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Synthesis, Characterization and Cytotoxic activity of New Indole Schiff Bases, Derived From 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-Malonaldehyde with Substituted aniline

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ABSTRACT

Series of new compounds of indole Schiff base derivatives have been synthesized by reaction of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde with aniline substituted. The chemical structures of the synthesized compounds were characterized by TLC, FT-IR, ^{1}H, ^{13}C NMR and APT ^{13}C NMR.The *in vitro* anticancer activity of the new synthesized compounds tested against– AMJ breast cancer cell line. The revealed data showed that compounds have promising anticancer activity against AMJ13 cell line at low concentrations.

Keywords: Schiff bases, Aniline substituted, Aldehyde, Cytotoxic Activity.

INTRODUCTION

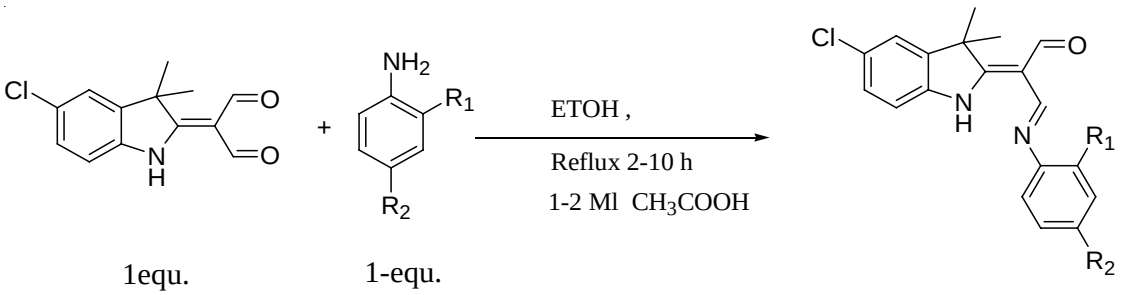
Schiff bases are important compounds in the chemistry and biochemistry fields due to their biological activities[1]. Schiff bases which are a class of compounds containing an azomethine group (-C=N-) as functional group have focus attention for a long time due to their medicinal and pharmaceutical activities[2]. Schiff bases were first reported by Hugo Schiff in 1864, they are formed by the condensation reaction of aldehydes or ketones with primary amines in the presence of acid as a catalyst [3]. They have many applications in different fields for example: antibacterial, antifungal, and antitumor activity[4].

Schiff bases obtain from different organic heterocyclic compounds chiefly those consisting of indole molecule, There is an important class of organic heterocyclic compounds because they have abroad applications for example, antioxidant, anticancer[5], Antibacterial, antifungal, anti-inflammatory and antiviral properties[6].

Cancer is a disease that make cells growth in the body out of control; the most common types of cancer are breast, lung, colon and prostate cancer[7]. When it is start in the breast then it is called breast cancer (BC)[8].

BC is one of the most common diagnosed types of cancer in women around the world an inducing to cancer death with almost 1.67 million new cases of cancer and more than 500,000 BC deaths predicted to have appeared in 2012[9].

Thus, in our current research we were aim to synthesis three new indole Schiff bases derivatives as illustration in Fig.1, and tested their cytotoxic activity against – AMJ13 breast cancer cell line[10]



1equ. 1-equ.

R_1= CH_3, H

R_2 = Br, CH_3, H

Fig. 1. The synthetic pathway to a new Schiff bases

EXPERIMENTAL

Chemistry part

Instruments

Melting points were measured by using open capillary melting point device and the purification were taken by using Thin layer Chromatography was performed by using Silica gel sheets and the spots were observed using florescence analysis cabinet model CM-10. IR spectra were recorded on Perkin-Elmer spectrum version 1 in Diyala University,^{1}H,^{13}C NMR and APT^{13}C NMR spectra were recorded in DMSO on a Bruker 400 MHz spectrometer, at 400 MHz for^{1}H NMR and at 100 MHz for^{13}C NMR and APT spectra in Jordan, University of science and technology, College of science, Irbid city .

Chemicals and solvent

All the chemicals and solvents used in this research were obtained from different companies; they were used as received without further purification Such 4-chlorophenyl hydrazine hydra chloride**,** methyl isobutyl ketone, Dimethyl formamide, and Dimethyl sulfoxide were obtained from Aldrich. Phosphoryl chloride, 4-Bromoaniline and 2,4-dimethyl aniline were obtained from Merck & Co. Aniline and Sodium hydroxide were obtained from Thomas baker. Glacial acetic acid was obtained from BDH. All organic solvent obtained from Scharlau. 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was synthesized with modification of a procedure described by[8]. As shown in Figure (2)

Synthetic methods

Synthesis of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-phenylimino-Propionaldehyde(1). As illustrated in Figure. 3.

A solution of (0.75g, 3 mmol) of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 30 mL and (0.28 g, 3 mmol) of phenyl amine was dissolved in ethanol 10 mL and then added glacial acetic acid 2 mL to the solution. The mixture was stirring in a water bath at 78 °C for 2 h. Yellow crystals precipitate was formed, filtered off, washed with ethanol and dried in oven at 78 °C. The purity of this compound was determined by using TLC with pre-coated silica

gel, which gave one spot. . IR data in (cm^{-1}): 2968υ (CH aromatic), 2705υ(CH aldehyde, 1672 υ (CH=O), 1628υ (C=C), 1599υ (CHN), 1402υ (CH_3), 1230υ (C-N), 820 υ (C-Cl) and 754 υ(C-H bending). ^{1}HNMR (400MHz,DMSO,δppm). 13.99(s, 1H, N*H*), 9.42 (s,1H,*H*CO), 8.69(s,1H,*H*CN), 7.63-7.23 (8H,Ar-*H*), and1.60 (s,6H, 2x CH_3). ^{13}CNMR (100MHz, DMSO, δ ppm):188.32 (*C*=O), 183.62 (NH-*C*=C),156.76 (*C*H=N), 149.60, 147.64, 139.51, 129.83, 129.53, 127.44, 125.45, 121.77, 119.98 and 118.17(*Ar*-*C*H),108.19 (O=C-*C*=C), 54.41 (CH_3 *C*CH_3), 21.64 (s, 6H, 2x*C*H_3). APT ^{13}CNMR shown signals for CH and CH_3 appeared at negative side (below base line of the spectrum) 188.32, 156.47,129.56, 127.17, 125.18,121.50, 119.70, 117.90 and 21.38 whereas quaternary carbons and carbons deuterated DMSO solvent were observed at positive side (above base line of the spectrum) 183.62, 149.33, 147.38, 139.25, 129.27, 107.92 and 54.13.

Synthesis of 3-(4-Bromo-phenylimino)-2-(5-chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-Propionaldehyde(2). As shown in Figure. 4.

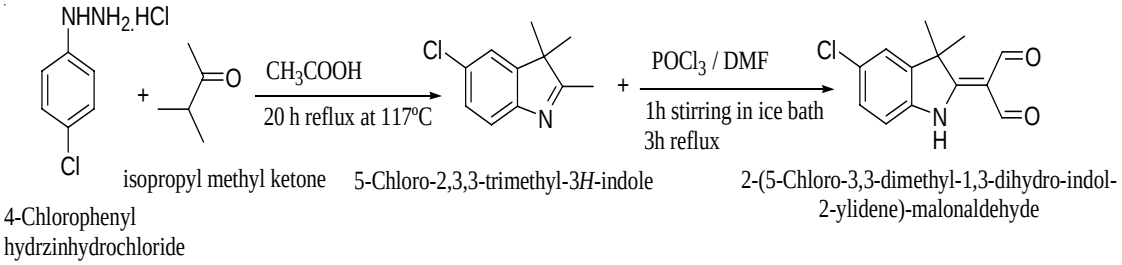


Fig. 2. The synthetic pathway of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde

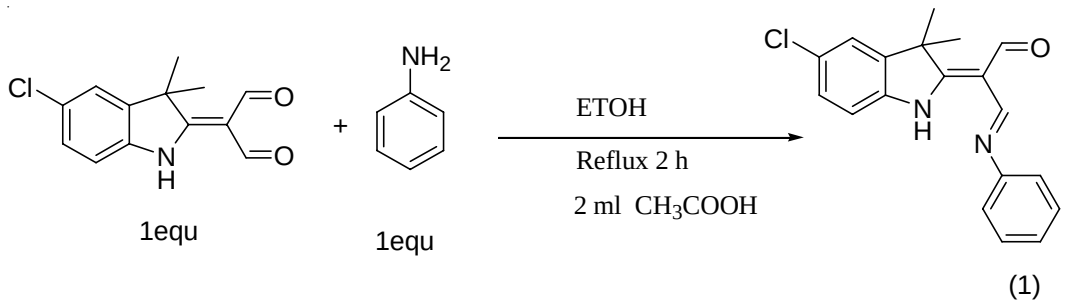


Fig. 3. Synthetic pathway of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-phenylimino-propionaldehyde (1).

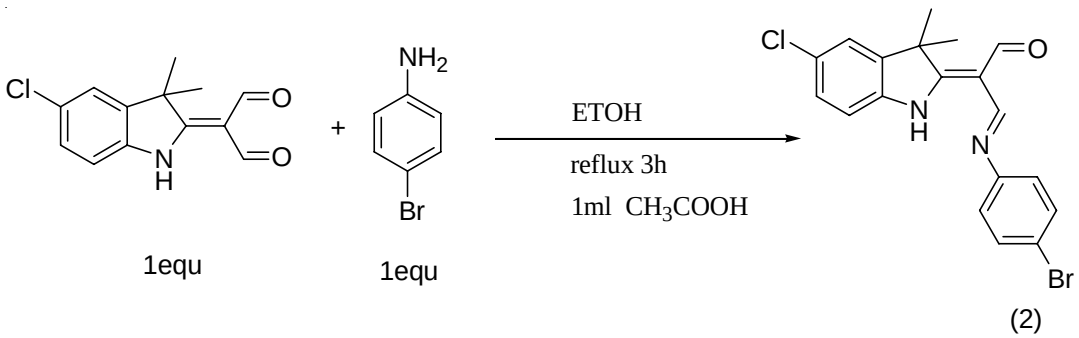


Fig. 4. Synthetic pathway of 3-(4-Bromo-phenylimino)-2-(5-chloro-3,3- dimethyl-1,3-dihydro-indol-2-ylidene)- Propionaldehyde (2)

A solution of (0.75 g, 3 mmol) of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 30 mL and (0.51 g, 3 mmol) of 4-Bromo-phenylamine was dissolved in ethanol 10 mL and then added glacial acetic acid 1 mL to the solution. The mixture was refluxed in a water bath at 78 °C for 3 hours. A solvent was reduced to one quarter; yellow precipitate was formed, filtered off, washed with ethanol and dried in oven at 78 °C. The purity of this compound was determined by using TLC with pre-coated silica gel, which gave one spot. IR data in (cm^{-1}): 2961 υ (CH aromatic), 2712 υ(CH aldehyde), 1665 υ(CH=O), 1621 υ(C=C), 1581 υ(CHN), 1394 υ(CH_3), 1233 υ (C-N), 816 υ(C-Cl) and 769 υ(C-H bending). ^{1}HNMR (400 MHz,DMSO, δppm). 13.95(s, 1H, N*H*), 9.43 (s,1H,*H*CO), 8.65(s,1H, *H*CN), 7.66-7.33(7H,Ar-*H*), and1.59 (s,6H, 2x CH_3). ^{13}CNMR (100MHz, DMSO, δppm):187.98 (*C*=O), 182.96 (NH-*C*=C), 156.56 (*C*H=N), 148.92, 147.20, 139.07, 132.17, 129.21, 127.14, 121.48, 120.08, 119.53, 117.03 (*Ar*-*C*H), 108.11(O=C-*C*=C), 53.92(CH_3*C*CH_3) and 21.46 (2x*C*H_3).APT ^{13}CNMR shown signals for CH and CH_3 appeared at negative side (below base line of the spectrum) 187.87, 156.61,132.18, 127.14, 121.47, 120.17, 119.46 and 116.96 whereas quarternary carbons and carbons deuterated DMSO solvent were observed at positive side (above base line of the spectrum) 183.03.149.05, 147.26, 132.43, 129.09, 116.96, 108.08 and 53.90.

Synthesis of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dimethyl-phenylimino)-propionaldehyde (3). As shown in Figure 5.

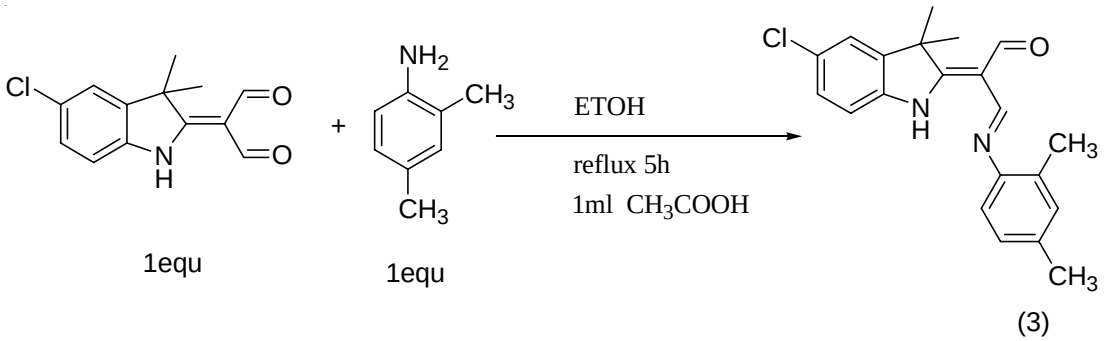


Fig. 5. Synthetic pathway of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dimethyl phenylimino)-propionaldehyde (3)

A solution of (0.7 g, 2.8 mmol) of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 25 mL and (0.33 g, 2.8 mmol) of 1,4-dimethyl aniline was dissolved in ethanol 10 mL and then added glacial acetic acid 1 mL to the solution. The mixture was refluxed in a water bath at 78 °C for 5 hours. A solvent was reduced to one quarter; yellow precipitate was formed, filtered off, washed with ethanol and dried in oven at 78 °C. The purity of this compound was determined by using TLC with pre-coated silica gel, which gave one spot. IR data in (cm^{-1}):-3049 υ (NH),2961 υ(CH aromatic), 2720 υ(CH aldehyde, 1669 υ(CH=O), 1625 υ(C=C), 1603 υ(CHN), 1398 υ(CH_3), 1239 υ(C-N),809 υ(C-Cl) and780υ (C-H bending), ^{1}HNMR(400MHz,DMSO,δppm). 14.09 (s,1H,N*H*),9.40(s,1H,*H*CO),8.64(s,1H,*H*CN),7.56-7.11(6H,Ar-*H*), 2.40 and 2.27 (s, *6H*, 2x CH_3) and 1.59(s,6H, 2x CH_3);^{13}CNMR (100MHz,DMSO, δppm):187.81 (*C*=O), 183.60 (NH-*C*=C), 155.71 (*C*H=N),149,45, 147.41, 135.21, 134.23, 131.42, 129.25, 127.56, 127.23, 126.86, 121.61, 119.05 and 115.09 (*Ar*-*C*H), 108.01 (O=C-*C*=C), 54.23 (CH_3*C*CH_3), 21.26 (2x*C*H_3), 20.17 (*o*-*C*H_3) and 17.34(*p*-*C*H_3). APT ^{13}CNMR shown signals for CH and CH_3 appeared at negative side (below base line of the spectrum) 187.81, 155.66,127.21, 121.58, 119.03 and 115.13, 21.26, 20.17 and 17.34 whereas quaternary carbons and carbons deuterated DMSO solvent were observed at positive side (above base line of the spectrum) 183.60, 149.43, 147.49, 135.23, 134.23, 131.40, 127.55, 126.87, 108.00 and 54.20.

Biological part

Two types of cell lines have been used in this Study. Breast cancer cell line (AMJ13) and broblastic and epithelial cells with normal chromosomal pictures (REF) as normal murine cell

lines were used. Both of them are locally established in ICCMGR and they are maintained for use.

Determination of solubility of compounds tested for *in vitro* cytotoxicity

Solubility assessment of synthesized compounds was carried out according to standard test method protocol[12]. The three new compounds were dissolved in dimethyl sulfoxide and diluted with nutrition medium RPMI-1640 to the desired concentrations (10 µg/ml, 20 µg/ml , 40 µg/ml and 60 µg/ml). Only freshly prepared solutions were used in experiments.

Both cell lines(AMJ13 and REF) are cultured in RPMI-1640 media that contains 10% fetal bovine serum, glutamine (2 mmol/L), streptomycin (100 U/ml) and penicillin (100 U/ml), then incubated in 5% co_2 at 37 °C for 24 hours. In this time the cells will grow and become monolayer. The confluent monolayer cells treated with 1 ml of trypsin/versine to provide suspension of cells, then add 10 ml of prepared media. About 200 µl of the cells were culture on clean sterile 96- well microtiter plate then let the cells for 24 hours. to make single monolayer to be ready to be treated with the our three new compounds.

Exposure day, decant the media from the cells and add 200 µl from the dilutions new compounds. Each concentration was triplicate and returns the microtiter plates to the incubator. Leave wells contains only cells without treatment contains serum free media representing control cells. Three different exposure times of the cells were included in this research, 24, 48 and 72 hours. The protocol of handing and treating the cells was prepared as described by Butler, 2004[13].

Cell Viability Assay

The cytotoxicity was determined after each exposure time using crystal violet. Decant the contents of microliter plate, add 200 ìl of the crystal violet to each wells of the treated cells for 20 min. in the incubator at 37 °C. The crystal violet will stain the nuclei of the viable cell and the color will be visible to the eye. Then the plates were read by ELISA reader at 495 nm. And then the inhibition rate was calculated using the following equation as recommended by[14].

$$\% \text{ Growth Inhibition} = (C-T)/C \times 100 \%$$

Were, C represent absorbance of control and T absorbance of sample.

Statistical Analysis

In this study, we used *student t-test* to determine the differences between the concentrations in each cell line and to determine the differences between two cells in each exposure time. The probability p was determined to be $p \leq 0.05$. Graph Pad Prism V6 was used to determine this statistical test. Excel 2010 sheet was used to draw the curves.

RESULTS AND DISCUSSION

The new synthesized compounds were subjected to TLC; spectral studies like HNMR, ^{13}CNMR, APT ^{13}CNMR and FTIR, and their results are discussed below. The physical properties such as the percentage yield and melting point of the compounds (1, 2 and 3) are represented in Table No.1

Table. 1: Physical properties of the synthesized compounds (1-3)

Compound No.	Molecular formula	Molecular weight	Percentage Yield	Melting Point °C
1	$C_{19}H_{17}ClN_2O$	(324.80)	82%	279-281^{0}C
2	$C_{19}H_{16}BrClN_2O$	(403.70)	75%	171-173^{0}C
3	$C_{21}H_{21}ClN_2O$	(352.86)	84%	169-170^{0}C

IR Study

The IR results of the synthesized compounds were shown absorption bands in the 4,000 - 400 cm^{-1} range, especially the new functional group (azomethine group CH=N) at 1599 cm^{-1}, 1581 cm^{-1} and 1603 cm^{-1} for synthesized

compounds (1, 2 and 3) respectively[15]. which approved to formation the accuracy chemical structure of synthesized compounds. Also strong absorption band at 1672 cm^{-1}, 1665 cm^{-1},1669 cm^{-1} for compounds(1, 2and 3) respectively which were belonged to (C=O)of the carbonyl group[16]. As well as stretching frequency at 1628 and 1621 cm^{-1} for compounds (1 and 2) respectively, 1625 cm^{-1} for compounds (3) were referred to (C=C) group[17]. at the same time the synthesized compounds were appeared an absorption bands at 1230 cm^{-1}, 1233 cm^{-1} and 1239 cm^{-1} which attributed to (C-N) groups of synthesized compounds (1, 2 and 3) respectively[18].

Finally, absorption band at 1402 cm^{-1} and 1394 cm^{-1} for compounds (1 and 3) respectively and 1398 cm^{-1} for compounds (2) were appointed to (CH_3) group[19]. All these main absorption bands are approved the chemical structures of the synthesized compounds (1, 2 and 3).

NMR Study

^{1}H-NMR, ^{13}C-NMR and APT ^{13}C-NMR spectra were reported in DMSO (dimethyl sulfoxide) with chemical shifts in ppm and using TMS (tetramethylsilane) as standard.

^{1}H-NMR

The ^{1}H-NMR results for compound (3) Fig. (6) shown single signals at 14.09 ppm was belonged to proton of (NH) of indole ring[20]. A singlet signal at 9.40 ppm was referred to proton atom of carbonyl group (C=O)[21]. As well as, single signal at 8.64 ppm was attributed to proton of Schiff base group (CH=N)[22] Signals were appeared in the region between (7.56-7.11) ppm were assigned to protons of aromatic ring for (3) compound[23]. Also singlet signals at 2.40 and 2.17 ppm were assigned to two methyl groups at positions para and ortho on aromatic ring. Finally peak at 1.59 ppm was belonged to six protons of two methyl groups[24]. ^{1}H NMR results of other compounds (1 and 2) are discussed and listed in table (2).

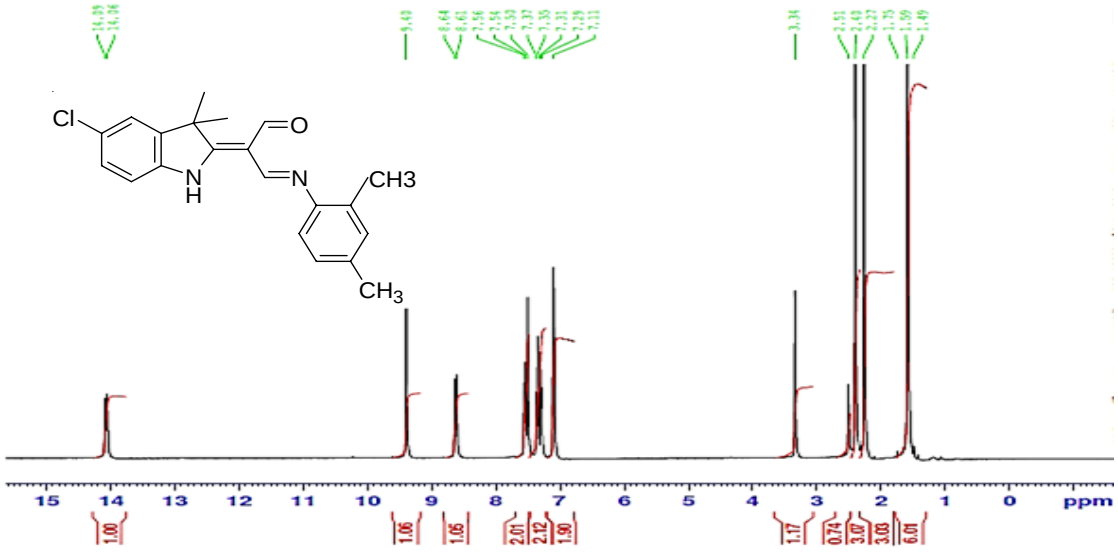


Fig. 6. ^{1}H NMR spectrum of2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dimethylphenylimino)-propionaldehyde (3

Table. 2: The chemical shift in ppm to^{1}H NMR results of compounds (1-3)

Compound No.	NH-	C=O	CH=N-	Ar-H	Ortho CH_3	para CH_3	2xCH_3
1	13.99	9.42	8.69	7.63-7.23	-	-	1.60
2	13.95	9.43	8.65	7.66-7.33	-	-	1.59
3	14.09	9.40	8.64	7.56-7.11	2.40	2.17	1.59

$_{13}$CNMR study

The ^{13}C NMR results supported ^{1}H NMR results for compound (3) as shown on Fig. 7. A signal at 187.81 ppm and at 155.71 ppm which belonged to the carbon atom of the carbonyl group *C*=O and the carbon atom of the azomethine group (*C*H=N) respectively[25]. The signals were appear in range between (149.45, 147.41, 135.21, 134.23, 131.42, 129.25, 127.56, 127.23, 126.86, 121.61, 119.05 and 115.09 ppm) assigned to the carbon atoms of aromatic ring[26]. While the signal at 108.01 ppm was referred to carbon atom of (O=C-*C*=C) group[27], as well as a signal of carbon atom of (CH_3*C*CH_3) group was observed at 54.23. In addition, signal two groups of methyl were observed at 21.26 ppm. Finally the signals of *p*-*C*H_3 and *o*-*C*H_3 were appeared at 20.17 and 17.34 ppm[28]. The ^{13}C NMR results of the rest compounds (1 and 2) were given in the Table. 3.

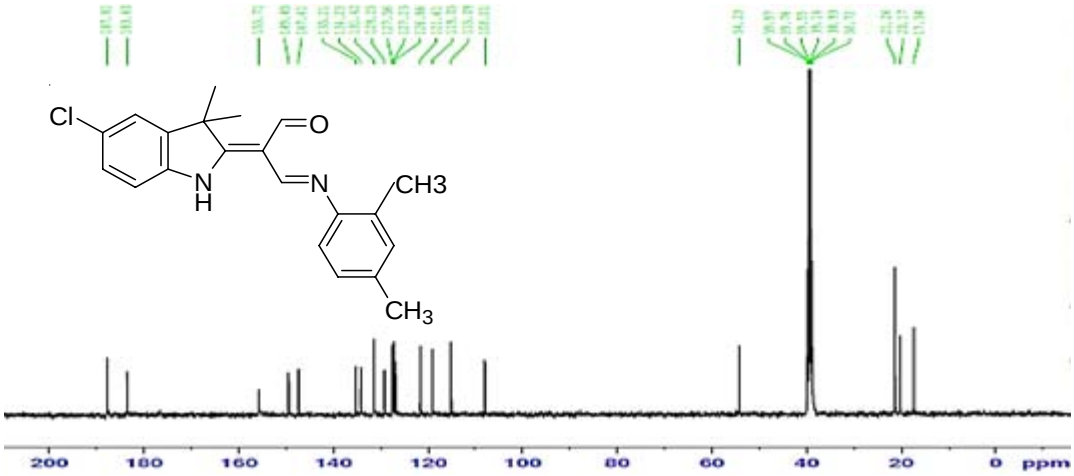


Fig. 7. ^{13}C NMR spectrum of2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dimethylphenylimino)-propionaldehyde (3)

Table. 3: the chemical shift in ppm to^{13}C NMR results of compounds (1-3)

Compound No.	*C*=O	*C*H=N-	*Ar*-CH ring	O=C-*C*=C)	CH_3*C*CH_3	Para *C*H_3	Ortho CH_3	2x *C*H_3
1	188	156	149-118	108	54	-	-	21
2	187	156	148-117	108	53	-	-	21
3	187	155	149.-115	108	54	17	20	21

APT ^{13}CNMR

APT ^{13}C NMR results were further used to characterize the new three compounds. For example APT ^{13}C NMR results of compound (**3**) Fig. 8 shown signals for quaternary carbons and solvent which appeared at positive side (above of the spectrum). While CH and CH_3observed at negative side (below of the spectrum).^{1}H, ^{13}C NMR and APT spectrum results for the new three compounds correspond well with the expected signals and are regular with the formation of these compounds.

***In Vitro* cytotoxicity effects of three indole derivatives**

Cytotoxic activity against AMJ13 cell line

The synthesized of three new compounds (1, 2 and 3) were tested in vitro to determine their cytotoxicity toward breast human cancer cell line AMJ 13. Our results indicated that the cytotoxic activity of Compound(1) (Fig. 9) at high concentration 60 µg/ml appears higher cytotoxicity against AMJ13 cells gradually increase in its cytotoxicity according to exposure time at 72 h to inhibit 70% of the cells. While values of anticancer activity were obtained

with the three concentrations 10, 20 and 40 µg/ml at 72 h of exposure to be 60-70% as well. Further concentrations decreased, however, did not lead to the total cell death, since the cell viability was more than 90% compared to untreated cells, even at the lower concentration tested (2-10 µg/mL). Higher concentrations more than 60 µg/mL could not be prepared.

In case of compound (2) as presented in Fig. 10, the AMJ13 cells was sensitive to this derivative also at concentration 60 µg/ml and the lower concentrations at 72 h of exposure, the inhibition of AMJ13 cells was range to be 70%. While at exposure time for 24 and 48 h the inhibition rate of cells were lowers than 60% for all the concentrations tested.

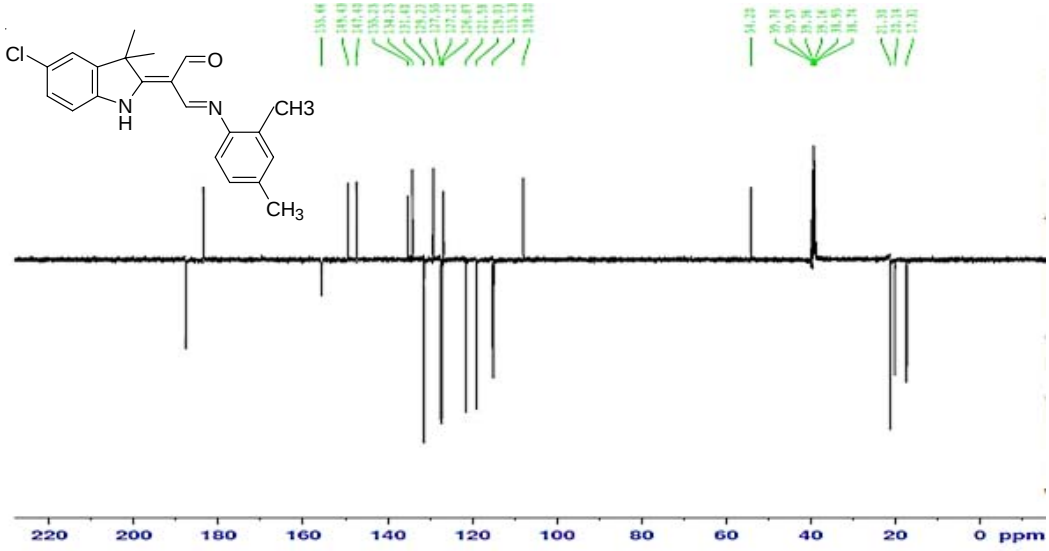


Fig. 8. APT ^{13}C NMR spectrum of2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dimethylphenylimino)-propionaldehyde

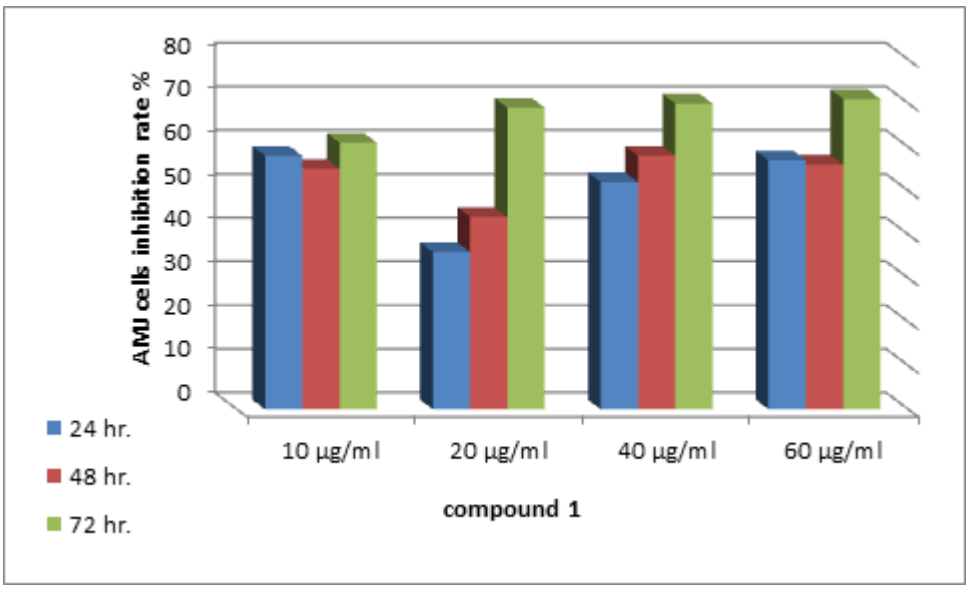


Fig. 9. Cytotoxic activity of compound (1) against AMJ13 cancer cell line from prepared different concentrations for three different exposure times

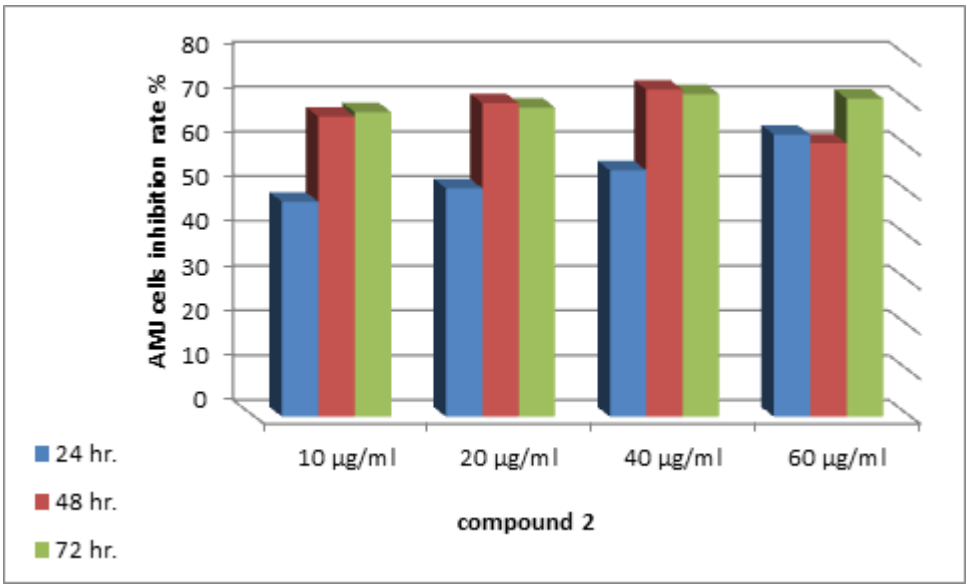


Fig. 10. cytotoxic activity of compound (2) against AMJ13 cancer cell line from prepared different concentrations for three different exposure times.

The cytotoxic activity of compound 3 was also observed to inhibit AMJ13 cells at concentrations 60, 40 and 20 µg/ml for 48 and 72 h of exposures shows 70% range of cells inhibition rate. While at lower concentration 10 µg/ml also gives 60% inhibition rate at 72 h of exposure against AMJ13 cells as presented in Figure. 11.

Totally talking about the ability of all the compounds tested and concentration 10 µg/ml to be nominated against AMJ13 cell line growth at 72 h of exposure, choosing the lowest concentration that has the same ability to inhibit AMJ13 cells to higher ones let us to think again in using it as anticancer drug.

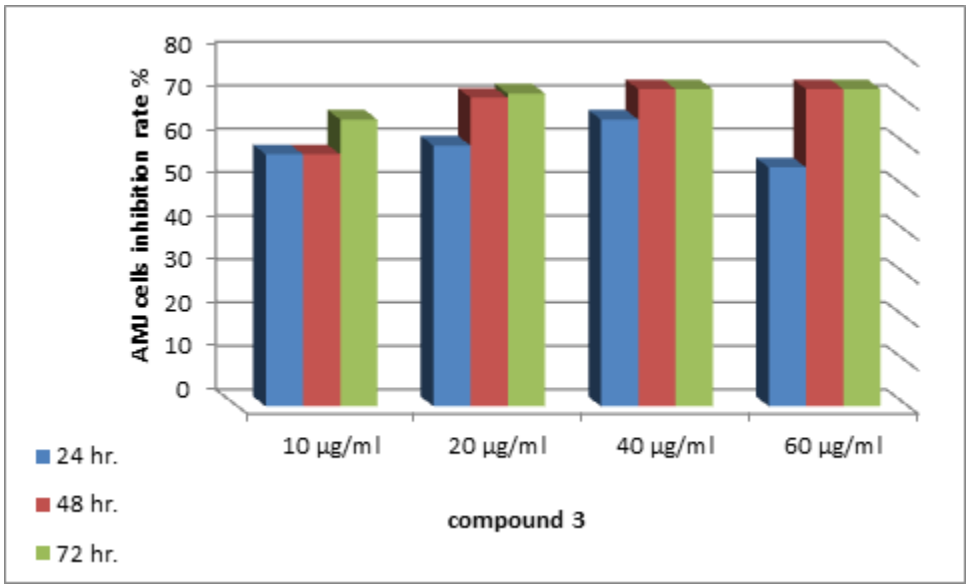


Fig. 11. cytotoxic activity of compound (3) against AMJ13 cancer cell line from prepared different concentrations for three different exposure times.

Cytotoxic activity against REF cell line

The prepared of three new compounds (1, 2 and 3) also tested their cytotoxic activity *in vitro* against REF cell line. For compound 1, all tested concentrations were appear to inhibit cell growth specially at high concentration 60 µg/ml as time dependent to give 25 % inhibition rate at 72 h of exposure while at lower concentrations the inhibition rates were lower means it could be safe to cell and let the REF cells grow normally specially at concentration 10 µg/ml as expressed in Figure 12.

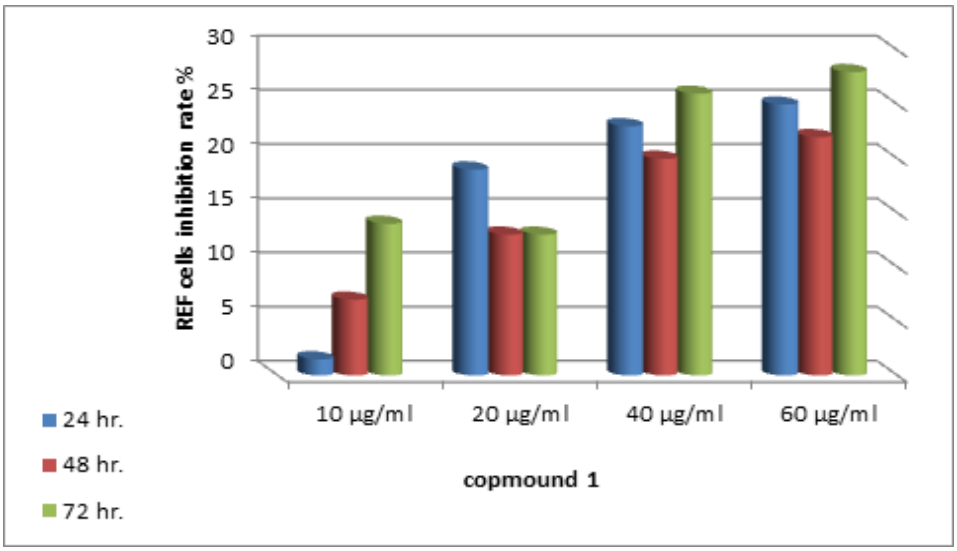


Fig. 12. Cytotoxic activity of compound (1) against REF cell line from prepared different concentrations for three different exposure times

While REF cells when exposed to different concentrations of prepared compound (2) the cells after 24 h of exposure and at higher concentrations than 10 µg/ml started to be very sensitive and inhibition rate was time dependent also in this case and concentration dependent. Only in concentrations 10 and 20 µg/ml and at 24 h of exposure the cells were live normally and did not affected by this derivative to range in its inhibition from 10-20% respectively as in Figure. 13.

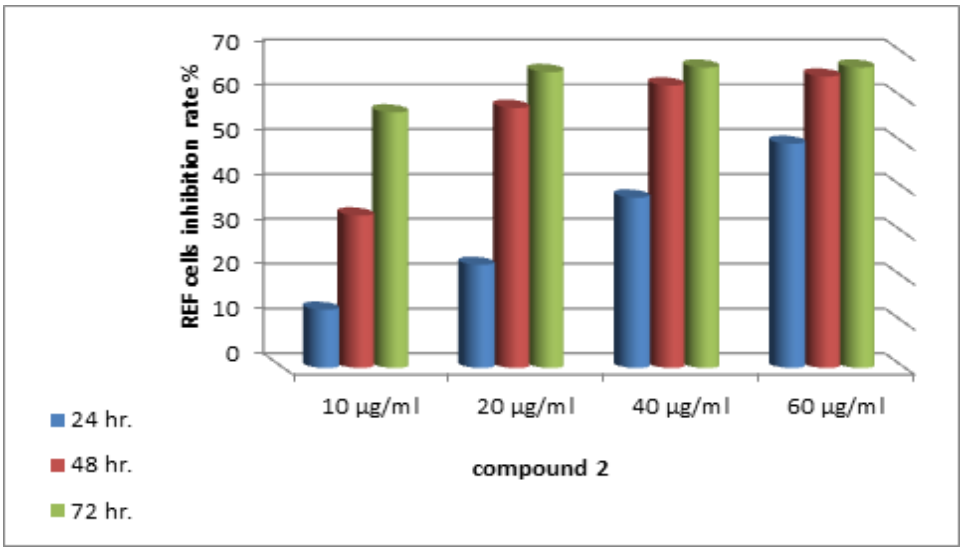


Fig. 13. cytotoxic activity of compound (2) against REF cell line from prepared different concentrations for three different exposure times.

In compound 3, concentration 10 µg/ml represented the safest concentration in the three exposure times to REF cells compared to other higher concentrations to give less than 15% inhibition rate at 72 h of exposure. Concentrations 20, 40 and 60 µg/ml at 24 h of REF cells exposure also gives lower inhibition rate 33-37% respectively as presented in Figure. 14.

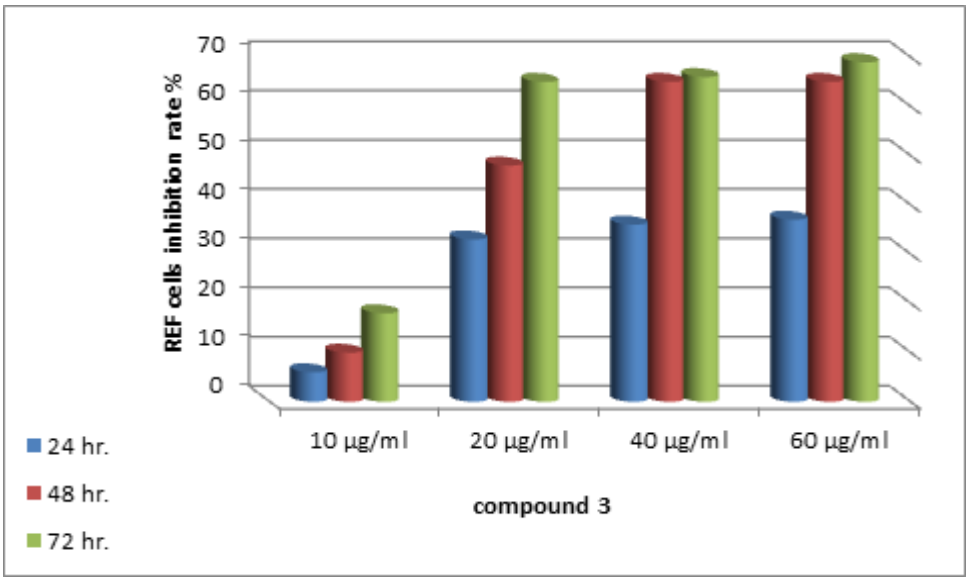


Fig. 14. cytotoxic activity of compound (3) against REF cell line from prepared different concentrations for three different exposure times

Reflecting from these results that compound (1) and its concentrations represent the safest to REF cell line growth did not show high inhibition rate during the exposure times. While only the lower concentration 10 µg/ml from compound (2) and (3) at 24 h of exposure give lower activity against REF cells.

CONCLUSIONS

Three new Schiff base indole derivatives [2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-phenylimino-propionaldehyde(1),3-(4-Bromo-phenylimino)-2-(5-chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-propionaldehyde (2),and 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,4-dimethyl-phenylimino)-propionaldehyde (3)] have been synthesized by reaction of 2-(5-Chloro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde with aniline derivatives, The chemical structure of the synthesized compounds have been characterized and approved by TLC, FT-IR, ^{1}H NMR, ^{13}C NMR and APT ^{13}C-NMR techniques. The *in vitro* cytotoxicity of the compound prepared against breast cancer cell line AMJ13 revealed that the lowest concentration 10 µg/ml of 1, 2 and 3 compounds has the ability to inhibit AMJ13 cells and in the same time safe to normal cell growth REF cell line. Let us to put a big highlight for further research on these compounds in cancer therapy models.

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