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Original Research Article

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

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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 (T_1, T_2, and T_3 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 T_1, T_2, and T_3 test compounds exhibited significant cytotoxic action. The principal derivatives T_1 and T_2 are the compounds with the most promising cytotoxic activity. Furthermore, when compared to the standard 5-FU drug, the IC_{50} values for the compounds T_1, T_2, and T_3 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]

,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]

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The click reaction of azido derivatives and alkynes can easily form 1 Recently, various synthetic methods have been reported for the synthesis of 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.

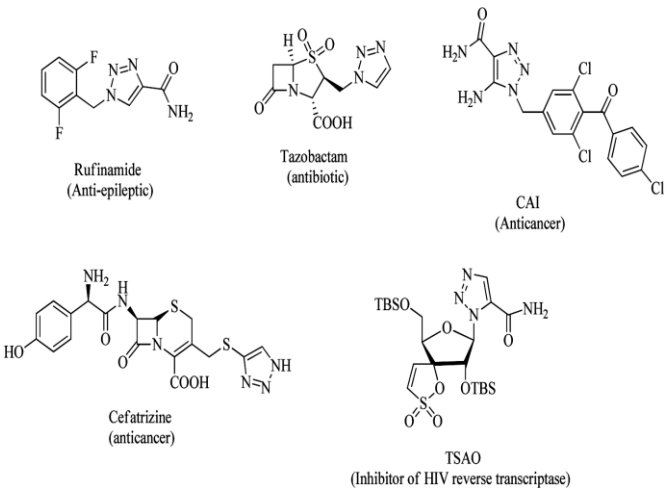
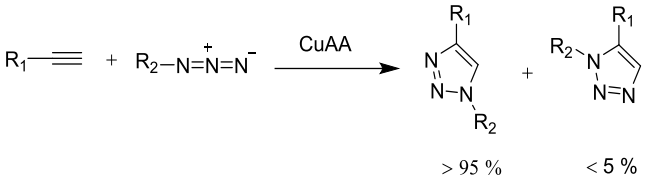


Figure 1: 1,2,3-triazole-containing drugs.



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% CO_2. 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 ($MgSO_4$), 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 (A_1- A_3)

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 NaN_3 (2 mmol) dissolved in 10 mL of water was added dropwise to the resulting solution at 5^0C with stirring for 30 minutes. The solid product was filtered and recrystallized.[20-22]

Synthesis of 1,4-disubstituted-1,2,3-triazole (T_1, T_2, and T_3)

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 H_2O-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 CH_2Cl_2 (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 H_2O-EtOH as a solvent compared to the solvent chloroform.

Spectra analyses

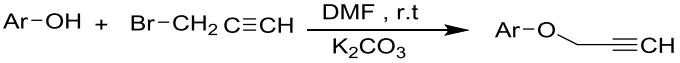
Infrared spectra of most compounds were recorded using FT-IR Shimadzu-model affinity (Japan) at room temperature using the KBr-disk in the range 400-4000 cm^{-1}. The analysis was conducted in the Department of Chemistry, College of Education for Pure Science, University of Basrah. The nuclear magnetic resonance spectra were obtained in the deuterated solvent DMSO-d6 using Gemini-400 MHz and 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}CNMR. 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 1.4×10^4 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% CO_2. 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 IC_{50}, which represents the concentration of substances that resulted in a 50% decrease in cell viability was calculated based on the dose-response curves.

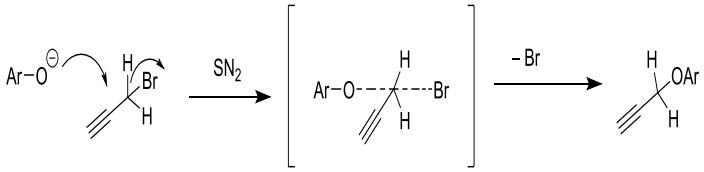
Results and Discussion

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 SN_2 mechanism (Scheme 3). The second stage involves the synthesis of azide compounds, A_1, A_2, and A_3 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 $–N_3$ 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 T_1, T_2, and T_3 were synthesized by reacting the first-step-obtained aryl propargyl ether with the second-stage-obtained azide derivatives A_1, A_2, and A_3, 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.

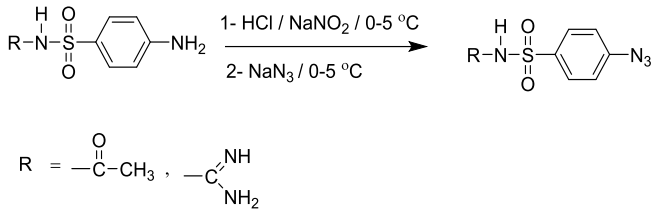


Ar–OH = p-hydroxy benzaldeyde

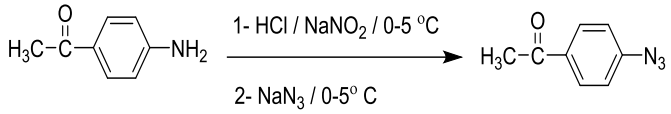
Scheme 2: Synthesis of aryl propargyl ether compounds.



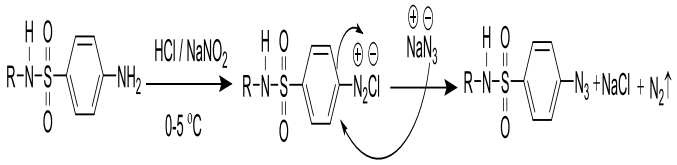
Scheme 3: SN_2 mechanism for the synthesis of aryl propargyl ether compound.



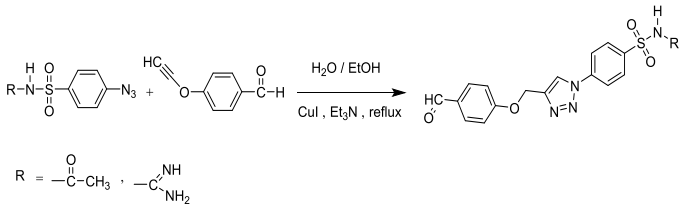
Scheme 4: Synthesis of azide derivatives.



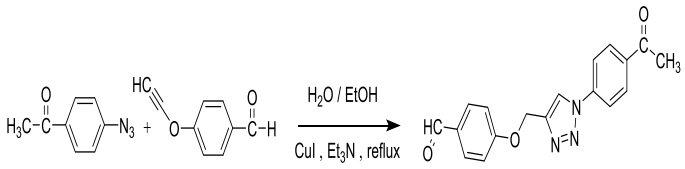
Scheme 5: Synthesis of 4-amino acetophenone azide.



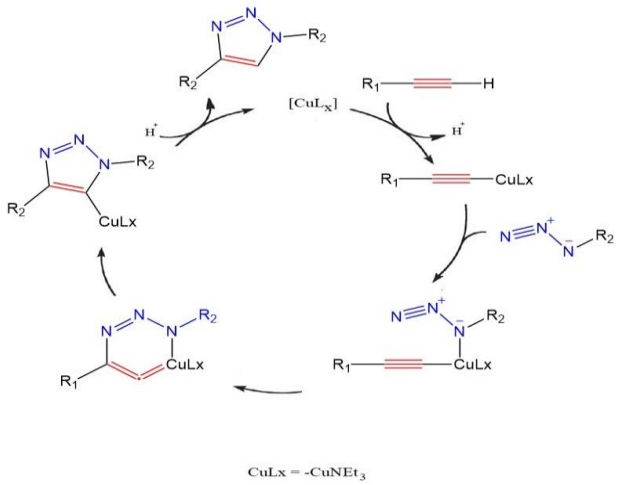
Scheme 6: S_NAr mechanism for the synthesis of azide derivatives.



Scheme 7: Synthesis of 1,4-disubstituted 1,2,3-triazole compounds (T_1 and T_2).



Scheme 8: Synthesis of compound T_3.



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

Through the presence of a single singlate at δ 9-9.9 ppm for 1,2,3–triazole derivatives for protons (CH groups) at the anticipated region, their ^{1}HNMR analysis clearly demonstrated the success of the synthesis of new 1,2,3–triazole derivatives T_1–T_3. The ^{1}HNMR and ^{13}CNMR spectra confirmed that the absence of CH protons (—C≡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 T_1–T_3. 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 T_1 through T_3 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 (T_1)

The compound is a light-yellow powder, yielding 60%, mp = 240 decompose, FT-IR (ν/cm^{-1}): 1315, 1163 (SO_2), 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). ^{1}H-NMR (DMSO-d_6): H_6 (3H, δ=1.95 ppm), H_2 (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), H_1 (1H, δ=9.13 ppm), H_9 (1H, δ=9.91 ppm), H_5 (1H, δ=12.26 ppm).^{13}CNMR (DMSO-d_6): C_{14}(δ=23.81 ppm), C_6(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), C_8(δ=124.01 ppm), C_2(δ=128.00 ppm), C_9(δ=130.48 ppm), C_{12}(δ=140.20 ppm), C_7(δ=144.17 ppm), C_5(δ=163.30 ppm), C_{13}(δ=169.88 ppm), C_1(δ=191.87 ppm). ESI-MS: *m/z* 399.1 $[M]^+$ observed for $C_{18}H_{16}N_4O_5S$, as shown in Figures 8-10.

N-(diaminomethylene)-4-(4-((4-formyl phenoxy) methyl)-1H-1,2,3-triazol-1-yl) benzenesul fonamide (T_2)

The compound is a light-yellow powder, yielding 70%, mp = 166-167 °C FT-IR (ν, cm^{-1}): 1390, 1166 (SO_2), 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 (NH_2). ^{1}H-NMR (DMSO-d_6): H_2 (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), H_1 (1H, δ=9.09 ppm), H_8 (1H, δ=9.91 ppm). ^{13}CNMR (DMSO-d_6): C_6(δ=61.81 ppm), $C_{4,4'}$(δ=115.72 ppm), $C_{10,10'}$(δ=120.78 ppm), C_8(δ=123.85 ppm), $C_{11,11'}$(δ=127.87 ppm), $C_{3,3'}$(δ=132.31 ppm), C_{13}(δ=158.66 ppm), C_9(δ=138.54 ppm), C_{12}(δ=143.97 ppm), C_7(δ=144.93 ppm), C_5(δ=163.30 ppm),

C_1(δ=191.89 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 (T_3)

The compound is a white powder, yielding 70%, mp = 185-187 °C FT-IR (*v*, 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-$H_{triazole}$). ^{1}H-NMR (DMSO-d_6): H_5 (3H, δ=2.65 ppm), H_2 (2H, δ=5.42 ppm), $H_{3,3'}$ (2H, δ=7.30 ppm, *J*=10Hz), $H_{6,6'}$ (2H, δ=7.915 ppm, *J*=5Hz), $H_{4,4'}$ 2H, δ=8.115 ppm, *J*=10Hz), $H_{7,7'}$ (2H, δ=8.19 ppm, *J*=10Hz), H_1 (1H, δ=9.16 ppm), H_8 (1H, δ =9.90 ppm). ^{13}CNMR(DMSO-d_6):C_{14}(δ=27.36 ppm), C_6(δ=61.78 ppm), $C_{4,4'}$(δ=115.72 ppm), $C_{10,10'}$(δ=120.38 ppm), C_8(δ=123.81 ppm), $C_{11,11'}$, C_2(δ=130.59), 130.45 ppm), $C_{3,3'}$(δ=132.33 ppm), C_{12}(δ=136.95 ppm), C_9(δ=139.94 ppm), C_7(δ=144.07 ppm), C_5(δ=163.28 ppm), C_1(δ=191.90 ppm), C_{13}(δ=197.48p pm). 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 IC_{50} 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 IC_{50} values (Table 1 and Figures 2-7). Based on the IC_{50} 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. T_1 had significant cytotoxic effects on the cellular pathways of PC-3 and MCF-7. Nevertheless, T_1 displayed the highest efficacy against PC-3 and MCF-7 cell lines, with IC_{50} measurements of 273.947 and 91.4766 µM, respectively. Compounds T_2 and T_3 were also observed to have good activity against the aforementioned cell, with IC_{50} values of 406.303 and 314.368, respectively, against the PC-3 cell line and 132.658 and 116.232, respectively, against MFC-7 breast cancer. Generally, most of the derivatives showed lower cytotoxicity compared to the parent compound 5-FU (IC_{50} 2.2.3e60 µM) and adriamycin (IC_{50} 2.2.3e60 µM). The position and mode of the substituents may have contributed to the difference in IC_{50} values. For example, the T_1 compound contains an amide group linked with sulfonyl, while T_2 and T_3 compounds contain imine and acyl groups respectively, which may reduce the activity of the prepared compounds compared to the amide group in compound T_1.

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 T_1 being the most active of the series of derivatives. Specifically, compound T_1 showed strong growth suppression against PC-3 and MCF-7 with IC_{50} values of 91.476 and 273.947 µM, respectively. In contrast, compounds T_2 and T_3 displayed less activity toward the test cancer cell lines. According to their functional groups, the two chemicals T_2 and T_3 showed less activity toward the cancer cells than the chemical T_1. 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.

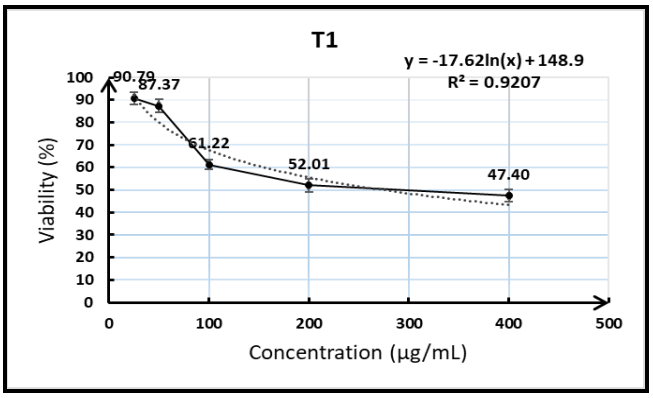


Figure 2: Cell viability assay on PC-3 cell line for compound T_1.

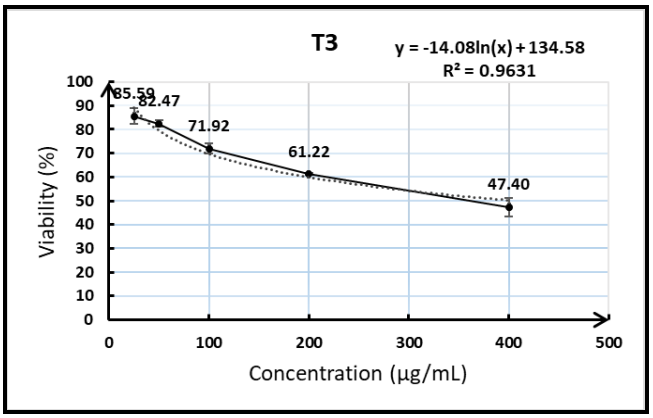


Figure 3: Cell viability assay on PC-3 cell line for compound T_2.

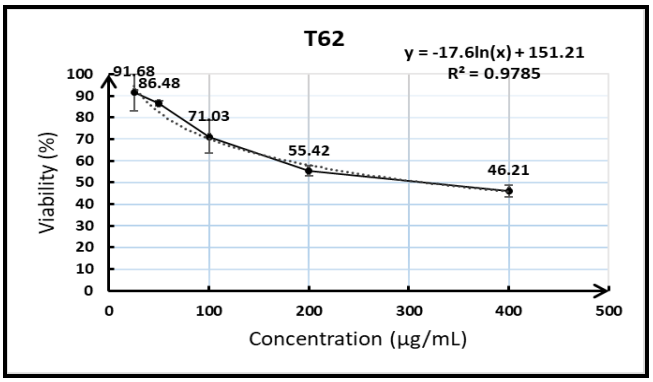


Figure 4: Cell viability assay on PC-3 cell line for compound T_3

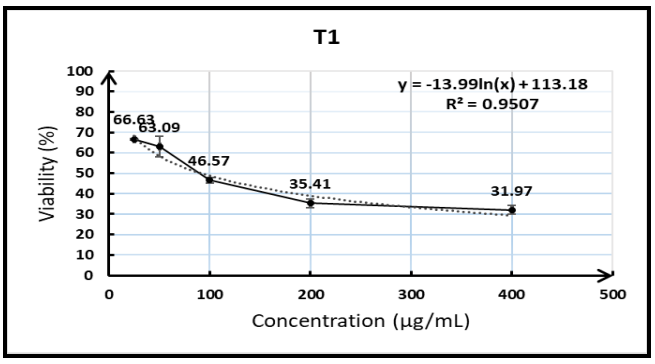


Figure 5: Cell viability assay on MCF-7 cell line for compound T_1

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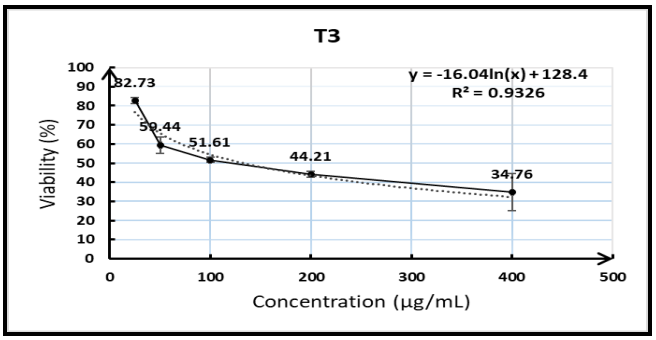


Figure 6: Cell viability assay on MCF-7 cell line for compound T_2.

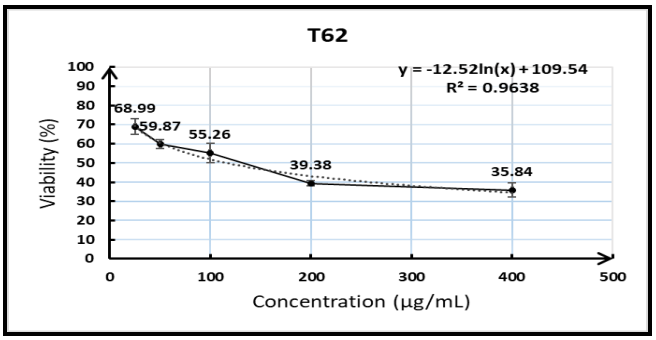


Figure 7: Cell viability assay on MCF-7 cell line for compound T_3.

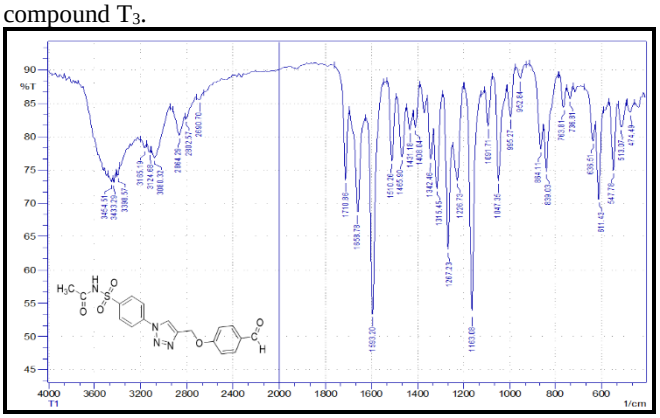


Figure 8: IR spectrum of compound T_1.

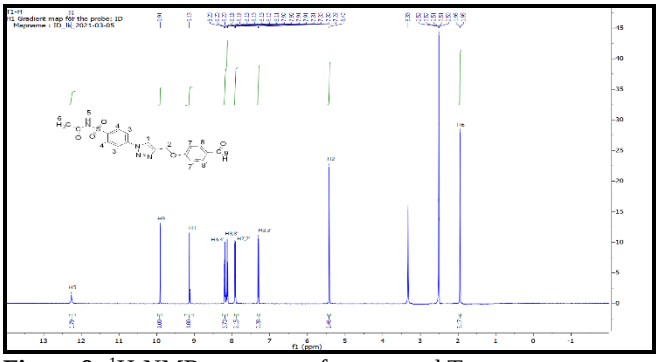


Figure 9: ^{1}H-NMR spectrum of compound T_1.

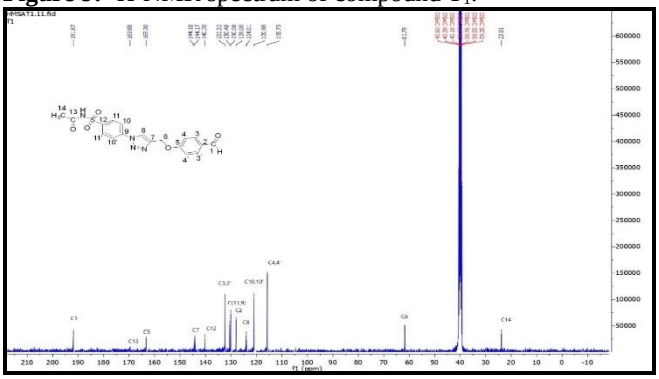


Figure 10: ^{13}C-NMR spectrum of compound T_1

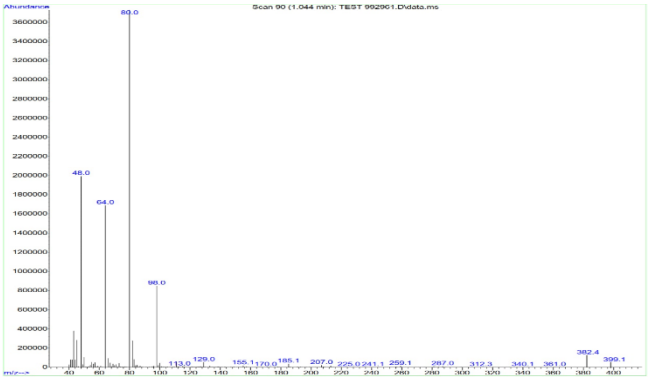
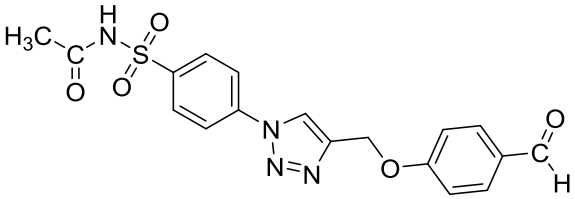
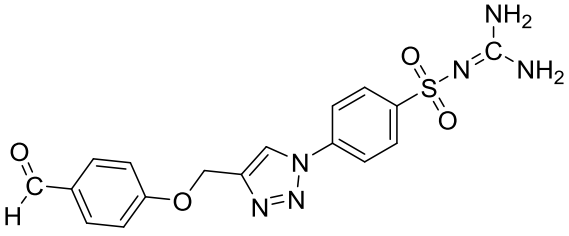


Figure 11: Mass spectrum of compound T_1.

Table 1: *In vitro* anti-proliferative activities IC_{50} of the synthesized compounds and reference drugs against two PC-3 and MCF-7 cell lines.

		IC_{50} PC-3	**IC_{50} MCF-7**
T_1		273.947	91.476
T_2		406.303	132.658

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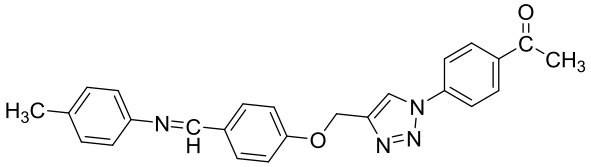
T_3		314.368	116.232
	Adriamycin	-	0.5
	5-FU	2.2	-

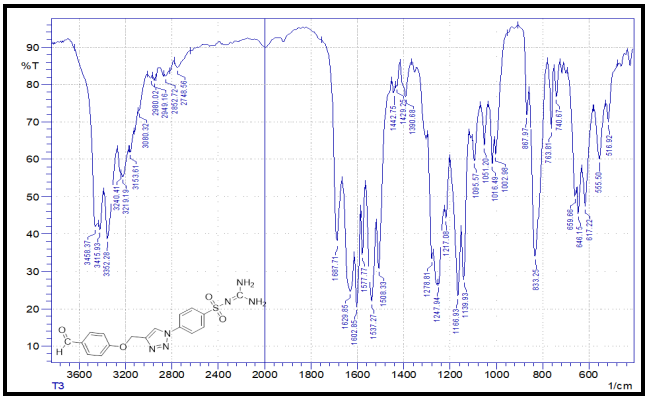


Figure 12: IR spectrum of compound T_2.

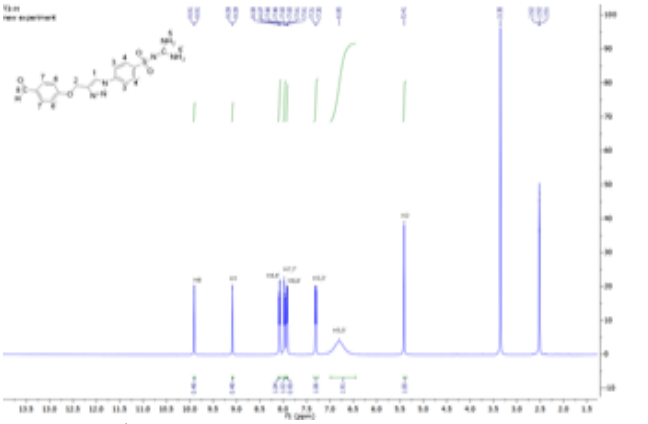


Figure 13: ^{1}H-NMR spectrum of compound T_2.

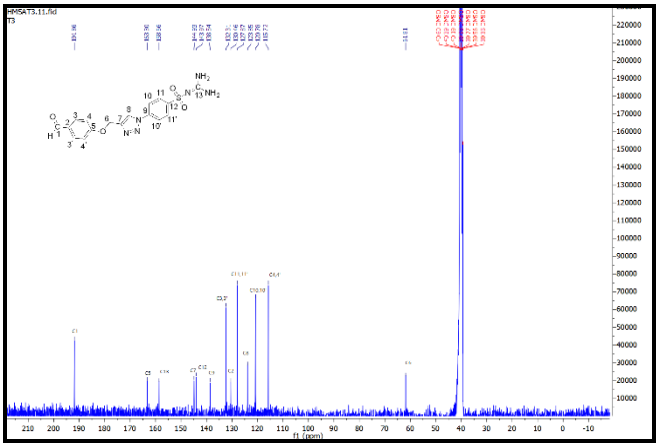


Figure 14: ^{13}C-NMR spectrum of compound T_2.

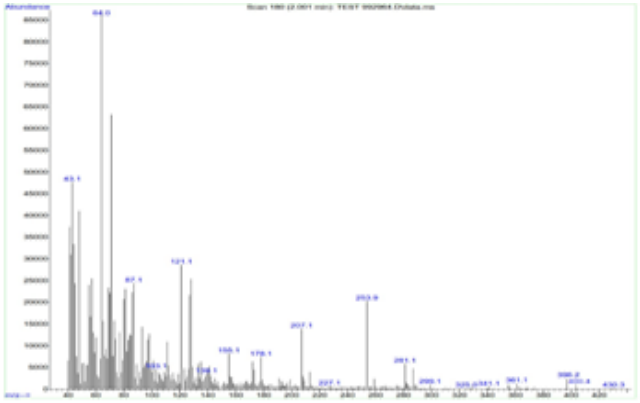


Figure 15: Mass spectrum of compound T_2

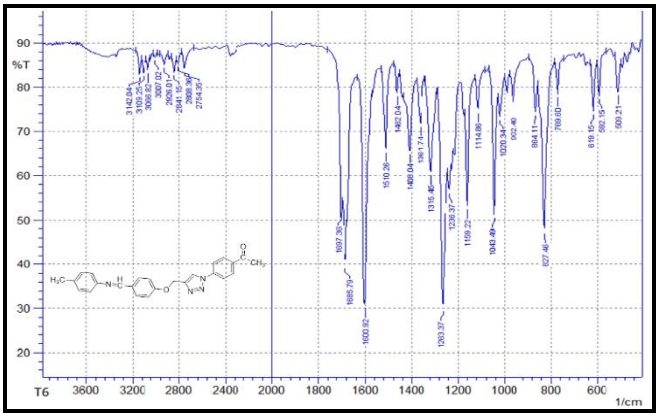


Figure 16: IR spectrum of compound T_3.

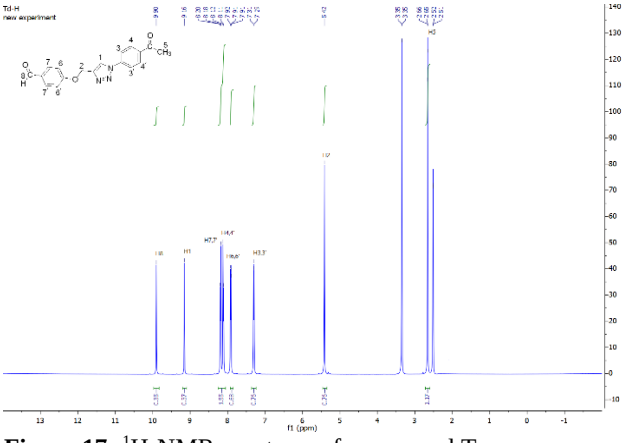


Figure 17: ^{1}H-NMR spectrum of compound T_3.

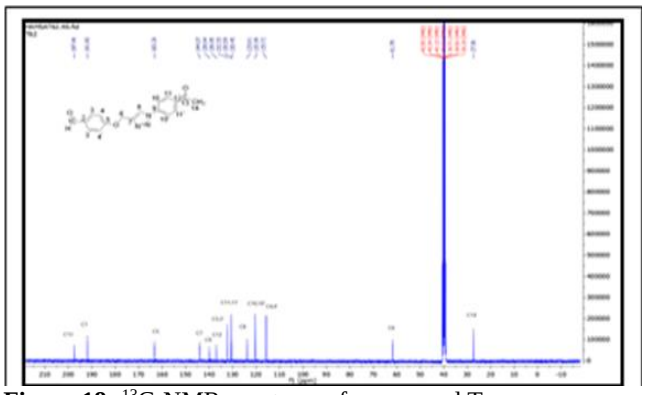


Figure 18: ^{13}C-NMR spectrum of compound T_3.

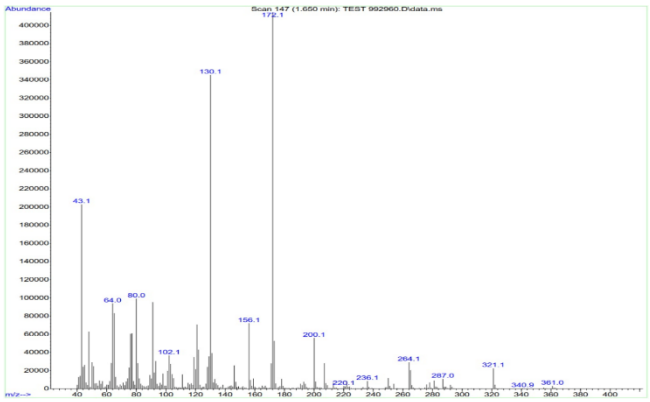


Figure 19: Mass spectrum of compound T_3.

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