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Synthesis, Characterization, and Activity Related to Cytotoxicity Study of Some Indole-Derived Chalcones

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Abstract

In this work, series of new chalcones derived from indole compounds were synthesized. In the first the compound indole with Phosphoryl chloride in in the presence of (DMF). Schiff base [C2] was prepared by reaction of with 3-amino acetophenone and then the compounds [C3-C6] were synthesized by reacting compound [C2] with a different aryl aldehyde in the presence of potassium hydroxide. Spectroscopic techniques were used to confirm and describe the compounds' chemical makeup (FT-IR spectroscopy, 1H-NMR spectroscopy). Target compounds' cytotoxic effects at different concentrations on the human breast cancer cell line MCF7 were investigated. According to the findings, compound (2) in particular showed potential cytotoxic action against the MCF7 cell line. This compound was tested at various dosages and demonstrated the highest inhibition at a rate of 100 g/ml among the compounds.

Keywords
indol derivatives, chalcones, Schiff base, cytotoxicity activity

Many naturally occurring and synthesized compounds contain the crucial scaffold chalcone, which has a wide range of pharmacological actions. Recent decades have seen a variety of chalcone compounds exhibit powerful anticancer action by preventing tubulin polymerization or depolymerization. Indoles, which are frequently found as the skeleton in both natural and synthetic compounds, have received a lot of interest in medicinal chemistry during the past 10 years. It is crucial to note that, according to the literature, the indole moiety is a crucial pharmacophoric component for the design and development of antitubulin medicines. [1-7]

Interest in medicinal chemistry has also been sparked by the pharmacological and therapeutic capabilities of indole derivatives, such as depressed, antiplatelet, antihypertensive, herbicidal, and plant growth regulating actions. In the current study, we report the productive synthesis of indole derivatives containing heterocyclic moieties such as pyrazole, thiazole, and thiophen. As antioxidant, antiproliferative, and anticancer medicines, indoles with additional heterocyclic moieties, notably those having pyrazole and thiazole moieties, are extremely important. In this study, the newly synthesized heterocyclic compounds were first investigated for their capacity to suppress microorganisms biochemically. [8-18]

Fischer initially offered a method for making indole when he suggested that an acidic reaction between an altered phenylhydrazine and an aldehyde or ketone may produce aromatic heterocycle indole derivatives. [15] Hermann Emil Fischer discovered the reaction in 1883. Today, this process is frequently used to create triptan-class antimigraine medications. [19-20]

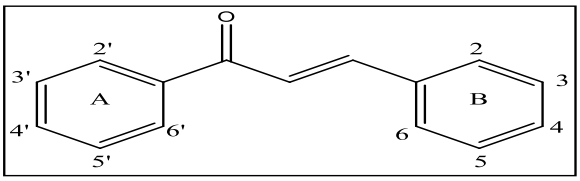

Figure 1. Molecular scaffold of chalcone.

Experimental Part

Materials and methods

All of the chemicals and solvents required to produce the compounds came from several businesses, including Merck, BDH, Fulka, 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 SPEACTUM-65, JASCO, Infrared spectrophotometer, within the range [4000-400] using KBr Disc, Diyala University, College of Science.

Deuterated DMSO was applied as the solvent and a Varian 400 MHz spectrophotometer was utilized to record the 1H-NMR spectra. In Iran's University of Tehran's College of Science, the tests were carried out in the Central Lab of the School of Chemistry.

General Methods

Synthesis of indol [C_1]

N,N-dimethyl formamide (DMF) (3ml) was cooled in an ice bath then added drop wise of (1.3ml) Phosphoryl chloride ($POCl_3$) with stirring under 5°C, then a solution of (1 g, 4.7 mmol) 1,1,2-trimethyl-1H-benzo[e]indole in DMF (3ml) was cooled under 5°C and added dropwise, the reaction mixture was heated to 88 ºC and refluxed for 3 hours while being stirred in an ice bath. Following the addition of the resultant solution to chilly distilled water and neutralization with 25% aqueous NaOH, the yellow precipitate was generated, filtered out, and dried in an oven. ethanol recrystallized to produce pure yellow precipitate. Yield: (1.243 g, 98%). M.P. 202-203 єC.

Synthesis of [C_2]

3-amino acetophenone (0.011 mol) was combined with a solution of chloride (0.011 mol) in (20 mL ethanol + 5 mL DMF), to which 5−6 drops of glacial acetic acid were added. The reaction mixture was then refluxed for 12 hours. Using TLC (3:1) hexane: ethyl acetate and pre-coated silica gel, which produced one spot, the reaction's completion was verified. The product was filtered and recrystallized from a suitable solvent after cooling at room temperature. (2.8g, 64%), M.P. (220 ºC), yield.

Synthesis of Compounds [C_3-C_6]

Equimolar quantity (0.0013 mol) of (2E)-3-((3-acetylphenyl) imino)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo [e] indol-2-ylidene) propanal and appropriate aryl aldehyde mixed and dissolved in 25 mL of ethanol absolute. A 1 mL slowly added 40% potassium hydroxide solution was then mixed intermittently for three hours at room temperature. the final mixture was refluxed for 6 to 8 hours at 78 degrees Celsius. By utilizing TLC and Silica gel-G. (3:1) hexane: ethyl acetate, which produced one spot, the reaction's completion was determined.

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) [12]. 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 105 cell/ml) were sown in a 96-well microplate and grown there for 24 hours at 37°C before the outdated medium was changed out for a brand-new serum-free medium (SFM) that had measurements of each ingredient. Plate was incubated for 24 hours at 37°C with 5% CO2 in a humidified incubator. After incubation, the culture medium was discarded and 100 мl of crystal violate was into each well and re-incubated 20 min at 37oC. The formula below was used to estimate the inhibition percentage:

Inhibition (%) = (A − B / A) Ч 100

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

Results and Discussion

The table lists the physical parameters of the novel compounds, including melting point and yields (1). Spectrum analyses were used to create fresh Schiff bases and chalcones that contained indoles (1H-NMR spectroscopy and FT-IR spectroscopy).

Table (1): Physical properties of the synthesized compounds

Comp. No.	%Yield	Melting Point ϵC
1	98%	207
2	64%	220
3	52%	154
4	62%	180
5	59%	203
6	68%	140

FT-IR spectroscopy study

The FT-IR spectra of the five newly synthesized compounds revealed the novel functional group's absorption band (imine group CH=N) at 1621, 1609, 1609, 1612, and 1610 cm-1 for compounds 2, 3, 4,5, and 6, respectively, which supported the compounds' chemical structures. All compounds (2, 3, 4, and 6) connected to the carbonyl group CH=O of aldehyde showed a prominent absorption band at 1681-1688 cm^{-1}[21]. Whereas 1655-1656 cm^{-}№ for stretching of C= O due to the conjugated with double bonds for the mentioned compounds. There was also a C=C group absorption band at 1598-1400 cm^{-1}. These bands all support the [C2-C6] chemical structures of the produced molecules.
[C_2] FT-IR spectroscopy data (cm^{-1}): 3458 υ (N-H), 3049 υ (CH aromatic),2932 υ (C-H aliphatic), 1688 υ (CH=O), 1668 υ ($COCH_3$) 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).
FT-IR data in (cm^{-1}) of compound [C_3]: 3131 υ (NH), 2885 υ (CHaliphatic),1681 υ (CHO),1656 υ (COCH=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 (COCH=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),1681υ(CHO),1655υ (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, COCH), , 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 (COCH=CH), 1610 υ (CH=N),1598-1455 υ (C=C), 1210 υ (CN),and750 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).

NMR spectroscopy Study

Using TMS (tetramethylsilane) as a standard, 1H-NMR spectra in DMSO (dimethyl sulfoxide) were reported with chemical shifts in ppm. The proton of the (NH) ring of the indole compound (1)'s 1H-NMR measurements showed a single signal at 13.14 ppm. A proton atom from an aldehyde (CH=O) group was identified by a singlet signal at 9.79 ppm. Protons of the aromatic ring for compound (2) were attributed to signals that appeared in the range of 7.69−7.38 ppm. The last peak, which was at 1.96 ppm, was caused by six protons from two methyl groups. The 1H-NMR data for compound (C2)

Figure (2) showed a single signal at 14.24 ppm that corresponded to an indole ring proton (NH). A singlet signal at 9.56 ppm was referred to proton atom of aldehyde (CH=O) group. A proton of the Schiff base group (CH=N) was assigned to a singlet signal at 8.81 ppm. Singlet signals at 2.69 ppm and signals in the range of 7.82 to 8.17 ppm were ascribed to aromatic protons, respectively ($COCH_3$). The peak at 1.89 ppm was belonged to six protons of two methyl groups. Whereas the ^{1}H-NMR of compound (C_3) Fig. (3) shown single signals at 13.46 ppm was belonged to proton of (NH) of indole ring. A singlet signal at 9.84 ppm was referred to proton atom of 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 [22].

^{1}H NMR results of other compounds are listed in table (2).

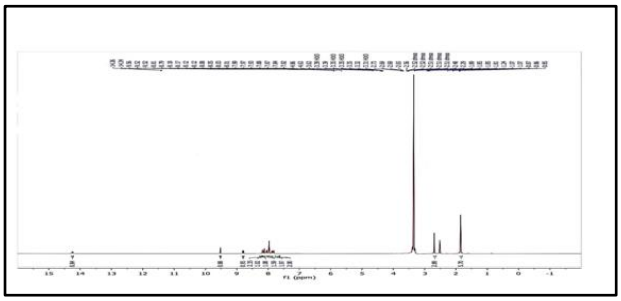

Figure 2. 1H-NMR spectra of [C1]

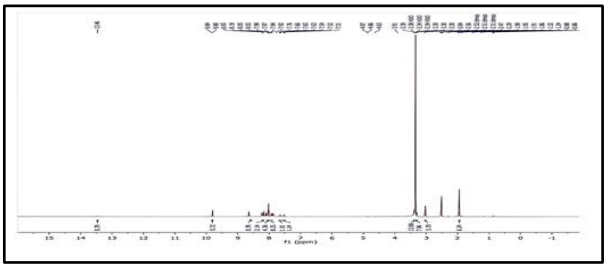

Figure 3. 1H-NMR spectra of [C2]

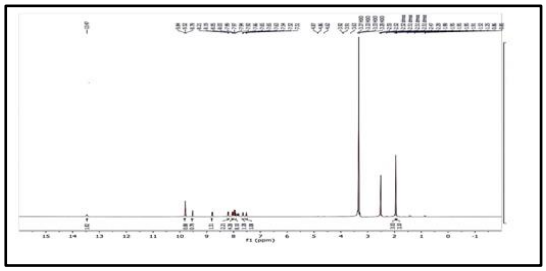

Figure 5. 1H-NMR spectra of [C4]

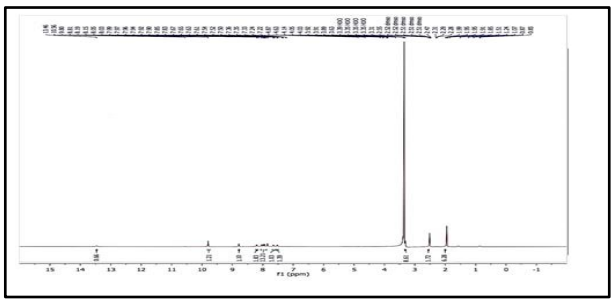

Figure 4. 1H-NMR spectra of [C3]

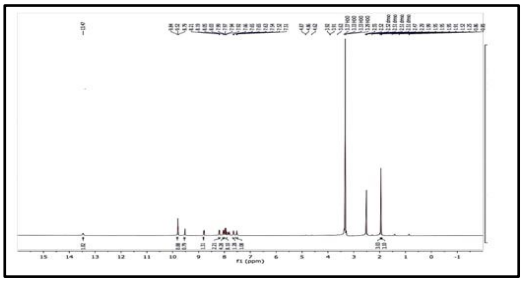

Figure 6. 1H-NMR spectra of [C5]

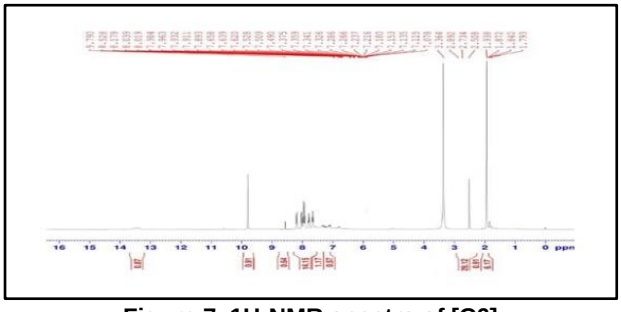

Figure 7. 1H-NMR spectra of [C6]

Table. (2): The chemical shift in ppm to1H 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 $COCH_3$
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	-

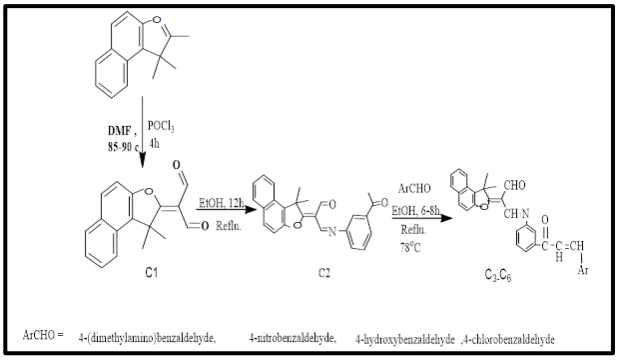

Figure 8. Synthesis of (C1,C2,(C3-C6))

In vitro cytotoxic activity

The three new composite vehicles (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 a.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 3: The in vitro cytotoxicity effect of prepared organic compounds on different cell lines at 50and 100 µg/ml after 24 hr incubationat 370C

Derivatives No.	Inhibition ratio100% Normal Cell Line MEF		Inhibition ratio100% Cell Line cancer MCF7	
	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 effort, novel derivatives of indole compounds classified C1−C6 were produced. A variety of spectroscopic techniques, including FT-IR and 1H-NMR, as well as measurements of some of these compounds' physical properties, were used to characterize them. The cytotoxic efficacy of target substances against the human breast cancer cell line MCF7 was examined. The findings indicated that the substances had potential cytotoxic effects on the MCF7 cell line, particularly compound [C2], which had the greatest inhibition at a rate of 100 g/ml among the substances examined at various doses.

Acknowledgment

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