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Synthesis and cytotoxicity evaluation of pyrazole compounds bearing oxazepine core against breast cancer cells

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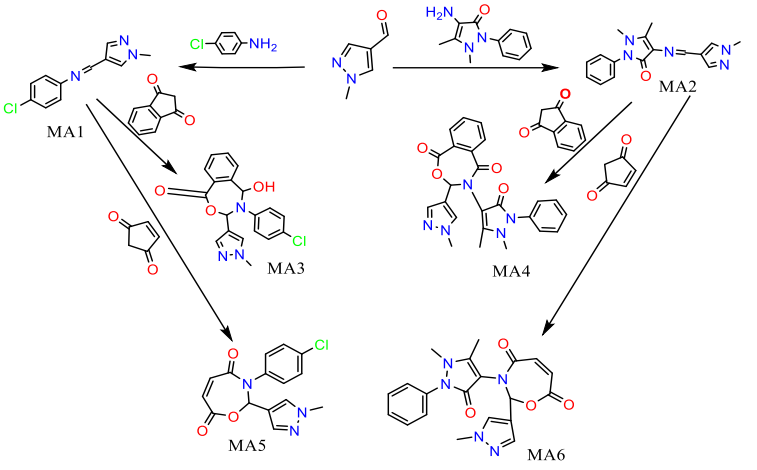
***Abstract*---**The research included the preparation of two Schiff bases MA1,2 starting from1-methyl-pyrazole-4-carbaldehyde with 4-chloroaniline or 4-amino antipyrine in equal moles in the presence of glacial acetic acid. Then 6984xazepane rings MA (3-6) were prepared from MA1,2 with phthalic anhydride or maleic anhydride. The prepared chemical formulas were confirmed using FTIR, 1HNMR, and 13CNMR spectroscopy. The toxicity activity of the compound MA2 against breast cancer MDA and REF cells was tested at seven concentrations (400, 200, 100, 50, 25, 12.5, 6.25) ml/ µg. The study also showed the lowest inhibition rate for MDA cancer line cells at 6.25 µg/ml by (4%) and the highest inhibition rate at 400 µg/ml by 77%, and the study showed the compound with the highest toxicity in healthy cells (64%-80%).

***Keywords*---**1-methyl-pyrazole-4-carbaldehyde, schiff base, 4-amino antipyrine, breast cancer cell line MDA-MB231, REF 52 cell line.

Introduction

Schiff bases are compounds prepared from the reaction of aldehydes or ketones with amines. The azomethine group has replaced the carbonyl group, and the nitrogen atom is attached to an aryl or alkyl (1). They are antimicrobial (2), anti-tuberculosis (3), anti-cancer (4), antioxidant (5), anti-inflammatory (6), antifungal (7), and corrosion inhibitors (8). Oxazepines are a class of heterocyclic compounds that own seven-membered rings with two heteroatoms (O, N). Oxazepines -1,3 can be synthesized from the reaction of Schiff bases with carboxylic acid anhydride (9). These compounds have a broad biological activity with distinctive

pharmacological characteristics (10). They are antipsychotic (11), anticoagulant (12), antibacterial (13), antifungal (14), antitumor (15), anti-cancer (16), and anxiolytic (17) . Due to the importance of this class of organic compounds, many researchers were able to prepare many oxazepine derivatives from Schiff bases to obtain more than one heterogeneous ring of varying size (18-21). Therefore, our research aims to prepare compounds containing the pyrazole ring with the oxazepine ring and to investigate their anti-cancer activity and how much in Scheme- 1: which shows the progress and steps of the reactions:



Scheme 1. The progress and steps of the reactions to prepared MA (1-6)

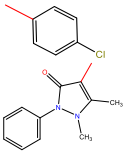
Practical part
The materials and equipment used

Solvents and chemicals were purchased from international companies and used without any purification. Infrared spectra were recorded, using an FTIR-8400S spectrometer supplied by Shimadzu Japan and using potassium bromide discs. Perkin/400MHz NMR spectra measured at Basrah University, College of Education, Department of Chemistry, using DMSO solvent. The biological efficacy was tested at the Department of Biomedical and Molecular Technologies, Al-Nahrain University, using an ELISA device from the Austrian company ASYS.

Preparation of two compounds from Schiff bases MA (1-2)

(1 g, 0.01 mol) of 1-methyl-pyrazole-4-carbaldehyde was dissolved in 5 ml of absolute ethanol. Then, added the stoichiometric moles of 4-aminoantipyrene or 4-chloroaniline, with two drops of glacial acetic acid. The mixture was inversely refluxed for 3 hours, and the completion of the reaction was confirmed using TLC technology. After the end of the reaction, the reaction mixture was left for 24 hours at a temperature of 10 ° C to form a precipitate, then filtered, collected the precipitate, and dried until stable weight. Table -1 shows the physical properties and product ratios of MA compounds (1-2).

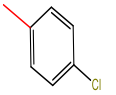
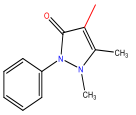
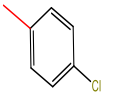
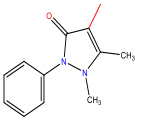
Table 1
The physical properties and product ratios of MA (1-2) compounds

Comp. No.	-Ar	M.Wt. (g/mol)	Molecular Formula	m.p ^{O}C	Color	Yield (%)
MA1		219.67	$C_{11}H_{10}ClN_3$	106	white	90
MA2		295.35	$C_{16}H_{17}N_5O$	173	Orange	92

Preparation of derivatives of 3.1-oxazepine 7.4-dione, MA (3-6)

Stoichiometric moles of prepared Schiff bases MA (1-2) were dissolved in 10 ml of dry dioxane with maleic anhydride and phthalic anhydride. It was recrystallized with ethanol. Table -2 shows some physical properties, percentages, and molecular formulas of the prepared compounds MA (3-6).

Table 2
Some physical properties, percentages and molecular formulas of the prepared compounds MA (3-6)

Comp. No.	-Ar	M.Wt. (g/mol)	Molecular Formula	m.p ^{O}C	Color	Yield (%)
MA3		369.8	$C_{19}H_{16}ClN_3O_3$	121	White	65
MA4		443.46	$C_{24}H_{21}N_5O_4$	184-187	White	70
MA5		317.7	$C_{15}H_{12}ClN_3O_3$	170-172	Yellow	65
MA6		393.40	$C_{24}H_{21}N_5O_4$	195	Yellow	74

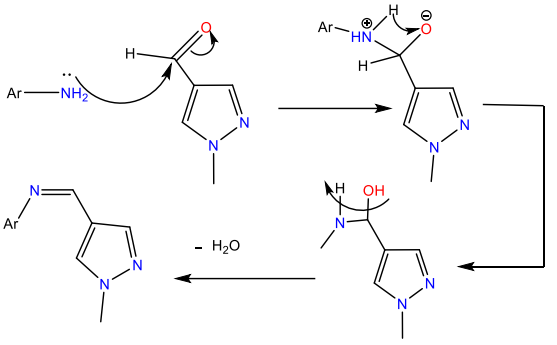
Inhibition of Cancer Cells and Evaluation of Compound Toxicity on Compounds

The biological activity of MA(2) was examined by using a sensitivity test of MTT dye on cancer cells. The culture medium containing RPMI 1640 was prepared as a nutrient medium by adding 200 µm of cancerous isolates for each case in a hole on 21 holes with a capacity of 400 µm. Incubated with a temperature of 36.8, cells were treated with 200 µmL of the prepared compound for each pit at different concentrations of seven half concentrations (400, 200, 100, 50, 25, 12.5, 6.25) mL/µg. Single concentration assays were performed to obtain the exact results in the plate, the plate was left in the incubator at 36.8 °C for 24 h, then

the pits were washed with phosphate-buffered saline (PBS), and then MTT dye was added to the adherent cells in each hole The third episode of the previous episode.

Results and Discussion

Schiff bases MA (1,2) was prepared from condensation 1-methyl-pyrazole-4-carbaldehyde with 4-chloroaniline or 4-amino antipyrine in the presence of glacial acetic acid. The reaction followed the mechanism nucleophilic addition then, elimination water molecule, to give the product. Scheme- 2 displays general mechanism:



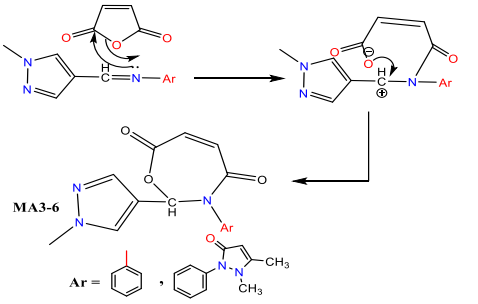
Scheme 2. The general mechanics of the Schiff base MA (1,2)

The two compounds MA1 and MA2 were confirmed by infrared spectrophotometry, and the spectrum showed the expected absorption bands, as the two stretching bands of the amine group and the carbonyl group of aldehyde had disappeared, and the aromatic CH stretching band appeared at (3066-3124) cm-1, the stretching band of aliphatic CH at (2947-2871) cm-1, a stretching bundle of lactam carbonyl -N-C=O at 1640 cm-1, a stretching bundle C=C at (1620-1599) cm-1 and a medium-strength bundle belonging to the azomethine binding bundle at (1544-1542) cm-1, Figures- 1,2 refer to the infrared spectrum of the two compounds, respectively. The MA1 compound was confirmed by proton nuclear magnetic resonance spectroscopy, as the spectrum showed a single 3Ha signal at the range of 3.91ppm belonging to the methyl group, two binary signals for phenyl ring protons 2Hb, 2Hc in the range (7.20-7.17), (7.44-7.40) ppm, a signal of ring protons Pyrazole has a single sign for each of Hd and He at the range (7.92), (8.25) ppm respectively, the single sign of the Hf proton goes back to the proton of the azomethine group at the range (8.45).ppm and the solvent and water sign appears at 2.5ppm and 3.38 ppm respectively as It is shown in Figure-3.

The MA2 compound was confirmed by 1HNMR spectroscopy. The signal of 3Ha and 3Hb protons appears in the spectrum at (3.11,2.38)ppm respectively, a single signal belonging to the methyl group linked to the pyrazole ring of antipyrine, and a single signal of the three Hc protons appears in the spectrum at 3.87 ppm belonging to the methyl group, the phenyl group signals showed triple signals for each of Hd, He in the range at (7.33-7.38), (7.50 - 7.54) ppm, the signals of the main pyrrole ring protons appeared as a single signal for each of the Hf, Hh in

respectively .The spectrum is at (7.83) ppm (9.48), and a single sign of the Hg proton appears at 8.13 ppm belonging to the azomethine proton, the two signs of the DMSO and water appear as in 2.5 ppm,3.39 respectively ppm as shown in the Figure-4.

The MA2 compound was identified by 13CNMR, fourteen signals appeared, the carbon signal belonging to the methyl groups C1, C2, and C3 appears in the spectrum at (10.25, 36.11) ppm respectively, and the carbon signal of C3 overlapped with the DMSO solvent at 40.17 ppm. The carbon signals of the pyrazoline ring C4, and C12 appeared in the spectrum at (148.89,177.66) ppm respectively. The carbon signals C11, C9, and C5 appear in the spectrum at (122.67,131.68,138.22) ppm, respectively, belonging to the pyrazole ring. . The C13 carbon signal appeared at (160.5)ppm belonging to the carbonyl group, and the C14 carbon signal appeared at (151.78)ppm. The sign of the as in Figure -5.Oxazepines ring MA (3-6) were prepared from the reaction of Schiff bases MA (1,2) with maleic anhydride or phthalic anhydride by cyclization addition reaction (2+5) profits conferring to the following mechanics to form a heptagon (22), in a reflux condition for (6-7) hours using dry dioxane as a solvent. Scheme- 3 displays general mechanism:



Scheme 3. The general mechanics of the Oxazepines MA (3-6)

The prepared Oxazepines were confirmed by FTIR spectroscopy. The disappearance of the azomethine bond and the appearance of stretching bands of lactone carbonyl groups in the range (1724-1701) ppm. Stretching of lactam carbonyl at (1649-1640) ppm. The spectra showed the appearance of a band at (3321- 3213) ppm The stretching of the enol tautomer for the carbonyl group and the appearance of the rest of the expected bands as in Table -3 and as in Figure -6,7.

Table 3
Infrared absorption bands for MA compounds (3-6)

Comp. No.	IR (KBr) cm^{-1} ν (C-H) Arom. &Aliph.	ν (C=O)	ν(C=C) Arom.	ν (C-O)
MA3	3132	1720	1600	1396
	2931	1643	1542	1244
MA4	3085	1724	1606	1380

	2923	1645	1596	1353
MA5	3080	1701	1577	1350
	2943	1640	1537	1321
MA6	3045	1716	1593	1373
	2974	1649	1562	1299

The MA3 compound was confirmed by ^{1}HNMR spectroscopy, where the 3Ha signal appeared as a single signal belonging to the methyl group at 3.57 ppm, and the binary Hb and He signal and the triple Hf signal appeared for the phenyl ring protons in the spectrum at the range (7.39-7.42) (7.61-7.61). 7.57 (7.68-7.65) ppm, respectively. The Hc proton signal appeared in the spectrum at the range (7.38) ppm and the Hc proton signal appeared at (7.54) ppm for the Hd proton of the pyrazole ring. (7.69-7.67) (7.75-7.73) ,(7.92-7.90) ppm, respectively, due to the phenyl ring bound to oxazapine. The last proton (C-H) of the oxazepine ring appeared at (10.49) ppm, a single signal. The sign of the solvent DMSO appeared at 2.51 ppm, and the water was 3.47 ppm Fig. 8.

The MA3 was diagnosed by ^{13}CNMR, in which eighteen signals appeared, and the C1 signal of the methyl group appeared in the band at 40.33 ppm overlapping with DMSO solvent atoms. The C2 signal belonging to the oxazapine ring appeared at (66.82) ppm. The C16, C5, and C3 signals appeared in the spectrum at (121.49) (128.23) (139.10) ppm, respectively, due to the pyrazole ring. The C13, C12, C8, and C4 signals appeared at (127.38,130.06,133.44,134.99) ppm, respectively, due to the oxazapine-binding loop, and the C6, C9, C9, C7, and C15 signal appeared in (129.04, 130,130.31,136.02,138.94) ppm respectively belongs to the phenyl ring, C11 and C10 signals appear at (132.28,132.61) ppm respectively. The carbon atoms of the C17 and C18 carbonyl amide groups appeared at (167.85 (168.02) ppm respectively, and the solvent signal was at 40.52 ppm in Figure- 9.

Compound MA6 was confirmed by ^{13}CNMR spectroscopy. The C3, C2, and C1 carbon signals appeared in the (11.78,36.35,40.35) ppm range of methyl groups. C12 and C5 signals appeared in the spectrum at (106.75,131.50) ppm belonging to the second pyrrole ring. The first C15, C9, and C6 pyrazole ring signals appeared at (117.1,128.05,152.64) ppm, respectively, and the C13 and C11 carbon marks appeared. and C10 and C8 at (126.88,129.62,130.69,131.65) ppm, respectively, belong to the phenyl ring, and carbon signals for the C14, C7, and C4 oxazepine ring appeared at (66.81,124.16,135.5) ppm respectively, the carbon atoms of the carbonyl group C16, C17, and C18 appeared at (161.76,164.52,167.06) ppm, respectively. The solvent signals appeared as shown in Figure -10.

Evaluation of the biological activity of the prepared compound MA2

The biological activity of the prepared MA2 compound was evaluated using seven half concentrations (400, 200, 100, 50, 25, 12.5, 6.25) µg / ml. Against two types of cells, the first cancer cells, which are breast cancer MDA cells, and the second normal healthy cells Ref, for a period of 24 hours, and comparing the results of the effect of the compound between them through the MTT dye chromatography absorption spectrum test. Where it showed that the percentage of the developing

live cells of the cancer bio-line MDA cells ranged between (96%-23%), while the percentage of growth of the normal healthy cell line Ref was between (36%-20%) and the table -4- shows the effect of The MA2 compound on the growth of MDA cancer cells and healthy cells REF. Figure -11 represents the relationship of the biological activity of cell growth between cancer cells and healthy cells. The study also showed the lowest inhibition rate for MDA cancer line cells at 6.25 µg/ml by (4%), and the highest inhibition rate at 400 µg/ml by 77%, and the study showed the compound with high toxicity on healthy cells. (64%-80%) and Figure -12 shows the relationship to the effect of the prepared compound on MDA and Ref cells and compare the results between them.

Table 4
The effect of The MA2 compound on the growth of MDA cancer cells and healthy cells REF

Con. (µg.ml^{-1})	MA2 chiff base Compound			
	Cancer line cells of MDA		Normal line cells of REF	
	Mean	SD	Mean	SD
6.25	96	0.28	36	0.14 4
12.5	94	0.11	34	0.10 9
25	91	0.52	42	0.09
50	60	0.21	37	0.042
100	55	0.07	27	0.037
200	30	0.16	23	0.022
400	23	0.01	20	0.05

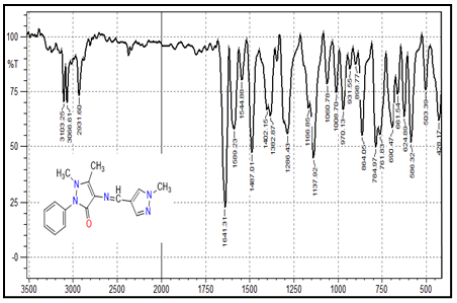


Figure 1. IR spectrum of compound MA1

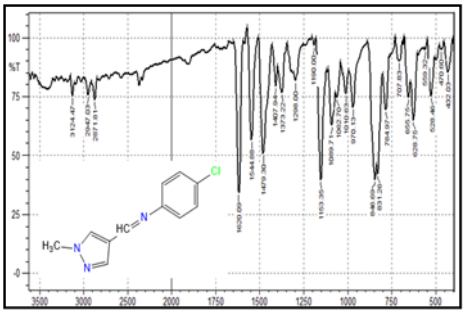


Figure 2. IR spectrum of compound MA2

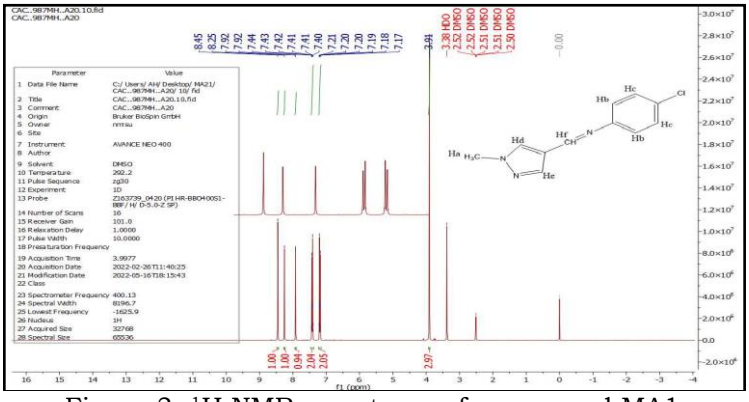


Figure 3. ^{1}H NMR spectrum of compound MA1

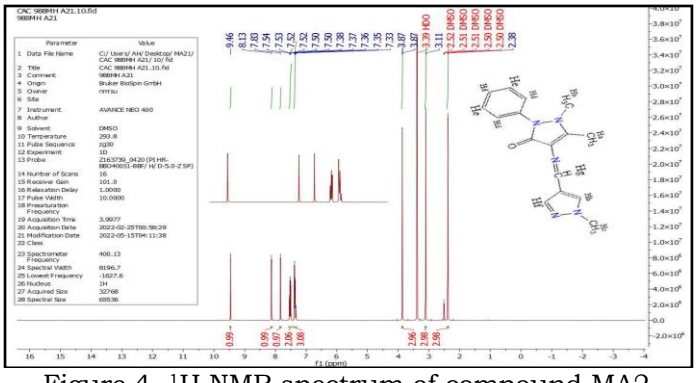


Figure 4. ^{1}H NMR spectrum of compound MA2

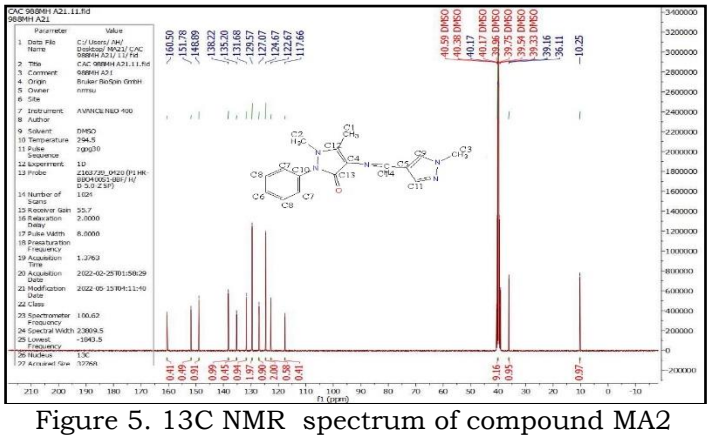


Figure 5. 13C NMR spectrum of compound MA2

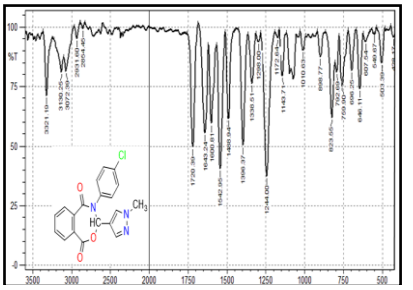


Figure 7. IR spectrum of compound MA6

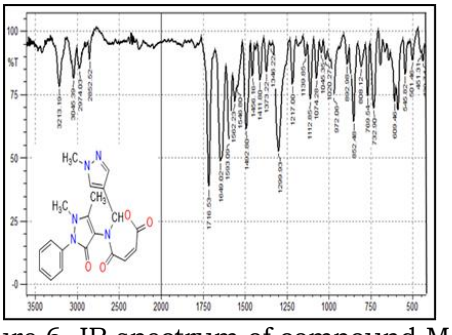


Figure 6. IR spectrum of compound MA3

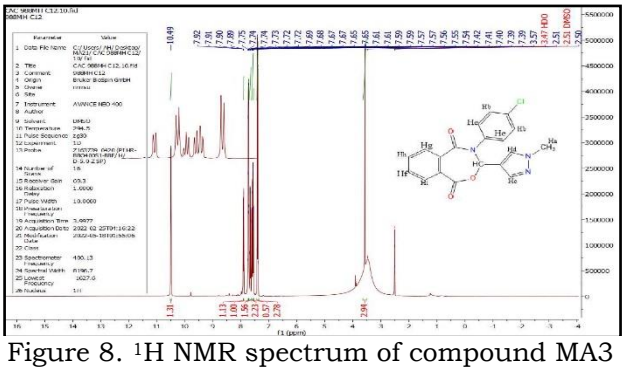


Figure 8. ^{1}H NMR spectrum of compound MA3

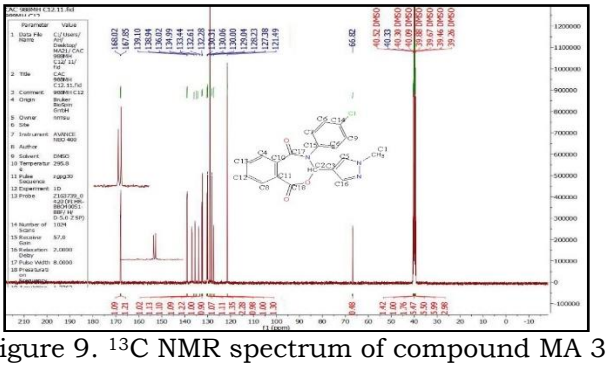


Figure 9. ^{13}C NMR spectrum of compound MA 3

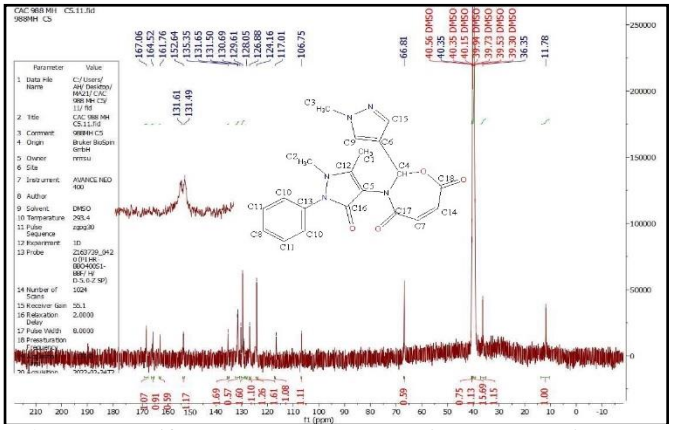


Figure 10. ^{13}C NMR spectrum of compound MA 6

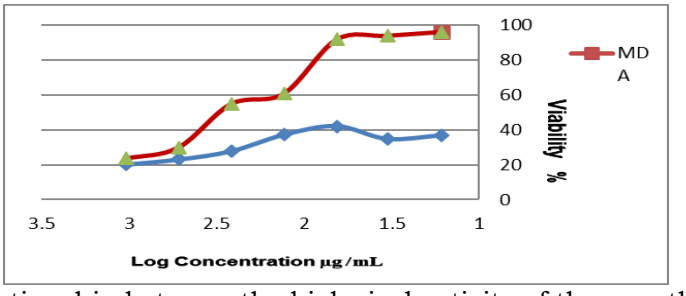


Figure 11. Relationship between the biological activity of the growth of the breast cancer line MDA and the growth of healthy cells of the REF line versus the logarithm of the concentration of compound MA2

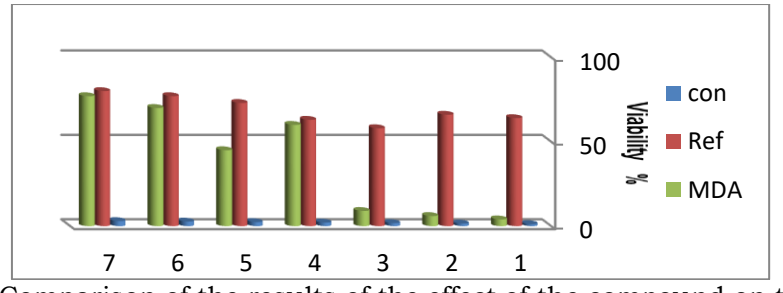


Figure 12. Comparison of the results of the effect of the compound on the cell line of MDA cancer cells and healthy cells REF

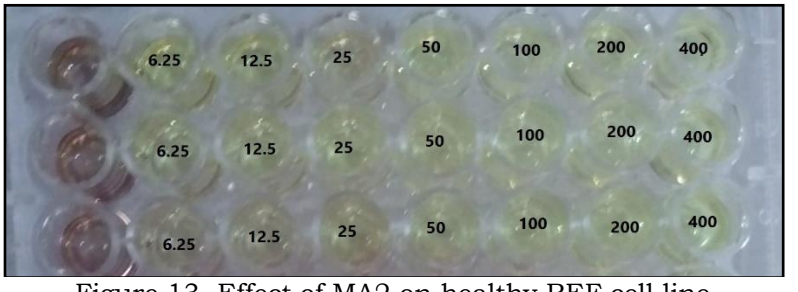


Figure 13. Effect of MA2 on healthy REF cell line

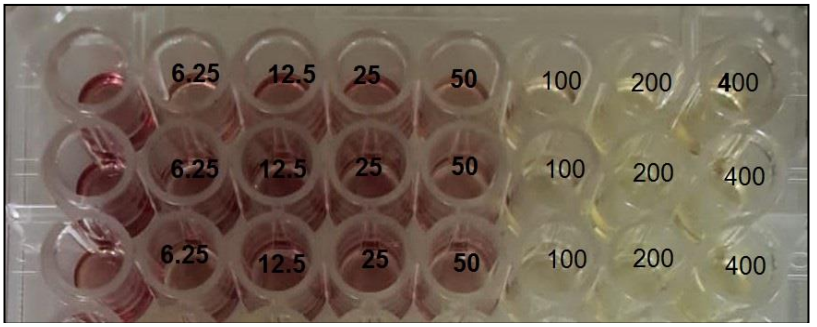


Figure 14. Effect of MA2 on the MDA cancer cell line

Conclusions

The compounds of the Schiff bases containing the pyrazole ring and the corresponding oxazapine rings obtained in good product ratios, and the Schiff bases showed high toxicity against cancer cells at high concentrations and moderate toxicity against healthy cells, which indicates good preliminary results as anti-cancer future breast cancer cells.

Acknowledgments

We extend our thanks and appreciation to the Department of Medical and Molecular Technologies, Biotechnology Research Center, Al-Nahrain University for its facilities for conducting toxicological examinations. We also thank the Department of Chemistry, College of Education for Pure Sciences, University of Basra, for conducting NMR examinations.

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