

Click Chemistry-Based Synthesis of Novel 1,2,3-Triazole Derivatives and Cytotoxic Activity on Breast and Prostate Cancer Cell Lines

Hamsa H. Al-hujaj¹, Faeza A. Almashal^{1*}, Aqeel T. Kadum², Mohammed K. Mohammed¹, Kawkab Ali Hussein¹, Ahmed M. Jassem¹

¹Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

²The General Company for the Petrochemical Industry, Basrah, Iraq

ARTICLE INFO

Article history:

Received 27 Mar 2023

Revised 14 June 2023

Accepted 10 July 2023

Published online 01 August 2023

Copyright: © 2023 Al-hujaj *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Breast and prostate cancers are a major cause of death each year. Most available anticancer drugs are not very effective and can cause side effects. Identifying a safe and effective alternative drug with fewer side effects for long-term anticancer therapy is therefore necessary. The present study was aimed at synthesizing 1,2,3-triazole derivatives and evaluating their activity against human breast cancer (MCF-7) and prostate cancer (PC-3) cell lines. Novel series of three 1,2,3-triazole derivatives (T₁, T₂, and T₃ compounds) were synthesized. The compounds were produced by the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition process. They were subsequently subjected to IR, H-NMR, and ESI-MS spectroscopic analyses. An *in vitro* cytotoxicity assay was conducted on each newly synthesized compound against MCF-7 and PC-3 cells. The results showed that most of the T₁, T₂, and T₃ test compounds exhibited significant cytotoxic action. The principal derivatives T₁ and T₂ are the compounds with the most promising cytotoxic activity. Furthermore, when compared to the standard 5-FU drug, the IC₅₀ values for the compounds T₁, T₂, and T₃ against the PC-3 cell line were 273.947, 406.303, and 314.368 M, respectively, while they were 91.476, 132.658, and 116.232 against breast cancer cells when compared to the standard drug adriamycin. The findings of this study demonstrated that the novel synthesized compounds could be used as potential anticancer drugs.

Keywords: 1,2,3-triazole, Anticancer, Breast cancer, Click chemistry, Prostate cancer.

Introduction

Breast and prostate cancers are prevalent malignancies that claim many lives each year. In recent years, many anticancer drugs have been developed. However, most of the anticancer drugs developed are not very effective, and side effects may occur at the same time as drug-induced impedance. Therefore, it is necessary to discover a safe and effective alternative drug with fewer side effects for long-term anticancer therapy.^{1,2} The basic building block of many medicinal drugs is 1,2,3-triazole (Figure 1), and these analogs have attracted interest in medicinal and pharmaceutical chemistry. Researchers are interested in lead compounds made of 1,2,3-triazoles with heterocycles because they have a variety of biological properties, including being antimicrobial, antibacterial, anticancer, antituberculosis, antiviral, anticonvulsant, anti-inflammatory, analgesic, and anti-HIV.³⁻¹¹

1,2,3-triazoles as heterocyclic compounds with excellent yield, and this reaction is valuable because azides and alkynes are simple to assemble into a single structure. The wide range of copper (I) and azide-alkyne catalyzed cycloaddition (CuAAC) (Scheme 1) is demonstrated by its use in various fields of material and life sciences, such as drug discovery,¹² DNA labeling,¹³ and oligonucleotide synthesis.¹⁴

*Corresponding author. E-mail: faeza.nasser@uobasrah.edu.iq
Tel: 009647717563147

Citation: Al-hujaj HH, Almashal FA, Kadum AT, Mohammed MK, Hussein KA, Jassem AM. Click Chemistry-Based Synthesis of Novel 1,2,3-Triazole Derivatives and Cytotoxic Activity on Breast and Prostate Cancer Cell Lines. Trop J Nat Prod Res. 2023; 7(7):3306-3313 <http://www.doi.org/10.26538/tjnpr/v7i7.6>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The click reaction of azido derivatives and alkynes can easily form 1,2,3-triazole scaffolds, demonstrating advancements in click chemistry. The click reaction is crucial for numerous processes, including the synthesis of 1,2,3-triazole scaffolds, chemical crosslinking, and polymer grafting, according to reviews. The aim of the present study was to synthesize 1,2,3-triazole derivatives and evaluate their cytotoxic activity against the MCF-7 breast cancer cell line and PC-3 prostate cancer cells.

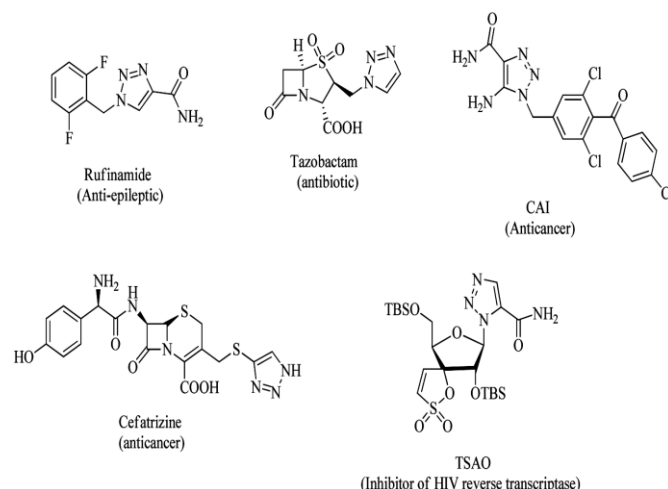
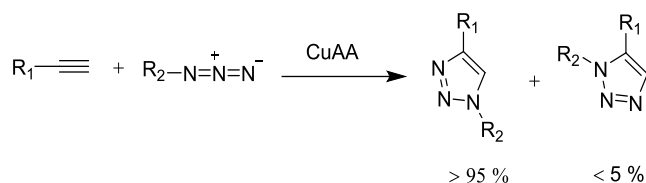


Figure 1: 1,2,3-triazole-containing drugs.



Scheme 1: Copper-catalyzed alkyne-azide cycloaddition

Materials and Methods

Sources of cell lines and culture maintenance

The National Cell Bank of Iran (Pasteur Institute, Iran) supplied the cell lines MCF-7 (a human breast cancer cell line) and PC-3 (a human prostate cancer cell line). The antibiotics (100 U/ml penicillin and 100 µg/mL streptomycin) were added to the RPMI-1640 medium (Gibco), where the cells were grown. Trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solutions were used to passage the cells, which were kept at 37°C in humidified air containing 5% CO₂. The conditions and culture medium used to grow the cells into 3D colonies were the same as for monolayer culture.

Synthesis of the aryl propargyl ether 4-(ethynyl oxy) benzaldehyde

A mixture of 0.122 g of 4-hydroxybenzaldehyde (1 mmol) and 0.276 g of potassium carbonate anhydrous (2 mmol) was placed in 15 mL of dimethylformamide (DMF) and stirred for 30 minutes.^{18,19} Then, 0.118 g propargyl bromide (1 mmol) was added to the aforementioned contents while being stirred at room temperature. Following a 24-hour period of reaction monitoring with TLC, the reaction mixture was poured into a separation funnel with 30 ml of water and extracted twice with 30 mL of diethyl ether. The resulting organic layers were then separated, dried with anhydrous magnesium sulfate (MgSO₄), filtered, and concentrated under a vacuum. A 1:2 solution of chloroform and hexane was used to recrystallize the powder that resulted from the process. A light brown powder with an 84% yield and an m.p. of 82–83 °C was obtained.

Synthesis of azides (A₁-A₃)

An aliquot of 2 mL of concentrated HCl (2 mmol) was dissolved in sulfa derivatives. The solution was cooled to between 0 and 5°C using an ice bath, and then an aqueous solution of sodium nitrite (2 mmol in 10 mL of water) was gradually added while stirring for 30 minutes. An aqueous solution of sodium azide NaN₃ (2 mmol) dissolved in 10 mL of water was added dropwise to the resulting solution at 5°C with stirring for 30 minutes. The solid product was filtered and recrystallized.²⁰⁻²²

Synthesis of 1,4-disubstituted-1,2,3-triazole (T₁, T₂, and T₃)

The mixture of propargyl ether (1 mmol), copper (I) iodide (3 mmol), three drops of trimethylamine,^{23,24} and 1 mmol of azide was dissolved in 20 mL of H₂O-EtOH (1:1), and 20 mL chloroform. The reaction mixture was heated under reflux while stirring in a 100 mL round bottom flask. TLC was used to monitor the reaction using chloroform: ethanol (8:2) as an eluent. When the suspension mixture was extracted with CH₂Cl₂ (3×20) mL, the collected organic solvent was removed, and the final product was recrystallized from a mixture of THF: hexane (8:2). A significant reduction in reaction time with comparable higher yields, high efficiency, and selectivity were observed when using H₂O-EtOH as a solvent compared to the solvent chloroform.

Spectra analyses

Infrared spectra of most compounds were recorded using FT-IR Shimadzu-model affinity (Japan) at room temperature using the KBr-disk in the range 400-4000 cm⁻¹. The analysis was conducted in the Department of Chemistry, College of Education for Pure Science, University of Basrah. The nuclear magnetic resonance spectra were obtained in the deuterated solvent DMSO-d₆ using Gemini-400 MHz and 500 MHz for ¹H-NMR and 125 MHz for ¹³CNMR. All chemical shifts were measured in relation to the internal reference, which was tetramethylsilane (TMS). The nuclear magnetic resonance spectra

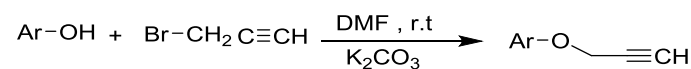
were captured in Iran and at the College of Education for Pure Science, University of Basrah. The mass spectra were recorded for compounds using the electron impact (EI) technique by an Agilent spectrometer model 5973 at 70 eV at Tehran University.

MTT cell viability assay in MCF7 and PC3 cells

The MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (Sigma-Aldrich) test was utilized to measure cell proliferation and cell survival. The MCF7 and PC3 cells were enzymatically dissociated using trypsin, collected, and then standardized to a density of 1.4×10⁴ cells/well to establish a monolayer culture. The standardized cells were then seeded into 96-well plates containing 200 µl of fresh medium per well, and incubated for 24 hours. After the cells had formed a single layer, they were exposed to compounds ranging from 500-31.25 µg/mL for 24 hours at 37°C with 5% CO₂. Upon completion of the therapy (24 hours), the liquid above was removed, and 200 µl/well of MTT solution (0.5 mg/mL in phosphate-buffered saline [PBS]) was added. The monolayer culture remained undisturbed in its original container. After that, the dish was maintained at 37°C for an additional 4 hours. The MTT solution was substituted with dimethyl sulfoxide (100 µl per well) after the removal of the cell supernatant. The cells were cultured on a shaking apparatus at 37°C until the crystals were fully dissolved. Using an ELISA reader (Model wave xs2, BioTek, USA), the cells' viability was evaluated by measuring their absorbance at 570 nm. The IC₅₀, which represents the concentration of substances that resulted in a 50% decrease in cell viability was calculated based on the dose-response curves.

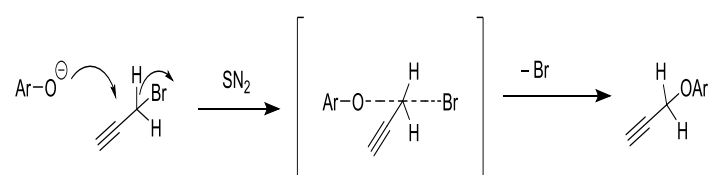
Results and Discussion

The synthesis of 1,4-disubstituted 1,2,3-triazoles involves three steps. In the first stage, aryl propargyl ether is made by reacting propargyl bromide with substituted phenol using DMF as a solvent, potassium carbonate as a catalyst, and stirring for 24 hours at room temperature.²⁵ This reaction is described in Scheme 2. The above reaction occurs based on the S_N2 mechanism (Scheme 3). The second stage involves the synthesis of azide compounds, A₁, A₂, and A₃ from the reaction of diazonium salts at a temperature range (0-5°C) with sodium azide solution.²⁶ This reaction is described in Schemes 4 and 5. As illustrated in Scheme 6, the process of replacing the azide ion -N₃ on the aromatic ring of diazonium salts in the above reaction occurs with released nitrogen gas based on S_NAr.²⁷ In the third step, 1,4-disubstituted 1,2,3-triazoles T₁, T₂, and T₃ were synthesized by reacting the first-step-obtained aryl propargyl ether with the second-stage-obtained azide derivatives A₁, A₂, and A₃, the presence of ethanol and water (1:1, v/v) as solvents, copper (I) iodide, and triethyl amine as the catalytic agents (Scheme 7 and 8).²⁸ When copper (I) iodide is used as a catalyst, the aforementioned reaction proceeds in accordance with the 1,3-dipolar cycloaddition mechanism,²⁹ as illustrated in Scheme 9. The above reaction occurs according to the mechanism of the 1,3-dipolar cycloaddition using copper (I) iodide as a catalyst,²⁹ as presented in Scheme 9.

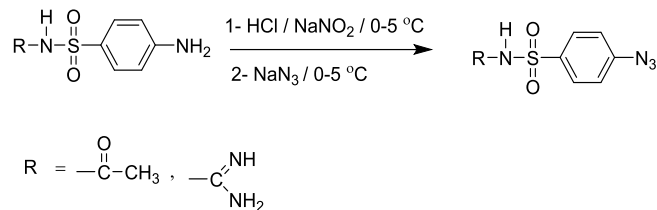


Ar-OH = p-hydroxy benzaldehyde

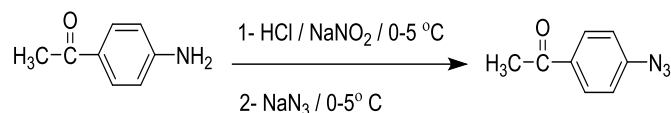
Scheme 2: Synthesis of aryl propargyl ether compounds.



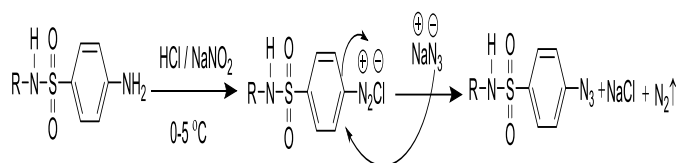
Scheme 3: SN₂ mechanism for the synthesis of aryl propargyl ether compound.



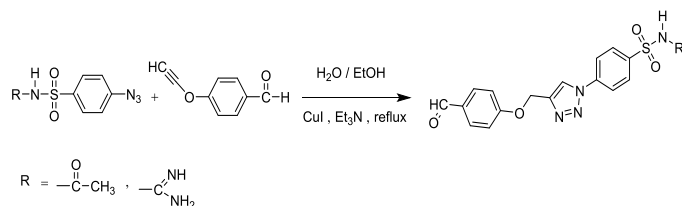
Scheme 4: Synthesis of azide derivatives.



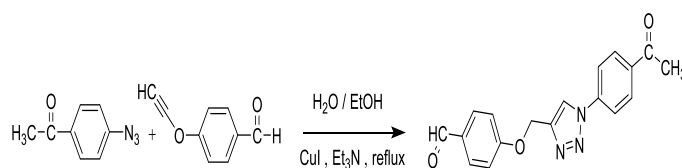
Scheme 5: Synthesis of 4-amino acetophenone azide.



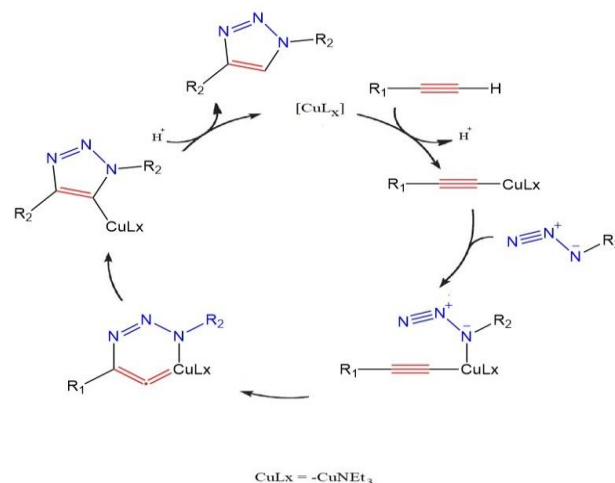
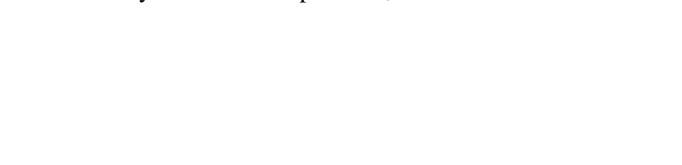
Scheme 6: S_NAr mechanism for the synthesis of azide derivatives.



Scheme 7: Synthesis of 1,4-disubstituted 1,2,3-triazole compounds (T₁ and T₂).



Scheme 8: Synthesis of compound T₃.



Scheme 9: Mechanism for the synthesis of 1,2,3-triazole compound.

Through the presence of a single singlet at δ 9-9.9 ppm for 1,2,3-triazole derivatives for protons (CH groups) at the anticipated region, their ¹H-NMR analysis clearly demonstrated the success of the synthesis of new 1,2,3-triazole derivatives T₁–T₃. The ¹H-NMR and ¹³C-NMR spectra confirmed that the absence of CH protons ($\text{C}\equiv\text{CH}$ groups) in propargyl was good evidence of the success of the 1,3-dipolar cycloaddition reaction. The remaining aromatic protons were observed where it was expected. The presence of absorption bands in the anticipated region of FTIR spectra further supported the creation of derivatives T₁–T₃. A prominent absorption band at 1315–1361 cm⁻¹, attributable to the azo group (N=N) of the 1,2,3-triazole moiety, was observed in the FTIR spectra, which clearly demonstrated the absence of an azido benzene group at 2150–2250 cm⁻¹. All 1,2,3-triazole derivatives T₁ through T₃ had mass spectra that were consistent with the predicted structures.

Characteristics of the synthesized compounds

N-((4-(4-((4-formyl phenoxy) methyl)-1H-1,2,3-triazol-1-yl) phenyl) sulfonyl) acetamide (T₁)

The compound is a light-yellow powder, yielding 60%, mp = 240 decompose, FT-IR (v/cm⁻¹): 1315, 1163 (SO₂), 1267 (C-N), 1593 (C=C), 1465 (N=N), 1510 (C=N), 1710,1658 (C=O), 2864 (C-H_{alpha}), 3080 (C-H_{arom}), 3124 (C-H_{triazole}), 3398 (N-H). ¹H-NMR (DMSO-d₆): H₆ (3H, δ =1.95 ppm), H₂ (2H, δ =5.42 ppm), H_{3,3'} (2H, δ =7.30 ppm, J =10Hz), H_{7,7'} (2H, δ =7.951 ppm, J =5Hz), H_{8,8'} (2H, δ =8.12 ppm, J =5Hz), H_{4,4'} (2H, δ =8.19 ppm, J =10Hz), H₁ (1H, δ =9.13 ppm), H₉ (1H, δ =9.91 ppm), H₅ (1H, δ =12.26 ppm). ¹³C-NMR (DMSO-d₆): C₁₄(δ =23.81 ppm), C₆(δ =61.79 ppm), C_{4,4'}(δ =115.73 ppm), C_{10,10'}(δ =120.98 ppm), C_{11,11'}(δ =130.08 ppm), C_{3,3'}(δ =132.32 ppm), C₈(δ =124.01 ppm), C₂(δ =128.00 ppm), C₉(δ =130.48 ppm), C₁₂(δ =140.20 ppm), C₇(δ =144.17 ppm), C₅(δ =163.30 ppm), C₁₃(δ =169.88 ppm), C₁(δ =191.87 ppm). ESI-MS: m/z 399.1 [M]⁺ observed for C₁₈H₁₆N₄O₅S, as shown in Figures 8-10.

N-(diaminomethylene)-4-(4-((4-formyl phenoxy) methyl)-1H-1,2,3-triazol-1-yl) benzenesul fonamide (T₂)

The compound is a light-yellow powder, yielding 70%, mp = 166-167 °C FT-IR (v, cm⁻¹): 1390, 1166 (SO₂), 1247 (C-N), 1537 (C=C), 1442 (N=N), 1508 (C=N), 1687 (C=O), 2949 (C-H_{alpha}), 3080 (C-H_{arom}), 3153 (C-H_{triazole}), 3415,3352 (NH₂). ¹H-NMR (DMSO-d₆): H₂ (2H, δ =5.41 ppm), H_{5,5'} (4H, δ =6.80 ppm), H_{3,3'} (2H, δ =7.305 ppm, J =5Hz), H_{6,6'} (2H, δ =7.92 ppm, J =10Hz), H_{7,7'} (2H, δ =7.97 ppm, J =10Hz), H_{4,4'} (2H, δ =8.07 ppm, J =5Hz), H₁ (1H, δ =9.09 ppm), H₈ (1H, δ =9.91 ppm). ¹³C-NMR (DMSO-d₆): C₆(δ =61.81 ppm), C_{4,4'}(δ =115.72 ppm), C_{10,10'}(δ =120.78 ppm), C₈(δ =123.85 ppm), C_{11,11'}(δ =127.87 ppm), C_{3,3'}(δ =132.31 ppm), C₁₃(δ =158.66 ppm), C₉(δ =138.54 ppm), C₁₂(δ =143.97 ppm), C₇(δ =144.93 ppm), C₅(δ =163.30 ppm),

$C_1(\delta=191.89 \text{ ppm})$. ESI-MS: m/z 400.4 $[M]^+$ observed for $C_{17}H_{16}N_6O_4S$, as depicted in Figures 11-13.

4-((1-(4-acetylphenyl)-1H-1,2,3-triazol-4-yl) methoxy) benzaldehyde (T₃)

The compound is a white powder, yielding 70%, mp = 185-187 °C FT-IR (ν , cm^{-1}): 1315, 1159(SO_2), 1263 (C-N), 1600 (C=C), 1462 (N=N), 1510 (C=N), 1697, 1685 (C=O), 2926 (C-H_{alpha}), 3066 (C-H_{arom}), 3109 (C-H_{triazole}). ¹H-NMR (DMSO- d_6): H₅ (3H, $\delta=2.65 \text{ ppm}$), H₂ (2H, $\delta=5.42 \text{ ppm}$), H_{3,3'} (2H, $\delta=7.30 \text{ ppm}$, $J=10\text{Hz}$), H_{6,6'} (2H, $\delta=7.915 \text{ ppm}$, $J=5\text{Hz}$), H_{4,4'} (2H, $\delta=8.115 \text{ ppm}$, $J=10\text{Hz}$), H_{7,7'} (2H, $\delta=8.19 \text{ ppm}$, $J=10\text{Hz}$), H₁ (1H, $\delta=9.16 \text{ ppm}$), H₈ (1H, $\delta=9.90 \text{ ppm}$). ¹³C-NMR(DMSO- d_6): C₁₄($\delta=27.36 \text{ ppm}$), C₆($\delta=61.78 \text{ ppm}$), C_{4,4'}($\delta=115.72 \text{ ppm}$), C_{10,10'}($\delta=120.38 \text{ ppm}$), C₈($\delta=123.81 \text{ ppm}$), C_{11,11'}, C₂($\delta=130.59$), 130.45 ppm), C_{3,3'}($\delta=132.33 \text{ ppm}$), C₁₂($\delta=136.95 \text{ ppm}$), C₉($\delta=139.94 \text{ ppm}$), C₇($\delta=144.07 \text{ ppm}$), C₅($\delta=163.28 \text{ ppm}$), C₁($\delta=191.90 \text{ ppm}$), C₁₃($\delta=197.48 \text{ ppm}$). ESI-MS: m/z 321.1 $[M]^+$ observed for $C_{18}H_{15}N_3O_3$, as presented in Figures 14-16.

In vitro anti-cancer activity of synthesized compounds

All the newly synthesized compounds were examined in PC-3 and MCF-7 cell cultures. The majority of the compounds demonstrated considerable inhibitory effects,^{30,31} exhibiting good IC₅₀ inhibition values (Table 1). Every chemical compound was evaluated for its *in vitro* cytotoxicity against two types of human cancer cells, namely PC-3 (prostate) and MCF-7 (breast). Adriamycin and 5-FU were used as benchmark substances, and the results are presented in terms of IC₅₀ values (Table 1 and Figures 2-7). Based on the IC₅₀ measurements, it is evident that a majority of the substances exhibit significant antitumor effects on prostate and breast cancer cell lines. However, the MCF-7 cell line was the one against which the chemicals were most effective. T₁ had significant cytotoxic effects on the cellular pathways of PC-3 and MCF-7. Nevertheless, T₁ displayed the highest efficacy against PC-3 and MCF-7 cell lines, with IC₅₀ measurements of 273.947 and 91.4766 μM , respectively. Compounds T₂ and T₃ were also observed to have good activity against the aforementioned cell, with IC₅₀ values of 406.303 and 314.368, respectively, against the PC-3 cell line and 132.658 and 116.232, respectively, against MCF-7 breast cancer. Generally, most of the derivatives showed lower cytotoxicity compared to the parent compound 5-FU (IC₅₀ 2.2.3e60 μM) and adriamycin (IC₅₀ 2.2.3e60 μM). The position and mode of the substituents may have contributed to the difference in IC₅₀ values. For example, the T₁ compound contains an amide group linked with sulfonyl, while T₂ and T₃ compounds contain imine and acyl groups respectively, which may reduce the activity of the prepared compounds compared to the amide group in compound T₁.

Conclusion

By using an alkyne-azide click reaction, a novel 1,2,3-triazole was successfully synthesized. Most of the compounds demonstrated considerable cytotoxicity when tested against human cancer cell lines, with compound T₁ being the most active of the series of derivatives. Specifically, compound T₁ showed strong growth suppression against PC-3 and MCF-7 with IC₅₀ values of 91.476 and 273.947 μM , respectively. In contrast, compounds T₂ and T₃ displayed less activity toward the test cancer cell lines. According to their functional groups, the two chemicals T₂ and T₃ showed less activity toward the cancer cells than the chemical T₁. Therefore, the novel synthesized compounds have the potential of being developed into anticancer drugs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

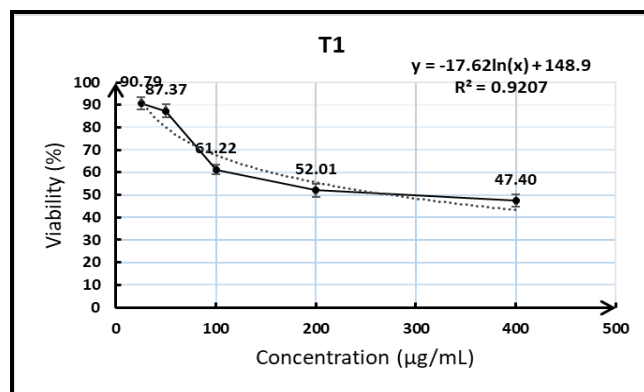


Figure 2: Cell viability assay on PC-3 cell line for compound T₁.

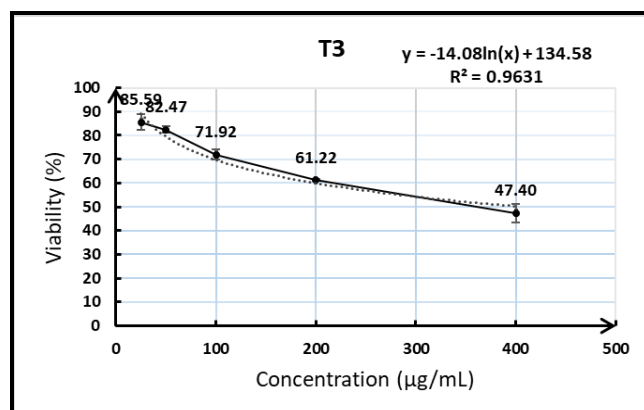


Figure 3: Cell viability assay on PC-3 cell line for compound T₂.

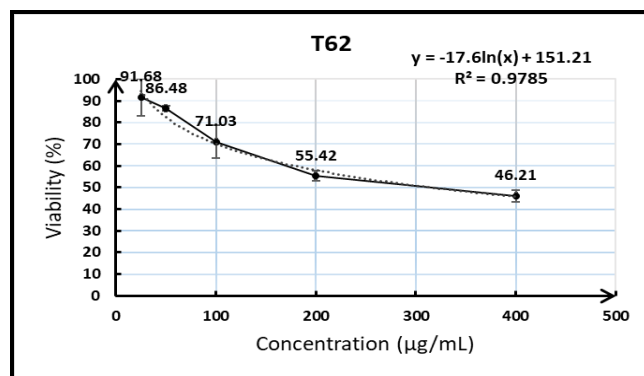


Figure 4: Cell viability assay on PC-3 cell line for compound T₃

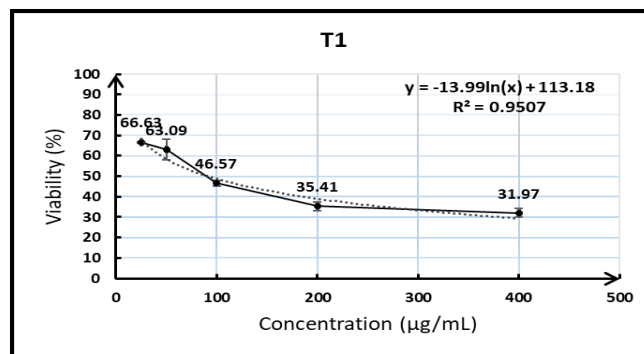


Figure 5: Cell viability assay on MCF-7 cell line for compound T₁

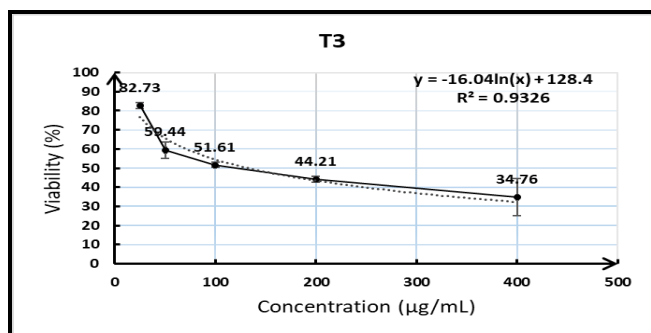


Figure 6: Cell viability assay on MCF-7 cell line for compound T₂.

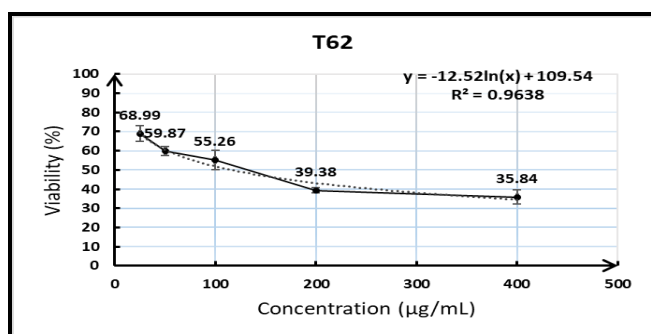


Figure 7: Cell viability assay on MCF-7 cell line for compound T₃.

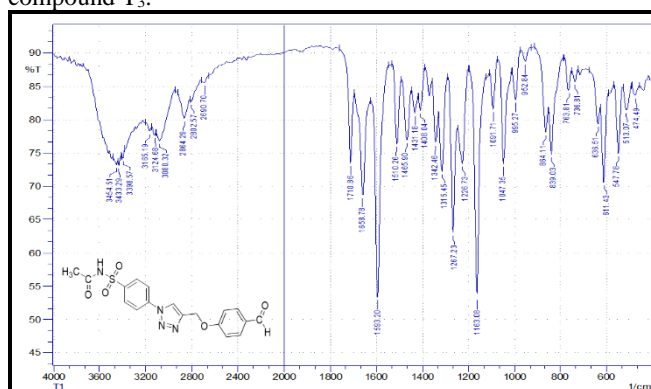


Figure 8: IR spectrum of compound T₁.

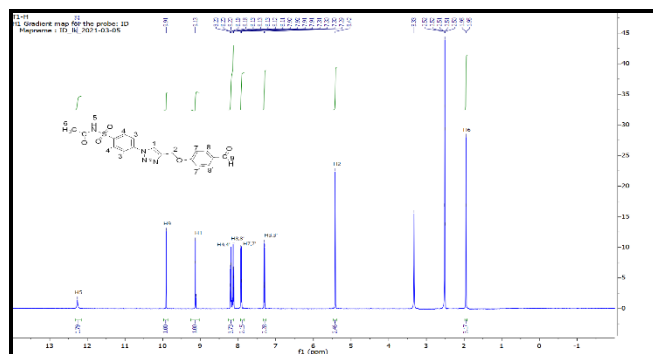


Figure 9: ¹H-NMR spectrum of compound T₁.

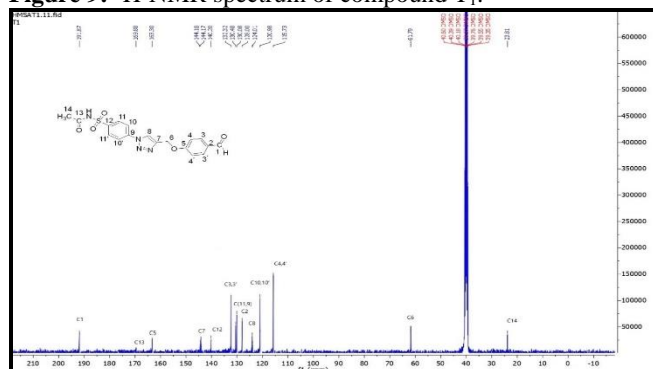


Figure 10: ¹³C-NMR spectrum of compound T₁.

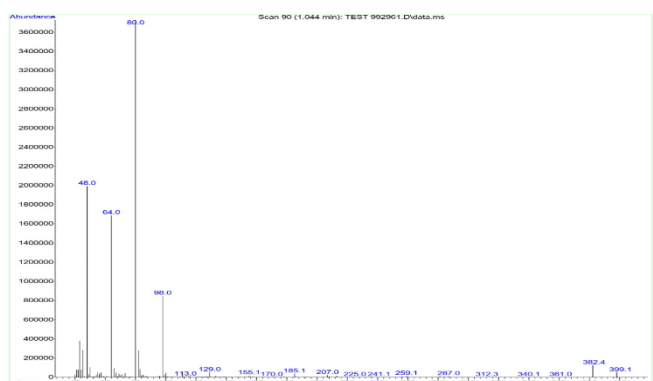
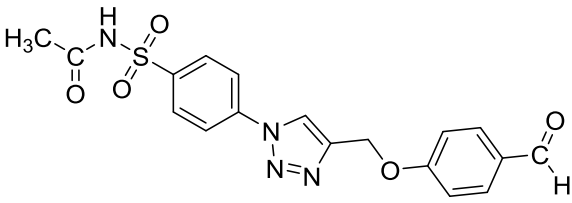
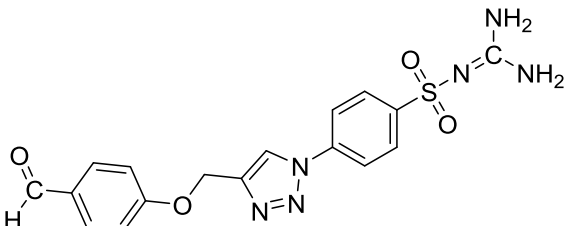
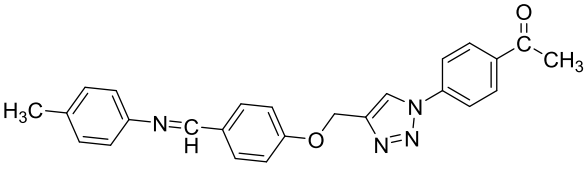
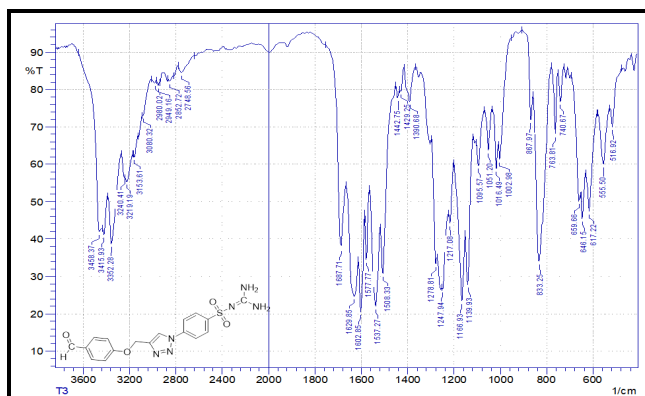
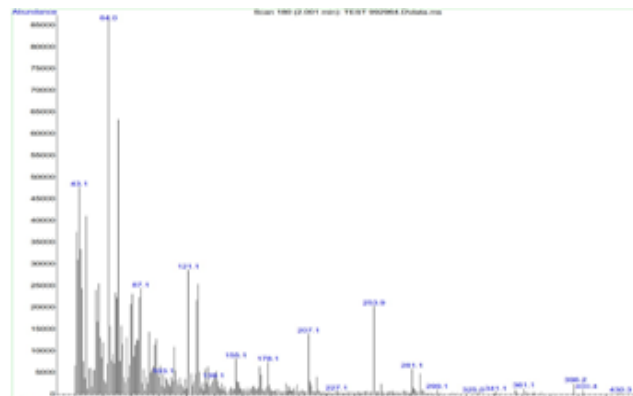
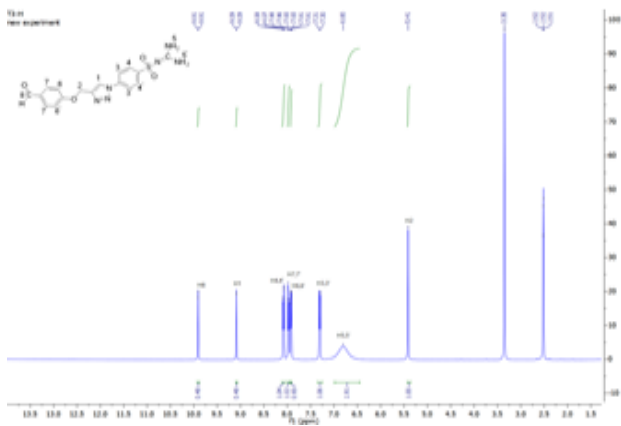
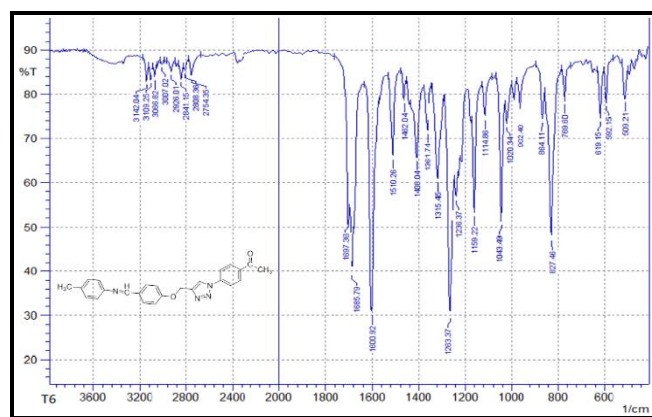
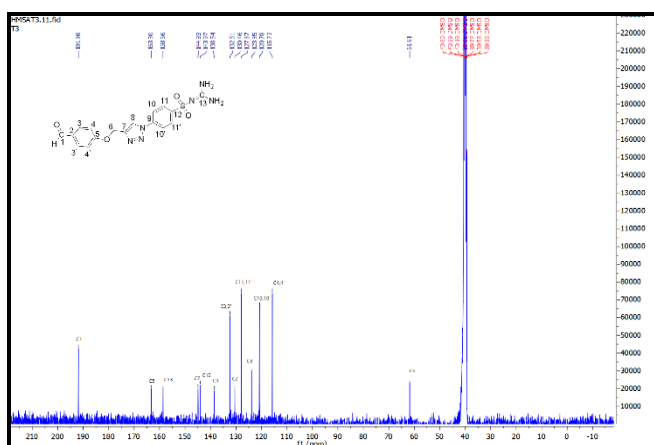
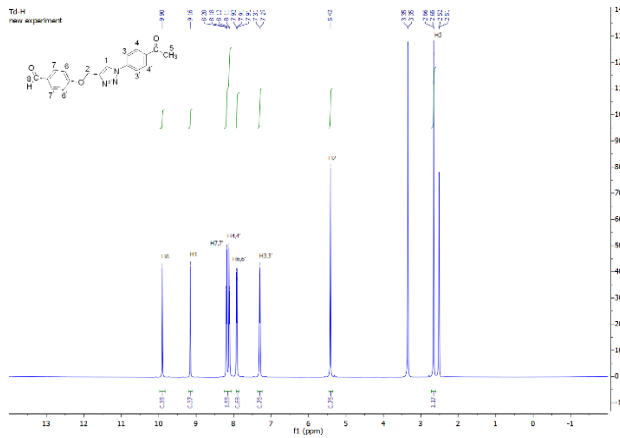


Figure 11: Mass spectrum of compound T₁.

Table 1: *In vitro* anti-proliferative activities IC₅₀ of the synthesized compounds and reference drugs against two PC-3 and MCF-7 cell lines.

T ₁		IC ₅₀ PC-3	IC ₅₀ MCF-7
		273.947	91.476
T ₂		406.303	132.658

T ₃		314.368	116.232
	Adriamycin	-	0.5
	5-FU	2.2	-

Figure 12: IR spectrum of compound T₂.Figure 15: Mass spectrum of compound T₂.Figure 13: ¹H-NMR spectrum of compound T₂.Figure 16: IR spectrum of compound T₃.Figure 14: ¹³C-NMR spectrum of compound T₂.Figure 17: ¹H-NMR spectrum of compound T₃.

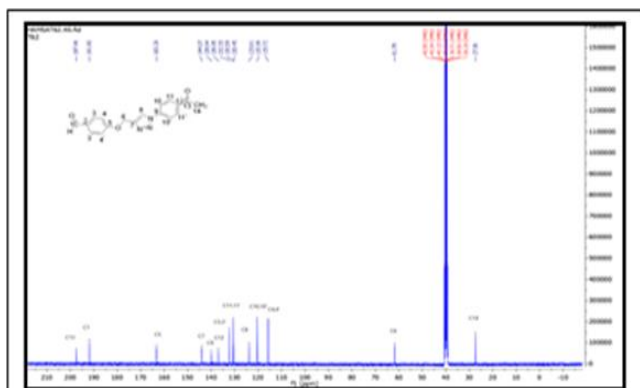


Figure 18: ^{13}C -NMR spectrum of compound T_3 .

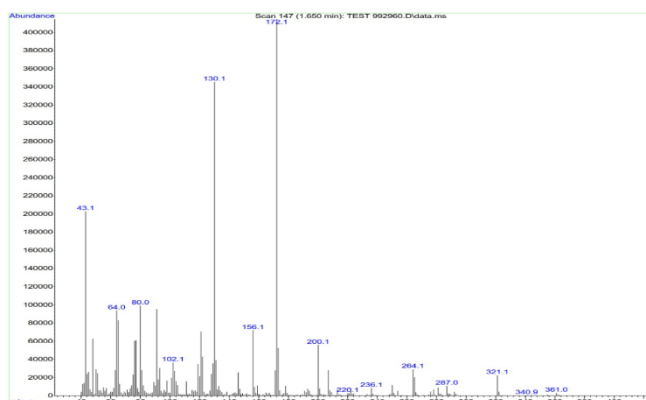


Figure 19: Mass spectrum of compound T_3 .

References

- Sangthong S, Krusong K, Ngamrojanavanich N, Vilaivan T, Puthong S, Chandchawan S, Muangsin N. Synthesis of rotenoid derivatives with cytotoxic and topoisomerase II inhibitor activities. *Bioorg. Med. Chem. Lett.* 2011; 21:4813-4818.
- Popiolek L, Biernasiuk A, Paruch K, Malm A, Wujec M. Synthesis and *in vitro* antimicrobial activity screening of new pipemidic acid derivatives. *Arch. Pharm. Res.* 2018; 41:633-645.
- Almashal FA, Khalaf M. 1,3-dipolar cycloaddition for 4-azidobenzene sulfonamide with acetylenic and olefinic compounds. *Basrah J Sci.* 2014; 32(1):49-69.
- Kapro B, Luszczki JJ, Plazi-Nnska A, Siwek A, Karcz T, Gribo A, Nowak G, Makuch-Kocka A, Walczak K, Langner E. Development of 1,2,3-triazole-based anticonvulsant drug candidates acting on the voltage-gated sodium channels. Insights from *in-vivo*, *in-vitro*, and *in-silico* studies. *Eur. J. Pharm. Sci.* 2019; 129:42-57.
- Xu Z, Zhao SJ, Liu Y. 1,2,3-triazole-containing hybrids as potential anticancer agents: Current developments, action mechanisms, and structure-activity relationships. *Eur. J. Med. Chem.* 2019; 183:111700-111713.
- Aouad MR, Mayaba MM, Naqvi A, Bardaweel SK, Alblewi FF, Messali M, Rezki N. Design, synthesis, *in silico* and *in vitro* antimicrobial screenings of novel 1,2,4-triazoles carrying 1,2,3-triazole scaffold with lipophilic side chain tether. *Chem. Cent. J.* 2017; 11:117-123.
- Kumar KA, Kalluraya B, Kumar SM. Synthesis and *in-vitro* antioxidant activities of some coumarin derivatives containing 1,2,3-triazole ring. *Phosp Sulf Relat. Elem.* 2018; 193:294-299.
- Timur I, Kocyigit ÜM, Dastan T, Sandal S, Ceribasi AO, Taslimi P, Gulcin I, Koparir M, Karatepe M, Çiftçi MJ. *In vitro* cytotoxic and *in vivo* antitumoral of some aminomethyl derivatives of 2,4-dihydro-3H-1,2,4-triazole-

- 3-thiones-Evaluation of their acetylcholinesterase and carbonic anhydrase enzymes inhibition profiles. *Biochem. Mol. Toxicol.* 2019; 33:22239-22250.
- Savanur HM, Naik KN, Ganapathi SM, Kim KM, Kalkhambkar RG. Click chemistry inspired design, synthesis, and molecular docking studies of coumarin, and quinolinone linked 1,2,3-triazoles as promising antimicrobial agents. *Chem. Select.* 2019; 3:5296-5303.
- Rajavelu K, Subaraja M, Rajakumar P. Synthesis, optical properties, and antioxidant and anticancer activity of benzoheterazole dendrimers with triazole bridging unit. *New J Chem.* 2018; 42:3282-3292.
- Santosh R, Selvam MK, Kanekar SU, Nagaraja GK. Synthesis, characterization, antibacterial and antioxidant studies of some heterocyclic from triazolelinked chalcone derivatives. *Chem. Select.* 2018; 3:6338-6343.
- Golas PL, Matyjaszewski K. Click chemistry and ATRP: A beneficial union for the preparation of functional materials. *QSAR Comb. Sci.* 2007; 26:1116-1134.
- Gierlich J, Burley GA, Gramlich PME, Hammond DM, Carell T. Click chemistry as a reliable method for the high-density postsynthetic functionalization of alkyne-modified DNA. *Org Lett.* 2006; 8: 3639-3642.
- Nuzziz A, Massi A, Dondoni A. Model studies toward the synthesis of thymidine oligonucleotides with triazole internucleosidic linkages via iterative Cu(I)-promoted azide-alkyne ligation. *QSAR Comb Sci.* 2007; 26: 1191-1199.
- El Malah T, Nour HF, Satti AAE, Hemdan BA, El-Sayed WA. Design, synthesis, and antimicrobial activities of 1,2,3-triazole glycoside clickamers. *Molec.* 2020; 25: 790.
- Kollaschinski M, Sobotta J, Schalk A, Frischmuth T, Graf B, Serdjukow S. Efficient DNA click reaction replaces enzymatic ligation. *Bioconj Chem.* 2020; 31: 507-512.
- Savaş B, Öztürk T, Meyvacı E, Hazer B. Synthesis and characterization of comb-type graft copolymers by redox polymerization and “click” chemistry method. *SN Appl Sci.* 2020; 2:18-26.
- Castillo JC, Bravo NF, Tamayo LV, Mestizo PD, Hurtado T, Macias M, Portilla J. Water-compatible synthesis of 1,2,3-triazoles under ultrasonic conditions by a Cu(I) complex-mediated click reaction. *ACS Omega.* 2020; 5:30148-30159.
- Pramod SP, Rajubai DB, Ravibhushan SK, Sambhaji TD, Prashant PD, Vagolu SK, Dharmarajan S, Vijay MK, Kishan PH. Design and synthesis of new indanol-1,2,3-triazole derivatives as potent antitubercular and antimicrobial agents. *J Pre-proofs.* 2020.
- Al-Hujaj HH, Hassan QMA, Almashal FA, Sultan HA, Dhumad AM, Jassem AM, Emshary CA. Benzenesulfonamide-thiazole system bearing an azide group: Synthesis and evaluation of its optical nonlinear responses. *Optik Int J Light Elect.* 2022; 265: 169477.
- Almashal FA, Al-hujaj HH, Ramadhan UH. Synthesis, characterization and biological study of two azide and two bis 1,2,3-triazole acyclonucleoside analogues of thimen. *Basrah J Sci.* 2015; 44(1):84-100.
- Mohammed MK, Al-Shuhaib Z, Al-Shawi AAA. Synthesis, characterization and cytotoxicity appraisal of original 1,2,3-Triazole derivatives, against breast cancer cell line (MIDA-MB-231). *Med J Chem.* 2019; 9(4):305-310.
- Ali AA, Gogoi D, Chaliha AK, Buragohain AK, Trivadi T, Saikia PJ, Gehlot PS, Kumar A, Chaturvedi V, Sarma D. Synthesis and biological evaluation of novel 1,2,3-triazole derivatives as anti-tubercular agents. *Bioorg Med Chem Lett.* 2017; 27:3698-3703.
- Almashal FAK, Al-Hujaj HH, Jassem AM, Al-Masoudi NA. A click synthesis, molecular docking, cytotoxicity on breast cancer (MDA-MB 231) and anti-HIV activities of new 1,4-disubstituted-1,2,4-triazole thymine derivatives. *Russ J Bioorg Chem.* 2020; 46: 360.

25. Ibraheem TK, Abdul Razak WAR, Shneshil MK. Synthesis of 1,2,3-triazole derivatives from azidoacetamide via cycloaddition reaction. *J Pharm Sci Res.* 2019; 11:540-544.
26. Almashal FA, Al-hujaj HH, Ramadhan UH. Synthesis, characterization and biological study of two azide and two bis 1,2,3-triazole acyclonucleoside analogues of thimen. *Basrah J Sci.* 2015; 33:84-100.
27. Tanimoto H, Kakiuchi K. Recent applications and developments of organic azides in total synthesis of natural products. *Natur Prod Comm.* 2013; 8:1021-1034.
28. Phatak PS, Bakale RD, Kulkarni RS, Dhupal ST, Dixit PP, Krishna VS, Sriram D, Khedkar VM, Haval KP. Design and synthesis of new indanol-1,2,3-triazole derivatives as potent antitubercular and antimicrobial agents. *Bioorg Med Chem Lett.* 2020; 30: 127579.
29. Worrell BT, Malik JA, Fokin VV. Direct evidence of a dinuclear copper intermediate in Cu(I)-catalyzed azide-alkyne cycloadditions. *Sci.* 2013; 340-457.
30. Ihmaid SK, Alraqa SY, Aouad MR, Aljuhani A, Elbadawy HM, Salama SA, Rezki N, Ahmad EA. Design of molecular hybrids of phthalimide-triazole agents with potent selective MCF-7 /HepG2 cytotoxicity: Synthesis, EGFR inhibitory effect, and metabolic stability. *Bioorg. Chem.* 2021; 111: 104835.
31. Madasu C, Shailaja K, Sangaraju R, Sistla R, Uppuluri VM. Synthesis and biological evaluation of some novel 1,2,3-triazole hybrids of myrrhanone B isolated from *Commiphora mukul gem resin*: Identification of potent antiproliferative leads active against prostate cancer cells (PC-3). *Eur J Med Chem.* 2019; 188: 111974.