenyl groups in compound 2, which may be characterized by their weak ability to bind or bond with proteins in cells, which reduces their ability to inhibit cell growth.

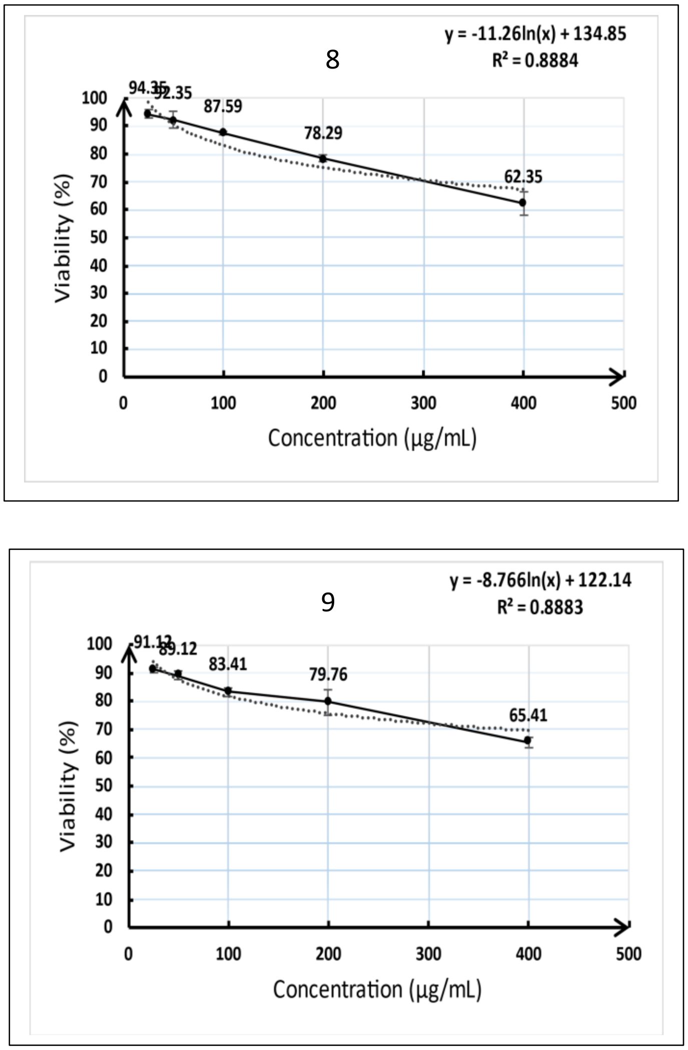

Figure 1: Graph Pad Prism 8.1 for estimation of IC_{50} value of compounds 1and 2 against breast cancer cells after 24

3. Conclusion

1,2,3-Triazoles can be prepared by the reaction of sulfonamide derivatives with beta-diketones using a basic medium of sodium carbonate via a 1,3-dipolar cycloaddition reaction. The synthesized compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectra. The compounds 1 and 2 demonstrated weak cytotoxicity when tested against human cancer cell lines. Therefore, these compounds can be developed and their effectiveness increased.

References

[1] Supuran C. T. [2017]: Special issue: Sulfonamides, Oct. 01, MDPI AG.

[2] Carta F., Supuran C. T., and Scozzafava A. [2014]: Sulfonamides and Their Isosters As Carbonic Anhydrase Inhibitors. Future Med Chem, vol. 6, no. 10, pp. 1149–1165.

[3] Supuran C. T. [2017]. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. Expert Opin Drug Discov, vol. 12, no. 1, pp. 61–88.

[4] Ahmed M., Qadir M. A., and Shafiq M. I. [2017] ‘Diazotized Curcumin Containing Sulfonamides: Synthesis, Characterization and Antibacterial Activities. Lat. Am. J. Pharm. 36 (9): 1789-95.

[5] Rudy B. C. and Senkowski B. Z. [1973] Sulfisoxazole in Analytical Profiles of Drug Substances. Ed., Academic Press. 2. pp. 487–506.

[6] Faeza A. Almashal and Mohammed Khalaf. [2014]. 1,3-Dipolar Cycloaddition for 4-Azidobenzene Sulfonamide with Acetylenic and Olefinic Compounds. Basrah Journal of Science (C). Vol.32(1). 49-69.

[7] Agouram. N. [2023]. 1,2,3-triazole derivatives as antiviral agents. Medicinal Chemistry Research. 01. 2.

[8] Zhang. B. [2019]. Comprehensive review on the anti-bacterial activity of 1,2,3-triazole hybrids. Eur J Med Chem. 168. 357–372.

[9] Krajczyk A. *et al.* [2014]. Antivirally active ribavirin analogues–4, 5-disubstituted 1, 2, 3-triazole nucleosides: biological evaluation against certain respiratory viruses and computational modelling. Antivir Chem Chemother, 23. 4. 161–171.

[10] Wu B. W. *et al.* [2024]. Design, synthesis and biological evaluation of 1,2,3-triazole benzothiazole derivatives as tubulin polymerization inhibitors with potent anti-esophageal cancer activities. Eur J Med Chem. 265. 116118.

[11] Venugopala K. N. *et al.* [2022]. 1,2,3-Triazolyl-tetrahydropyrimidine Conjugates as Potential Sterol Carrier Protein-2 Inhibitors: Larvicidal Activity against the Malaria Vector Anopheles arabiensis and In Silico Molecular Docking Study. Molecules. 27(9). 2676.

[12] Mohammed K., Almashal F. A., and Jassem A. M. [2021]. 1,3-dipolar cycloaddition: Free catalytic synthesis and esophageal cancer activity of new 1,2,3-triazole-oxydianiline-maleimide hybrids. Egypt J Chem. 64. 1. 47–53.

[13] Mohammed K., Al-Shuhaib Z., and Al-Shawi A. A. A. [2019]. Synthesis, characterization and cytotoxicity appraisal of original 1, 2, 3-Triazole derivatives, against breast cancer cell lines (MDA-MB-231). Mediterranean Journal of Chemistry. 9. 4. 305–310, 2019.

[14] Al-Hujaj H. H., Almashal F. A., Kadum A. T., Mohammed K., Hussein K. A., and Jassem A. M. [2023]. Click Chemistry-Based Synthesis of Novel 1,2,3-Triazole Derivatives and Cytotoxic Activity on Breast and Prostate Cancer Cell Lines. Tropical Journal of Natural Product Research. 7. 7. 3306–3313.

[15] Rashdan R. M., Abdelrahman M. T., De Luca A. C., and Mangini M. [2024]. Towards a New Generation of Hormone Therapies: Design, Synthesis and Biological Evaluation of Novel 1,2,3-Triazoles as Estrogen-Positive Breast Cancer Therapeutics and Non-Steroidal Aromatase Inhibitors. Pharmaceuticals. 17(1). 88.

[16] Nasir F. A. K. and Al-Hujaj H. H. K. [2023]. Synthesis, and Biological Activity Study of New 1,2,3-Triazole Compounds a Partial Fulfillment of the Requirements for The Degree of Doctor of Philosophy in Chemistry Synthesis, and Biological Activity Study of New 1,2,3-Triazole Compounds.