RESEARCH ARTICLE

Quantitative Estimation of Few Novel Indole Derivatives using Spectroscopic Technique

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ABSTRACT

The reaction of 2- (1, 1-dimethyl-1, 3-dihydro-2H-benzo[e]indol-2-ylidene) malonaldehyde with various substituted o-phenylinediamine resulted in a number of new indole Schiff base derivatives. Total leukocyte count (TLC), Fourier-transform spectroscopy (FT-IR), Proton nuclear magnetic resonance (^{1}HNMR), characterized the chemical structures of the synthesized compounds and quantitatively estimated using FT-IR spectroscopy, the results showed linear in concentration ranges 10.0 to 50.0 ppm. The biological activity of some novel synthesized compounds was tested against two types of bacteria. Compounds exhibit antibacterial action, according to the provided data.

Keywords: Biological activity, Quantitative estimation, Schiff base, o-phenylinediamine.

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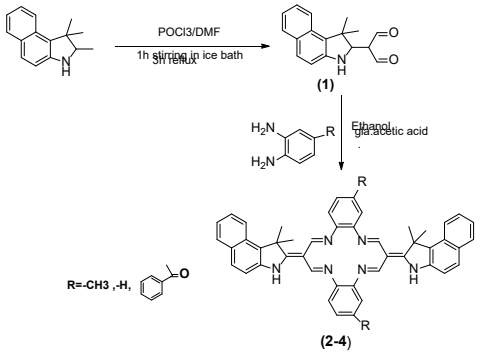
INTRODUCTION

When any primary amine reacts with an aldehyde or ketone under particular conditions, Schiff bases are produced. It is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (C=O) has been substituted by an azomethine or imine group (also known as azomethine or imine) .[1, 2] It is also an essential group of chemicals in the medical and pharmaceutical industries since they have been demonstrated to have a wide range of biological actions, such as antifungal, [3] antibacterial, [4] anti-inflammatory, [5] anticancer, [6] antimicrobial.[7, 8] Similarly, indole derivatives are produced for a range of biological functions over a lengthy period of timeincluding Central nervous system (CNS) depressive, anticancer, antibiotic, antihistaminic, anticonvulsant, and more.[9] Indole is also very popular as a pharmacophore in a variety of pharmacological situations. Its intriguing molecular architecture piques the curiosity of organic and medicinal chemists who want to create therapeutic derivatives.[10] The aim of this study is to synthesize novel indole-schiff base derivatives from the synthesized 2- (1, 1-dimethyl-1, 3-dihydro-2H-benzo[e]indol-2-ylidene) malonaldehyde as shown in scheme 1, which was reported by, [11] quantified, and then test them for biological activity against two gram-positive (G+ve) isolates, *Staphylococcus aure* and *Staphylococcus Epidermidis*, and two gram-negative (G-ve) *Escherichia coli, Klebsiella sp.* by disk diffusion, the results were acceptable .



Scheme 1: The three novel indole Schiff bases derivatives are synthesized using a generic scheme.

EXPERIMENTAL

Instruments

The melting points were determined with open capillary melting point equipment, and the purification was done with a thin layer purification method. Silica gel sheets were used for chromatography, and the spots were viewed using a florescence analysis cabinet type CM-10. Interventional radiology (IR) spectra were taken using a Perkin-Elmer spectrum version 1, Proton nuclear magnetic resonance (^{1}HNMR) spectra were taken with a Bruker 300 MHz spectrometer in Dimethyl sulfoxide (DMSO) and $CHCl_3$.

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Solvents Chemicals

The chemicals and solvents utilized in this study came from a variety of sources, such as Merck, Thomas Baker, Fulka, BDH, Sigma Aldrich, and Scharlau. They were used as received without further purification.

Synthetic Methods

2- (1, 1-dimethyl-1, 3-dihydro-2H benzo[e]indol-2-ylidene) malonaldehyde (1) synthesis using a ginyral method As illustrated in Scheme 2.

N, N-dimethyl formamide (DMF) (3 mL) was cooled in an ice bath, then (1.3 mL) Phosphoryl chloride ($POCl_3$) was added dropwise with stirring under 5°C, then a solution of (1 g, 4.7 mmol) 1, 1, 2-trimethyl-1H-benzo [e] indole in DMF (3 mL) was added dropwise, and the reaction mixture was stirred on an ice path for 1-hours. The resultant solution was mixed with ice distilled water and neutralized with aqueous NaOH at a concentration of 25%. The yellow precipitate was filtered and dried in the oven. The product is recrystallized by ethanol, resulting in a pure yellow precipitate yield:92%, m.p=203-204°C.

Synthesis of (5E, 7E, 8E, 14E, 16E, 17E) -7- (1, 1-dimethyl-1, 3-dihydro-2H-benzo[e]indol-2-ylidene) -16- (3, 3-dimethyl-1, 3-dihydro-2Hbenzo[g]indol-2-ylidene) -7, 16 dihydrodibenzo[b, i][1,4,8,11] tetraazacyclotetradecine comp. (2) . As illustrated in Scheme 3.

A 0.43 gm (2 mmol) solution of benzene-1, 2-diamine dissolved in ethanol (10 mL) was added to a 0.25 gm (2 mmol) solution of 2- (1, 1-dimethyl-1, 3-dihydro-2H-benzo [e] indol-2-ylidene) malonaldehyde dissolved in distilled ethanol (20 mL) The mixture was then added 1-mL of glacial acetic acid. In a water bath at 75°C, the mixture was refluxed. The result was an orange precipitate. After 20 hours of refluxing, the mixture was filtered, washed with ethanol, and dried in a 50°C oven. TLC was used to verify the reaction's completion (3:1) hexane: ethyl acetate with silica gel that has been pre-coated. Yield (0.53g, 85%), mp:<300°C, IR data in (cm^{-1}): 3149 (NH), 3054 (CH aromatic), 2960-2865 (C-H) Aliphatic, 1633 (CH=N), 1594 (C=C aromatic), 1314 (CH_3), 1251 (C-N), 731 (CH) bending

^{1}HNMR (300 MHz, CDCl3, δ in ppm): δ =14.90 (s, 2H, 2xNH indol ring), 9.26 (s, 4H, 4x CH=N), 7.28-8.12 (m, 20H, 6x Ar-H), 1.27 (s, 12H, 4xCH3) .

Synthesis of (5E, 7E, 8E, 14E, 16E, 17E) -7- (1, 1-dimethyl-1, 3-dihydro-2H-benzo[e]indol-2-ylidene) -16- (3, 3-dimethyl-1, 3-dihydro-2H-benzo[g]indol-2-ylidene) -2, 12-dimethyl-7, 16-dihydrodibenzo[b, i][1,4,8,11] tetraazacyclotetradecine comp. (3) As illustrated in scheme 4.

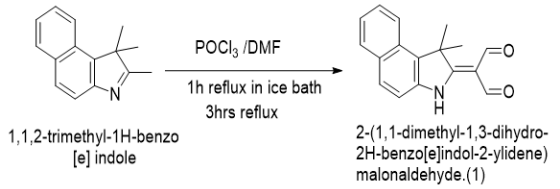
In distilled ethanol (10 mL), a solution of (0.48 gm, 2 mmol) of 4-Methyl-1, 2-phenylenediamine was added to a solution of (0.25 gm, 2 mmol) of (1, 1-dimethyl-1, 3-dihydro-2H-benzo [e] indol-2-ylidene) malonaldehyde (20 mL). The mixture was then given 1-mL of glacial acetic acid. In a water bath at 75°C, the mixture was refluxed. After 5 minutes, a dark brown precipitate appeared. The refluxing was continued for another 18 hours before being filtered, rinsed with ethanol, and dried in a 50°C oven. TLC (4:1) hexane: ethyl acetate with pre-coated silica gel was used to check for reaction completion. Yield (0.58 g, 87%), m.p: <300°C. IR data in (cm^{-1}): 3122 (NH), 3060 (CH aromatic), 2958-2864 (C-H) Aliphatic, 1637 (CH=N), 1562 (C=C aromatic), 1312 (CH_3), 1253 (C-N), 708 (CH) bending ^{1}HNMR (300 MHz, DMSO, δ in ppm): δ =14.93 (s, 2H, 2xNH indol ring), 9.13 (s, 4H, 4x CH=N), 7.27-8.27 (m, 18H, 6x Ar-H), 1.96 (s, 6H, 2x ArCH3), 1.24 (s, 12H, 4x CH3) .

Synthesis of (5E, 7Z, 8E, 14E, 16Z, 17E) -7, 16-bis (1, 1-dimethyl-1, 3-dihydro-2H-benzo[e]indol-2-ylidene) -2, 12-dimethyl-7, 16 dihydrodibenzo[b, i][1,4,8,11] tetraaza cyclotetradecine comp. (4) as illustrated in Scheme 5.

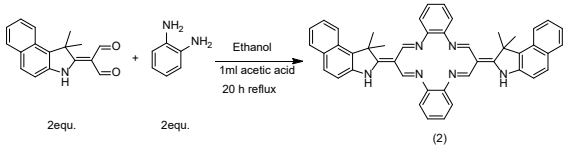
A solution of (0.85 gm, 2 mmol) 4-Methyl-1, 2-phenylenediamine dissolved in ethanol (10 mL) was mixed with a solution of (0.25 gm, 2 mmol) (1, 1-dimethyl-1, 3-dihydro-2H-benzo [e] indol-2-ylidene) malonaldehyde (8) dissolved in distilled ethanol (20 mL) . The mixture was then given 1-mL of glacial acetic acid. In a water bath at 75°C, the mixture was refluxed. After 5 minutes, a brawn precipitate developed. The refluxing was continued for another 25 hours before being filtered, rinsed with ethanol, and dried in a 50°C oven. TLC (3:1) hexane: ethyl acetate with pre-coated silica gel was used to check for reaction completion. Yield (0.58 g, 87%), m.p: <300°C. IR data in (cm^{-1}): 3141 (NH), 3062 (CH aromatic), 2960-2873 (C-H) aliphatic, 1633 (CH=N) and (C=O keton), 1590 (C=C aromatic), 1310 (CH3), 1251 (C-N), 708 (CH) bending. ^{1}HNMR- (300 MHz, DMSO, δ in ppm): δ =14.89 (s, 2H, 2xNH indol ring), 9.20 (s, 4H, 4x CH=N), 7.28-7.96 (m, 28H, 8x.Ar-H), 1.28 (s, -H, 4xCH3) .

Quantitative Estimation of Compounds (2, 3 and 4) by FT-IR Spectroscopy Standard Stock Solutions Preparation

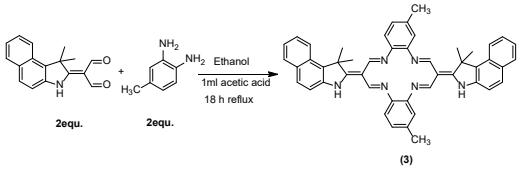
In separate 50 mL volumetric flasks, standard stock solutions containing 500 ppm of (2-4) were prepared. These solutions



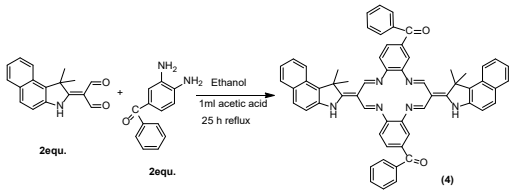
Scheme 2: Synthesized 2- (1, 1-dimethyl-1, 3-dihydro-2H-benzo[e] indol-2-ylidene) malonaldehyde (1).



Scheme 3: Compound syntheses (2).



Scheme 4: Compound syntheses (3)



Scheme 5: Compound syntheses (4).

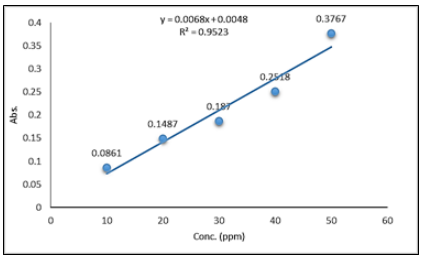


Figure 1: Calibration curve of compound (2).

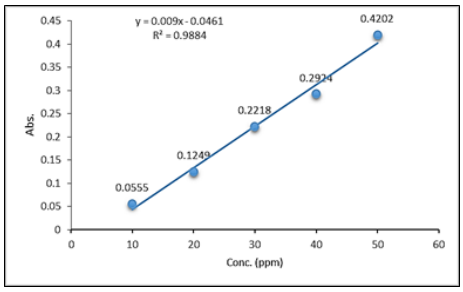


Figure 2: Calibration curve of compound (3).

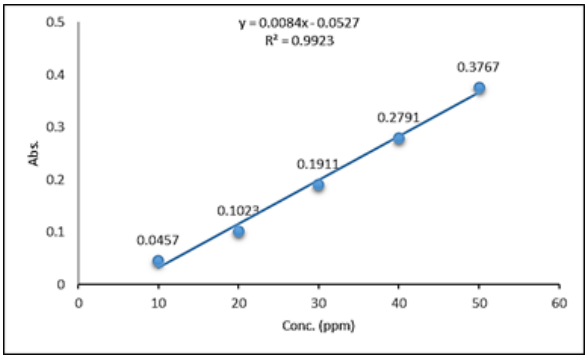


Figure 3: Calibration curve of compound (3) .

were made by dissolving 25 mg of each organic component in ($CHCl_3$, DMSO solvent) and diluting to the desired concentration using solvent. Light was kept out of the stock solutions. Further dilution was done to achieve (100 ppm) of each organic constituent for the creation of working solutions.

Sample Preparation for Linearity

Solutions comprising various concentrations of the referenced chemical were generated by serial dilutions at concentration levels of (10–50) ppm for the tested compounds in order to determine if there is a direct proportional connection between the analyte response and its concentration.

RESULTS AND DISCUSSION

Novel indoles containing Schiff bases were synthesized and characterized using spectral measurements (^{1}HNMR, and FT-IR). Physical characteristics of the new compounds, such as melting point and yields, are shown in Table 1.

Study using IR

The three novel synthesized compounds' infrared spectra indicated a substantial absorption band of the new functional group. (imine group CH=N) at 1633, 1637, and 1633 cm^{-1} for compounds 2, 3, and 4, [12], respectively, confirming the chemical structure. There was also an absorption band at 1594, 1562, and 1590 cm^{-1} for the stated compounds 2, 3, and 4, which belonged to the C=C group.[13] The absorption bands at 1251, 1253, and 1251, on the other hand, belonged to the C-N group.[14] The chemical structures of the produced substances have been confirmed in all of these bands (2, 3 and 4) .

Study using ^{1}H-NMR

^{1}H-NMR spectra in DMSO (dimethyl sulfoxide) and chloroform were published with chemical shifts in ppm and TMS (tetramethylsilane) as the standard.

^{1}H-NMR For compound (2), the ^{1}H-NMR measurements revealed a single signal at 14.90 ppm that corresponded to the proton of the indole ring's (NH),[15] with a single signal at 9.26 ppm attributable to a Schiff base group proton (CH=N).[16] For compound, a signal arose in the range of (7.28-8.12 ppm) that corresponded to protons of the aromatic ring (2).[17,18] Finally, at 1.27 ppm, the signal was attributed to six protons from two methyl groups.[19] Other compounds' ^{1}H-NMR findings (2 and 3) are reviewed and mentioned in Table 2 .

Table 1: Physical properties of the synthesized compounds

Comp. no.	*Molecular formula*	*M. wt (g/mol)*	*Yield %*	*Appearance*	*M.P°C*	*Rec. solvent*
2	C46H38N6	674.85	85	Orange	<300	Chloroform
3	C48H42N6	702.91	87	Dark brown	<300	DMSO
4	C60H46N6O2	883.07	90	Brown	<300	DMSO

Table 2: The chemical shift in ppm of ^{1}HNMR results of compounds (2-4)

Comp. no.	*NH*	*HC=N*	*Ar-H*	*6H 2×Ar-CH_3*	*12H 4×CH_3*
2	14.90	9.26	7.28-8.12	-	1.27
3	14.93	9.13	7.27-8.27	1.96	1.24
4	14.89	9.20	7.28-7.96	-	1, 28

Table 3: The selection wavenumber of each organic compound

Comp. no.	*Wavenumber*
2	1620
3	1690
4	1680

Table 4: Analytical parameters

Organic compound	*Beer's law limit (ppm) con.*	*Regression Equation*	*Slope*	*Correlation coefficient*
2	10-50	Y= 0.0068x-0.0048	0.0068	0.9523
3	10-50	Y= 0.009x-0.0461	0.0090	0.9884
4	10-50	Y= 0.0084x-0.0527	0.0084	0.9923

Table 5: Accuracy and precision of the method.

comp. no.	*Calculated Conc. (ppm)*		*RSD%*	*RE%*
	Taken	*Mean**		
9	25	24.9481	0.1028	-0.2076
10	25	24.8592	0.0755	-0.5632
11	25	25.0079	0.0635	0.0316

*Average three replicates

Table 6: Antibacterial activity of the synthesized compounds.

Comp. symbol	*Inhibition zone diameter (mm) for compounds*			
	Gram-positive		*Gram-negative*	
	S. aureus	*S. epidermidis*	*E. coli*	*Klebsiella sp.*
3	14	11	16	14
4	15	12	17	15
Gentamicine	36	35	35	42
DMSO	0	0	0	0

Quantitative Analysis of Organic Compounds Wavenumber Selection

To determine the correct wavenumber for recognizing the concentration bands of the studied compounds that yielded satisfactory absorbance values for each of the three analytes independently.

The absorption was calculated using the equation:

A= Log (1/T)

The spectrum of (30 ppm) solution of each compound in the wavenumber range of (400–4000) cm^{-1} was recorded. Shows the IR spectra of the three compounds from which the best wavenumber was chosen for qualitative evaluation and quantification of the cited compounds Table 3 .

Construction of Calibration Curve

Percent transmittance and absorbance values of the studied compounds were calculated of different concentrations (10–50) ppm. As a result, a calibration plot was created for each component's test by plotting the observed absorbance values against the compound concentration. A linear relationship was found Figures (1-3), This was used to compute the regression equation and coefficient of determination (R2) .

Analytical Characteristics

For the determination of these compounds, analytical properties such as Beer's law limit, slope, and correlation coefficient were computed, and the findings are presented in Table 4 .

Precision and Accuracy

The precision of the suggested technique was determined by calculating the values of relative standard deviation percentage (RSD percent) of the obtained findings for the analyzed compounds, and the accuracy of the method was determined by calculating the values of relative error percentage (RE percent) . For each chemical, three replicate studies were performed at one level of concentration within the linearity range. The obtained findings show that the proposed approach is accurate and precise at the concentration level examined Table 5 .

Antibacterial Activity

Some synthesis compounds (3 and 4) were tested for biological activity by the agar diffusion method. These compounds were tested, and the plates were incubated at 37°C for 24 hours. These compounds were evaluated for antibacterial activity against different bacterial strains (*S. aureus, S. epidermidis, E. coli, Klebsiella sp.*) These bacteria were selected because of their importance in the medical field, since they cause many diseases and have resistance to many chemical drugs and antibiotics. The concentration of the compounds was 10 mg/mL, inhibition zones were measured in millimeters and were compared with (Gentamicine) as a standard antibiotic reference. The results of the antibacterial activity tests are shown in Table 6, which shows that compounds 3 and 4 showed good antibacterial activity, especially against *E. coli.*

CONCLUSION

Novel indole-containing Schiff basse derivatives (2-4) were synthesized from 2- (1, 1-dimethyl-1, 3-dihydro-2H-benzo[e] indol-2-ylidene) malonaldehyde and three distinct o-phenylene diamine derivatives. FT-IR and ^{1}H-NMR spectrum methods were used to characterize the produced derivatives. The examined substances were measured using infrared technology, recording the absorption spectra of the compounds in question, and it was discovered that they were applied at concentrations of 10-50 ppm. According to the pharmacological analysis, several compounds had good efficacy against gram-negative (*E.coli* and *Klebsiella sp*) and gram-positive (*S. epidermidis, S. aureus*) bacteria.

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