DOI : 10.35124/bca.2019.19.2.4517

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 4517-4524, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

SYNTHESIS OF NEW INDOLE SCHIFF BASES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITY ON LYMPHATIC CELL IN METAPHASE IN HUMAN BLOOD

Fadhil Lafta Faraj[1], Luma Salman Abd[1], Ahmed Hatem Alwan[2] and Rusul Adnan Nafia[1]

[1]Department of Chemistry, Faculty of Science, University of Diyala, Diyala Governorate, Iraq.
[2]Department of Biology, Faculty of Science, University of Diyala, Diyala Governorate, Iraq.
*e-mail: fadhillaftafaraj@gmail.com

(Received 16 April 2019, Revised 24 July 2018, Accepted 29 July 2019)

ABSTRACT : Six 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 structures of the new synthesized compounds were characterized by TLC, FT-IR, ^{1}H, 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 compared with Colchicine.

Key words : Schiff Bases, aniline substituted, lymphatic cell in Metaphase in human blood.

INTRODUCTION

Azomethine group (–CH=N–) containing compounds typically known as Schiffbases have been synthesized by the condensation of primary amines with active carbonyls (Essa ***et al***, 2012). The common structural feature of these compounds is the azomethine group with a general formula $R_1HC=N-R_2$, where R_1 and R_2 are alkyl, aryl, cyclo alkyl or heterocyclic groups whichmay be variously substituted. These compounds are also known as anils, imines or azomethines (Ashraf ***et al***, 2011).

Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a benzene ring and a pyrrole nucleus are fused in 2, 3 positions of the pyrrole ring (Lalit ***et al***, 2012).

Indole compounds include the plant hormone Auxin, the anti-inflammatory drug indomethacin, the β-blocker pindolol and the naturally occurring hallucinogen dimethyltryptamine (Sai ***et al***, 2015). For these reasons, they have been brought the attentions of organic chemists, medicinal chemists, pharmacists and biologists and encouraged them to compete to synthesize new biologically active substances. Our target in the present study synthesis six new compounds (1-6) according to synthetic pathway as shown in scheme 1 and then screening their biological activity on Lymphatic Cell in Metaphase in Human Blood.

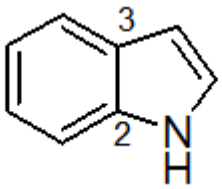


Fig. 1 : The structural formula of isomeric benzopyrrole.

Experimental section

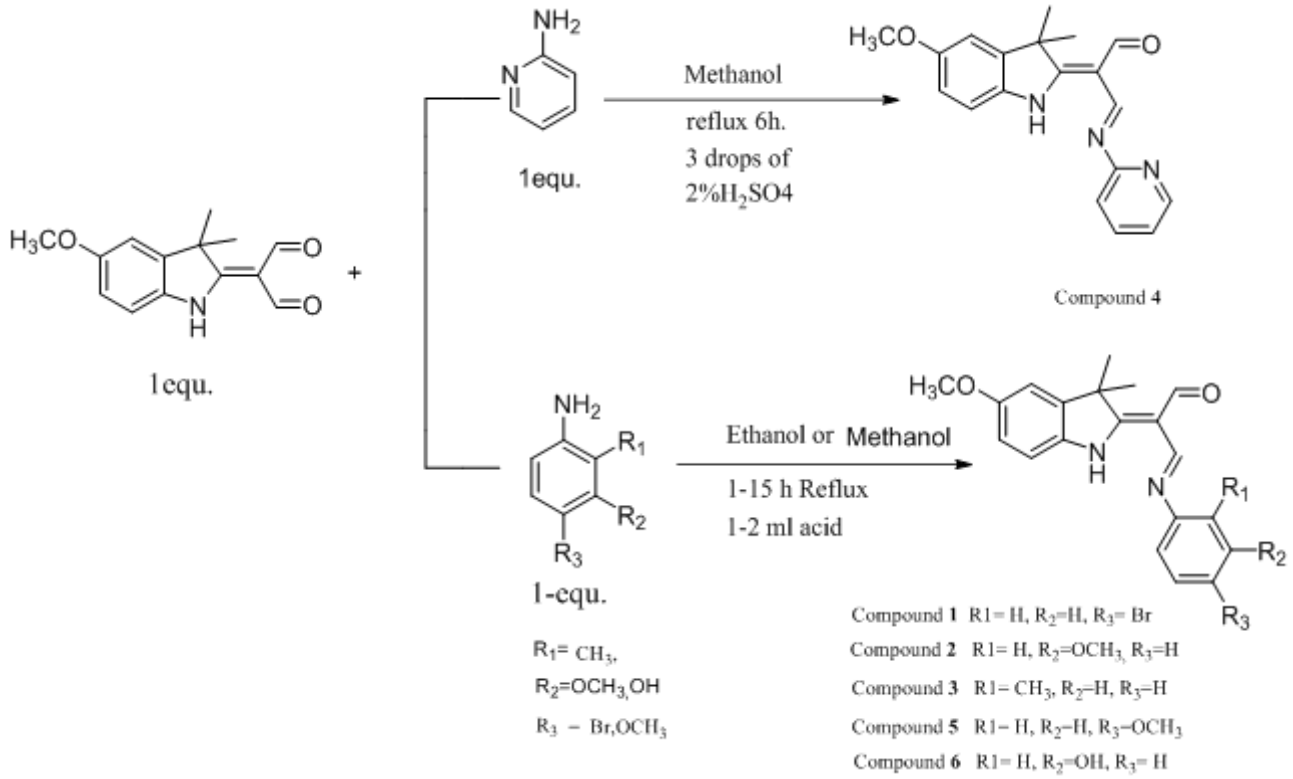
Chemistry part

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 Aghdam ***et al*** (2013). The purity of the synthesized compounds was checked it by TLC sheet and the melting points were determined by open capillary melting point apparatus.

Synthetic methods

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

A solution of (0.3g, 1.2mmol) of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 10ml and (0.206g, 1.2mmol) of 4-Bromo-phenylamine was dissolved in ethanol and then 1 ml of glacial acetic added to this solution. The mixture



Scheme 1 : The synthetic pathway of the new synthesized compounds (1-6).

was refluxed in an oil bath at 78ºC for 10h. A solvent was reduced to one quarter; brown precipitate was formed, filtered off, washed with water and dried in oven. The purity of this compound was determined by using TLC (4:2) hexane: ethyl acetate, which gave one spot. Yield (0,45g 91.8%), m.p. (122-123ºC). IR data in (cm^{-1}) : 3061 υ(CH aromatic), 2931 υ(CH aliphatic), 2865 υ(CH aldehyde), 1618 υ(CHN), 1523 υ(C=C), 1472 υ(C-C), 1334 υ(CH_3), 1287 υ(C-N), 1076 υ(C-O) and 814 υ(C-H bending), ^{1}HNMR(400MHz, DMSO, δinppm): δ = 13.25(s,1H,N***H***),10.07(s,1H,***H***C=O),9.71(s,1H, ***H***C=N), 7.58-6.88(7H,Ar-***H***), 3.80 (s, 3H, OCH_3), 2.52 and 2.52 (6H,s, 2x C***H***$_3$); APT ^{13}C NMR was displayed signals for quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum). The chemical shifts at 176.73 for ***C***H=O, 157.50 for ***C***H=N, 55.33 for OCH_3 and 22.78 for the two methyl groups were appeared at a negative side and aliphatic carbon atoms such as 168.10 for NH-***C***=C and 108.80 for O=C-***C***=C were appeared at a positive side, the other chemical shifts positive side and negative side between (132.67-108.48) for ***Ar***-***C***H.

Synthesis of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(3-methoxy–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 10 ml with heating and (0.250g, 2mmol) of 3-methoxy–phenylimino was dissolved in ethanol 5ml and then added glacial acetic acid 2ml to the solution. The mixture wasrefluxedin a water bath at 78ºC for 10h. A solvent was reduced to one quarter, brown precipitate was formed, filtered off, dried in oven at 78ºC. The purity of this compound was determined by using TLC (4:1) hexane: ethyl acetate with, which gave one spot. Yield (0, 62g, 87%), m.p. 149-150°C. IR data in (cm^{-1}):3063 υ(CH aromatic), 2961 υ(CH aliphatic), 2859υ (CH aldehyde), 1614 υ(CHN), 1530 υ(C=C), 1471 υ(C-C), 1332 υ(CH_3), 1281 υ(C-N), 1076 υ(C-O) and 813 υ(C-H bending). ^{1}HNMR (400MHz, DMSO, δinppm): δ = 9.57(s,1H,***H***CO), 8.85(s,1H, ***H***C=N), 6.90-8.10(7H, Ar-***H***), 3.82 (s, 6H, OCH_3) and 1.61(6H,s, 2x C***H***$_3$); APT^{13}C NMR was displayed signals for quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) like 178.73 for NH-***C***=C, 119.63 for O=C-***C***=C and 53.22 CH_3-***C***-CH_3. While CH and CH_3 observed at a negative side (below of the spectrum) such as 149.81 for ***C***H=N, 55.41 for OCH_3, 24.22 for the two methyl groups, the other chemical shifts positive side and negative side between (159.74-107.50) for ***Ar***-***C***H

Synthesis of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-o-tolylimino-propionaldehyde (3)

A solution of (0.2g, 0.81mmol) of 2-(5-Methoxy-3,3-

dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in methanol 20ml and (0.085g, 0.81mmol) of ***o***-tolylamine was dissolved in methanol 20ml and then added 3 drops of 2% H_2SO_4 to the solution. The mixture was stirring in a water bath at 65ºC for 7h. A solvent was reduced to one quarter, orange precipitate was formed, filtered off, washed with hexane and dried in oven at 65ºC. The purity of this compound was determined by using TLC (4:1) hexane: ethyl acetate, which gave one spot. Yield (0,264g, 97%), m.p 240-242ºC.

IR data in (cm^{-1}): 2924 υ(CH aromatic), 2610 υ(CH aldehyde), 1669 υ(CH=O), 1621 υ(CHN), 1603 υ(C=C), 1471 υ(C-C) 1332 υ(CH_3), 1288 υ(C-N), 1061 υ(C-O) and 762 υ(C-H bending), ^{1}HNMR(400MHz, DMSO, δ in ppm): 14.27(s, 1H, N***H***), δ = 9.41 (s, 1H, ***H***CO), 8.68(1H, s,***H***C=N), 7.72-6.85 (7H,Ar-***H***), 3.79 (s, 3H, OCH_3), 1.59 (6H,s, 2x C***H***$_3$) and 1.21 ortho CH_3. APT^{13}C NMR was displayed signals for quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) 180.70 for NH-***C***=C, (147.47-112.44) for***Ar***-***C***, 108.50 for O=C-***C***=C and 54.38 CH_3-***C***-CH_3. While CH and CH_3 observed at a negative side (below of the spectrum) 185.71 for ***C***H=O, 146.6 for ***C***H=N, (126.52-107.56) for ***Ar***-***C***H, 55.37 for OCH_3, 21.64 for the two methyl groups and 17.16 for Ortho-***C***H_3.

Synthesis of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(pyridin-2-ylimino)-propionaldehyde (4).

A solution of (0.5g, 2mmol) of 2-(5-methoxy-3,3-dimethyl-1,3dihydro-indol-2-ylidene)-malonaldehyde was dissolved in methanol 10ml with heating and (0.19g, 2mmol) of Pyridin-2-ylamine was dissolved in methanol 5ml and then added 3 drops from 2% H_2SO_4 to the solution. The mixture was refluxed in a water bath at 65ºC for 6h. then stirring at room temperature for 2h. brown precipitate was formed, filtered off, washed with methanol and dried in oven at 65ºC. The purity of this compound was determined by using TLC (4:1) hexane: ethyl acetate, which gave one spot. Yield (0.5g 83.3%), m.p.(138-139)ºC. IR data in (cm^{-1}): 3331 υ(NH), 3061 υ(CH aromatic), 2960 υ(CH aliphatic), 2727 υ(CH aldehyde), 1672 υ(CH=O), 1614 υ(CHN), 1567 υ(C=C), 1476υ(C-C), 1323 υ(CH_3), 1287 υ(C-N), 1058 υ(C-O) and 770 υ(C-H bending), ^{1}H NMR(400MHz, DMSO, δ inppm): δ = 13.95 (s,1H, N***H***), 9.68 (s,1H, ***H***CO), 8.87 (s,1H, ***H***C=N), 7.57-6.87 (7H, Ar-***H***), 3.79 (s, 3H, OCH_3), and 1.50 (s, 6H,2x C***H***$_3$), APT ^{13}C NMR was displayed signals for quaternary carbons, methylene CH_2 and carbons of DMSO solvent, which appeared at a positive side (above of the spectrum). The chemical shifts at 188.70 for ***C***H=O, 153.37 for ***C***H=N, 55.35 for OCH_3 and 21.80 for the two methyl groups were appeared at a negative side and aliphatic carbon atoms such as 180.93 for NH-***C***=C and 107.65 for O=C-***C***=C were appeared at a positive side, the other chemical shifts positive side and negative side between (157.86-109.38) for ***Ar***-***C***H

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

A solution of (0.250g, 1.01mmol) of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 10ml with heating and (0.12g, 1.0mmol) of 4-methoxy aniline was dissolved in ethanol 5ml and then added glacial acetic acid 2ml to the solution. The mixture wasrefluxedin water bath at 78ºC for 15h. Solvent was reduced to one quarter, green precipitate was formed, filtered off, washed with hot water and dried in oven at 78ºC. The purity of compound was determined by using TLC (4:1) hexane : ethyl acetate with pre-coated silica gel, which gave one spot. Yield (0,35g 97%), m.p. 138-139°C. IR data in (cm^{-1}):3107 υ(CH aromatic) 2932 υ(CH aliphatic), 2844 υ(CH aldehyde), 1647 υ(C=O), 1606 υ(CHN), 1544 υ(C=C), 1486υ(C-C), 1365 υ(CH_3), 1252 υ(C-N), 1179 υ(C-O) and 798 υ(C-H bending). ^{1}HNMR(400MHz, DMSO, δ in ppm): δ = 13.25 (s,1H,N***H***), 9.69(s,1H,***H***CO), 8.44(s,1H, ***H***CN), 7.52-6.82(m, 7H, Ar-***H***), 3.77 (s, 6H, OCH_3) and 1.66(s,6H, 2x C***H***$_3$); APT ^{13}C NMR was displayed signals for quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) 181.16 NH-C=C for, (157.69-131.43) for ***Ar***-***C***, 108.26 for O=C-***C***=C and 55.35 CH_3-***C***-CH_3, while CH and CH_3 observed at a negative side (below of the spectrum) 187.54 for ***C***H=O, 157.69 for ***C***H=N, (119-112.) for ***Ar***-***C***H, 55.51 for OCH_3, 21.30 for the two methyl groups.

Synthesis of 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-3-(3-hydroxy-phenylimino)-propionaldehyde (6)

A solution of (0.3g, 1.2mmol) of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 20ml and (0.131g, 1.2mmol) of 3-hydroxy-phenylamine was dissolved in ethanol 10ml and then added glacial acetic acid 2ml to the solution.The mixture wasr efluxed in a water bath at 78ºC for 8h. A solvent was reduced to one quarter; dark brown precipitate was formed, filtered off, washed with water and dried in oven at 78ºC. The purity of this compound was determined by using TLC (3:1) hexane: ethyl acetate, which gave one spot. Yield (0,38g, 92.7%), m.p 135-

136°C. IR data in (cm^{-1}): 3301 υ(N-H), 3120 (CH aromatic) 2923 υ(CH aliphatic), 2865 υ(CH aldehyde), 1640 υ(C=O), 1607 υ(CH=N), 1541 υ(C=C), 1487 υ(C-C), 1371 υ(CH_3), 1254 υ(C-N), 1076 υ(C-O) and 800 υ(C-H bending). ^{1}HNMR (400MHz, DMSO, δ in ppm): δ = 9.65(s,1H,**H**C=O), 8.87(s,1H, **H**C=N), 8.12-6.94 (7H, Ar-**H**), 3.84 (s, 3H, OCH_3) and 1.63(6H,s, 2x C**H**$_3$); APT^{13}C NMR was displayed signals for quaternary carbons, methylene CH_2 and carbons of DMSO solvent which appeared at a positive side (above of the spectrum) 178.52 for (NH-**C**=C), (128.46-112.67) for (**Ar-C)**, 101.17 for O=C-**C**=C and 53.02 CH_3-**C**-CH_3. While CH and CH_3 observed at a negative side (below of the spectrum) 134.00-107.32 for (**Ar-C**H), 55.23 for OCH_3 24.03 for the two methyl groups.

Biological part

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, Giemsa stain:

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.

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 refrigerate until use

Study of Compound Effectin 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.

Blood transplantation with complete compound : A-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.

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

C. 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.

D. Left a group of pipes without adding any extract and promised this transaction control

Cell Harvesting : A- (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.

B. 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.

C. 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.

D. Incubated pipes in a water bath (37) m for a period of (30) minutes.

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

Fixation : A - 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.

B. Mix the samples with Vortex and then heat the tubes (4) m for 30 minutes.

Washing : A. 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. B. 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) m.

Dropping

I attended clean glass slides free of wet and cold fat, 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.

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.

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:

$$\text{Coagulation factor (MI)} = \frac{\text{Number of split cells}}{\text{Total number of cells1000}} \times 100$$

Table 1 : The chemical shift in ppm to ^{1}H NMR results for compounds (2, 3, 4, 5 and 6).

Compound No.	N-H	C=O	CH=N	Ar-H	OCH_3	*ortho*-CH_3	2x CH_3
2	13.25	9.57	8.85	7.66-6.90	3.82		1.61
3	14.27	9.41	8.68	7.72-6.85	3.79	2.21	1.59
4	13.95	9.68	8.88	7.57-6.87	3.79	-	1.50
5	13.25	9.69	8.44	7.52-6.82	3.77		1.66
6	13.95	9.65	8.87	8.12-6.94	3.84	-	1.63

Table 2 : The biological results of the compounds (1-6) on Lymphatic Cell.

Compounds and control	Cell rate in tropical phase	Percentage of control 24h	Cell rate in tropical phase	Percentage of control 48h
Control	0.00±4.20		0.00±4.20	
Compound (1)	0.17±2.43B	57.82	0.40±3.50b	83.33
Compound (2)	0.20±1.10	26.19	0.11±1.60	30.09
Compound (3)	0.00±2.29	36.68	0.00±2.67	55.49
Compound (4)	0.11±0.30	7.14	0.56±0.56	13.33
Compound (5)	0.11±2.53C	50.00	0.08±3.33	79.28
Compound (6)	0.17 ±2.21	36.42	0.08 ±2.33	55.47

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

RESULTS AND DISCUSSION

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 Department of Chemistry, College of Science, University of Diyala, while ^{1}H and APT ^{13}C NMR spectroscopy were shown on a Bruker 400 MHz spectrometer in University of Science and technology, College of science, Irbid city, Jordan.

IR Spectroscopy

IR spectral study of compounds (1, 2, 3, 4, 5 and 6) revealed the presence of characteristic of new azomethine group absorption bands appeared at 1618, 1614, 1621, 1614, 1606, and 1607 cm^{-1}, respectively (Ahmed ***et al***, 2009), 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 1647, 1669, 1672, and 1640 cm^{-1} respectively (Abbas and Saafan, 2007). In addition, abroad absorption bands of (NH) appeared at 3331, 3301 cm^{-1} respectively (Fischer and Hess, 1884), another strong absorption bands were observed at 1287, 1281, 1288, 1287, 1248, and 1254 cm^{-1} of (C-N) groups (Kumar ***et al***, 2010) and at 1076, 1076, 1061, 1058, 1076, 1179 cm^{-1} (C-O) groups (Amir ***et al***, 2013) respectively. All these main absorption bands confirmed the formation of the new compounds.

NMR Spectroscopy

^{1}H-NMR and APT ^{13}CNMR spectra of new Schiff bases were recorded in dimethylsulfoxide DMSO.$d_6$with chemical shifts expressed in ppm.^{1}H-NMR spectra of compound (1). Fig. 2 displayed signal at 13.25ppm which assigned to proton atom of N***H*** group (Faraj ***et al***, 2014). Sharp single signal at 10.07 ppm were attributed to proton atoms of C***H***O groups for compound (Misra ***et al***, 1996). As well as signal at 9.71, which assigned to proton atom of azomethine group (Matar ***et al***, 2015). Signals were appeared in region between 7.58-6.88 ppm which attributed to seven proton atoms of aromatic rings (Al-Omar ***et al***, 2005). Furthermore, single signal of methoxy groups was observed at 3.80ppm attributed to three proton atoms of OCH_3 groups (Li ***et al***, 2007). Finally, single signal was appeared at 1.50ppm which belonged to six proton atoms of two groups of methyl (Alamgir, 2007).

The ^{1}H NMR results of the other new compounds (2, 3, 4, 5 and 6) are listed in Table 1.

Biological results

The biological results of the compounds (1-6) on Lymphatic Cell in Metaphase in Human Blood showed different results of each compound as demonstrated in Table 2 compared with colchicine.

For compound (2), Fig. 5 showed less control ratio compared to (1 and 5) compounds, Figs. 5 and 6, which showed a higher control ratio due to its containing electron donating group such as, methoxy groups OCH_3 substituted in ***meta*** positionand OCH_3 substituted in ***para*** positionon

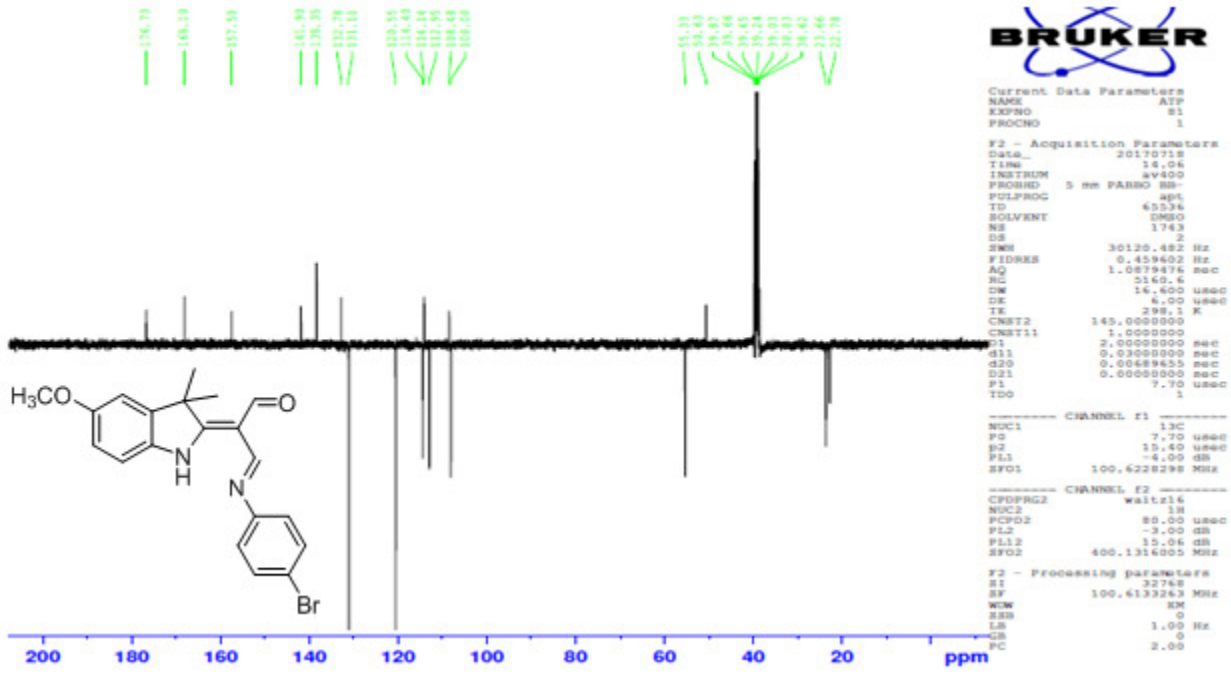


Fig. 3 : APT ^{13}C NMR spectrum of compound (1).

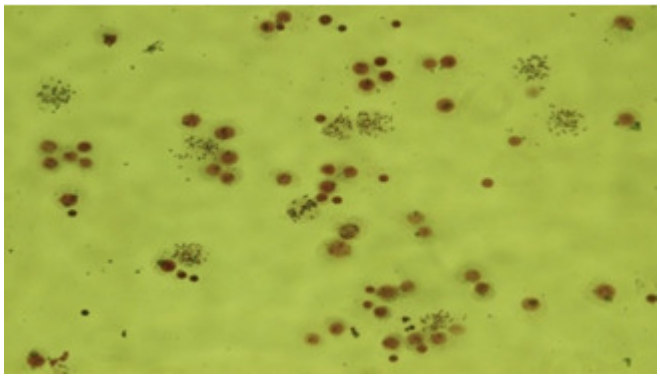


Fig. 4 : A tropical phase suspended for lymphocytes of human blood when used 0.2ml of compound (1).

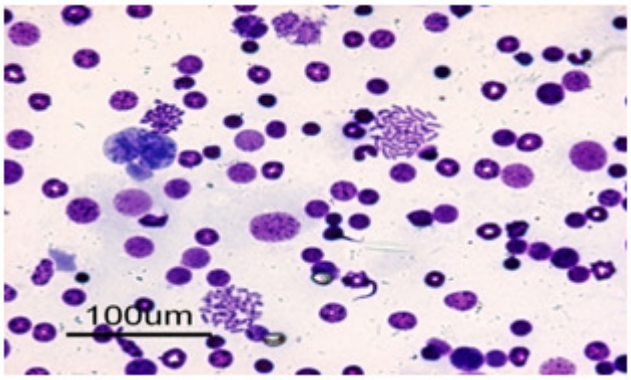


Fig. 5 : A tropical phase suspended for lymphocytes of human blood when used 0.2ml of compound (2).

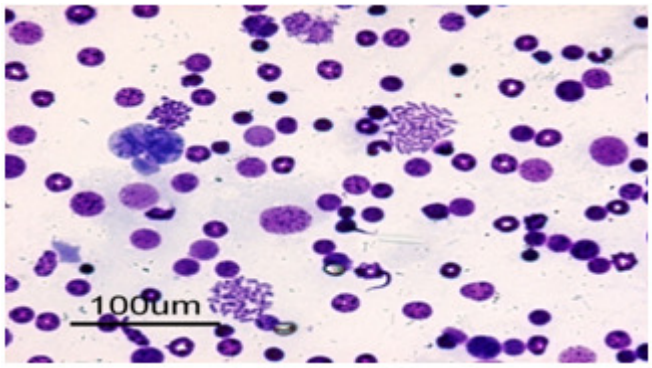


Fig. 6 : A tropical phase suspended for lymphocytes of human blood when used 0.2ml of compound (4).

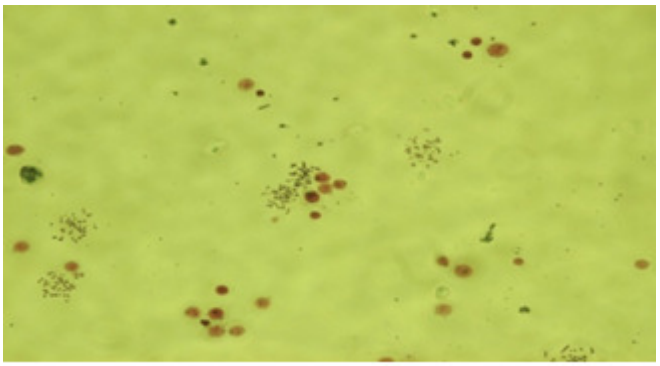


Fig. 7 : A tropical phase suspended for lymphocytes of human blood when used 0.2ml of compound (5).

benzene ring, while low control ratio of compound 2 due to its containing electron withdrawing like bromine atom. Also the position of OCH_3 substituted group on benzene ring effect on the activity. As conclusion, difference in site of methoxy group on benzene ring affects the effectiveness of the compound, as well as the activity increase with time as explain for compound (2). It was 26.19 after 24h and became 30.09 after 48h, also the compound (1). It was 57.82 after 24h and became 83.33 after 48h and compound (5). It was 50.00 after 24h and

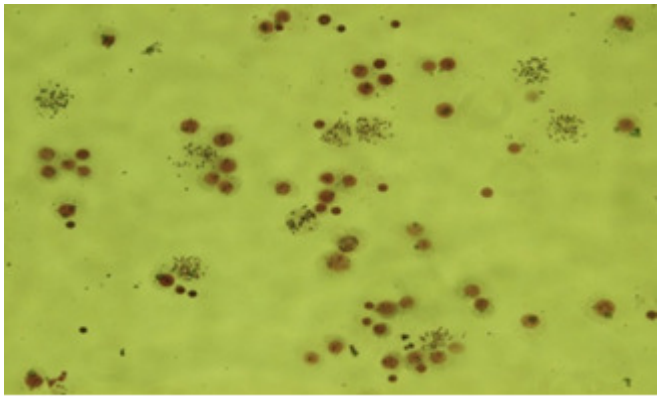


Fig. 8 : A tropical phase suspended for lymphocytes of human blood when used 0.2ml of compound (3).

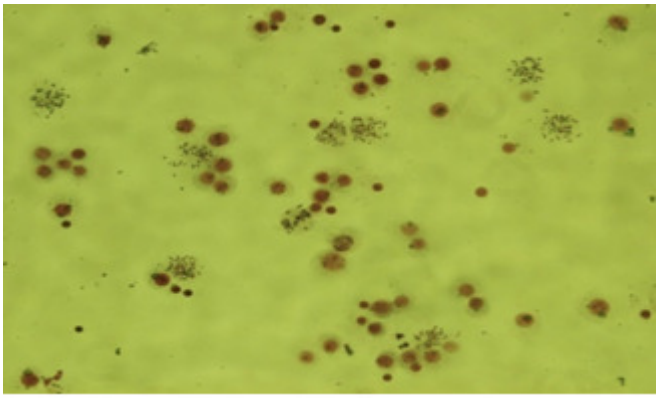


Fig. 9 : A tropical phase suspended for lymphocytes of human blood when used 0.2ml of compound (6).

became 79.28 after 48h. For compound (4), Fig. 7 showed less control ratio compared to other compounds due to its containing pyridine ring. For compound (3), it was 36.68 after 24h and became 55.49 after 48h. The compound (3 and 6) Figs. 8 and 10 showed moderate control ratio due to presence electron donating groups such as hydroxyl and methyl group in meta position on benzene ring.

Thus. the difference of biological activity of the compounds (1-6) on Lymphatic Cell in Metaphase in Human Blood depend on nature, number and position of the substituted group on benzene ring.

CONCLUSION

Six new compounds (1-6) 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, ^{1}H 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.

ACKNOWLEDGMENTS

The authors thank Department of Chemistry, Faculty of Science, University of Diyala, Iraq, for supporting this work and thank the Iraqi Center for Cancer and Medical Genetic Research, Mustansiriyah University.

Conflict of interests

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

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