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SYNTHESIS, CHARACTERIZATION AND ANTICANCER ACTIVITY OF NOVEL MANNICH BASES

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ABSTRACT : Mannich bases, also known as beta-amino ketone carrying compounds, are the end products of the Mannich reaction. The Mannich reaction is a nucleophilic addition reaction that forms carbon-carbon bonds and is used to synthesize a wide range of natural products, medicines and other compounds. For the production of nitrogen-containing molecules, the Mannich reaction is crucial. This study including synthesis, characterisation, biological activity and anticancer estimate of a novel series of Mannich base derivatives including the 1,3,4-oxadiazole system are all part of this research. The structures of the newly synthesized compounds have been confirmed using FTIR and ^{1}HNMR spectrum data obtained from the interaction of 1,3,4-oxadiazole-2-thiol derivatives with different secondary amines and formaldehyde. The biological activity of Mannich bases is investigated, with a focus on the relationship between structure and biological activity. Mannich bases are a structurally heterogeneous class of chemical compounds that are generated from various substrates through the introduction of an aminomethyl function via the Mannich reaction. In vitro,an anticancer activity against human cancer cell lines were investigated for these compounds: breast cancer (MCF7), (AMJ13) and FER cells. Compounds 3a showed anticancer activity.

Key words : Mannich bases, oxadiazole, biological activity, anticancer.

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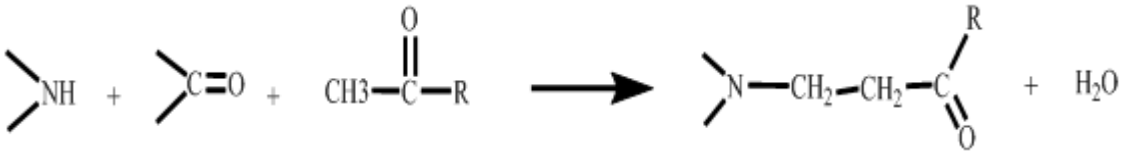
INTRODUCTION

Cancer is a severe disease defined by uncontrolled cell development, which results in the inclusion of surrounding tissue and, in many cases, the spread of the illness throughout the body. This disease considered as a group of diseases described by the uncontrolled growth, in addition to the spread of untypical cells. So, for the case of the not controlled spread, it can result in final failure and death (Rahman, 2013). Many studies have been conducted on cancer, but no medication molecules have been identified that can entirely treat it, and it continues to cause significant rates of morbidity and mortality (Jemal *et al*, 2008). Compounds with heterocyclic ring structures are extremely useful in both medicine and industry. Five-membered ring heterocycles with two members, for example, there are two of each carbon atoms, nitrogen atoms and finally one oxygen atom in each molecule that recognized as oxadiazoles (Boström *et al*, 2012). Mannich bases, also known as beta-amino ketone-carrying compounds, which consider the products of the Mannich reaction (Belinelo *et al*, 2002 and Saba *et al*, 2021). The Mannich reaction is a nucleophilic addition process in which a is condensed with active hydrogen (ketones, nitrolycans, b-ketoster), formaldehyde (aldehyde of any kind) and an amine (primary or secondary) (Scheme 1) (Joshi *et al*, 2004).

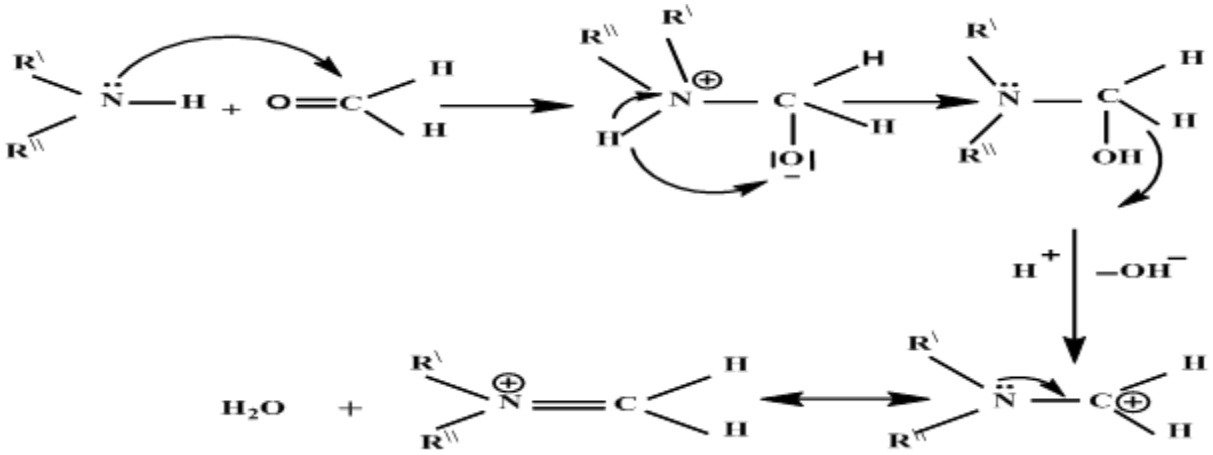
The condensation process takes place in two stages : 1) addition of a nucleophilic by the amine to the carbonyl group to form the electrophilic Mannich base (Scheme 2) (Esthar *et al*, 2004).

Alkylation of amines with a hydrogen-containing compound (Scheme 3) (Notz *et al*, 2003).

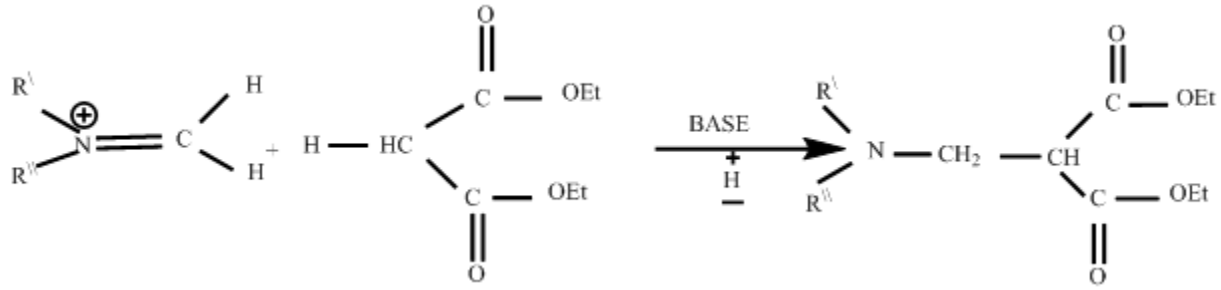
Although, primary amines and even ammonia may be employed as an amine reagent, secondary aliphatic amines (R_2NH) are more commonly encountered as an amine reagent in the Mannich reactions (Roman *et al*, 2015). Mannich bases are derivatives of substrates that are generated by substituting an amino alkyl moiety (Roman *et al*, 2015). Heterocycles Mannich bases are noteworthy compounds with a variety of therapeutic characteristics, including antibacterial (Ashok *et al*, 2007), anticancer (Ivanova *et al*, 2007), analgesic (Malinka *et*



Scheme 1 : Mannich Reaction.



Scheme 2 : Reaction between amine and formaldehyde.



Scheme 3 : Alkylation of amine.

al, 2005), anti-HIV (Sriram *et al*, 2009).

According to the above facts, as an effort to obtain a novel potent antitumor agents, this work explains the synthesis of new series of 5-(4-((3,4-dimethoxy benzylidene) amino) phenyl)-1,3,4-oxadiazole-2(3H)-thione and Mannich bases of 5-(4-amino phenyl)-1,3,4-oxadiazole-2(3H) thione. Furthermore, the anti-tumor characterization assessment to these compounds versus human tumor breast" cancers cell lines (AMJ13, FER cells and MCF7).

MATERIALS AND METHODS

Chemicals

Open-ended capillary tubes were used to calculate melting points on an electronic melting point apparatus**.** The synthesized compounds structure was elucidated by FT-IR spectra in ν_{max} (cm^{-1}) on FT-IR (Shizmadu-8400 series) using KBR disc technique. ^{1}HNMR spectra in δ units (ppm) relative to an internal standard of tetra methyl silane on ^{1}HNMR (Brucker 400 MHz) in DMSO-d6.

Synthesis of 5 (4-amino phenyl)1,3,4 oxadiazole-2thiol (1)

The compound 4-amino benzohydrazide was dissolved (0.006 moles,1g) in 10 ml of ethanol and added to a (0.5 g) of KOH doped in solution. dissolved in 10 ml of water. Carbon disulfide. It was added with the reaction mixture and it was refluxed for 8 hours. The resulting residue was dissolved in water and acidified by HCL 10% (pH= 3) (Subramani *et al*, 2009).

Synthesis of 5-(4-((3,4-dimethoxybenzylidene) amino)phenyl)-1,3,4-oxadiazole-2(3H)-thione (2)

The compound (1) (0,003 moles, 0.579g) is dissolved in (15 mL) ethanol, then (0.003 moles,0.33 g) of aldehyde is added to the mixture with stirring for 10 minutes, followed by 5 drops of G.A.A after 5 minutes of reflux. The precipitated solid was filtered, dried, and distilled using ethanol recrystallization (Taher *et al*, 2001).

Synthesis of Mannich bases (3a-3c)

After dissolving (0.003 moles, 0.86g) of compound (2) in (15 ml) of absolute ethanol, add formalin (0.003

mole, 0.1ml) and then amines (0.003mols) for each type of amine used gradually to the reaction mixture with continuous stirring in an ice bath for one hour, the precipitate was left in the refrigerator for 24 hours, after which it was purified and recrystallized using absolute ethanol (Jaber *et al*, 2004).

RESULTS AND DISCUSSION

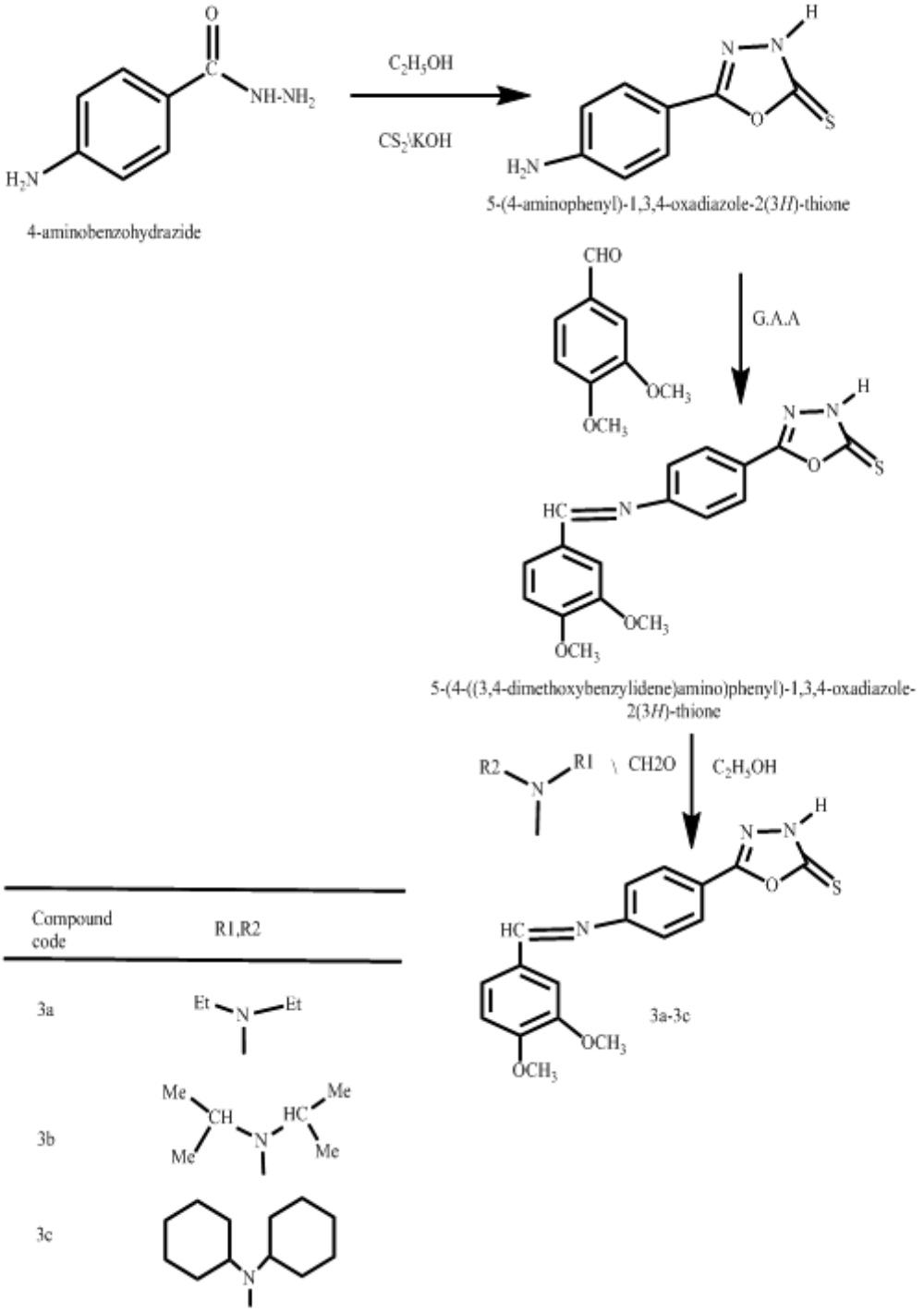
5 (4-amino phenyl)1,3,4 oxadiazole-2-thione

$C_8H_7N_3O_1S_1$; Yield: 80%; m.p: 240-242^{0}C;FT.IR (KBr, ν_{max} cm^{-1}): (3446.91-3350.64) cm^{-1} (NH_2, NH), (1606.76) cm^{-1} (C=C), (1620.26) cm^{-1}(C=N); ^{1}H NMR (δ ppm): 6.65 (s, NH2, 2H), 9.285 (s, NH, 1H), 6.524 – 7.571 (D, 4H, ArH).

5-(4-((3,4-dimethoxybenzylidene) amino) phenyl)-1,3,4-oxadiazole-2(3H)-thione

$C_{17}H_{15}O_3N_3S_1$; Yield: 81%; m.p: 228-230^{0}C; FT.IR (KBr, ν_{max} cm^{-1}):

(3242.54) cm^{-1} (NH), (1606.76) cm^{-1} (C=C),



Scheme 4 : Synthesis of Compounds 4a-4c.

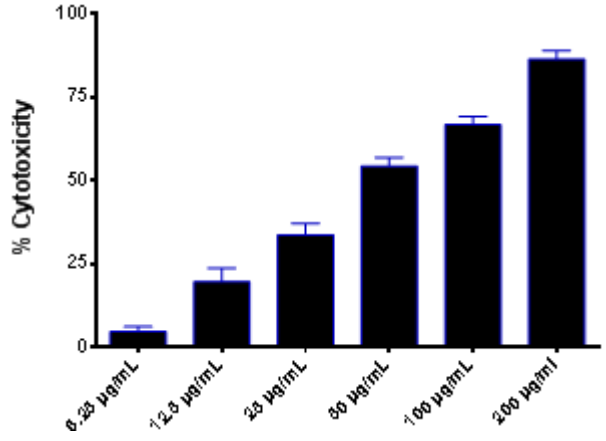


Fig. 1 : Cytotoxic effect of 3a in MCF7 cells. IC50 = 57.43 µg/ml.

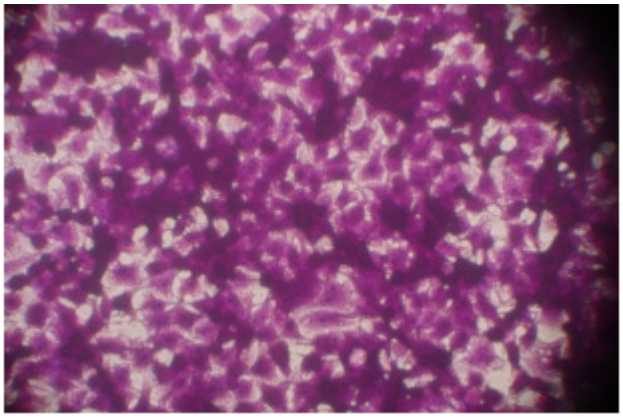


Fig. 2 : Control untreated MCF7 cells.

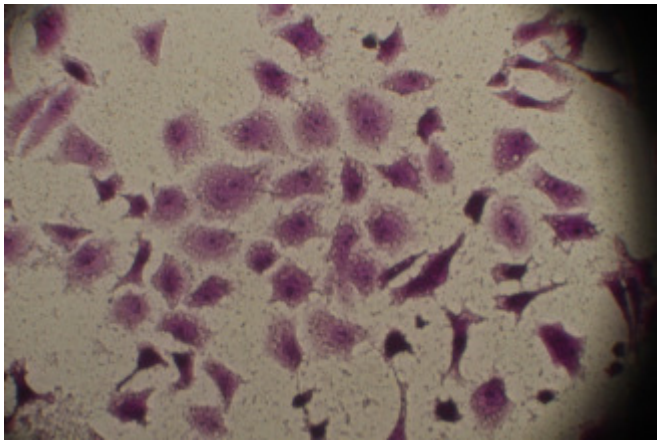


Fig. 3 : Morphological changes in MCF7 cell after treated with 3a.

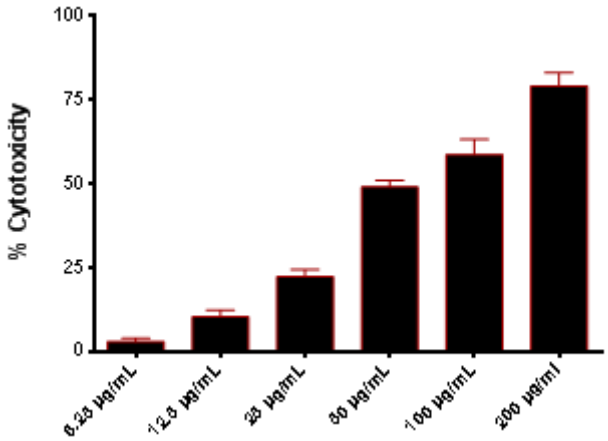


Fig. 4 : Cytotoxic effect of 3a in AMJ13 cells. IC50=62.48 µg/ml.

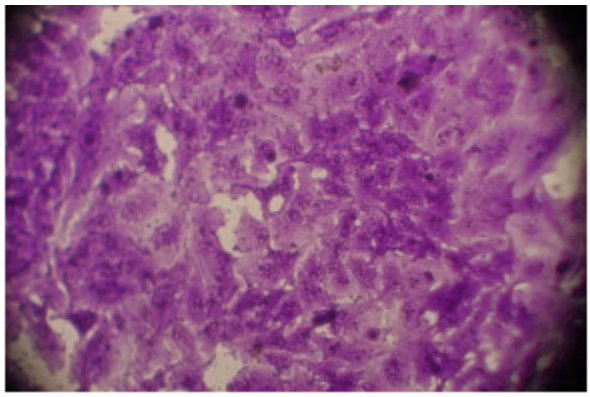


Fig. 5 : Control untreated AMJ13 cells.

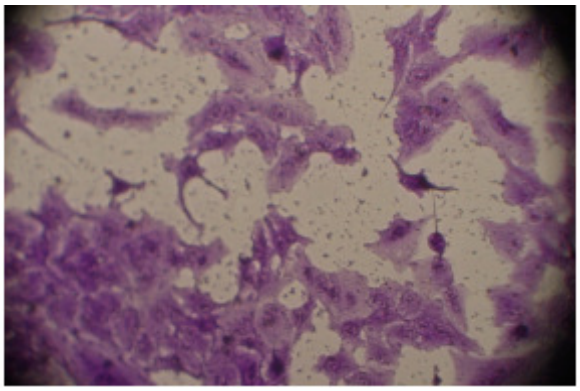


Fig. 6 : Morphological changes in AMJ13 cells after treated with 3a.

Table 1 : Antimicrobial activity-sensitivity testing of compounds.

Compound Code	Zone of inhibition(mm)				
	Antibacterial Activity				Antifungal Activity
	S. aureus	*S. epidermidis*	*E. coli*	*K. aerogenes*	*C. albicans*
3a	16	15	-	11	14
3b	13	15	-	-	-
3c	-	-	-	12	11

(1629.90) cm^{-1}(C=N); ^1H NMR (δ ppm): 9.97 (s, 1H, NH), 8.58(s, 1H, HC=N), 7.52 – 7.93 (D, 4H, ArH), 3.84 ($_{S,}$ 6H, OCH_3).

3-((diethylamino)methyl)-5-(4-((3,4-dimethoxybenzylidene) amino) phenyl)-1,3,4-oxadiazole-2(3H)-thione

$C_{22}H_{26}O_3N_4S_1$; Yield:50%; m.p: 247-249 °C; FT.IR (KBr, $\nu_{max}cm^{-1}$): (2931.90) cm^{-1}(CH), 1604.83 cm^{-1} (C=N), (1583.83) cm^{-1} (C=C),(1338.644) cm^{-1} (C-N); ^1H NMR (δ ppm): 0. 90-1.06 (t, 6H, CH_3), 1.61-1.65 (q, 2H, CH_2), 4.10 ($_S$,2H, CH_2).

3-((diisopropylamino)methyl)-5-(4-((3,4-dimethoxybenzylidene) amino) phenyl)-1,3,4-oxadiazole-2(3H)-thione

$C_{24}H_{30}O_3N_4S_1$; Yield:27%; m.p: 243-246^{0}C; FT.IR (KBr, ν_{max} cm^{-1}): (2843.17) cm^{-1}(CH), (1604.83) cm^{-1} (C=N), (1583.61) cm^{-1} (C=C), (1345) cm^{-1} (C-N); ^1H NMR (δ ppm): 0.89-1.0 (d, 6H, CH_3), 2.70(q, 1H, CH), 4.10($_S$,2H, CH_2).

3-((dicyclohexylamino) methyl)-5-(4-((3,4-dimethoxy benzylidene) amino) phenyl)-1,3,4-oxadiazole-2(3H)-thione

$C_{30}H_{38}O_3N_4S_1$; Yield:88%; m.p: 129-131^{0}C; FT.IR (KBr, ν_{max} cm^{-1}): (2935.76) cm^{-1}(CH), (1624.12) cm^{-1} (C=N), (1587.47) cm^{-1} (C=C), (1377.22) cm^{-1} (C-N) ;^1H NMR (δ ppm): 1.07-1.99 (m, 22H, cyclohexyl), 3.12(s, 2H, CH_2), 4.10 ($_S$,2H, CH_2).

Biological activity

Depending on the antimicrobial activity that was carried out on Mannich compounds (3a-3c) as shown in the Table 1. The results showed that compound 3a is more effective against anti-bacteria, and for this reason, this compound was used to reduce the toxicity of breast and ovarian cancer.

In vitro cytotoxicity assay

Cell cultures servicing

RPMI-1640 accomplished with to Fetal bovine serum about 10%, 100 unit's/mL penicillin and 100 g/mL streptomycin was used to sustain AMJ13 cells, MCF7 cells, and REF cells. Cells were passaged twice a week with Trypsin-EDTA, reseeded at 80% confluence, and incubated at 37°C (Al-Ziaydi *et al*, 2020 and Hamzah *et al*, 2020).

Cytotoxicity assays

To evaluate the cytotoxic influence of (3a), MTT assay was performed by 96-well plates (AlSalman *et al*, 2020 and Maeh *et al*, 2020). Cell line were seeded at 1 × 10^4 cells/well. After 24 hrs. a confluent monolayer was carried out, cells were processed with tested compounds at various concentration. Cell viability was determined after 72 hours of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37°C. After that, MTT solution removed and the crystals left 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. The absorbency was measured on a microplate reader at 492 nm; the assay was applied in triplicate. cell growth

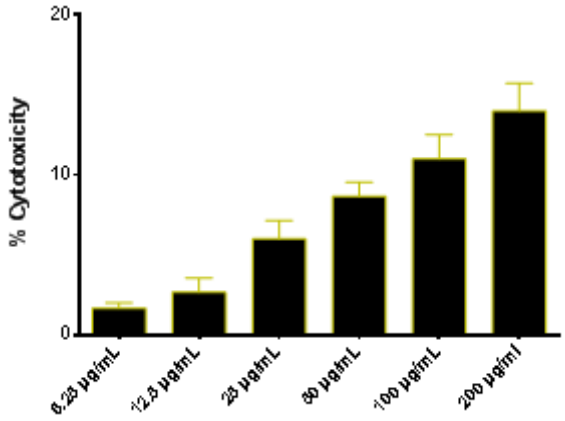


Fig. 7 : Cytotoxic effect of 3a in REF cells.

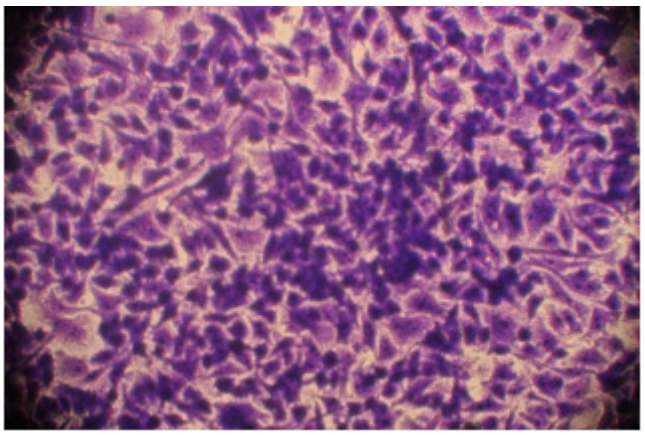


Fig. 8 : Control untreated REF cells.

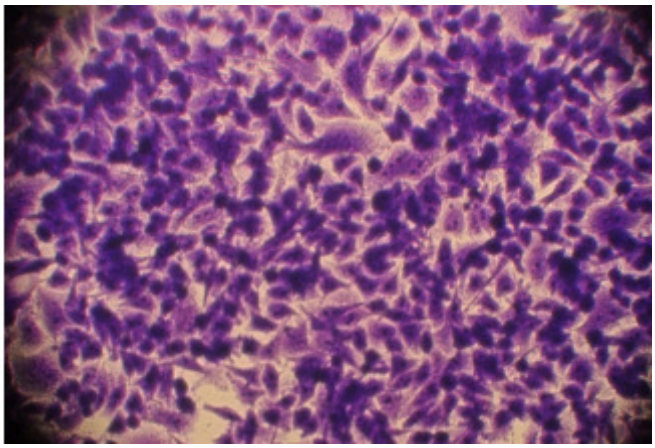


Fig. 9 : Morphological variation in REF cells after processes with 3a.

inhibition rate (cytotoxicity ratio) was calculated as the following equation (Al-Ziaydi *et al*, 2020 and Khashan *et al*, 2020).

Inhibition rate = A- B/A × 100

where, A is the control optical density and B is the samples optical density of the (Kareem *et al*, 2020 and Jabir *et al*, 2020). To visualize the cells shape using inverted microscope, cell seeded into 24-well micro-titration plates with 1×10^5 density cells mL^{-1} and incubated for 24 h at 37°C. Furthermore, cells were exposed to (3a) at IC50 for 24hr. later on, after exposure time the plates were stained with crystal violet stain and incubated at 37°C for 10–15 min (Waheeb *et al*, 2020 and Al-Shammari *et al*, 2020). Gently, the stain must have washed off with tap water until the dye was removed. Then, the cells were investigated under an inverted microscope at 40× magnification and the images were captured using the attached digital camera of the microscope (Majid *et al*, 2020 and Jabir *et al*, 2020).

Statistical analysis

Statically analysis was performed to the obtained data using an unpaired t-test with GraphPad Prism 6 (Khashan *et al*, 2020). The obtained results were presented as the mean ± SD of triplicate measurements (Sameen *et al*, 2020).

Anti-proliferative activity of Peptide against cancer cell line

The influence of cytotoxic (3a) against AMJ13, MCF7, and FER cells was investigated. The antitumor activity of the (3a) was evaluated through studying the ability to inhibit the proliferation of tested cells. The obtained results of the study showed cytotoxic activity with significant amount of (3a) against the cancer cell line, but not normal cell line as showed in Figs. 2-10. From the results, the ability of (3a) suggested to suppress the cell lines growth and this influence is dependent on the concentration. cancer cell line *in vitro* showed that the inhibition rate increased when the compound concentration.

CONCLUSION

In the present study, synthesis, characterization, anticancer have been performed on a series of the Mannich reaction was used to create a sequence 1,3,4-oxadiazole-2-thione derivatives against AMJ13, MCF7, and FER cells. Compounds 3a showed anticancer activity.

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