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Synthesis, Characterization and Cytotoxicity Activity Study of Some Chalcones Derived from 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)malonaldehyde

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Abstract: In this work, series of new chalcones derived from indole compounds were synthesized. In the first the compound 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)malonaldehyde was synthesized from the reaction of 1,1,2-trimethyl-1H-benzo[e]indole with Phosphoryl chloride in in the presence of (DMF). Schiff base (C_2) was prepared by reaction of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene) malonaldehyde with 3-amino acetophenone and then the compounds (C_3-C_6) were synthesized by reacting compound (C_2) with a different aryl aldehyde in the presence of potassium hydroxide. The chemical composition of the compounds was confirmed and characterized by spectroscopic techniques (FT-IR, ^{1}H-NMR and^{13}C-NMR). Target compounds with different concentrations were investigated for their cytotoxic activity against the human breast cancer cell line MCF7. The results showed that the compounds had promising cytotoxic activity against MCF7 cell line especially compound (2) which showed the highest inhibition at the rate of 100 µg/mL among the tested compounds at varied concentrations.

Keywords: Indole derivatives, Chalcones, Schiff base, Cytotoxicity activity

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INTRODUCTION

There are many biological active compounds that have been synthesized and reported from different heter°Cyclic compounds. As breast cancer has become dangerous for many people, the need to modify and find new biologically active compounds to overcome this problem has become important and has a wide interest of scientists. Generally, heter°Cycles °Ccupy a prominent place in chemistry due to their wide range of applications in the fields of drug design, phot°Chemistry, agr°Chemicals, dyes and so on. Among them, indole scaffolds have been found in most of the important synthetic drug molecules and paved a faithful way to develop effective targets. Privileged structures bind to multiple receptors with high affinity, thus aiding the development of novel biologically active compounds (1). Recently, indoles are considered interesting heter°Cyclic compounds due to their wide range of biological activities (2). The derivatives of indoles and indazoles exhibits antibacterial, anticancer, antionidants, anti-inflammatory, antidiabetic, antiviral, atniproliferative, antituberculosis, antispermetogenic activity, antipsychotic drugs etc (3). Schiff bases/imines are a substantial class of organic compounds achieved via condensation of carbonyl compounds (aldehydes or ketones) and primary amines to generate an azomethine (−C=N−) functionality (4) Compounds of this class have shown significant applications in the field of

analytical, coordination, and pharmaceutical chemistry (5) . It has several applications, including Nafia R. A. et. al.(2019). Synthesis and characterization of new indole schiff bases and study effect of the compounds on lymphatic cell in metaphase in human blood (6). Chalcones are known as α, β-unsaturated ketones, characterized by having the presence of two aromatic rings that are joined by a three-carbon chain, they are a class of compounds considered an exceptional model due to chemical simplicity and a wide variety of biological activities (7). Chalcones are a group of polyphenolic compounds derived from plants which belong to the flavonoids family and owna wide variety of modulatory and cytoprotective functions. They have been linked with anti-bacterial, anti-fungal, antiinflammatory, anti-oxidant, anti-cancer and anti-diabetic activities (8). a family of small molecules that are naturally abundant in edible plants, have been found to have antitumor properties for specific cancer cell lines and to interfere in each step of carcinogenesis, including apoptosis (9). Özdemir, Ahmet, et al. new indole-based chalcone derivatives were obtained via the reaction of 5-substituted-1H-indole-3-carboxaldehydes/1-methylindole-3-carboxaldehyde with appropriate acetophenones. The synthesized compounds were investigated for their in vitro inhibitory activity. According to in vivo studies, these compounds displayed antiinflammatory and antioxidant activities (10). In the present work, As a result of the importance of the compound indole, schiff and chalcone, and in order to continue to find a new modification to the compound (1,1,2-trimethyl-1H-benzo[e]indole). We therefore synthesized them as well as measure the biological effectiveness of these compounds to know their importance, also to allow the possibility of using them in other uses.

MATERIALS AND METHODS

Chemical Part

All chemicals and solvents used during synthesis compounds were obtained from a numeral of different companies such as Merck, BDH, Fluka and Sigma Aldrich.

Melting points were determined by utilizing the device Melting point SMP10. Diyala University, College of Science. FT-IR spectra was recorded using PERKIN ELMER SPECTRUM-65, JASCO, Infrared spectrometer, within the range (4000-400) using KBr Disc, Diyala University, College of Science.

The ^{1}H-NMR and ^{13}C-NMR spectra was recorded by Varian 400 MHz spectrometer with TMS as internal standard and deuterated DMSO was used as a solvent, measurements were made at Central Lab., School of Chemistry, College of Science, University of Tehran, Iran.

Synthesis of Malonaldehyde 2-(1,1-dimethyl-1,3-dihydro-2Hbenzo[e]indol-2-ylidene) (C_1).

N,N-dimethyl formamide (DMF) (3 mL) was cooled in an ice bath then added drop wise of (1.3 mL) Phosphoryl chloride ($POCl_3$) with stirring under 5°C, then a solution of (1 g, 0.0047 mole) 1,1,2-trimethyl-1H-benzo[e]indole in DMF (3 mL) was cooled under 5 °C and added dropwise, the reaction mixture was stirred in ice path for 1h. then reflux for 3h. at 88 °C. The resulting solution was added to icy distilled water and neutralized with 25% NaOH aqueous, the yellow precipitate was formed filtered off and dried in oven. Recrystallized from ethanol to afford pure yellow precipitate. Yield: (1.243 g, 98%). m.p. 202-203 ºC.(11,12)

Synthesis of (2E)-3-((3-acetylphenyl)imino)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)propanal. (C_2)

A solution of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene) malonaldehyde (3 g, 0.011 mole) in (20 mL of ethanol +5 mL of DMF) is mixed with 3-aminoacetophenone (1.48 g, 0.011 mole), to which 5-6 drops of glacial acetic acid was added, and the reaction mixture was refluxed for 12 hours. The completion of the reaction was checked by using TLC (3:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot. After cooling at room temperature the product was filtered and recrystallized from a suitable solvent. Yield: (2.8g, 64 %), M.P.(220 °C).

FT-IR data (cm^{-1}): 3458 υ (N-H), 3049 υ (CH aromatic),2932 υ (C-H aliphatic), 1688 υ (CH=O), 1668 υ (C°CH_3) 1621 υ (CH=N), 1579-1486 υ (C=C), 1208 υ (C-N), 759 υ (C-H bending). ^{1}H NMR (400 MHz, DMSO, δ in ppm): δ =14.24 (s,1H,NH) 9.56(s,1H, CHO), 8.81 (s, 1H, CH=N), 7.82-8.17 (m, 10 Ar-H), 2.69 (s, 3H, $COCH_3$) , 1.89 (s, 6H, 2xCH_3). The ^{13}C-NMR spectra of this compound, exhibits the signals (400 MHz, DMSO-d6, δ in ppm): δ = 198.15(C=O),147.98(C=N),140.86(C-NH), 109.11(C=C),(138.79-123.34) C-Ar, 27.52, 20.95 (2CH_3), 56.02(C(CH_3)$_2$).

Synthesis of Compounds (C_3-C_6)

Equimolar quantity (0.5g,0.0013 mole) of (2E)-3-((3-acetylphenyl)imino)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)propanal and appropriate aryl aldehyde (0.0013 mole) mixed and dissolved in 25 mL of ethanol absolute. To this, 40% potassium hydroxide solution (1 mL) added slowly and mixed occasionally for (3 hrs) at room temperature. the resulted mixture was refluxed for (6-8 hrs) at (78 °C). Completion of the reaction was identified by TLC using Silica gel-

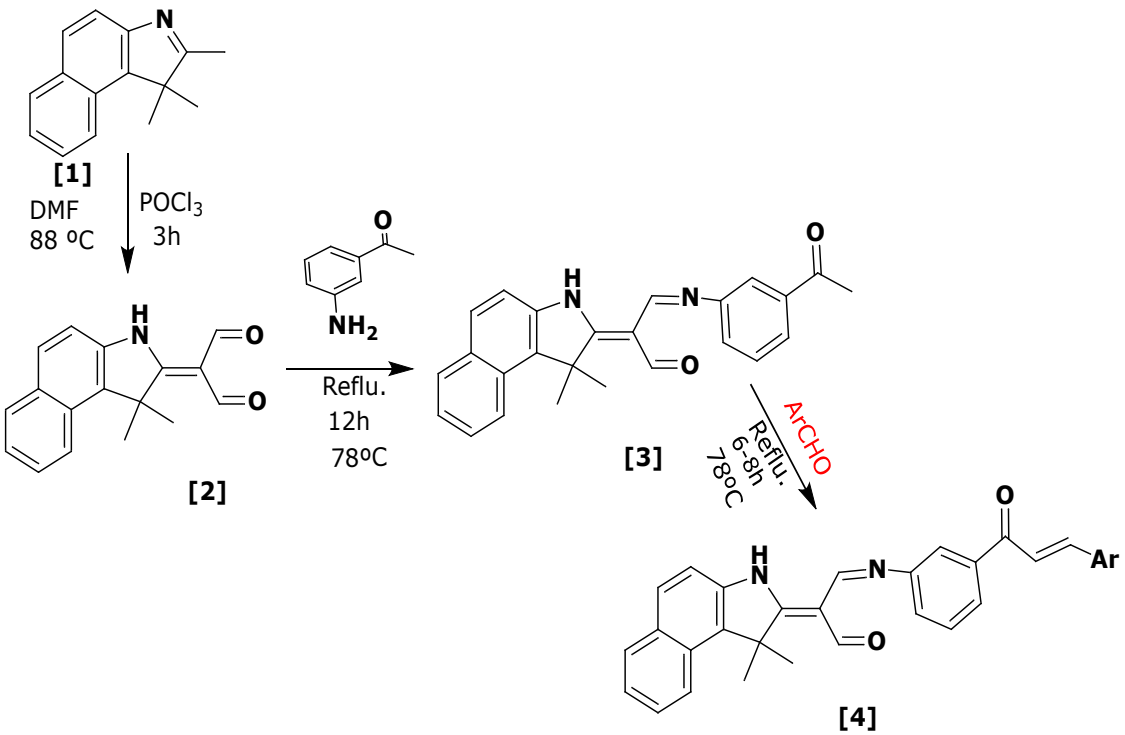
G. (3:1) hexane: ethyl acetate, which gave one spot.

FT-IR data in (cm^{-1}) of compound **(C_3)**: 3131 υ (NH), 2885 υ (CH aliphatic),1681 υ (CHO),1656 υ (C°CH=CH), 1609 υ (CH=N),1598-1457 υ (C=C),1210 υ (CN), and751 υ (CH bending).^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **(C_3)**: δ =13.46(NH), 9.84 (CHO), 8.65 (CH=N), 7.63(CH=C), 7.51 (CH=CO), 7.66-8.19 (Ar-H, 14H), 3.04 (s,6H,$N(CH_3)_2$), 1.99 (s, 6H, $2xCH_3$).

FT-IR data in (cm^{-1}) of compound **(C_4)**: 3134 υ (NH),2928 υ (CH aliphatic),2728 υ (CH aldehyde),1681 υ (CHO),1655 υ (C°CH=CH),1609 υ (CH=N),1598-1456 υ (C=C),1399-1512 υ (NO_2), 1210 υ (CN), and 751 υ (CH bending). ^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **(C_4)**: δ =13.46 (s,1H,NH), 9.80 (s,1H,CHO), 8.81 (s,1H, CH=N) ,7.61 (d, 1H, C=CH), 7.52 (d, 1H, COCH), 7.83-8.19 (m , 14H, Ar-H) , 1.99 (s, 6H, $2xCH_3$).

FT-IR data in (cm^{-1}) of compound **(C_5)**:3650 υ(OH),3131υ(NH),2968 υ(CH aliphatic),2740 υ(CH aldehyde),1681 υ(CHO),1655 υ (COCH=CH),1612 υ(CH=N),1598-1400 υ(C=C), 1210 υ(CN),and751 υ(CH bending). ^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **(C_5)**: δ =13.47 (s, 1H , NH) , 9.84 (s, 1H, CHO), 9.52 (S, 1H, OH) , 8.79 (s, 1H, CH=N), 7.63-8.19 (m. 14H, Ar – H) , 7.52 (d, 1H, C=CH) 7.51 (d, 1H, C°CH), , 1.99 (s, 6H, $2xCH_3$)

FT-IR data in (cm^{-1}) of compound **(C_6)**:3136 υ (NH),2978 (Charomatic), 2930 υ (Chaliphatic), 1681 υ (CHO),1656 υ (C-CH=CH),1610 υ (CH=N), 1598-1455 υ (C=C), 1210 υ (CN), and 750 υ (CH bending),714 υ (C-Cl).^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **(C_6)**:δ=13.47 (NH), 9.79 (CH=O), 8.20 (CH=N), 7.93 (CH=C), 7.50 (CH=CO), 7.63-8.17 (Ar-H,14H), 1.93 (s, 6H, $2xCH_3$). The ^{13}C-NMR spectra of this compound **(C_6)**, exhibits the signals (400 MHz, DMSO-d6, δ in ppm):δ=179.79(C=O), 138.05(C=N), 133.98(C-NH), 132.18-122.86(C-Ar), 114.74 (COC=C), 109.16 (C=C), 24.06, 22.25 ($2CH_3$), 52.96 ($C(CH_3)_2$).



ArCHO=4-(dimethylamino)benzaldehyde, 4-nitrobenzaldehyde, 4-hydroxybenzaldehyde, 4-chlorobenzaldehyde

Scheme 1: Synthesis of (C1-C6).

Biological Part

Determination of solubility of compounds tested for in vitro cytotoxicity. The cytotoxicity assay was carried out using the crystal violate stain according to the method of Freshney (2012) (13). In brief, the organic compounds were dissolved in DMSO and diluted by serum free media (SFM) to prepare different concentrations range of (50,100) µg/mL. Two types of cell lines were used in human breast cancer cell line MCF7, and normal human (MEF) cell lines. The tumor cells (1 x 10^5 cell/mL) were seeded in 96-well microplate and incubated for 24 h at 37 °C, then old media was changed with a new serum-free medium (SFM) containing concentrations of each compound. Plate was incubated for 24 h in humidified incubator at 37 °C containing 5% CO_2. After incubation, the culture medium was discarded and 100 mL of crystal violate was into each well and re-incubated for 20 min at 37 °C. The inhibition percentage was calculated by the following formula (1):

$$\text{Inhibition (\%)} = (A\text{-}B/A) \times 100 \qquad \text{(Eq. 1)}$$

Where,
A = Absorbance of the control
B = Absorbance of the sample

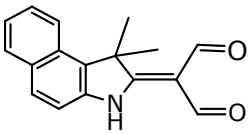
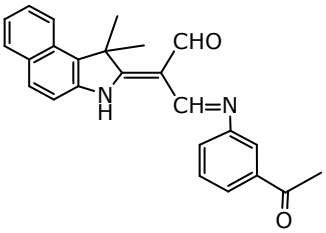
RESULTS AND DISCUSSION

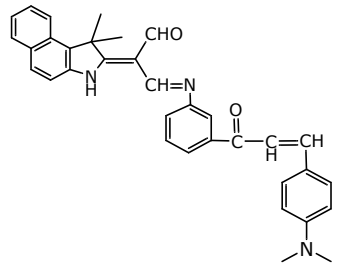
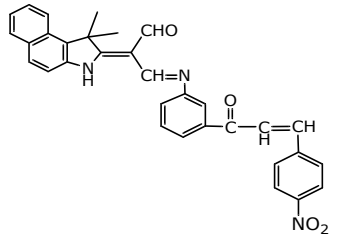
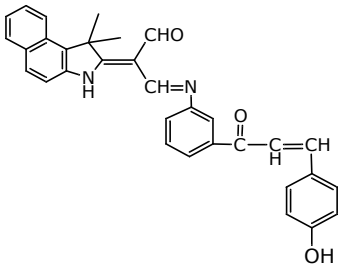
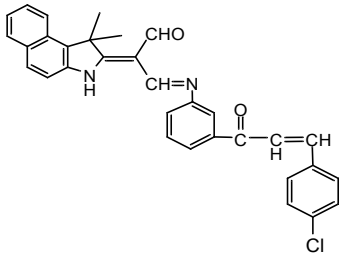
New indole containing Schiff bases and chalcones were synthesized and characterized with spectral studies (^{1}H-NMR,^{13}C-NMR, and FT-IR), physical properties such as melting point and yields of the new compounds are mentioned in Table 1.

Table 1: Physical properties of the synthesized compounds.

Comp. No.	Molecular formula	%Yield	Melting Point, °C
1	$C_{17}H_{15}NO_2$	98%	207
2	$C_{25}H_{22}N_2O_2$	64%	220
3	$C_{34}H_{31}N_3O_2$	52%	154
4	$C_{32}H_{25}N_3O_4$	62%	180
5	$C_{32}H_{26}N_2O_3$	59%	203
6	$C_{32}H_{25}ClN_2O_2$	68%	140

Table 2: The newly synthesized compounds.

Comp No.	Comp. Structure	Comp. Name
C_1		2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)malonaldehyde
C_2		3-((3-acetylphenyl)imino)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)propanal

C_3		2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)imino)propanal
C_4		2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-nitrophenyl)acryloyl)phenyl)imino)propanal
C_5		2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-hydroxyphenyl)acryloyl)phenyl)imino)propanal
C_6		3-((3-(3-(4-chlorophenyl)acryloyl)phenyl)imino)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)propanal

FT-IR study

The FT-IR spectra of the new five synthesized compounds showed the absorption band of the new functional group (imine group CH=N) at 1621, 1609, 1609, 1612, and 1610 cm^{-1} for the compounds 2, 3, 4, 5, and 6, respectively which approved the chemical structure of the synthesized compounds. A strong absorption band appeared at 1681-1688 cm^{-1} for all compounds (2, 3, 4, 5, and 6) related to the carbonyl group CH=O of aldehyde. Whereas 1655-1656 cm^{-1} for stretching of C= O due to the conjugated with double bonds for the mentioned compounds. Also an absorption band at 1598-1400 cm^{-1} which belonged to C=C group. All of these bands are confirmed the chemical structures of the synthesized compounds (C_2-C_6).

NMR Study

^{1}H-NMR spectra were reported in DMSO (dimethyl sulfoxide) with chemical shifts in ppm and using TMS (tetramethylsilane) as standard. The ^{1}H-NMR results for compound (1) shown single signals at 13.14 ppm was belonged to proton of (NH) of indole ring. A singlet signal at 9.79 ppm was referred to proton of aldehyde (CH=O) group. Signals were appeared in the region between (7.69-7.38) ppm were assigned to protons of aromatic ring for (2) compound. Finally, a peak at 1.96 ppm belonged to six protons of two methyl groups. The ^{1}H-NMR results for compound (C_2) Figure 1 showed single signals at 14.24 ppm which belonged to proton of (NH) of indole ring. A singlet signal at 9.56 ppm referred to the proton of aldehyde (CH=O) group. A singlet signal at 8.81

ppm was referred to the proton of Schiff base group (CH=N). Signals were appeared in the region between (7.82-8.17) ppm were assigned to protons of aromatic and singlet signal at 2.69 ppm was attributed to ($COCH_3$). The peak at 1.89 ppm belonged to six protons of two methyl groups. Whereas the ^{1}H-NMR of compound (C_3) Figure (3) showed singlets at 13.46 ppm that belonged to the proton of (NH) of indole ring. A singlet signal at 9.84 ppm was referred to proton of the aldehyde (CH=O) group. A singlet signal at 8.65 ppm was referred to proton of Schiff base group (CH=N). Signals was appeared in the 7.63 was attributed to (CH=C) and signals was appeared in the 7.51 was attributed to (CH=CO), Signals were appeared in the region between (7.66-8.19) ppm were assigned to protons of aromatic and singlet signal at 3.04 ppm was attributed to ($N(CH_3)_2$). Finally peak at 1.99 ppm was referred to six protons of two methyl groups. ^{1}H NMR results of other compounds are listed in Table (3).

^{13}C-NMR results were used to characterize this new compound and support the results of 1H- NMR. Figure (2) results of compound (C_2) A signal at 198.15 ppm were assigned to the carbonyl group C=O and, while a signal of CH=N group detected at 147.98 ppm. The signals were appear in the range between 123.34- 138.79 ppm were belonged to the carbon atoms of aromatic rings. In addition, two signals appeared at 109.11 ppm and 56.02 ppm were assigned to C=C and CH3-C-CH3 groups respectively. Finally, signal at(27.52 and 20.95) ppm was belongs to the rest two methyl groups.^{13}C NMR results of other compound (C_6) Figure (7) are discussed A signal at 179.79 ppm were assigned to the carbonyl group C=O and, while a signal of CH=N group detected at 138.05 ppm. The signals were appear in the range between 122.86- 133.08 ppm were belonged to he carbon atoms of aromatic rings. In addition, to appearing COC=C at 114.74 .two signals appeared at 109.16 ppm and 52.96 ppm were assigned to C=C and CH3-C-CH3 groups respectively. Finally, signal at (24.06 and 22.25) ppm was belongs to the rest two methyl groups.

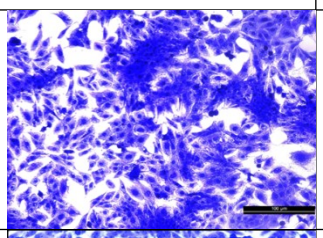
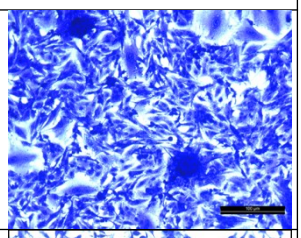
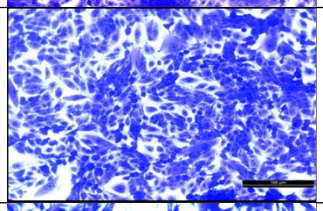
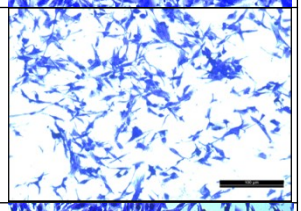
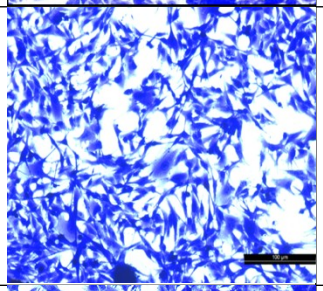
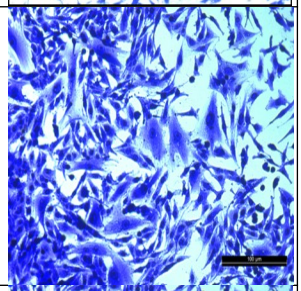
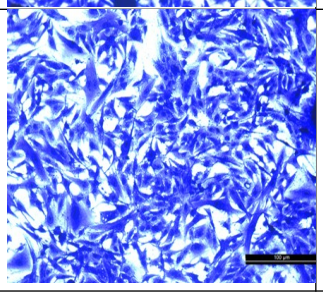
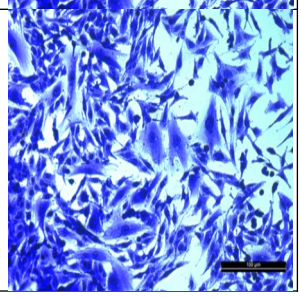
Table 3: The chemical shift in ppm to ^{1}H NMR results of compounds.

No	NH	HC=O	CH=N	CH=C	CH=CO	Ar-H	2xCH_3	Other
1	13.14	9.79	-	-	-	7.69-7.38	1.68	-
2	14.24	9.56	8.81	-	-	7.82-8.17		2.69 C-CH_3
3	13.46	9.84	8.65	7.63	7.51	7.66-8.19	1.99	3.04 $N(CH_3)_2$
4	13.46	9.80	8.81	7.61	7.52	7.83-8.19	1.99	-
5	13.47	9.84	8.79	7.52	7.51	7.63-8.19	1.99	9.52 OH
6	13.47	9.79	8.20	7.93	7.50	7.63-8.17	1.93	-

In vitro cytotoxic activity

The new Prepared compounds (2, 3 ,4 and 5) in vitro to study cytotoxicity Activity against the human breast cancer cell line MCF7 in two different concentrations 50 and 100 mcg/mL with an exposure time of 24 hours and a temperature of 37. Results that we obtained showed compound (C_2) Highest cytotoxic activity with inhibition rate 66.40% at a concentration of 100 µg/mL Among the rest of the vehicles installed with Diverse concentrations. In the case of compound (C_3), the results were revealed Reliance on them to focus regularly, the inhibition rates were 20.07 and 56.45% for 50 and 100 µg/mL concentrations, respectively. While compound (C_4) showed inhibition rate and the inhibition rates were (30.13 and 48.46%) for 50 concentrations and 100 µg/mL, respectively. The compound (C_5) gave inhibition rates of 40.17 and 46.33% for concentrations 50 and 100 µg/mL, respectively.

Table 4 : The in vitro cytotoxicity effect of prepared organic compounds on different cell lines at 50 and 100 µg/mL after 24 h incubation at 37 °C.

Derivatives No.	**Inhibition ratio 100% Normal Cell Line MEF**		**Inhibition ratio 100% Cell Line cancer MCF7**		**Normal cell line(MEF) in 100 µg/m Images**	**Cell line(MCF7) in 100 µg/m Images**
	Con. µg/mL		**Con. µg/mL**			
	50	**100**	**50**	**100**		
C_2	18.40	19.70	50.19	66.40		
C_3	9.12	11.78	20.07	56.45		
C_4	8.11	13.57	30.13	48.46		
C_5	10.12	16.22	40.17	46.33		

CONCLUSION

In the current work, synthesized new derivatives of Indole compounds categorized (C_1–C_6). These compounds were characterized utilizing diverse spectroscopic methods like FT-IR, ^{1}H-NMR and ^{13}C-NMR In addition to measurement some of their physical properties. Target compounds were investigated for their cytotoxic activity against the human breast cancer cell line MCF7. The results showed that the compounds had promising cytotoxic activity against MCF7 cell line especially compound (C_2) which showed the highest inhibition at the rate of 100 µg/mL among the tested compounds at varied concentrations.

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