

# Trilogy-Function Thiadiazole-Triazole-Pyridine Derivatives as Anticancer Activity

Ali D Hammed, Nasreen R Jber\* and Mehdi S Shihab

Chemistry Department, College of Science, Al-Nahrain University, Baghdad, Iraq

## Corresponding author:

Nasreen R Jber,  
Chemistry Department,  
College of Science,  
Al-Nahrain University, Baghdad,  
Iraq, Uzbekistan,  
E-mail: Nasreen.jber@nahrainuniv.edu.iq

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## Abstract

1-Methyl-4-(6-(substitutedphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-alkylpyridinium was synthesized in this study. The 4-Amino-5-pyridin-4-yl-4H-(1,2,4)triazole-3-thiol was combined with substituted phenacyl bromide in a condensation reaction to produce 4-Amino-5-pyridin-4-yl-4H-(1,2,4)triazole-3-thiol. 1-Methyl-4-(6-(substitutedphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)pyridinium was alkylated with three distinct alkyl bromides. The anticancer effects of the produced triazolo (3,4-b)(1,3,4)thiadiazole pyridine derivatives were investigated using FTIR and <sup>1</sup>H-NMR, and some compounds in the series showed promising anti-cancer activity when compared to standard medications.

**Keywords:** Triazole; Thiadiazole; Antibacterial; Antifungal

## Introduction

Cancer is a serious disease that is still a major medical concern around the world. It is the second most frequent sickness after cardiovascular diseases. As a result, the development of seriously strong and potent novel antineoplastic medications is one of the most certain goals of modern therapeutic science. The science of 1,2,4-triazoles and their intertwined heterocyclic subordinates has gotten a lot of attention because of its planned and significant organic applicability. Numerous 1,2,4-triazoles, for example, have been incorporated into a wide range of therapeutically intriguing antibacterial pharmaceutical up-and-comers, [1,2] reducing, [3] reducing pain [4] and anticancer workouts. [5] Mercapto 1,2,4-triazoles are also quite useful in preparative natural science as helpful intermediates in the design of several triazolothiadiazines and triazolothiadiazines, despite their major natural applications. The amino and mercapto groups provide immediate nucleophilic sites for the mixture of consolidated heterocyclic rings. [6] The triazolothiadiazoles and triazolothiadiazines, which have a broad natural profile, have now evolved into important heterocyclic platforms. [7,8] The term MCF-7 refers to the Michigan Cancer Foundation-7, a detroit-based organization where herbert soule and coworkers established the cell line in 1973. [9,10] MCF7 is a sort of malignant growth beginning from bosom tissue, most regularly from the internal covering of milk channels or the lobules that stock the pipes with milk. [11] MCF-7 cells are valuable for in vitro bosom disease contemplates on the grounds that the cell line has held a few ideal attributes specific to the mammary Epithelium. These incorporate the capacity for MCF-7 cells to deal with estrogen, as estradiol, through estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an Estrogen Receptor (ER) positive control cell line. [12] Apoptosis is a crucial component of cell programming that leads to cell death in long-lived vertebrates. [13] Because it assumes a critical role in the development and maintenance of homeostasis. [14] It is the process of removing excess or damaged cells from the body. Furthermore, the balance between current cell formation and cell passage is what maintains the appropriate number of cells in a tissue. [15] Apoptosis is a vital life measure for all living

things. Managed cell passage plays an important role in various natural cycles that occur in all species, such as cell turnover, tissue homeostasis, embryogenesis, acceptance and support of invulnerable resilience, and improvement of the sensory system, and endocrine ward tissue decay. [16]

## Experimental Study

### 1-Synthetic Methods of 4-Amino-5-pyridin-4-yl-4H-(1,2,4)triazole-3-thiol(A)

At ambient temperature, isonicotinic acid hydrazide (13.7 g, 0.1 mole) was dissolved in pure ethanol (200 mL) containing potassium hydroxide (11.2 g, 0.1 mole) and carbon disulphide (13.17 mL, 0.21 mole) was added in parts to a 500 mL round bottom flask. After 16 hours of agitation, the mixture was diluted with dry diethyl ether (100 mL). The substance was then filtered and vacuum dried at 65°C-70°C The potassium dithiocarbazinate salt was produced in approximately quantitative yields as described above. With stirring, hydrazine hydrate (0.01 mole, 99 percent) was gradually added to potassium dithiocarbazinate salt dissolved in water (100 mL) and refluxed over water bath until H<sub>2</sub>S was produced.

### Synthesis of 1-Methyl-4-(6-phenyl-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-pyridinium(B1-B2)

In 100% ethanol, a 0.01 mol mixture of 4-amino-5-pyridin-4-yl-4H-(1,2,4)triazole-3-thiol and the corresponding phenacyl bromide (0.012 mol) was heated under reflux for 8 hours. The reaction liquid was placed onto crushed ice, and the pH was adjusted with Na<sub>2</sub>CO<sub>3</sub>. Filtered, washed with water, dried, and crystallized from ethanol, the solid product was obtained.

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### Preparation of N-(Ethyl, propyl, pentyl) pyridinium bromide derivatives (C1-C6)

In a round-bottomed flask equipped with a reflux condenser, a solution of (10 mmol) of (B1-B2) and (10 mmol) of n-Ethyl bromide, n-Propyl bromide, and n-Pentyl bromide in 2 ml of 100% ethanol was allowed to stand overnight at room temperature. The mixture was then cooked for 10 hrs at reflux temperature. The pyridinium chloride derivative solution is then dried to remove the solvent and refined by washing with diethyl ether.

### Preparation of N-(Ethyl, propyl, pentyl) pyridinium bromide derivatives (C1-C6)

In a round-bottomed flask equipped with a reflux condenser, a solution of (10 mmol) of (B1-B2) and (10 mmol) of n-Ethyl bromide, n-Propyl bromide, and n-Pentyl bromide in 2 ml

of 100% ethanol was allowed to stand overnight at room temperature. The mixture was then cooked for 10 hours at reflux temperature. The pyridinium chloride derivative solution is then dried to remove the solvent and refined by washing with diethyl ether.

Compounds (A) also characterized using <sup>1</sup>HNMR. Figures 1-9 show the <sup>1</sup>HNMR spectrum of compound (IV) (DMSO-d<sub>6</sub>, δ in ppm): 7.43-7.62 (d, 4H, arom. H) for the pyridine ring, 12.93 (s, 2H, SH), 9.81 (s, 2H, N-H).

1-Methyl-4-(6-phenyl-(1,2,4)triazolo(3,4-b) The reaction of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (A) with two substituted phenacyl bromide yielded (1,3,4)thiadiazol-3-yl-pyridinium (B1-B2).

The NH<sub>2</sub> and S-H stretching bands vanished from the FTIR spectra of compounds (B1-B2). New bands at (3417, 3082, 2958, 2847, 1629, 1600, 1527, and 1346) cm<sup>-1</sup> could be assigned to

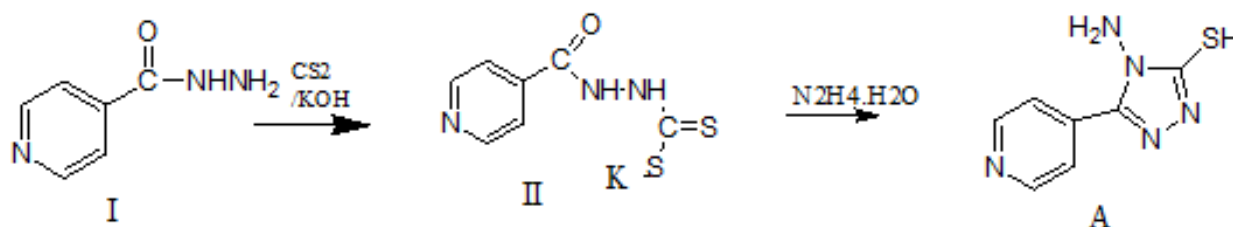


Figure 1: Synthesis of 5-pyridin-4-yl-4H-(1,2,4)triazole-3-thiol.

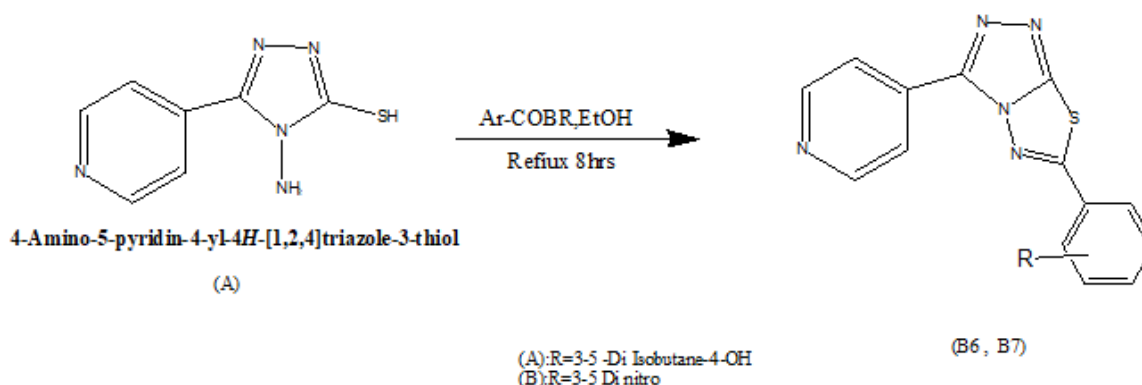


Figure 2: Synthesis of 1-Methyl-4-(6-phenyl-(1,2,4)triazolo(3,4-b)1,3,4)thiadiazol-3-yl-pyridinium (B1-B2).

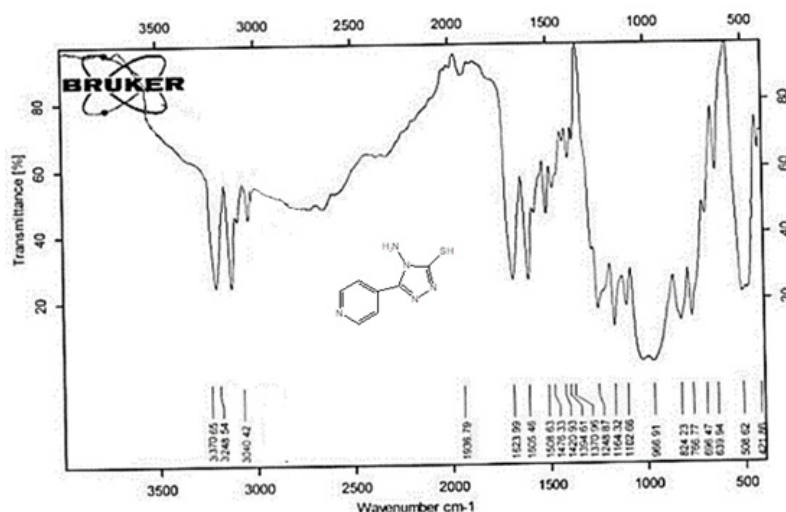
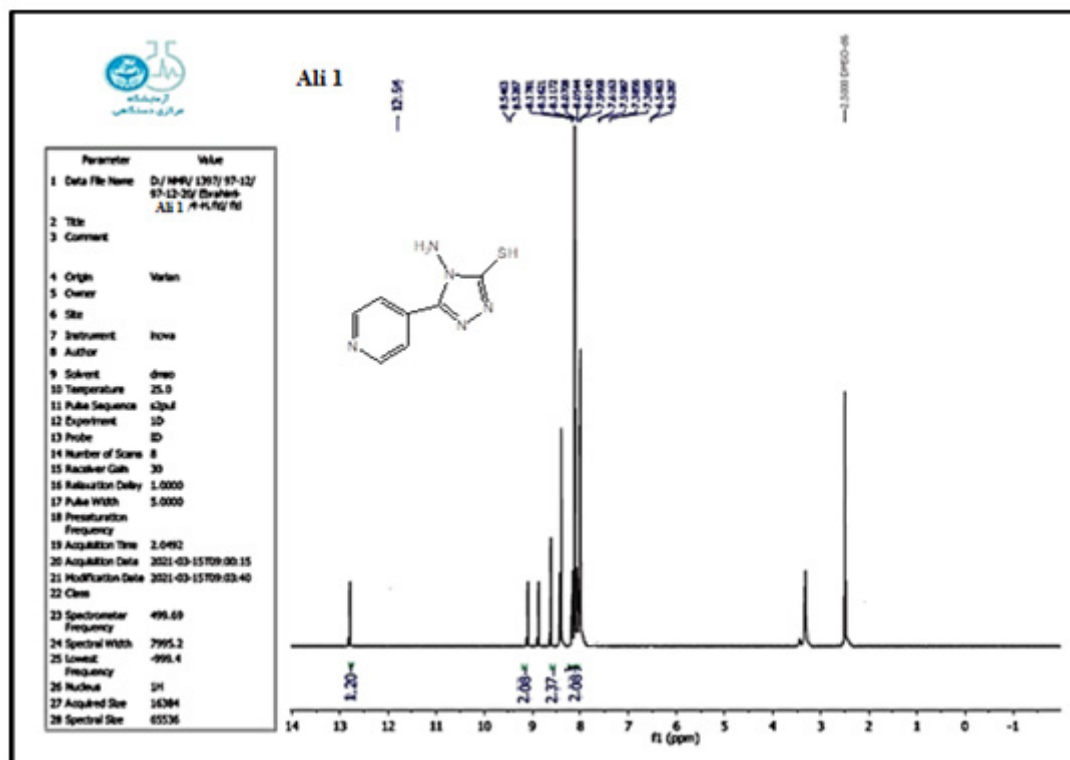
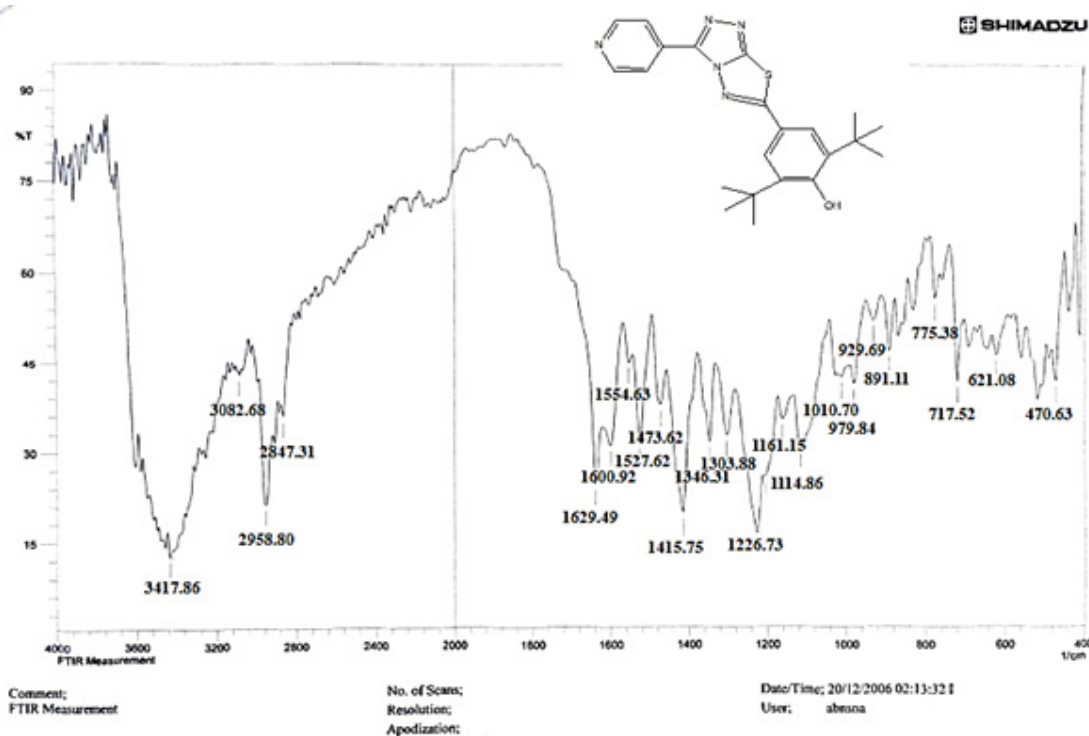


Figure 3: FTIR spectrum 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol.



**Figure 4:** <sup>1</sup>HNMR spectrum 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol.



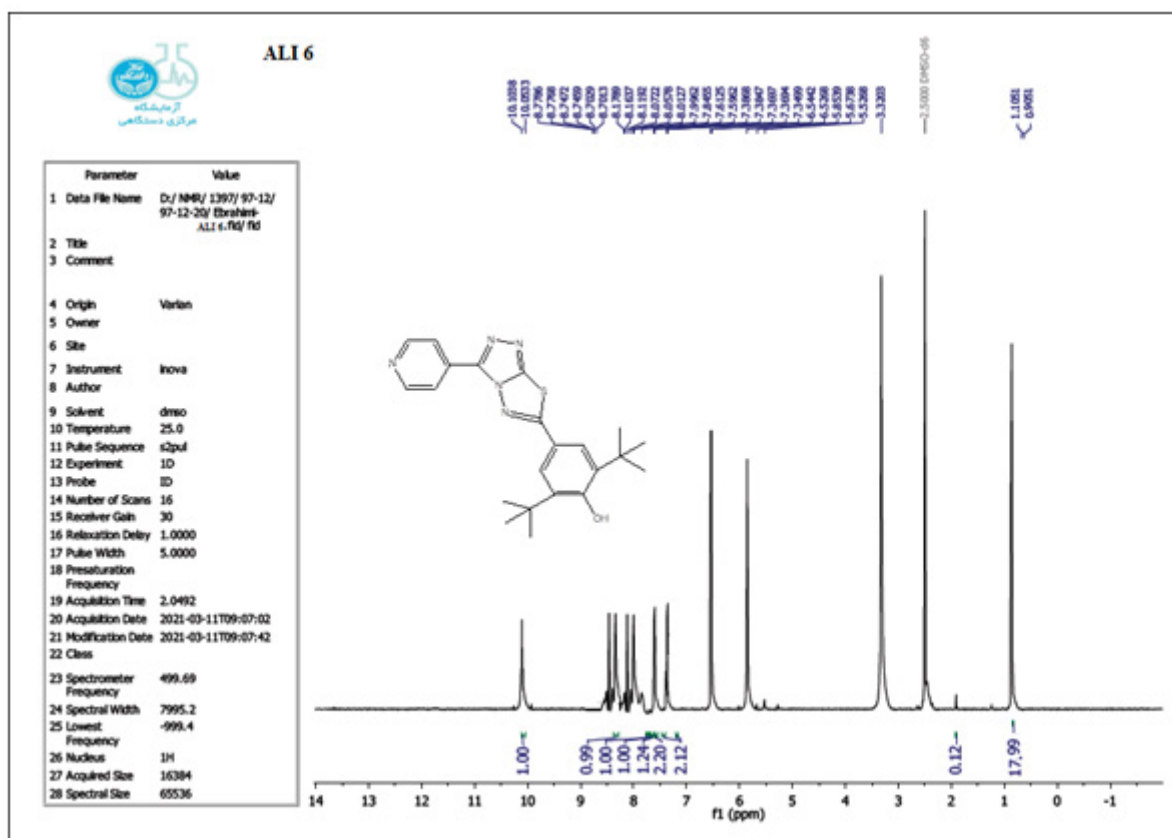
**Figure 5:** FTIR spectrum of 1-Methyl-4-(6-(3,5-diisobutyl-4-hydroxyphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-pyridinium.

O–H, aromatic C–H, aliphatic, C=N, C=C, and NO<sub>2</sub> stretching bands, respectively, in the FTIR spectrum of compound (B1), which was synthesized from reaction compound (A) with 3,5-diisobutyl-4-hydroxy phenacyl bromide. The FTIR spectra of compound (B2), which was produced by reacting compound (A) with 3,5-dinitro phenacyl bromide, exhibits bands at (3081, 1625, 166, 1552, and 1350 cm<sup>-1</sup> that may be ascribed to C–H

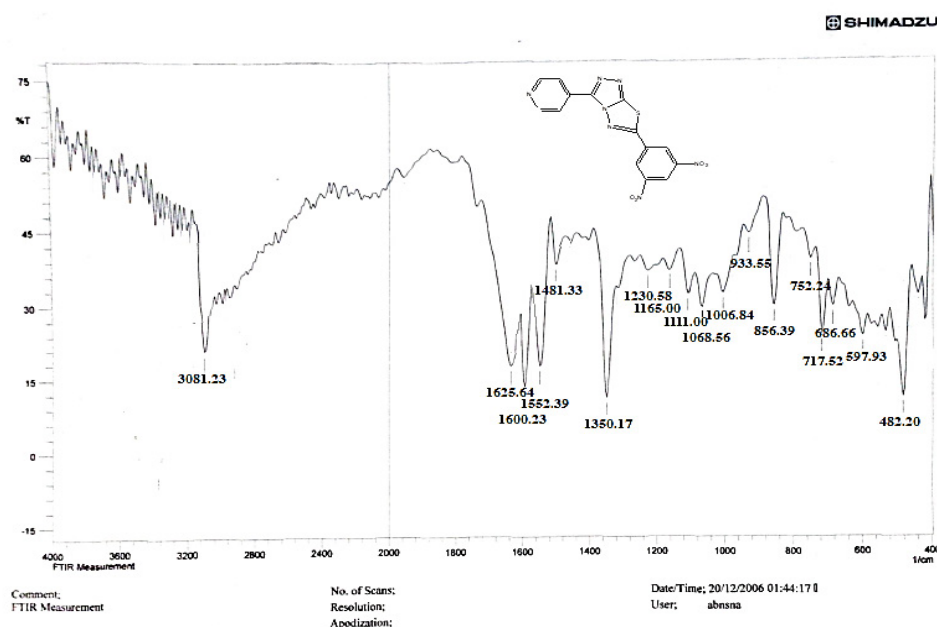
aromatic, C=N, C=C, and asymmetrical and symmetrical NO<sub>2</sub> stretching bands, respectively.

Alkylation of the pyridine nitrogen atom with ethyl, propyl, and pentyl bromides yielded N-(Ethyl, Propyl, Pentyl) pyridinium bromide derivatives (C1-C6).

The intensity of C–H aliphatic asymmetrical and symmetrical



**Figure 6:** HNMR spectrum of 1-Methyl-4-(6-(3,5-diisobutyl-4-hydroxyphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-pyridinium.



**Figure 7:** FTIR spectrum of 1-Methyl-4-(6-(3,5-dinitrophenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-pyridinium.

stretching increases in the FTIR spectra of compounds (C1-C3) synthesized from reaction compound (B1) with ethyl, propyl, and pentyl bromide at (2961, 2974) and (2861, 2873, 2858)  $\text{cm}^{-1}$ , respectively.

The structure of the produced compounds was also studied using the  $^1\text{H}$ NMR spectroscopic method. The  $^1\text{H}$ NMR spectra of chemical C1 is shown in Figures 10-13. Singlet at 10.10 for

hydroxy group (d6-DMSO, ppm). The six protons of the phenyl and pyridine rings are responsible for two signals at 7.5-8.7. The eighteen protons of the tert. butyl group are assigned to the signal at 1.10. The signal triplet at 0.97 (3 H) and quartet signal at 2.2 (2 H) on the  $^1\text{H}$ NMR may be referred to the ethyl group.

Compounds (C4-C6) produced from reaction compound (B2) with ethyl, propyl, and pentyl bromide display novel bands

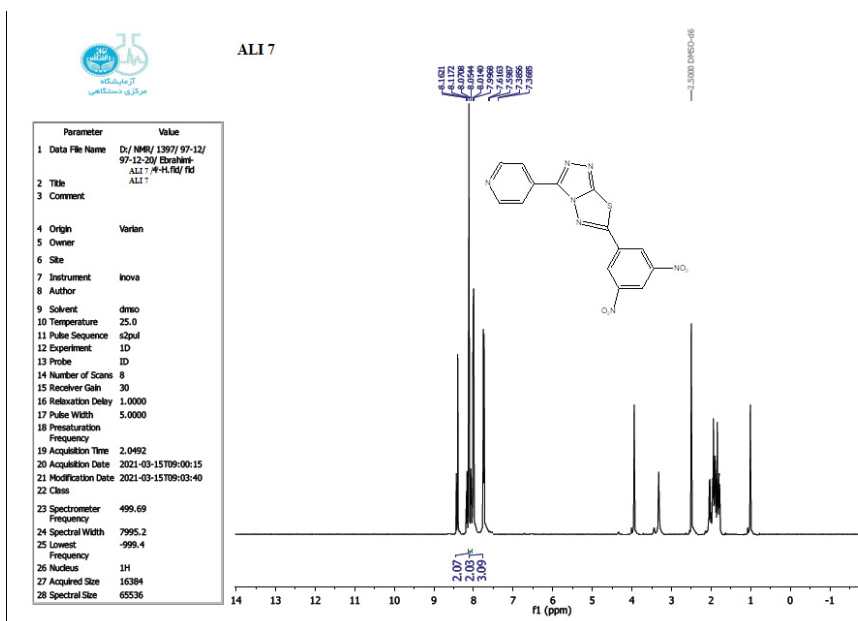


Figure 8: HNMR spectrum of 1-Methyl-4-(6-(3,5-dinitrophenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-pyridinium.

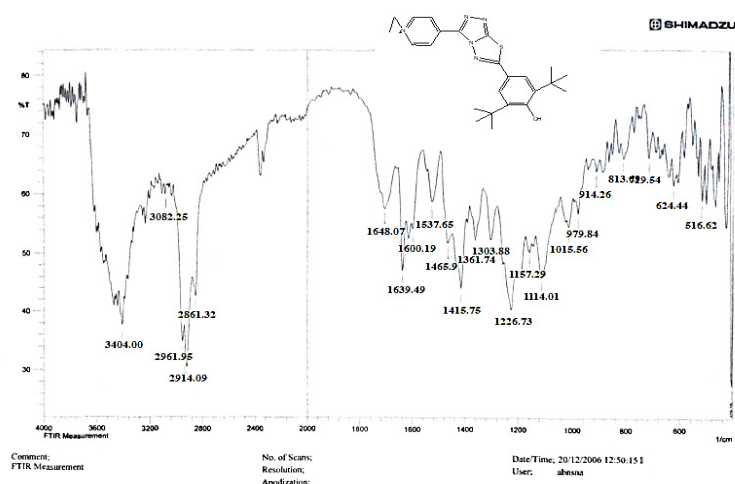


Figure 9: FTIR spectrum of 1-Methyl-4-(6-(3,5-diisobutyl-4-hydroxyphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-ethylpyridinium.

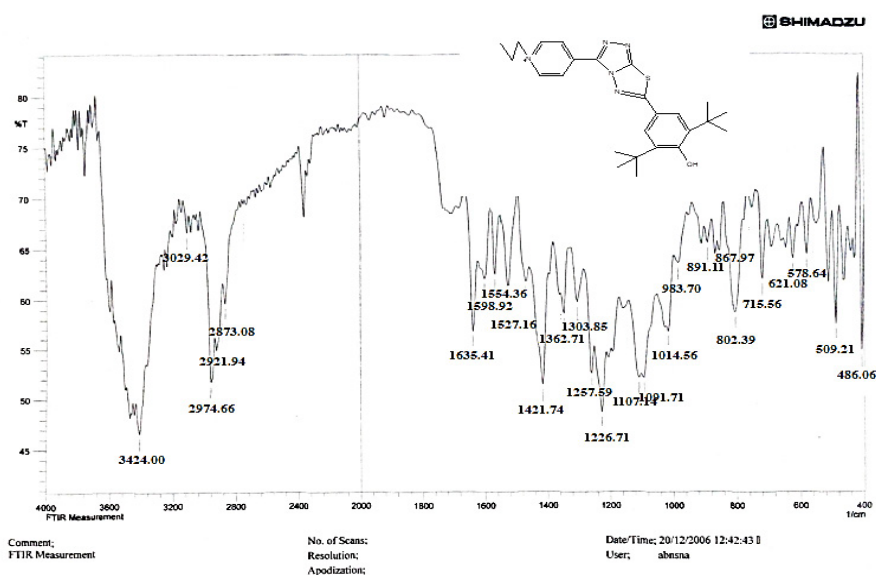
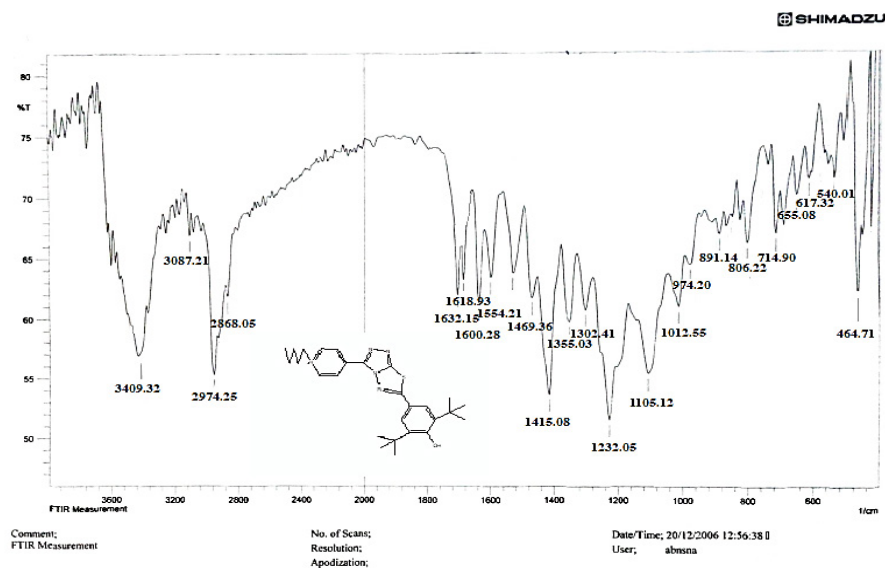
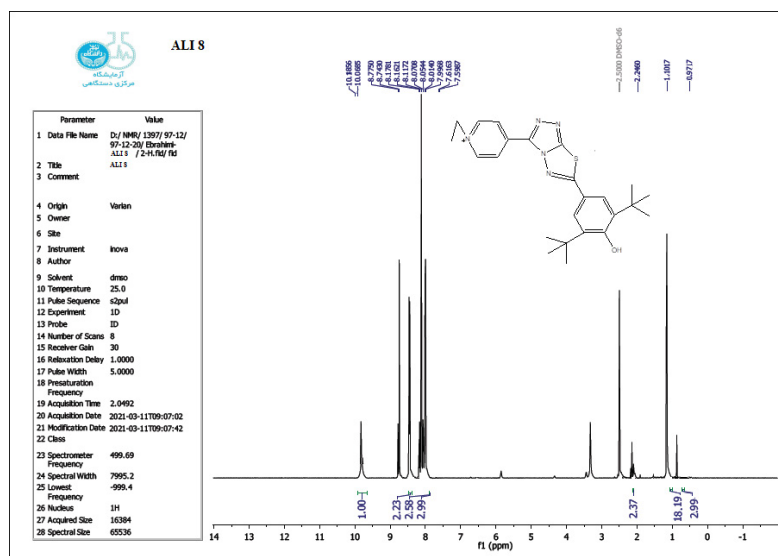


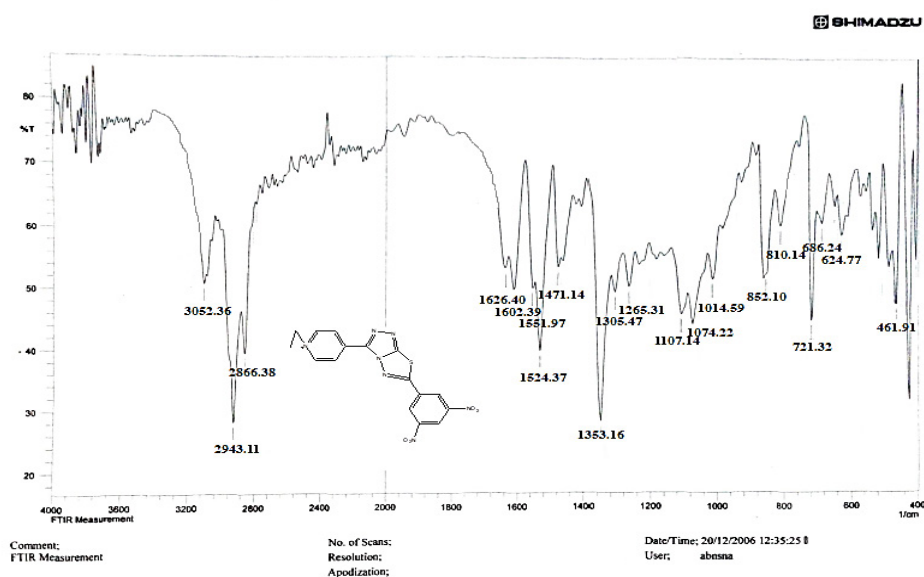
Figure 10: FTIR spectrum of 1-Methyl-4-(6-(3,5-diisobutyl-4-hydroxyphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-propylpyridinium.



**Figure 11:** FTIR spectrum of 1-Methyl-4-(6-(3,5-diisobutyl-4-hydroxyphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-pentylpyridinium.



**Figure 12:** <sup>1</sup>H NMR spectrum of 1-Methyl-4-(6-(3,5-diisobutyl-4-hydroxyphenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-ethylpyridinium.



**Figure 13:** FTIR spectrum of 1-Methyl-4-(6-(3,5-dinitrophenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-ethylpyridinium.

of C-H aliphatic asymmetrical and symmetrical stretching at (2943, 2935,) and (2866, 2870,)  $\text{cm}^{-1}$ , respectively, in their FTIR spectra.

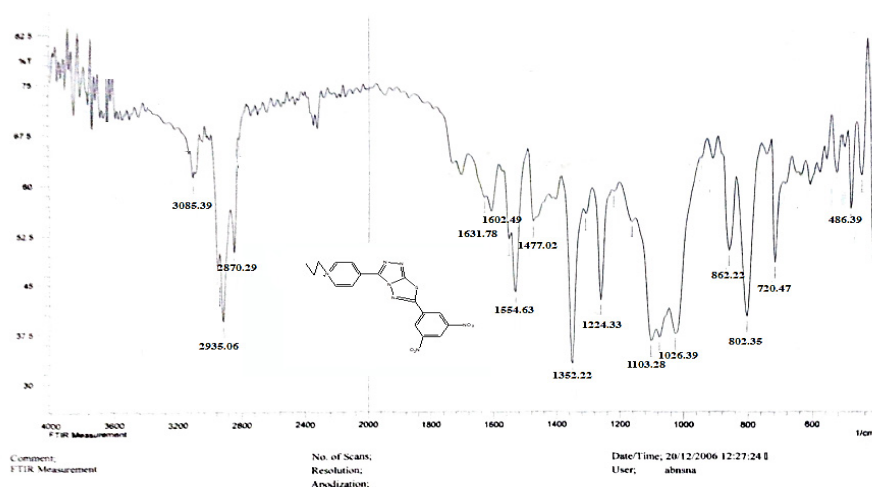
The  $^1\text{H}$ NMR spectrum of chemical C4 is shown in Figure 14 (d<sub>6</sub>-DMSO, ppm), the six protons of the phenyl and pyridine rings are responsible for two signals at 7.5-8.7. The eighteen protons of the tert butyl group are assigned to the signal at 1.10. The signal triplet at 0.97 (3 H) and quartet signal at 2.2 (2 H) on the  $^1\text{H}$ NMR may be referred to the ethyl group.

## Anticancer Activity

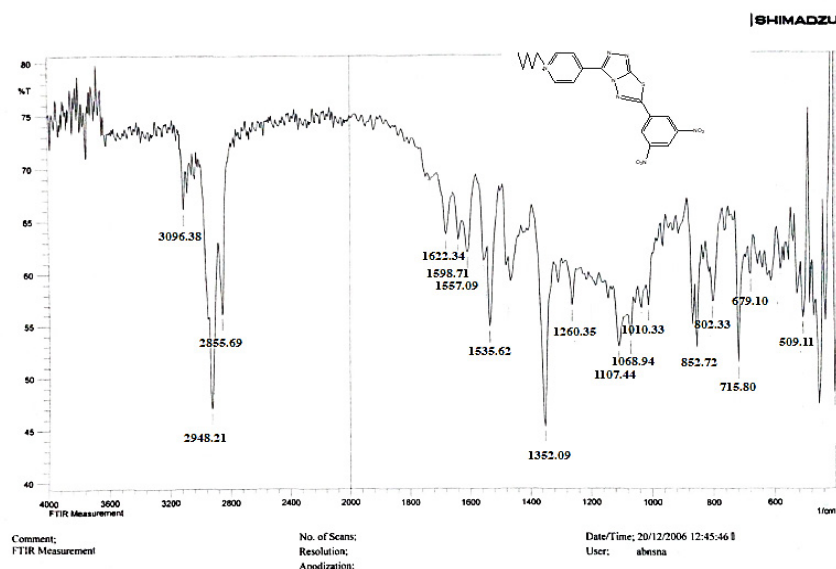
On two types of cancer cell lines, MCF-7 and WRL68, the MTT

assay was utilized to find chemicals with lethal effects. After employing varied amounts of each chemical on cancer cell lines, this experiment was used to detect cell viability.

The cytotoxic effect of chemical (C2) revealed that treating MCF-7 cells at concentrations ranging from 12.5 to 400 g/ml for 24 hours resulted in a considerable loss of cell viability, which increased in a dose-dependent pattern, reaching up to 70% death at 200 g/ml with an IC<sub>50</sub> of 110.4/ml [Figure 15, Tables 1 and 2]. The treatment of WRL68 cells with compound (C2), on the other hand, proved to be less affected when the same concentration range was used, with a detected IC<sub>50</sub> of 272.8/ml [Figure 15].



**Figure 14:** FTIR spectrum of 1-Methyl-4-(6-(3,5-dinitrophenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-propylpyridinium.



**Figure 15:** FTIR spectrum of 1-Methyl-4-(6-(3,5-dinitrophenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-pentylpyridinium.

**Table 1: The name and chemical formula of prepared compounds (C1–C6).**

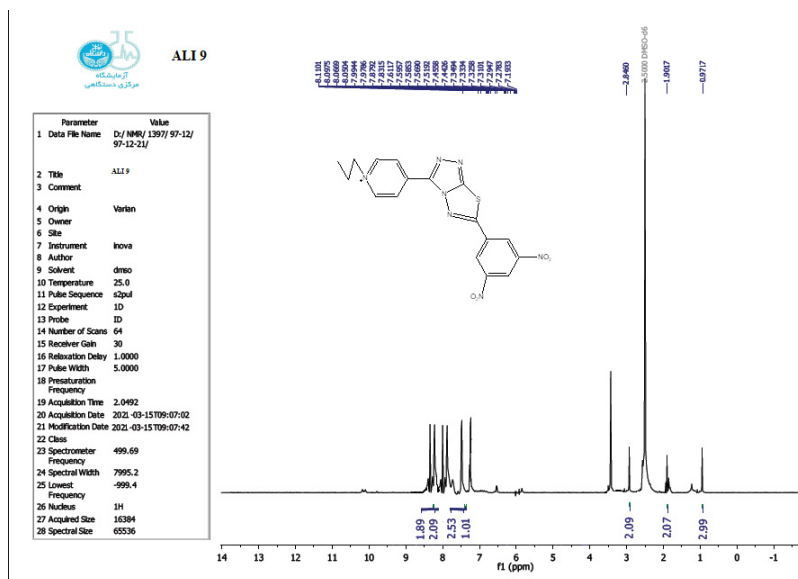
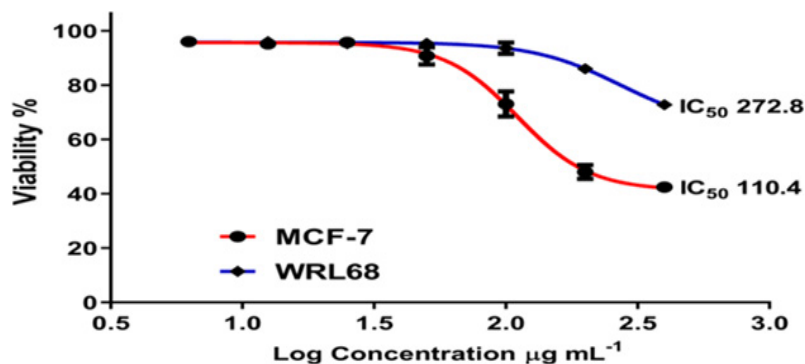
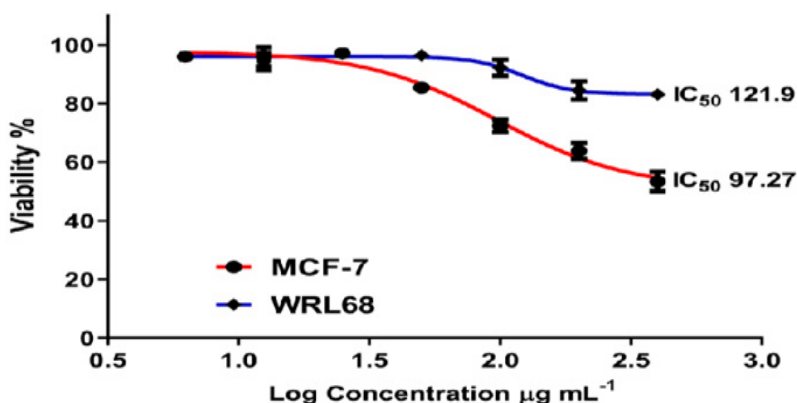
Comp. name and no.		Chemical formula
C1	4-(6-(3,5-Di-tert-butyl-4-hydroxy-phenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-1-ethyl-pyridinium	$\text{C}_{24}\text{H}_{32}\text{BrSN}_5\text{O}$
C2	4-(6-(3,5-Di-tert-butyl-4-hydroxy-phenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-1-propyl-pyridinium	$\text{C}_{25}\text{H}_{34}\text{BrSN}_5\text{O}$
C3	4-(6-(3,5-Di-tert-butyl-4-hydroxy-phenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-1-pentyl-pyridinium	$\text{C}_{27}\text{H}_{38}\text{BrSN}_5\text{O}$
C4	4-(6-(3,5-Dinitro-phenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-1-ethyl-pyridinium	$\text{C}_{16}\text{H}_{12}\text{BrSN}_7\text{O}_4$
C5	4-(6-(3,5-Dinitro-phenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-1-propyl-pyridinium	$\text{C}_{17}\text{H}_{14}\text{BrSN}_7\text{O}_4$
C6	4-(6-(3,5-Dinitro-phenyl)-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-1-pentyl-pyridinium	$\text{C}_{19}\text{H}_{18}\text{BrSN}_7\text{O}_4$

**Table 2: Multiple comparisons of compound (C2) between MCF-7 and WRL68 (p value=0.0001).**

	MCF-7		WRL-68	
	Mean	SD	Mean	SD
400	42.4383	1.4699	72.724	1.5059
200	48.0323	2.5543	85.956	0.8838
100	73.0323	4.6426	93.59	2.1
50	90.7023	3.1831	95.331	1.1825
25	95.7176	0.8099	95.216	0.8209
12.5	95.1773	1.2804	95.949	1.0283
6.25	95.949	0.5305	95.949	0.2003

On the normal cell line MCF-7, the other chemicals in Figures 16-18 showed weak to moderate cytotoxicity, with IC<sub>50</sub> values ranging from 190.0 to 90.02 g/mL. The IC<sub>50</sub> for WRL-68 was found to be between 267.9 and 121.9 g/mL.

Figure 19 shows that when the concentration of Z officinal's extract was 200 g/mL, the viable count of MCF-7 cells decreased compared to untreated cells. The drop in cell counts was substantially different from control in cells dealing with twenty five, fifty, one hundred, and two hundred g/mL of Z. officinal's compound, but in different ranges. This result strongly

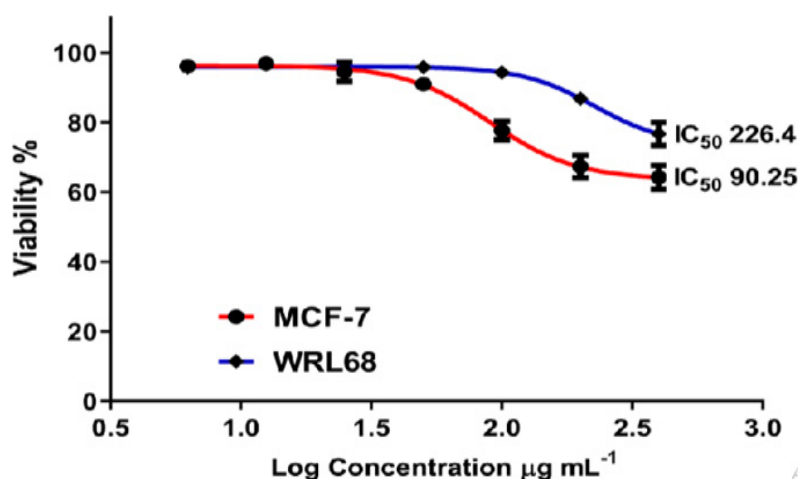

**Figure 16:** FTIR spectrum of 1-Methyl-4-(6-(3,5-dinitrophenyl)-(1,2,4)thiazolo(3,4-b)(1,3,4)thiadiazol-3-yl)-N-propylpyridinium.

**Figure 17:** After a 24-hour incubation period at 37°C, the chemical (C2) had a cytotoxic effect on MCF-7 and WRL-68 cells.

**Figure 18:** Compound (C1) had a cytotoxic effect on MCF-7 and WRL-68 cells following a 24-hour incubation period at 37°C.

suggests This suggests that landfills are effective in killing cells, as evidenced by the MTT assay, which revealed that the drop in cell count was dosage dependant, with the most significant reduction occurring after administration of high concentrations (P 0.001) dosage (200 g/mL).

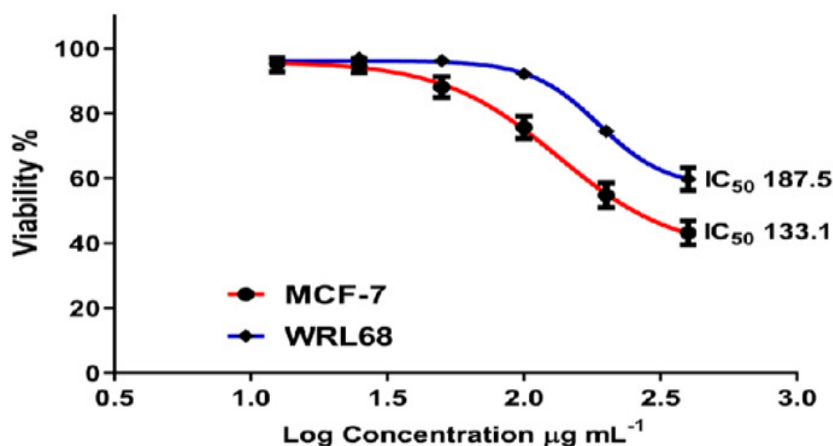
Figures 20-24 shows, on the other hand, that the intensity of MCF-7 cell membrane permeability (green) increased dose-dependently, with a considerable rise after exposure to 100 and

200 of the substance. Official g/mL. The fluorescence intensity of compound and doxorubicin (powerful anti-tumor agent) was revealed in comparison to untreated cells, with doxorubicin in comparison, the nuclear intensity increased greatly when exposed to 200 g/mL of compound solution. Chemotherapy embeds itself in DNA molecules, generating twice the amount of DNA damage.

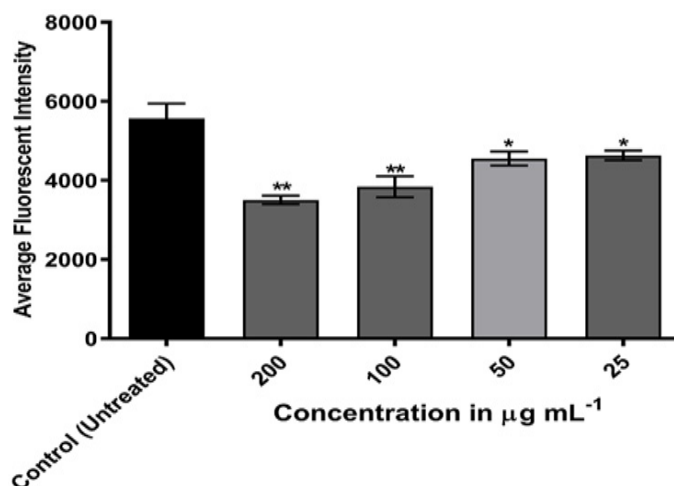
Cytochrome C plays an important function in apoptosis and can



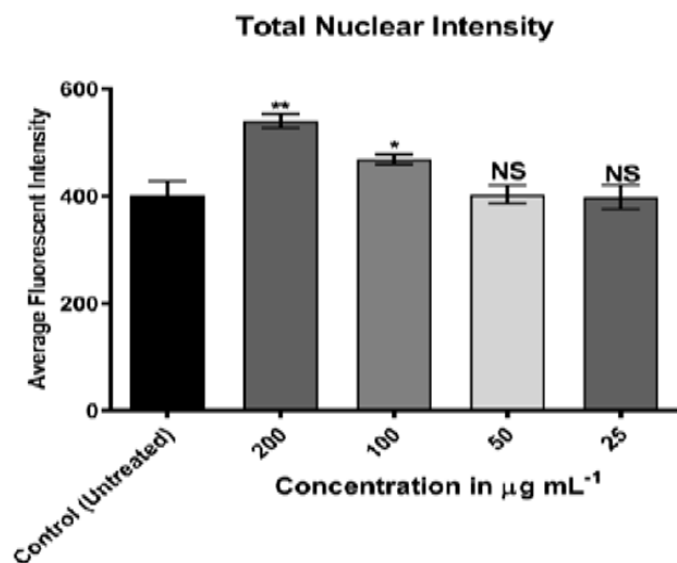
**Figure 19:** After a 24-hour incubation period at 37°C, the chemical (C3) had a cytotoxic effect on MCF-7 and WRL-68 cells.



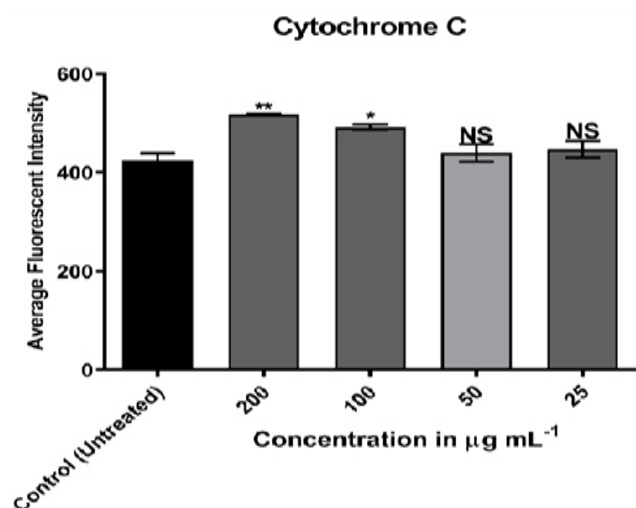
**Figure 20:** After a 24-hour incubation period at 37°C, the chemical (C5) had a cytotoxic effect on MCF-7 and WRL-68 cells.



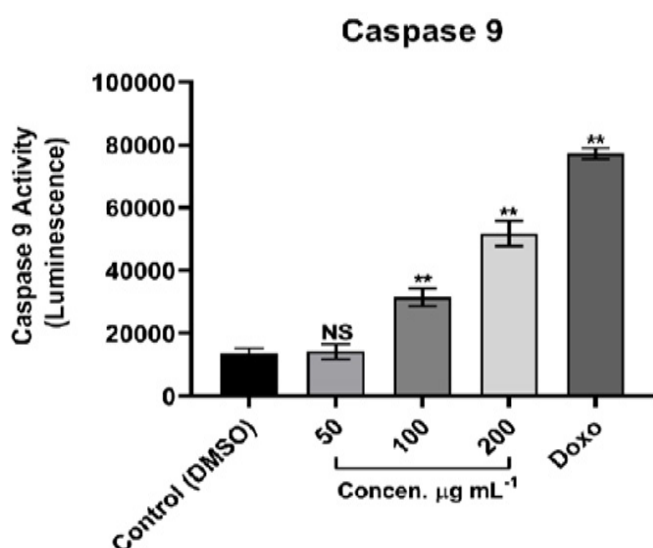
**Figure 21:** Treatment of MCF-7 cells with chemical (C2) has an effect on cell viability.



**Figure 22:** Treatment of MCF-7 cells with compound C 2 solution has an effect on nuclear intensity.



**Figure 23:** In MCF-7 cells, the effect of chemical solution treatment on cytochrome C.



**Figure 24:** Caspase 9 activity evaluated in luminescence versus various doses in compound (C2) MCF-7 cells. Data was compared to DMSO, a negative vehicle (p 0.001).

be released into the cytoplasm from mitochondria. <sup>[17]</sup> MCF-7 cells treated with compound (C2) solution displayed high nuclear cytochrome C staining when compared to doxorubicin, indicating that treatment of breast cancer cells with compound (C2) mimics cytochrome C from mitochondria to cytoplasm.

The Caspase-Glo 9 test is a homogenous luminescence assay that evaluates caspase-9 activity in human cells. <sup>[18]</sup> Caspase-9 is a major activator in the intrinsic apoptotic cascade. The findings suggest that MCF-7 cells treated with compound (C2) solution undergo apoptosis through breaking cell membrane and cytochrome C release, as these two events are linked to Caspase activation. <sup>[19]</sup> Caspase 9 and 3 activation are instances of initiators and effectors, respectively; it is also implicated in the intrinsic pathway of mitochondrial dependency. The intrinsic pathway is primarily regulated by mitochondria, which is not only the site where anti-apoptotic proteins and pro-apoptotic proteins interact and determine cell fate, but also the place where anti-apoptotic proteins and pro-apoptotic proteins interact and define cell fate. However, it is also the source of the signal that triggers Caspase activation *via* a variety of pathways. <sup>[20-22]</sup>

## Conclusion

This work involve the synthesis new trilogy compounds containing thiadiazole triazole-pyridine rings. The structures of the synthesized compounds were investigated by FTIR and <sup>1</sup>HNMR. The MTT assay was used to examine the cytotoxic effect of compounds on two types of tumor cell lines, MCF-7 and WRL68. The cytotoxic effect of chemical (C2) revealed that treating MCF-7 cells at concentrations ranging from 12.5 to 400 g/ml for 24 hours resulted in a considerable loss of cell viability, which increased in a dose-dependent pattern, reaching up to 70% death at 200 g/ml with an IC<sub>50</sub> of 110.4/ml. The treatment of WRL68 cells with compound (C2), on the other hand, proved to be less affected when the same concentration range was used, with a detected IC<sub>50</sub> of 272.8/ml

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