

Synthesis and Characterization of New Indole Schiff Bases and Study Effect of the Compounds on Lymphatic Cell in Metaphase in Human Blood

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Abstract

Three new Schiff Bases have been synthesized by reaction of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde with substituted aniline.

The chemical purity structures of the new synthesized compounds was observed by TLC and the chemical structures were characterized by FT-IR, ^1H , and APT ^{13}C NMR. The biological activity of the new synthesized compounds screened on Lymphatic Cell in Metaphase in Human Blood, which were revealed different results

Keywords: Schiff Bases, substituted anilines, lymphatic Cell in Metaphase in Human Blood.

1. INTRODUCTION

An azomethine group is a functional group that contains a carbon nitrogen double bond with the nitrogen atom linked to an aryl or alkyl group but not hydrogen. Stabilized Schiff bases are usually synthesized from the condensation of aromatic primary amines and active carbonyl group of aromatic aldehydes and ketones [1].

Indole ring is an aromatic heterocyclic compound, and it is a white powder at room temperature. The indole chemical formula is $\text{C}_8\text{H}_7\text{N}$. It has an aromatic bicyclic structure consisting of a five-membered pyrrole ring fused together with a benzene ring to form two isomeric benzopyrrole figure 1 [2]

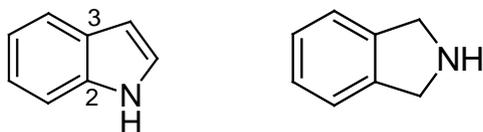


Figure 1: The structural formula of isomeric benzopyrrole

The name *indole* is merging of the words *indigo* and *oleum*, since indole was first isolated by treatment of the indigo dye with oleum. Indole chemistry began with the study of the dye indigo.

Indole derivatives are an important class of organic heterocyclic which have played prominent and vital role in curing so many ailments throughout the history of medicines. Also they are one of the most attractive frameworks with a wide range of biological and pharmacological activities [3] and one of the most active areas of heterocyclic chemistry which acquiring enormous significance in the field of medicinal chemistry in recent years, particularly due to their natural occurrence and pharmacological activities, There are several thousand indole derivatives known and many of these have important pharmacological activity [4, 5]. Indole derivatives also occur widely in many natural products such as those from plants, fungi and marine organisms. Moreover, a great interest which observed of combination of two or more heterocyclic moieties enhances the biological profile many folds than the parent indole nucleus [6] for these reasons, they have been brought the attentions of organic chemists, medicinal chemists, pharmacists and biologists and encouraged them to compete to synthesise new biologically active substances. Our target in the present synthesis three new compounds (1-3) according to synthetic pathway as shown in figure 2 and then screening the biological activity on Lymphatic Cell in Metaphase in Human Blood.

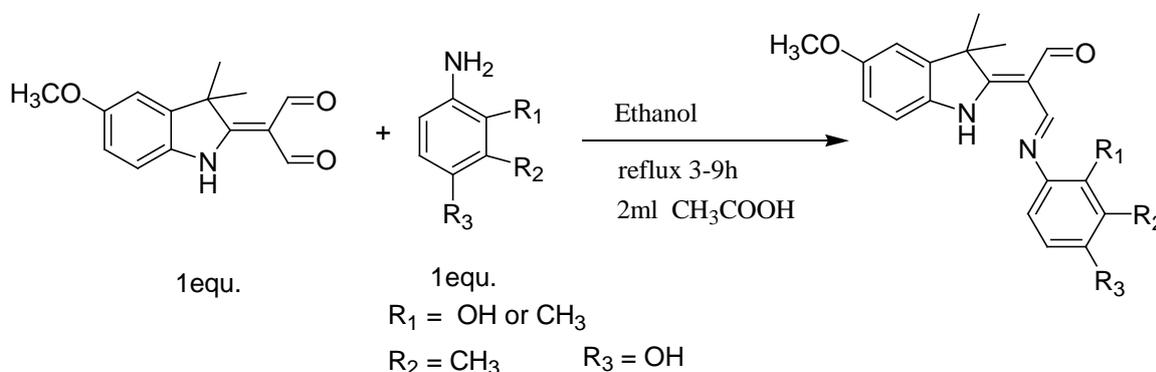


Figure 2: The synthetic pathway of the new synthesized compounds (1-3)

2. EXPERIMENTAL SECTION

2. 1. Chemistry part

All chemicals and solvents used in the chemistry part were purchased from a number of different companies such as Merck, BDH, Sigma Aldrich and Fulka. They were used as obtained without further purification. The starting material 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was synthesized with modification of a procedure defined by [7]. The purity of the synthesized compounds was checked it by TLC sheet and the chemical structures were characterized by FT-IR, ¹H, and APT ¹³C NMR as well as the melting points were determined by open capillary melting point apparatus.

2. 1. 1. Synthetic methods

2. 1. 1.1. Synthesis of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2-hydroxy- phenylimino)-propionaldehyde (1)

A solution of (0.15g, 0.6mmol) of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 10ml with heating and (0.066g, 0.6mmol) of 2-Amino-phenol was dissolved in ethanol 10ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed in a water bath at 78°C for 5h. A solvent was reduced to one quarter; brown 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(4:1) hexane : ethyl acetate with, which gave one spot. Yield (0.18g 90%), m.p. 118-119 °C.

IR data in (cm⁻¹): 3488 ν (O-H)· 3071 (CH aromatic) 2968 ν (CH aliphatic), 2865 ν (CH aldehyde), 1617 ν (CHN), 1588 ν (C=C), 1467 ν (C-C), 1347 ν (CH₃), 1277 ν (C-N), 1029 ν (C-O), and 747 ν (C-H bending).

¹H NMR (400 MHz, DMSO, δ in ppm): δ = 14.03 (s, 1H, NH), 9.37 (s, 1H, HC=O), 8.55 (s, 1H, HC=N), 7.65-6.84 (7H, Ar-H), 3.79 (s, 3H, OCH₃), and 1.57 (6H, s, 2x CH₃); APT¹³C NMR was displayed signals for quaternary carbons, methylene CH₂ and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) like 181.05 for NH-C=C, 108.75 for O=C-C and 54.07 CH₃-C-CH₃. While CH and CH₃ observed at a negative side (below of the spectrum) such as 188.07 for CH=O, 154.29 for CH=N, 55.36 for OCH₃, 21.71 for the two methyl groups, the other chemical shifts positive side and negative side between (157.57-107.52) for Ar-CH

2. 1. 1.2. Synthesis of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-hydroxy-phenylimino)-propionaldehyde (2)

A solution of (0.5g, 2mmol) of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 10ml with heating and (0.21g, 2mmol) of 4-Amino-phenol was dissolved in ethanol 5ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed in a water bath at 78°C for 8.5h. A solvent was reduced to one quarter; dark brown precipitate was formed, filtered off, washed with water and dried in oven at 78 °C. The purity of this compound was determined by using TLC (3:1) hexane: ethyl acetate, which gave one spot. Yield (0.5g 83%), m.p 165-166°C.

IR data in (cm⁻¹): 3246 ν (O-H)· 2976 (CH aromatic) 2829 ν (CH aliphatic), 1632 ν (C=O), 1611 ν (CH=N), 1511 ν (C=C), 1471 ν (C-C), 1354 ν (CH₃), 1277 ν (C-N), 1025 ν (C-O), and 831 ν (C-H bending).

¹H NMR (400 MHz, DMSO, δ in ppm): δ = 13.98 (s, 1H, NH), 9.31 (s, 1H, HC=O), 8.70 (s, 1H, OH), 8.41 (s, 1H, HC=N), 7.44-6.84 (7H, Ar-H), 3.77 (s, 3H, OCH₃), and 1.65 (6H, s, 2x CH₃);

APT¹³C NMR was displayed signals for quaternary carbons, methylene CH₂ and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) 179.73 for (NH-C=C), (156.87-118.7) for (Ar-C), 101.17 for O=C-C and 52.17 CH₃-C-CH₃. While CH and CH₃ observed at a negative side (below of the spectrum) 118.70-107.73 for (Ar-CH), 55.36 for OCH₃ 25.43 for the two methyl groups,

2. 1. 1.3. Synthesis of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,3-dimethyl-phenylimino)-propionaldehyde (3)

A solution of (0.5g, 2 mmol) of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 10 mL with heating and (0.24g, 2 mmol) of 1,3-dimethyl aniline was dissolved in ethanol 5ml and then added glacial acetic acid 2ml to the solution. Themixture was stirring 2h. A solvent was reduced to one quarter; yellow precipitate was formed, filtered off, and dried in oven at 78 °C. The purity of this compound was determined by using TLC (4:1.5) hexane: ethyl acetate, which gave one spot. Yield (0.66g 94.2%), m.p. 105-106°C. IR data in (cm⁻¹): 3341 ν (N-H), 3005 ν (CH aromatic), 2954 ν (CH aliphatic), 1716 ν (CH=O), 1621 ν (CHN), 1595 ν (C=C), 1471 ν (C-C), 1347 ν (CH₃), 1288 ν (C-N), 1061 ν (C-O) and 769 ν (C-H bending),

¹HNMR (400MHz, DMSO, δ in ppm): δ = 9.37 (s,1H, HC=O), 8.59 (s,1H, HC=N), 7.50-6.83(6H, Ar-H), 3.78 (s, 3H, OCH₃), 2.37 and 2.32 (s, 6H, 2x CH₃) and 1.57(s, 6H, 2x CH₃),

APT ¹³C NMR was displayed signals for quaternary carbons, methylene CH₂ and carbon atoms of DMSO solvent which appeared at a positive side (above of the spectrum). While CH and CH₃ observed at a negative side (below of the spectrum). The chemical shifts at 188.00 for CH=O, 147.07 for CH=N, 55.39 for OCH₃, 21.68 for the two methyl groups, 20.04 for *meta*-CH₃ and 13.28 for *Ortho*-CH₃ were appeared at a negative side and aliphatic carbon atoms such as 180.93 for NH-C=C and 107.50 for O=C-C were appeared at a positive side, the other chemical shifts positive side and negative side between (147.07-107.56) for Ar-CH

2. 2. Biological part

2. 2 . 1. Special solutions for testing cellular toxicity on lymphocytes:

Complete Growth Media, Fetal Calf Serum Phytohaemagglutinin (PHA), Colchicin Hypotonic solution, Fixative solution, Sorenson's buffer solution and Giemsa stain

2. 2. 2. Blood collection:

Human venous blood withdrawal from the AB + class by a 5 ml syringe that is pre-washed with heparin to prevent blood clotting. The total blood is used simultaneously.

2. 2. 3. Preparation of glass slides :

The glass slides were cleaned by immersing them with Chromic acid for 72 hours and then wash with hot water and then kept in refrigerator until use

2. 2. 4. Study of Compound Effect in the Mitotic Index in Human lymphocytes:

Study the effect of concentrations in lymphocyte-stimulating cells by method of Verma and Babu (1989) using short-term culture. Blood samples were randomly drawn from healthy individuals ranging in age (25-40 years) by means of a medical syringe containing heparin solution (5ml) of each person and use in the following tests

A. Blood transplantation with extract:

1- 0.2 mL of each compound was added to the whole plant medium PMRI-1640, taking into account the final volume of the mixture and by three replicates per concentration.

2. Add 0.5 ml of blood to each tube using a 5 ml syringe.

3. Add 0.1 ml of the lymphocyte and prepared lymphocyte and then mix gently with the medium and incubate at a temperature of 37 ° C in a tilted manner for 72 hours, taking into account the mixing of the piping content every 12 hours.

4 - left a group of pipes without adding any extract and promised this transaction control

B. Cell Harvesting:

1- (0.1) ml of collagen was added at a concentration of 10 mg / ml for each control tube 15 minutes before the end of the original implant time. The treated tubes were not added to the collagen. The tubes were then returned to the incubator.

2. The tubes containing the samples were placed in the centrifuge for 10 minutes and at a speed of 1500 cycles / minute. The leachate and the precipitate were well disposed of with the leaves of the plant medium.

3 - Add (10-5) ml of low voltage (0.075 M) solution and warm (37) m in increments of each tube gradually and quietly with shaking.

4 - incubated pipes in a water bath (37) m for a period of (30) minutes.

5. The pipes were centrifuged at a speed of 1500 cycles / min for 10 minutes.

C. Fixation:

1 - shake the deposit well and add a few drops of fixative cold fixer on the wall of the tube with constant shaking and complete the size to (5) ml. 2. Mix the samples with Vortex and then heat the tubes (4) m for 30 minutes.

D. Washing:

1-The tubes were placed in the centrifuge for 10 minutes at a speed of 1500 cycles / minute minutes. The leachate was disposed of and the cells were abandoned

2. The installation was repeated several times until the luminous color of the lumen was observed, the precipitate was suspended by (1) mL of the stabilizer and warmly preserved (-20).

E. Dropping:

Clean glass slides free of wet and cold fat is used, mix the cells well and drop the cells onto cold slices using a Pasteur pipette from a distance (1-0.5) meters and left to dry.

F. Pigmentation and microscopy:

Pour the slides in a prepared and diluted formula with the warm-up Sorensen solution and 4: 1 for 3-2.5 minutes. Then, wash with Sorensen and leave to dry.

2. 2. 5. Mitotic Index (MI):

The cell division was calculated according to King et al. (1982). The cells were divided into the total number of cells examined (1000) as in the following equation:

$$\text{Coagulation factor (MI)} = \frac{\text{Number of split cells}}{\text{Total number of cells}} \times 1000$$

Effect of compound as catalysts for the division of lymphocytes into human blood.

3. RESULTS AND DISCUSSION**3. 1. Chemistry results**

The synthesis of new compounds was first attempted by the reaction of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde with substituted aniline in ratio 1:1 under refluxing. The purity of synthesized compounds was checked by TLC and the chemical structures were characterized by FT-IR which recorded on a Perkin-Elmer. Spectrum version 10.02 by using a disk of KBr for solid material in the chemistry Department, College of Science, Diyala, University while ¹H and APT ¹³C NMR spectroscopy were shown on a Bruker 400 MHz spectrometer in Jordan, University of Science and technology, College of science, Irbid city.

3.1. 1. IR Spectroscopy

IR spectral study of compounds (**1**, **2** and **3**) revealed the presence of characteristic of new azomethine group absorption bands appeared at 1617, 1611 and 1621 cm⁻¹, respectively [8], these absorption bands were absent in starting materials, this is an evidence to formation Schiff bases as well as absorption bands of carbonyl groups at 1632 and 1716 cm⁻¹ respectively [9]. In addition, abroad absorption bands of (NH) appeared at 3341 cm⁻¹ respectively [10], another strong absorption bands were observed at 1277, 1277, 1288 of (C-N) groups [11] and at 1029, 1025, 1061 cm⁻¹ (C-O) groups [12] respectively. All these main absorption bands confirmed the formation of the new compounds.

3. 1. 2. NMR Spectroscopy

¹H-NMR and APT ¹³CNMR spectra of new Schiff bases were recorded in dimethylsulfoxide DMSO-d₆ with chemical shifts expressed in ppm using tetramethylsilane TMS as internal standard figures (3- 8).

¹H-NMR spectra of compounds (**1** and **2**) figures 3 and 4) displayed doublet signals at 14.02 ppm and 13.98 which assigned to proton atoms of NH group for compound (**1** and **2**) respectively [13]. Sharp single signals at 9.37 and 9.31 ppm were attributed to proton atoms of CHO groups for compound (**1** and **2**) respectively [14]. As well as

doublet signals at 8.56 ppm and 8.55 which assigned to proton atoms of azomethine groups for compound (1 and 2) respectively.[14]. Signals were appeared in region between 7.65-6.84ppm and 7.44-6.88ppm which attributed to seven proton atoms of aromatic rings for compound (1 and 2) respectively respectively [15]. Single signals of methoxy

groups were observed at 3.37 and 3.77ppm were attributed to proton atoms of OCH₃ groups for compound (1 and 2) respectively [16]. Finally single signals were appeared at 1.57 and 1.54ppm which belonged to six proton atoms of two groups of methyl for compounds (1 and 2) respectively [17].

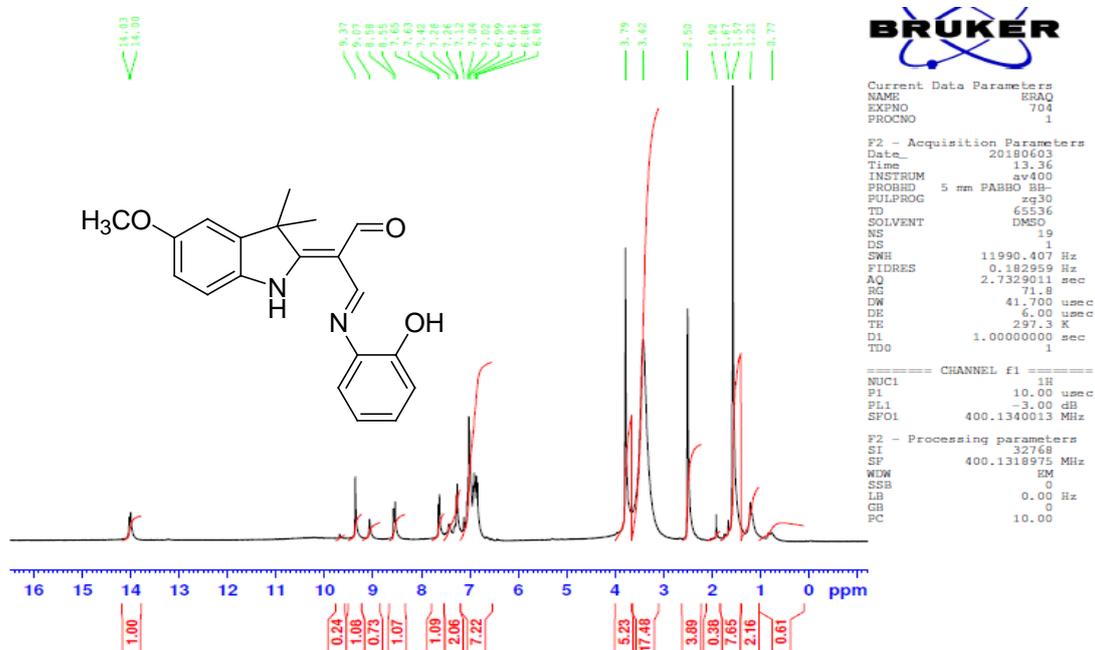


Figure 3: ¹H NMR spectrum of compound (1) in (400MHz, DMSO-d₆).

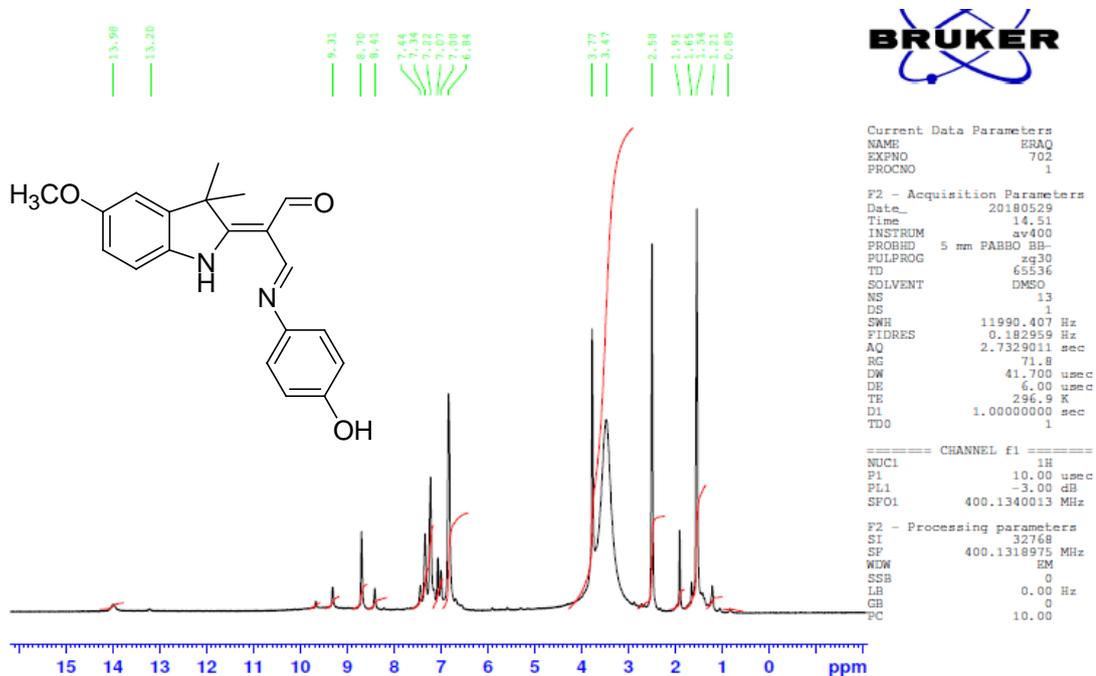


Figure 4: ¹H NMR spectrum of compound (2) in (400MHz, DMSO-d₆)

$^1\text{H-NMR}$ spectra of compound (3) figure 5 shows single signal at 9.37 ppm was attributed to proton atom of CHO group and doublet signal at 8.59 ppm which assigned to proton atom of azomethine group. In addition signals in region between 7.50-6.83ppm which belonged to six proton atoms of aromatic rings as well as two single signals observed at 2.37 and 2.32ppm which attributed to six proton atoms of two methyl groups. Finally single signals were appeared at 1.57 ppm which assigned to six proton atoms of two groups of methyl group [18].

3. 1. 3. APT ^{13}C NMR

APT ^{13}C NMR results of compounds (1, 2 and 3) figures (6- 8) were supported the $^1\text{H-NMR}$ results and confirmed the chemical structures of the new three compounds. For example APT ^{13}C NMR spectra shows signals for quaternary carbons and carbon atoms of solvent which appeared above of the base line of spectrum) While carbon atoms of CH and CH_3 observed below of the base line of spectrum. As results, ^1H , and APT ^{13}C NMR spectrum results for the new three compounds correspond well with the expected signals and confirmation of formation of these compounds.

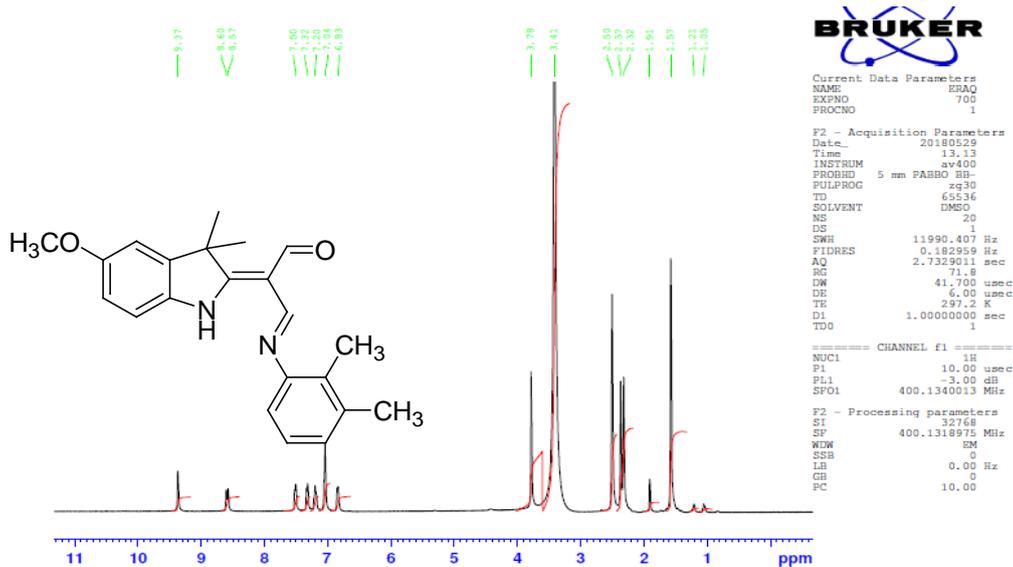


Figure 5: ^1H NMR spectrum of compound (3) in (400MHz, $\text{DMSO-}d_6$).

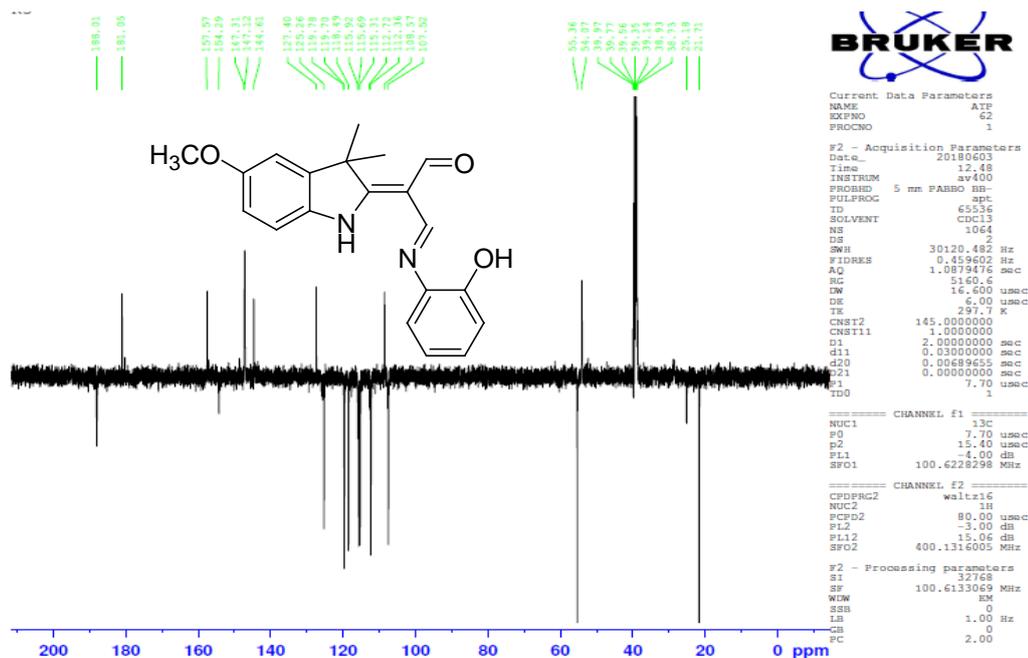


Figure 6: APT ^{13}C NMR spectrum of compound (1)

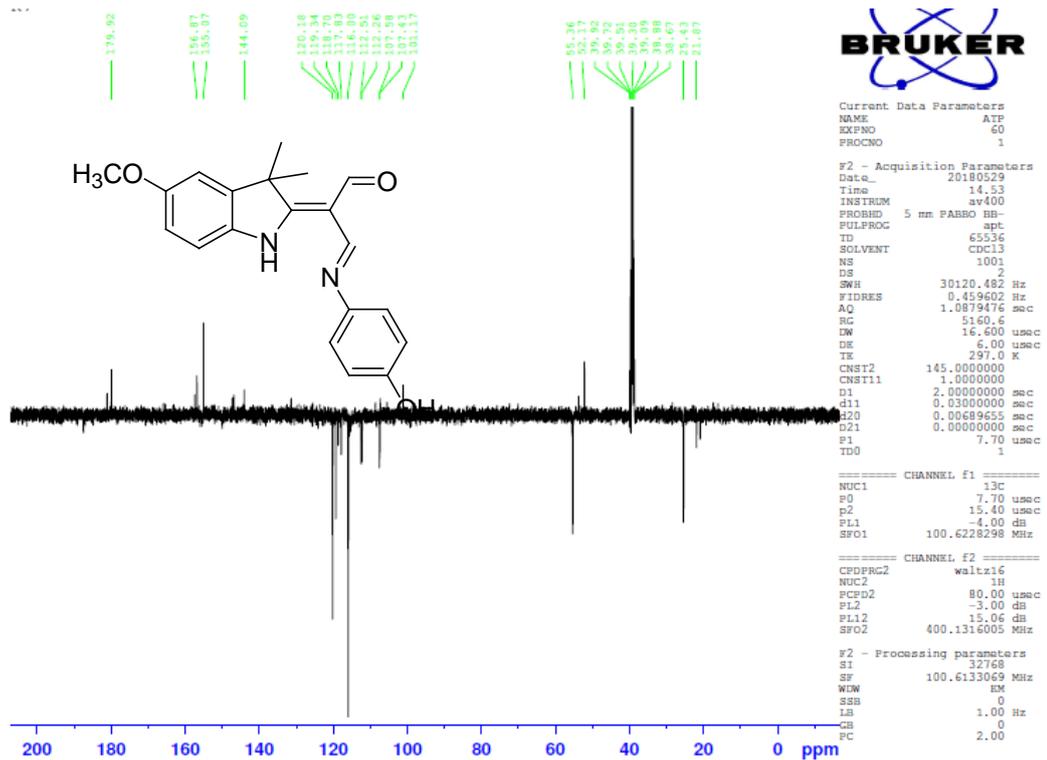


Figure 7: APT ¹³C NMR spectrum of compound (2)

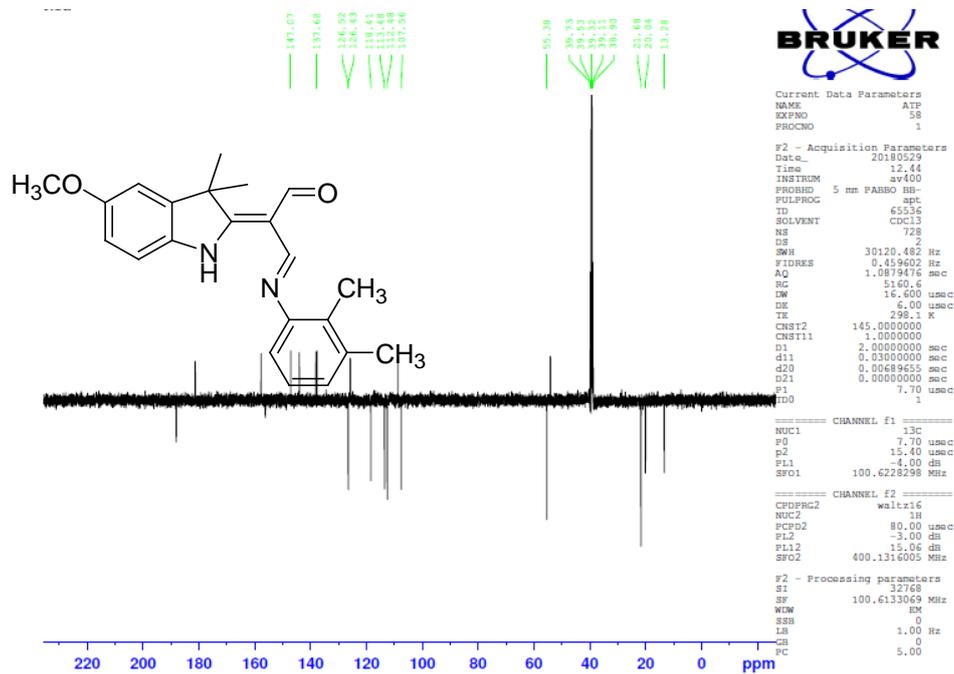


Figure 8: APT ¹³C NMR spectrum of compound (3)

Table 1: The biological results of the compounds (1, 2 and 3) on Lymphatic Cell

Percentage of control after 48h	Cell rate in tropical phase	Percentage of control after 24h	Cell rate in tropical phase	Compounds and control
	0.00±4.20		0.00±4.20	Control
13.33	0.56±0.56	7.14	0.11±0.30	Compound (1)
55.47	0.08±2.33	36.42	0.17±2.21	Compound (2)
91.19	0.03±3.83	69.04	0.051±2.90	Compound (3)

3. 2. Biological results

The biological results of the compounds (1, 2 and 3) on Lymphatic Cell in Metaphase in Human Blood showed different results of each compound as demonstrated in table 1

The compound (1) figure 9 showed less control ratio compared to compound (2) figure 10 which showed a higher control ratio due to its containing hydroxyl group OH substituted in *ortho* site on benzene ring. While the compound (2) showed a higher control ratio due to its containing hydroxyl group OH substituted in *para* site on benzene ring, as conclusion. Difference in site of hydroxyl group on benzene ring affects the effectiveness of the compound. As well as the activity increase with time as explain for compound (1) It was 7.14 after 24h and became 13.33 after 48h, also the compound (2) It was 36.42 after 24h and became 55.47 after 48h.

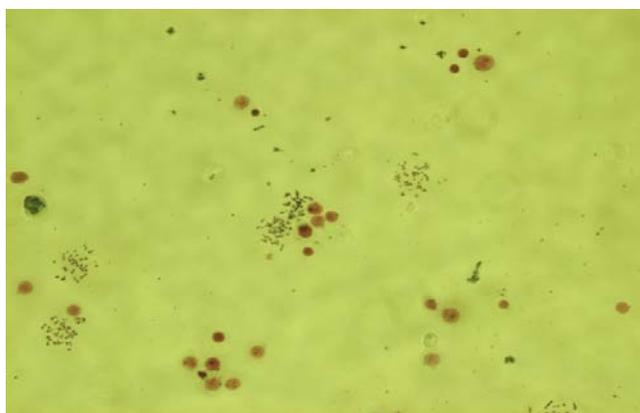


Figure 9: A tropical phase suspended for lymphocytes of human blood when used 0.2ml compound (1) (400X)

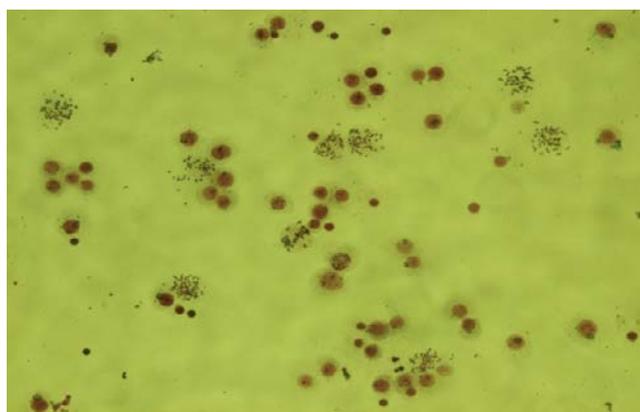


Figure 10: A tropical phase suspended for lymphocytes of human blood when used 0.2ml compound (2) (400X)

The compound (3) figure 10 showed a high control ratio compared with other compounds (1 and 2) it was 69.04 after 24h and became 91.19 after 48h because its contains two methyl groups CH₃ in sites *ortho* and *meta* on benzene ring.

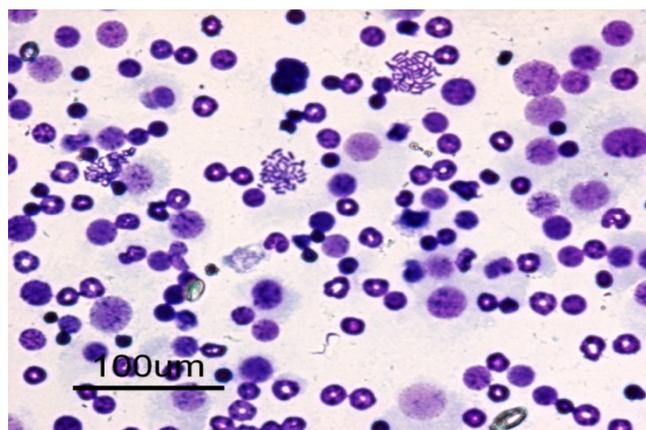


Figure 10: A tropical phase suspended for lymphocytes of human blood when used 0.2ml compound (3) (400X).

Thus. The difference of biological activity of the compounds (1, 2 and 3) on Lymphatic Cell in Metaphase in Human Blood depend on nature, number and position of the substituted group on benzene ring.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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CONCLUSION

Three new compounds [2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2-hydroxy-phenylimino)-propionaldehyde (1), 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(4-hydroxy-phenylimino)-propionaldehyde (2) and 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(2,3-dimethyl-phenylimino)-propionaldehyde (3)] have been synthesized by reaction of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde with substituted aniline. The purity of new synthesized compounds was checked by TLC and the chemical structures were characterized by some Spectroscopic techniques such as FT-IR, ¹H, and APT ¹³C NMR. The biological activity of the new synthesized compounds screened on Lymphatic Cell in Metaphase in Human Blood, which were revealed different results

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