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Synthesis, Antiproliferative, and Cytotoxic Effect of Two New Hydantoin Derivatives on Growth of Mouse Mammary Adenocarcinoma Cell Line (AMN3) *(In vitro)*

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Abstract

The present paper included a study of the inhibitory effect of two new hydantoin derivatives 3-((1-(4-methoxyphenyl) ethylidene)amino)-2-thioxoimidazolidin-4-one(3-3) and 3- (4-(dimethylamino)benzylidene)amino)-2-thioxoimidazolidin-4-one(4-4) as a standard anticancer on the inhibition of the growth of cancer cell line mouse mammary adenocarcinoma (AMN3) (In vitro). Statistically, have shown significant differences of a probability level ($p<0.05$), it was synthesize it from easily accessible starting materials were synthesized by two steps the first involves the reaction of carbonyl compounds with thiosemicarbazide, the second step, includes the recyclaziton of the product with chloroethylacetate in the presence of sodium acetate in absolute ethanol.

Then tested to study the effect of hydantoin derivatives compounds (3-3and 4-4) cellular toxicity and antiproliferative against mouse mammary adenocarcinoma (AMN3) cancer cell line with different concentrations (6.25 to100 µg / ml), the results showed that the new hydantion derivatives compound 3-3 showed the highest inhibition rate when the concentration was 100 µg / ml, while the compound 4-4 showed lowest inhibition rate at the same concentration, the following inhibition percentage 49.88%, 46.95 % respectively . The results showed of the inhibition rate at 6.25µg/ml that the compound (3-3) has the highest percentage of inhibition (43.82%) while the compound (4-4) has lowest inhibition percentage (34.43%). The other concentration showed different inhibition rate. Also, values of inhibition concentration of (IC50) were extracted. It has been found that the best activity was for (3-3) compound. Moreover, lot many things to be explored about these compounds. This review highlights the important things about the potential role, some chemical reactions and biological activities of hydantoin.

Key words: - hydantoin derivatives, cancer cells line, antiproliferative, cytotoxic.

INTRODUCTION

Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lump, but may be distributed diffusely [1,2] .

Cancer constitutes an enormous burden on society in more and less economically developed countries alike. The occurrence of cancer is increasing because of the growth and aging of the population, as well as an increasing prevalence of established risk factors such as smoking, overweight, physical inactivity, and changing reproductive patterns associated with urbanization and economic development [3, 4, 5].

Cancer cell lines serve as extremely useful *in vitro* models for a specific tumor type to study cancer biology and treatment response. Early collections of cancer cell lines included the NCI-60 cancer cell line panel, probably the most widely used model for studying cancer biology and the role of somatic alteration in drug response [6], as well as cancer-specific collections such as the breast cancer cell line panel and colorectal cancer (CRC) cell line panel. Many recent publications also included additional large-scale collections of various cancer cell lines with extensive genomic characterization and drug cytotoxicity profiles [7, 8].

Hydantoin derivatives possess a variety of biochemical and pharmacological properties and are used to treat many human diseases. They possess good anticonvulsant properties and depending on the nature of substitution on the hydantoin ring, a wide range of other pharmacological properties, including fungicidal, herbicidal, antitumor, anti-inflammatory, anti-HIV, hypolipidemic, antiarrhythmic and antihypertensive activities[9,10]. Although hydantoin compounds are studied extensively, there are not many studies about their anticancer properties. Recently, the cytotoxic activity of spirohydantoin derivatives was tested in ovarian and breast cancer cells [11]. It has been shown that a spirohydantoin derivative induces growth inhibition and apoptosis in leukemic cells [12]. Former studies demonstrated that 5-arylidene-2-thiohydantoins have *in vitro* antimycobacterial activity [13].

MATERIALS AND METHODS

Synthesis of Hydantoin derivatives

The precursors have been prepared according to [14, 15].

The compounds were prepared according to the literature procedure. Generally, the preparation of the new hydantoin derivatives can be demonstrated as follows:

(a)Synthesis of 2-arylidenehydrazinecarbothioamide compounds (A3, A4)

We dissolved (0.01mole) of carbonyl compounds in ethanol (30 ml) and (0.01 mole) of thiosemicarbazide was added, and then refluxed for 3 hrs. The resulting mixture was transferred into crushed ice and stirred for 15 min. Then we filtered the precipitated crystalline solids, washed with water, and were recrystallized from ethanol. Table (1) shows the physical properties for these compounds.

Table (1): Physical properties, and yield percentage of compounds (A3, A4).

Compound Symbol	Molecular Formula	Compound name	M.P ^{O}C	Color	Yield%
A3	C10H13N3OS	2-(1-(4-methoxyphenyl)ethylidene)hydrazine-1-carbothioamide	172-175	White	94
A4	C10H14N4S	2-(4-(dimethylamino)benzylidene)hydrazine-1-carbothioamide	220-222	Yellow	93

(b) Synthesis of 3-arylidene-amine-2-thioxoimidazolidin-4-one (3-3, 4-4). We dissolved (0.01mole) of compounds A3, A4 and (0.01 mole) of chloroethylacetate in 30 ml ethanol to obtain a mixture. The mixtures were stirred for a few minutes, and then sodium acetate (0.02mole) was added to the mixture. The mixtures were refluxed for 6hrs. After cooling, the precipitates found which are filtered off and recrystallization from dioxane. Table (2) showed the physical properties.

Table (2): Physical properties, and yield percentage of (3-3, 4-4)

Compound Symbol	Molecular Formula	Compound name	M.P ^{o}C	Color	Yield%
3-3	C12H13N3O2S	3-((1-(4-methoxyphenyl)ethylidene)amino)-2-thioxoimidazolidin-4-one	195-198	White	92
4-4	C12H14N4OS	3-((4-(dimethylamino)benzylidene)amino)-2-thioxoimidazolidin-4-one	256-258	Yellow	94

Cell line

Maintenance of cell cultures

AMN3 cell line(mouse mammary adenocarcinoma)[16] was obtained from the Iraq biotech Cell Bank Unit and maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C [17].

Combination Cytotoxicity and Antiproliferation Assays

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1×10^4cells/well. After 24 hrs or a confluent monolayer was achieved, cells were treated with tested compound. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT (and incubating the cells for 1.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [18]. The absorbency was determined on a microplate reader at 492 nm (test wavelength); the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was

calculated as the following equation:-

% **Cell viability** = (Absorbance of treated cell / Absorbance of non-treated cell) x 100

% **Cytotoxicity** = 100 – cell viability

RESULT AND DISCUSSION

Synthesis and Characterization of the New Hydantion Derivatives (3-3, 4-4and 5-5)

Compounds were synthesized by two steps the first involves the reaction of carbonyl compounds (aromatic aldehyde or keton) with thiosemicarbazide, the second step, includes the recyclaziton of the product with chloroethylacetate in the presence of sodium acetate in absolute ethanol, as shown in the following Fig. (1).

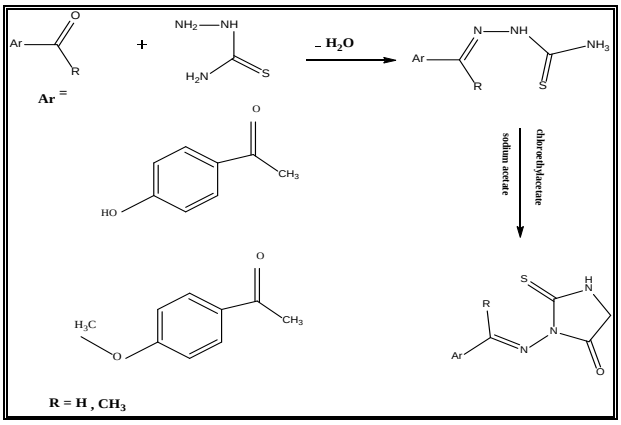


Figure (1): Synthetic diagram for compound (3-3and 4-4).

FTIR Spectra of New Hydantoin Derivatives

FTIR Spectrum of A3 and A4

FTIR spectrum of compound [A3, A4], Fig (2, 3), displays 2 bands at (3398cm -1) and (3377cm-1), represent υas and υs (NH2) vibrations [19, 20]. The spectrum similarly displays weak band at (3159 cm -1) was allocated to υ(NH). Whereas band appeared at (1591 cm -1) denote υ(C=N) of Schiff's base, bands at (1541, 1492cm -1) due to aromatic (C=C). The strong band noticed at (1446, 1375 cm -1), states to nitro group asymmetric and symmetric and lastly the absorption band of υ (C=S) seems at (1176cm -1)[20,21].While FTIR spectrum of compound [A4], Fig (3-3), displays 2 bands located at (3466 cm -1) and (3367 cm -1), denote υ as and υs (NH2) vibrations [20,22] and (3329 cm -1)for υas(NH) , the spectrum moreover displays band at (3248 cm -1) was allocated to υ (C-H) of vibrations of aromatic ring, bands at (3153 cm-1) and (3138 cm-1) because of methyl group, spectrum displays the appearing of the stretching vibration of υ (C=N) at (1583 cm -1), moreover spectrum displays other bands at (1585,1500 cm -1) because of aromatic (C=C) [20,23], and lastly the absorption band of υ (C=S) appears at (1089 cm -1).

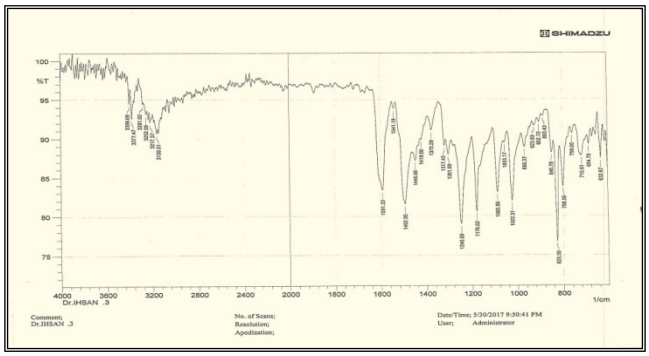


Figure (2): FTIR spectrum of (A3)

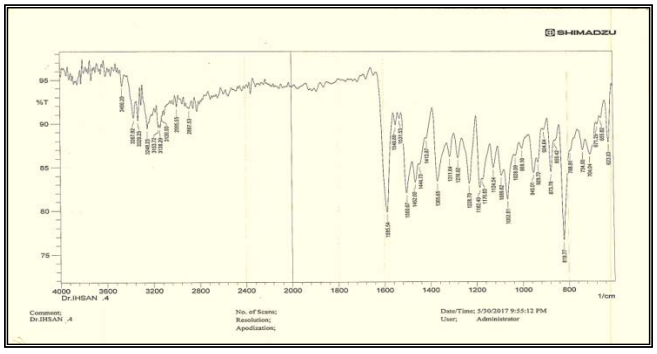


Figure (3): FTIR spectrum of (A4).

FTIR Spectrum of 3-3 and 4-4

FTIR spectrum of [3-3,4-4], Fig (4,5), displays band at (3282 cm -1), because of υ(NH) vibration [20,24], bands at (3188 cm-1) was allocated to υ(C-H) vibrations of aromatic ring, whereas the band at (3117 cm -1) accredited to symmetric (CH2) group. Spectrum moreover displays new distinctive band at (1714 cm -1), which is accredited to υ(C=O) vibration, band at (1620cm -1) accredited to υ(C=N) vibration of Schiff's base, whereas the absorption bands of υ(C=C) aromatic was seemed at (1593, 1570cm -1) [25,26].The strong band noticed at (1390, 1332cm -1), states to asymmetric and symmetric of nitro group, and lastly the absorption band of υ (C=S) seemed at (1085cm -1).

While FTIR spectrum of (4-4), Fig (4,5), shown weak band at (3182cm -1) which accredited to υ (NH) vibrations[27] ,weak band at (3030 cm -1) was allocated to υ (C-H) vibrations of aromatic ring, whereas the vibrations of υ (CH) aliphatic seems at

(2983 cm -1) and (2958 cm -1). The new band at (1708 cm -1), accredited to υ (C=O) vibration of carbonyl [25], whereas the υ (C=N) vibration seems as a strong band at (1635cm -1). Three bands noticed at (1600cm -1), (1531 cm -1) and (1519 cm -1), were allocated to υ (C=C) vibrations of benzene ring.
The vibration of υ (C=S) seemed at (1053cm -1 [26]. .

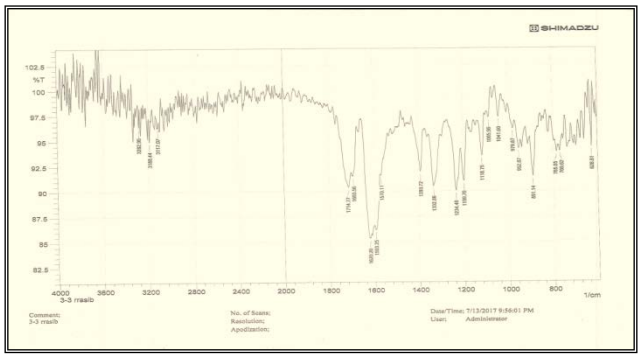


Figure (4): FTIR spectrum of (3-3).

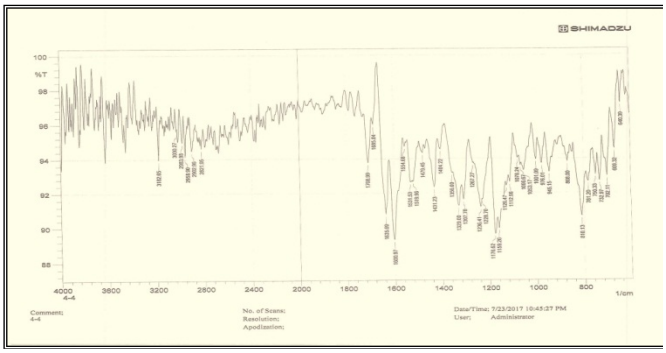
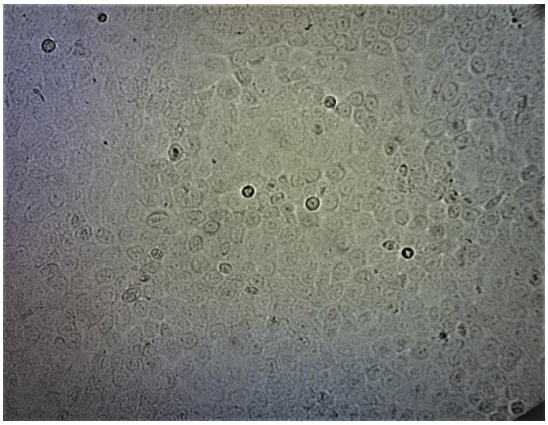


Figure (5): FTIR spectrum of (4-4).

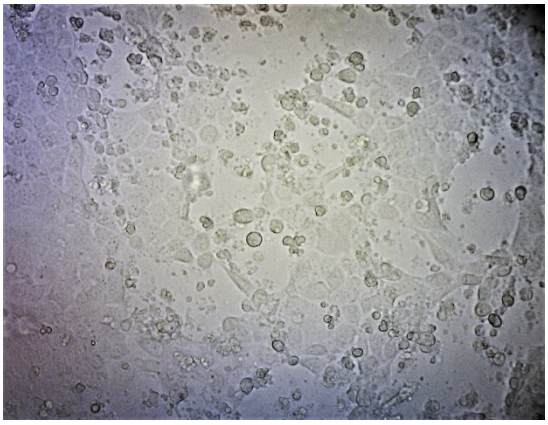
Antiproliferative and cytotoxic effects of new hydantoin derivatives on AMN3 cell line (mouse mammary adenocarcinoma)

Comparison between the control and new hydantion derivatives

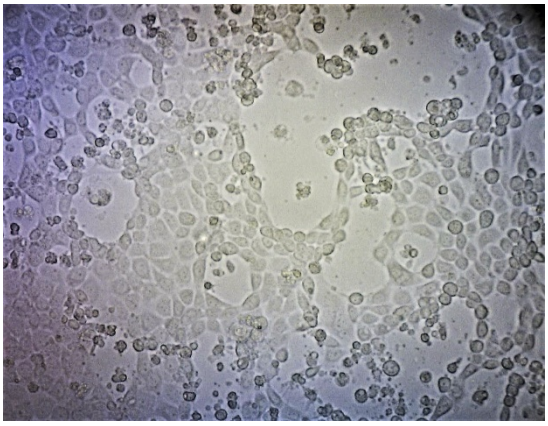
Statistical analysis showed a significant difference between the five new hydantion derivatives 3-3, 4-4 and 5-5) and the control in the terms of inhibition of mouse mammary adenocarcinoma cell line (AMN3) at (P< 0.05).All the new hydantion derivatives inhibited mouse mammary adenocarcinoma cell line (AMN3) in different percentages. The dose response effect of each new hydantion derivatives on mouse mammary adenocarcinoma cell line (AMN3) Fig. (6).



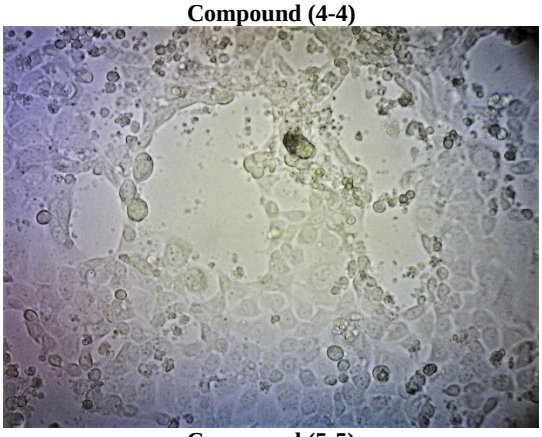
Untreated Amn3 control



Compound (3-3)



Compound (4-4)



Compound (5-5)

Figure (6): Antiproliferative and cytotoxic effects of new hydantoin derivatives against in vitro (AMN3) cell line.

3.3 Dose response curve of new hydantion derivatives (3-3and 4-4) on mouse mammary adenocarcinoma cell line (AMN3**):**

The antiproliferative and cytotoxic effects of new hydantion derivatives (3-3and 4-4) were studied against mouse mammary adenocarcinoma cell line (AMN3). This cell line was exposed to concentrations of these new hydantion derivatives (3-3, 4-4) ranged from (6.25 to100 µg/ml) for 72 hrs only. The optical density was measured under wavelength 570 nm with ELISA reader after their staining with MTT stain. The results showed that these new hydantion derivatives (3-3, 4-4) led to decrease the growth of mouse mammary adenocarcinoma cell line (AMN3) significantly as compared to untreated control cells as estimated by comparison of the optical density of the treated and control cell lines. The results showed of the inhibition rate at 6.25µg/ml that the compound (3-3) has the highest percentage of inhibition

(43.82%) while the compound (4-4) has lowest inhibition percentage (34.43%). When the concentration of new hydantion derivatives (3-3, 4-4) increased to 12.5µg/ml, the inhibition rates for these concentrations increase and the compound (3-3) stay at greatest inhibition percentage at 25µg/ml, the new hydantion derivatives (3-3, and 4-4) have the following inhibition percentage 46.90%, 45.35% respectively. Although at 50µg/ml compounds (3-3, 4-4) showed varying in the percentage of inhibition arranged as follows : 47.59 %, 46.17 %, respectively. Whereas at 100µg/ml the new hydantion derivatives (3-3and 4-4) have the following inhibition percentage 49.88 %, 46.95 %, respectively.

This study demonstrated that new hydantion derivatives (3-3, 4-4) have significant antiproliferative and cytotoxic effect and its inhibition effect was concentration manner as shown in Tables (3, 4) and Fig. (7, 8) . This may be due to its activity as potent inhibitor of Na^+/H^+ antiporter [27], this ubiquitous transport system participates in many important cellular functions, such as regulation of intracellular pH, trans cellular movement of acid and base equivalents, cell growth and proliferation, and regulation of cell volume. The mouse mammary adenocarcinoma cell line is a non-polarized epithelial cell which is known to express Na^+/H^+ exchanger activity[28,29] . The inhibition caused by new hydantion derivatives (3-3, 4-4) on mouse mammary adenocarcinoma cell line is mediated not through their receptors but through direct interaction with the Na+/H+ exchanger and bonded to nucleic acids in DNA. Determination of the interactions between new hydantion derivatives (3-3, 4-4) and DNA should be elucidated to help explain the mechanisms of apoptotic events and drug potential of these compounds [29, 30].

Table (3): Serial concentrations and their Cytotoxic for (3-3) compound

Concentration(µg/ml)	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL
Mean	49.88	47.59	46.90	45.79	43.82
P value	0.0016	0.0055	0.0014	0.0030	0.0026
Significant (alpha=0.05)	Yes	Yes	Yes	Yes	Yes

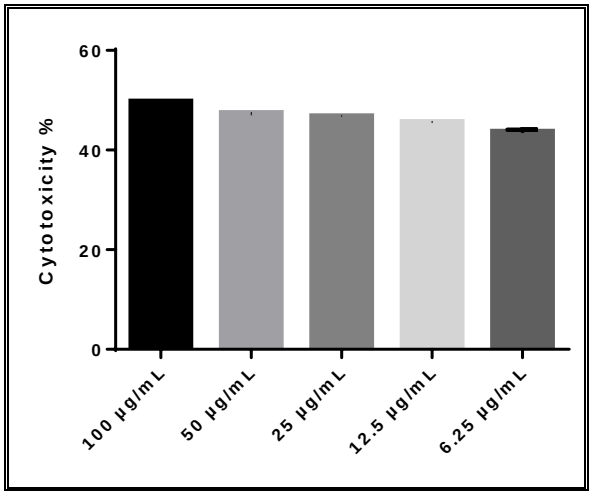


Figure (7): Cytotoxic effect of compound (3-3) on AMN3 cell line.

Table (4): Serial concentrations and their Cytotoxic for (4-4) compound

Concentration(µg/ml)	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL
Mean	46.95	46.17	45.35	43.25	34.43
P value	0.0007	0.0023	0.0049	0.0037	0.0105
Significant (alpha=0.05)	Yes	Yes	Yes	Yes	Yes

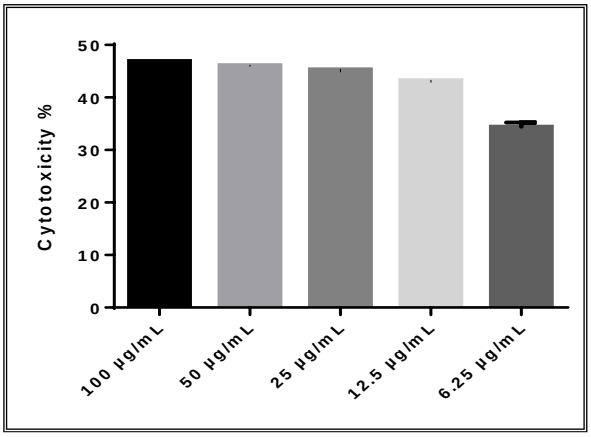
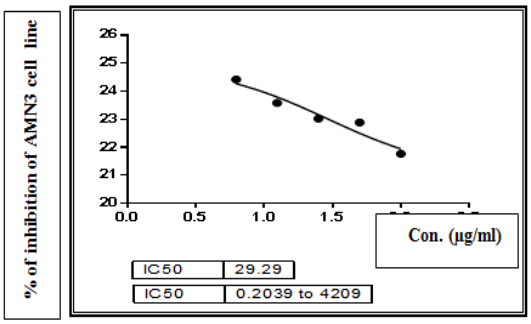


Figure (8): Cytotoxic effect of compound (4-4) on AMN3 cell line.

The IC_{50} (Dose concentration that inhibited cell growth by 50%) for new hydantion derivatives (3-3, 4-4) was determined from the logarithmic equation that is shown in figure (9), (10). Where Y equal the inhibition percentage and X equal the concentration.



Figure(9):Dose response curve of compound (3-3) against AMN3 cell line

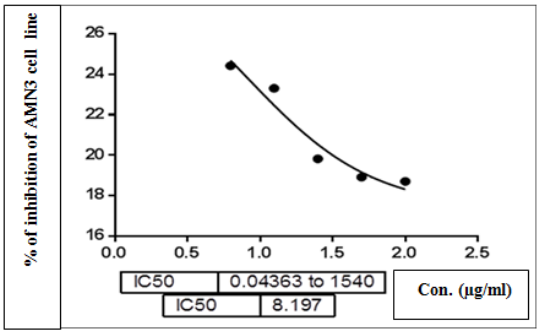


Figure (10): Dose response curve of compound (4-4) against AMN3 cell line.

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