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Synthesis, identification, and cytotoxicity effect of Oxazapine scaffolding

Mohammed Hekmat Azzawi
Chemistry Department, College of Education, University of Samarra, Iraq
*Corresponding author email: moohmo1968@gamail.com

Maha Salih Hussein
Chemistry Department, College of Education, University of Samarra, Iraq
Email: maha.s56@uosamarra.edu.iq

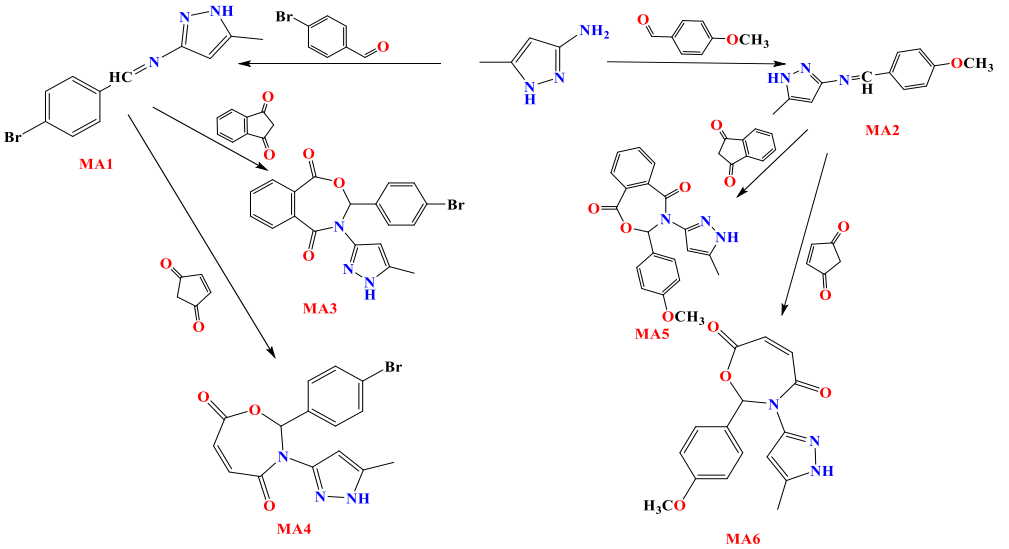
Abstract---The research includes the preparation of two Schiff bases MA (2-1) and some of their derivatives from the reaction of para-bromobenzaldehyde or para-methoxy benzaldehyde with stoichiometric 5-methyl pyrazole-3-amino in the presence of glacial acetic acid. Then the corresponding oxazepine rings MA (6-3) were prepared from each MA (2-1) with maleic anhydride or phthalic anhydride. The prepared chemical formulas were spectroscopically confirmed using FTIR and 1HNMR spectroscopy. The biological activity of MA5 had tested against MDA breast cancer and REF healthy cells at seven concentrations (500,250,125,62.5,31.5,15.5,7.5) µg / ml. The compound showed a high inhibition against cancer cells, so this makes it a candidate as a drug for future cytotoxicity.

Keywords---schiff base, 5-methylpyrazole-3-amino, MDA-231 breast cancer cells, REF 52 cell line.

Introduction

Schiff bases are compounds prepared by the reaction of aldehydes or ketones with amines, in which the carbonyl group is replaced by an azomethine group, and the nitrogen atom is attached to an aryl or alkyl (1). Oxazepine is a heterocyclic seven-membered compound containing two atoms (O, N): the oxygen atom is in position 1 and the nitrogen in position 3 (2). It had prepared by the condensation of Schiff bases with carboxylic acid anhydride (3,4). Their chemical structure has a distinctive feature in pharmacological chemotherapy drugs: antimicrobial (5), anti-cancer and anti-tuberculosis (6,7), anti-inflammatory (8), antioxidant (9), antifungal (10), and antibacterial. As auxiliary agents and in the manufacture of dyes as coloring materials, inhibitors of metal corrosion (11), and has a varied biological activity, its effectiveness used as an anticoagulant (12),

treatment of mental disorders (13,14), anti-tumor (15), (16). Based on the great importance of these compounds, the research aimed to prepare two compounds from Schiff bases with the derivation of the oxazepine ring and study their biological effectiveness on MDA breast cancer to know the extent of the toxicity of the compounds on healthy cells Ref. Scheme-1- shows the course of the reactions to prepare MA compounds (6-1):



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

Practical part

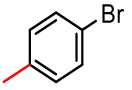
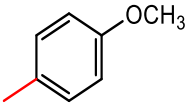
The materials and equipment used

Solvents and chemicals from international companies were prepared and used without any purification. Infrared spectra had recorded using an FTIR-8400S device supplied by Shimadzu Japanese company with KBr potassium bromide discs. The nuclear magnetic resonance (1H.NMR) spectra had measured at Basra University, College of Education, Department of Chemistry, using DMSO (400 MHz) solvent. 0.2 mm thick fluorescent activated, measured using several solvents. The biological efficacy was tested at the Department of Biomedical and Molecular Technologies, Al-Nahrain University, using an ELISA device from the Austrian ASYS company.

Preparation of Schiff MA (1-2)

(0.01 mol) 5-methyl pyrazole-3-amino had dissolved in (5 mL) absolute ethanol and added to para-bromobenzaldehyde or para-methoxy benzaldehyde with two drops of glacial acetic. The mixture had refluxed for 3 hours, and the completeness of the reaction was confirmed using TLC technology. The mixture was left for 24 hours at 10 °C to form a precipitate, then filtered, collected, and dried until the weight became stable. Table 1 shows the physical properties and product ratios of MA (1-2) compounds.

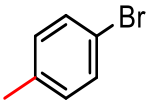
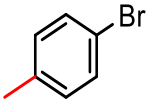
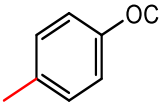
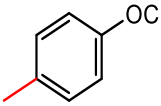
Table 1
Shows the physical properties and product ratios of MA(1-2) compounds

Comp. No.	-Ar	M.Wt. (g/mol)	Molecular Formula	m.p ^{O}C	Color	Yield (%)
MA1		264.13	$C_{11}H_{10}BrN_3$	179	White	94
MA2		215.26	$C_{12}H_{13}N_3O$	186	Light yellow	93

General synthesis of 1,3-oxazepine, 7.4-dione, MA (3-6) derivatives

(0.002 mol) of prepared Schiff bases (1,2) MA had mixed in 10 ml of dioxane with maleic anhydride or phthalic anhydride, then the mixture had infused (6-7) hours, and the reaction had confirmed with TLC after which the mixture had cooled in An ice bath, the precipitate is filtered, dried, and recrystallized by ethanol.

Table 2
Shows some physical properties, proportions and molecular formula of the prepared MA compounds (3-6)

Comp. No.	-Ar	M.Wt. (g/mol)	Molecular Formula	m.p ^{O}C	Color	Yield (%)
MA3		412.24	$C_{19}H_{14}BrN_3O_3$	235	Light Brown	73
MA4		362.18	$C_{15}H_{12}BrN_3O_3$	125-123	Light Brown	71
MA5		363.37	$C_{20}H_{17}N_3O_4$	201	Light Orange	69
MA6		313.31	$C_{16}H_{15}N_3O_4$	78	Brown	74

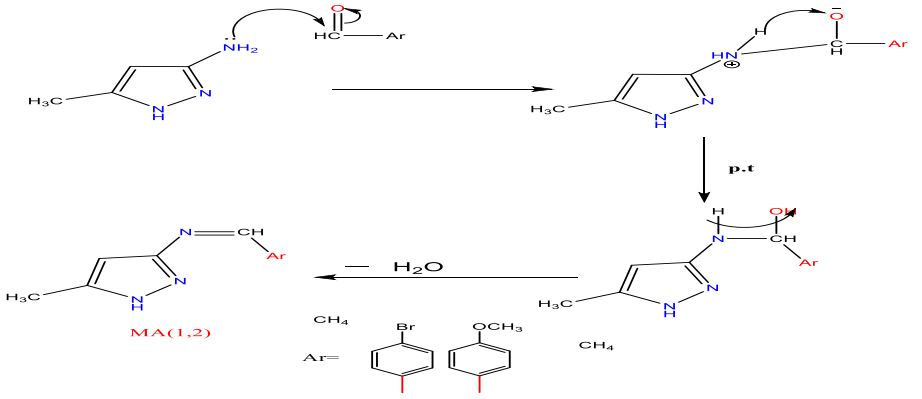
Test for inhibition of cancerous cells and evaluation of the toxicity of compounds on healthy cells

The bioactivity had examined using an MTT dye sensitivity test on breast cancer MDA, healthy cells, and reference. A culture medium containing RPMI 1640 had prepared as a nutrient medium by adding 200 µ ml of cancer isolates to each hole in a plate containing 21 holes with a capacity of 400 µ ml. Each hole has a different concentration of seven halves of concentrations

(500,250,125,62.5,31.5,15.5,7.5) mL/mcg. Three single concentration tests had performed to ensure the accuracy of the results on the same plate. The plate was left in the incubator at 36.8 °C for 24 h, then the holes had washed with phosphate-buffered saline (PBS), and then MTT dye had added to the adhesive cells remaining in each hole. Leave for 3 hours in the incubator at a temperature of 36.8 °C. Colorimetric sensitivity had read using an ELISA device at wavelength 620. The test was performed under the same conditions as before on healthy cells.

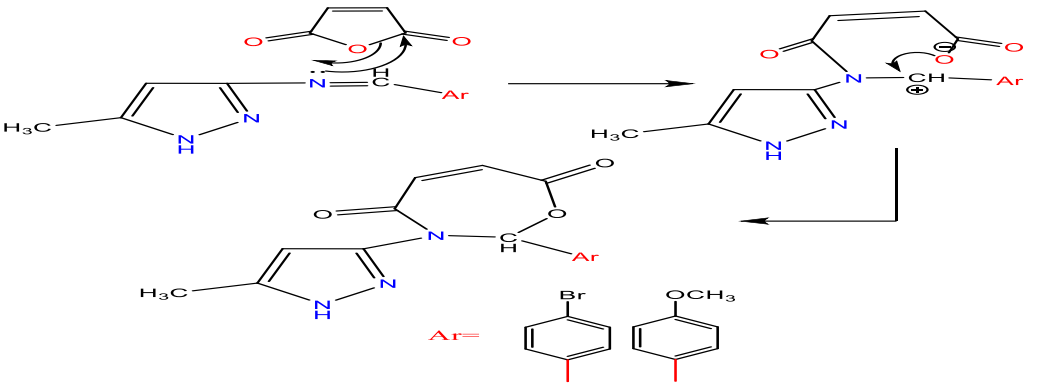
Results and Discussion

Schiff bases MA (1-2) had prepared according to condensation amine pyrazole with substituted benzaldehyde in acid catalysts, as nucleophilic addition then elimination molecular of water, as in Scheme 2 illustrates the proposed general mechanism:



Scheme 2. General mechanical to synthesis MA1 and MA2

The two Schiff bases MA1 and MA2 had confirmed by infrared spectrophotometry, and the spectrum showed the expected absorption bands where the stretching bands of the amine group and the carbonyl group of aldehydes had disappeared, and the aromatic CH stretching band appeared at (3124-3030) cm-1, the stretching band of aliphatic CH At (2933-2921) cm-1 and a medium-strength bundle belonging to the azomethine splint bundle at (1608-1593) cm-1. Figures (1,2) indicate the infrared spectrum. The chemical formula of MA2 had identified by 1HNMR spectroscopy. Where a single signal of Hb, Ha appeared in the spectrum at (2.21-3.87) ppm belonging to the methyl groups, and the signal of the Hc pyrazole ring proton at (6.25) ppm. The signals of the 2He and 2Hd phenyl ring protons appeared at (7.15–7.12) (7.89–7.86) ppm, respectively. A single sign of the azomethine proton appeared at (9.87) ppm, and the amine proton signal appeared at (11.25) ppm. The solvent signal appeared as in Figure (3). 4-Oxazepine rings MA (3-6) had prepared by ring-closing reaction of Schiff bases MA (1-2) with maleic or phthalic anhydride. Where the addition (2 + 5) takes place according to the following mechanics to form a heptagonal ring, as in Scheme 3:



Scheme 3. A fine mechanical structure of the oxazepine MA ring (3-6)

Table 3
Of absorption packages for MA compounds (3-6)

Comp. No.	IR (KBr) cm^{-1} V(N-H)	ν (C-H) Olefin. , Arom. &	ν (C=O)	ν(C=C) Arom.	ν (C-O)
MA3	3230	3064	1787	1595	1402
		2854	1724	1577	1253
MA4	3298	3100	1737	1598	1398
		2926	1720	1487	1263
MA5	3201	3010	1722	1589	1253
		2858	1629	1544	1213
MA6	3350	3020	1720	1544	1350
		2962	1623	1512	1301

The prepared compounds had confirmed by infrared spectrometry, and the spectrum showed the expected absorption bands where the azomethine band disappeared and the aromatic CH stretching band appeared at (3124-3030) cm-1, the aliphatic CH stretching bands at (2947-2887) cm-Table (3) Vehicle absorption packages. Figures (4,5 and 6) refer to the infrared spectrum of MA (3-6) compounds. The compound MA4 had identified by 1HNMR spectroscopy. The Ha signal of the methyl group appeared to overlap with the solvent at ppm (3.57). The Hb proton signal appeared as a singlet at ppm (6.28) belonging to the pyrazole ring. The Hc and Hd oxazepine ring appeared doublet at (6.44), (688-6.85), respectively. The signal of the phenyl ring protons, Hf, and He, appeared doublet at (7.37-7.35) and (7.09-7.07) ppm, respectively. The Hg proton of the oxazepine ring appeared with a single signal at (10.38) ppm. The N-H proton appeared at (13.08) ppm, as in Figure (7). The MA5 compound had identified by 1HNMR spectroscopy. The signal of protons 3Ha and 3Hb appeared at (3.57) (2.67) ppm, respectively, referring to the methyl groups. A singlet of Hc proton appeared at (6.82) ppm, referring to the pyrazole ring proton. The 2He.2Hd signal appeared at ppm (7.58-7.57) (7.15-7.13), respectively referring to the phenyl ring. The fused benzene ring signals showed a 3Hf triple signal and Hh single signal at (9.88)

(9.89-9.86) ppm, respectively. The Hg proton of the oxazepine ring appeared at 10.23 ppm. The solvent DMSO signal appeared at 2.51 ppm and the signal of the water at 3.36 ppm, as shown in Figure (8).

Vitality assessment of compound MA5

The biological activity of the MA5 compound had evaluated against two types of cells, the first cancer cells, which are MDA breast cancer cells, and the second normal healthy cells Ref, for 24 hours, using seven half concentrations (500,250,125,62.5,31.5,15.5,7.5) mcg/ mL, and comparing the results effect of the compound by MTT dye chromatography absorption spectrometry test. Where it showed that the percentage of live cells of the cancerous bio-line MDA cells at (32.89-71.51) %, while the percentage of the normal healthy cell line Ref showed a growth rate of (25-63) %, as shown in Table (3), and Fig. (9) represents the relationship of the biological activity of cell growth between cancer cells and healthy cells. These results indicate the possibility of using low concentrations as an antidote for breast cancer, as it is considered effective on cancer cells and has little toxicity on normal cells, and Figure (11 and 10) shows the effect of the compound MA5 on healthy and cancerous cells.

Table 3
Effect of MA5 on the growth of MDA cancer cells and healthy cells REF

Con. µg.mL^{-1}	Complex- MA1			
	Cancer line cells of MDA		Normal line cells of REF	
	Mean	SD	Mean	SD
7.5	71.51	0. 057	63	0.039
15.5	44.67	0. 049	60	0.058
31.25	33.83	0. 047	57	0.05
62.5	31.21	0. 030	51	0.032
125	32.33	0. 066	33	0.025
250	33.27	0.017	29	0.032
500	32.89	0.024	25	0.015

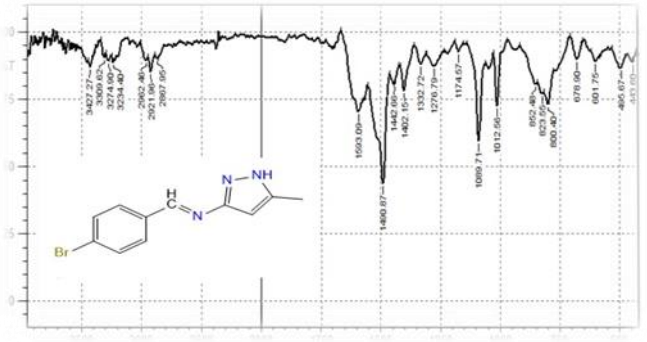


Figure 1. FTIR spectrum of compound MA1

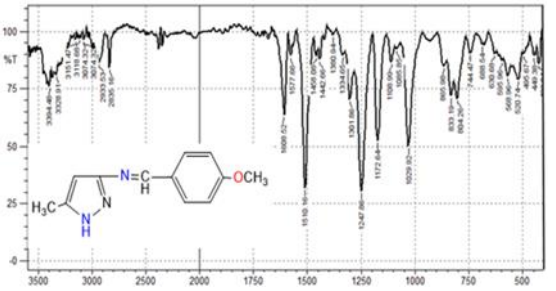


Figure 2. FTIR spectrum of compound MA2

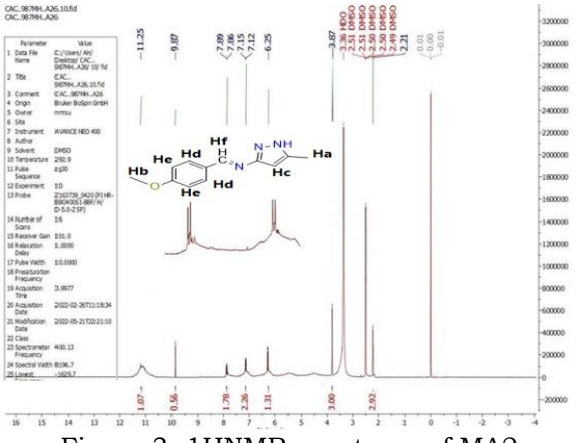


Figure 3. 1HNMR spectrum of MA2

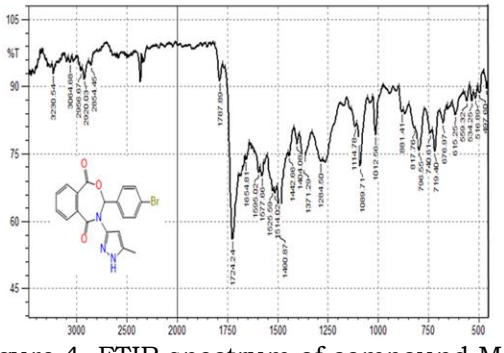


Figure 4. FTIR spectrum of compound MA3

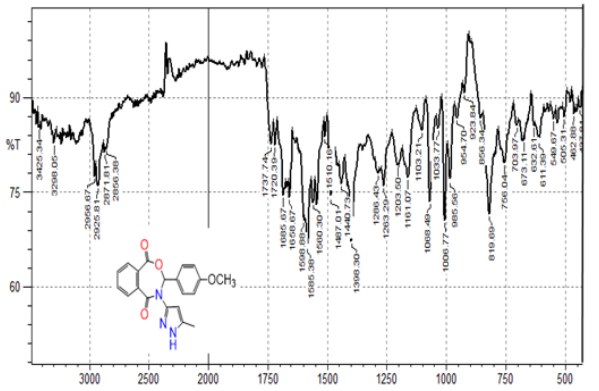


Figure 5. FTIR spectrum of compound MA5

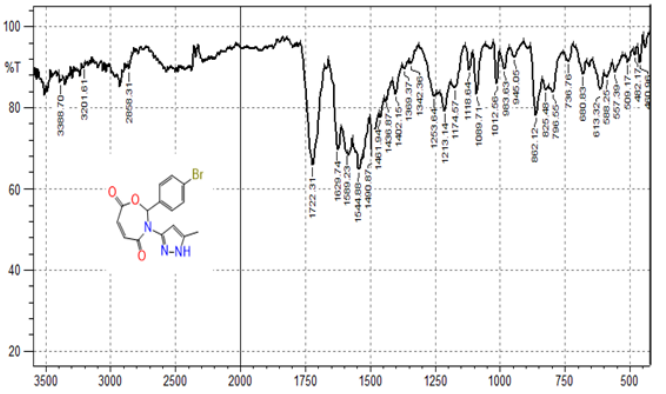


Figure 6. FTIR spectrum of compound MA4

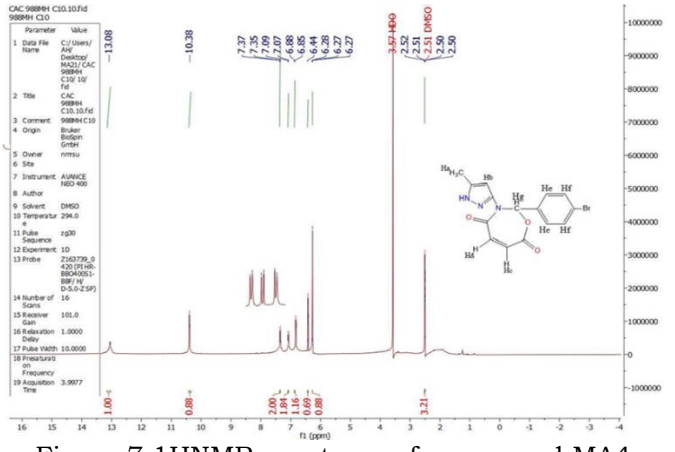


Figure 7.1HNMR spectrum of compound MA4

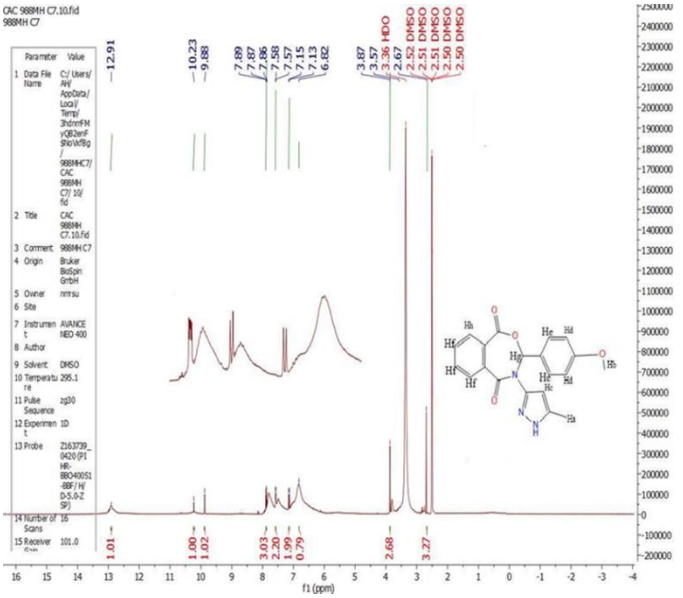


Figure 8. 1HNMR spectrum of MA5

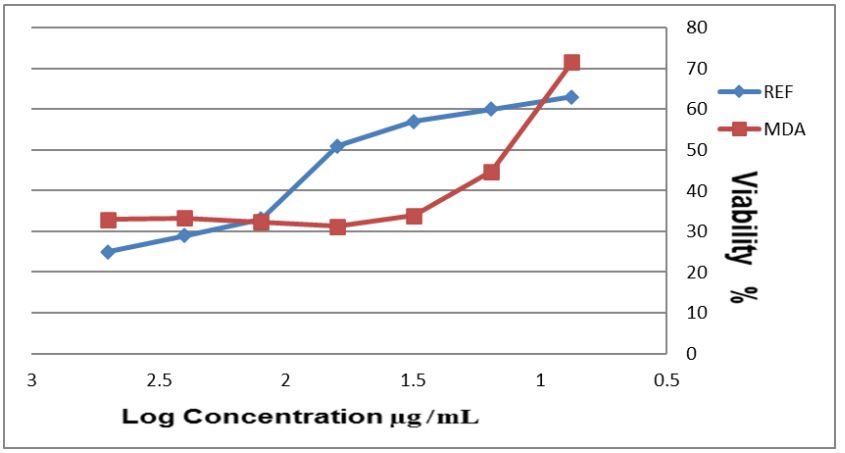


Figure 9. Represents the relationship of the biological activity of cell growth between cancer cells and healthy cells

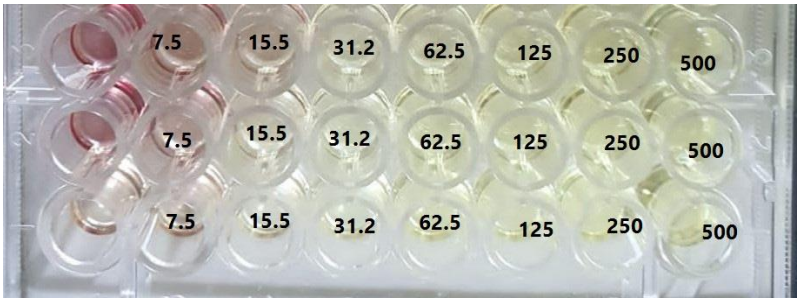


Figure 10. Effect of MA5 on the MDA cancer cell line

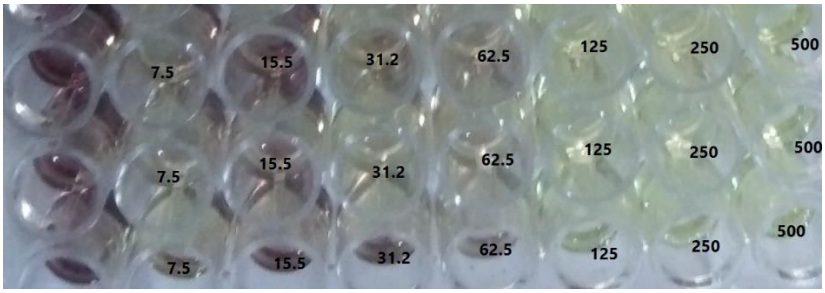


Figure 11. Effect of MA5 on healthy cell line REF

Conclusions

Oxazepine compounds containing the pyrazole ring and the corresponding Schiff bases compounds obtained with good product ratios showed high toxicity against cancer cells at high concentrations and high toxicity against healthy cells, which indicates good preliminary results as anticancer in future breast cancer cells using low concentrations.

Acknowledgments

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