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RESEARCH ARTICLE

New pyrazole derivatives and investigate their toxic effect in Hella and RD cancer cells lines

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ABSTRACT:

New pyrazole derivatives have been synthesized by reaction of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde with substituted phenyl hydrazine hydrochloride. The chemical purity of the new synthesized compounds was tested by TLC, and the chemical structures were characterized by, FT-IR, ^{1}H, and APT ^{13}C NMR. The biological activity of the new synthesized compounds was conducted to investigate the toxic effect of these compounds on the growth of tumor cells represented by Hela cell line and the human muscle cancer RD in the laboratory. The study included exposure period 24 and 48 hours and shows good results.

KEYWORDS: Substituted phenyl hydrazine hydrochloride, pyrazole derivatives, Hella and RD Cancer Cells lines.

INTRODUCTION:

The indole skeleton is represent the most important structure subunits for the discover of new drugs[1]. Indol and their derivatives present widely in many natural products for instant those obtained from plants, fungi, and marine organisms, and there are several thousand indole alkaloids including simple or more complex functionalized indole compounds[2] figure (1).

Of all these compounds, bis-indole alkaloids Ei-iii exhibit high degree of biological activity in vitro against P388 cell with IC_{50} (inhibitory concentration) values of 7-6, 7-8 and 1-7 Mg/mL respectively. The multiple biological and pharmacological properties of heterocyclic compounds has become of the most important aim of current research**.** Pyrazole is five-membered ring having two nitrogen as hetero atoms in the system. It is widely found as the core structure in a large variety of compounds that possess important agrochemical and pharmaceutical activities.[3,4]

It has been reported that indole derivatives bearing pyrazole nucleus has a broad range of biological activities and pharmacological activities such as antitumor anticancer, anti- inflammatory and anti-tubercula[5-9].

The fischer indol synthesis is a chemical reaction that afford aromatic heterocyolic indole from phenylhydrazine as a substituted and aldehyde or ketone, in hot acidic conditions[10]**.** In 1883 Hermann Emil Fischer reported the first synthesis method of indole· Today the most impressive application of Fischer indole reaction is synthesis of anti-migraine drugs and Fukuyama·s synthesis of haplophytine[6]. Formylation of various aromatic heteroaromatic *3-H*-indole with vilsmeier reagent formed from dimethyl formamide and phosphorusoxy chloride to produce aminomethylene malondialdehyde was described by Baradarani and co-workers [11,12].

In the present research new pyrazol derivatives have been synthesized by using combination of biologically active moieties into one molecule and synthesis of totally newer moieties.[13,14] 2-(5-Methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde with various nucleophiles yield a range of new heterocyclic compounds as illustrated in scheme 1**.**

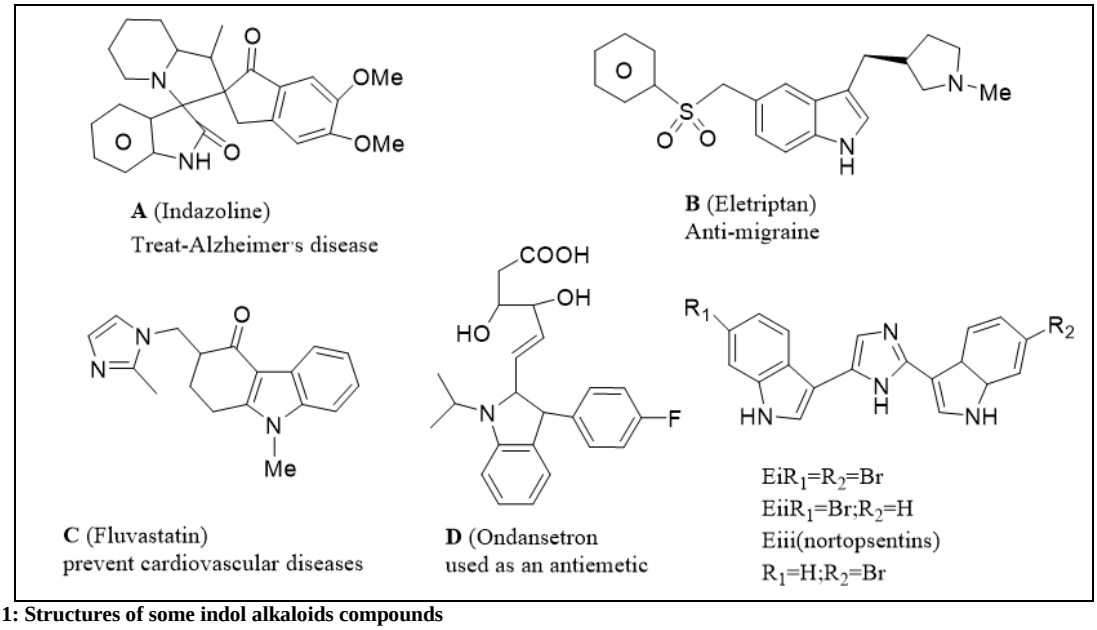
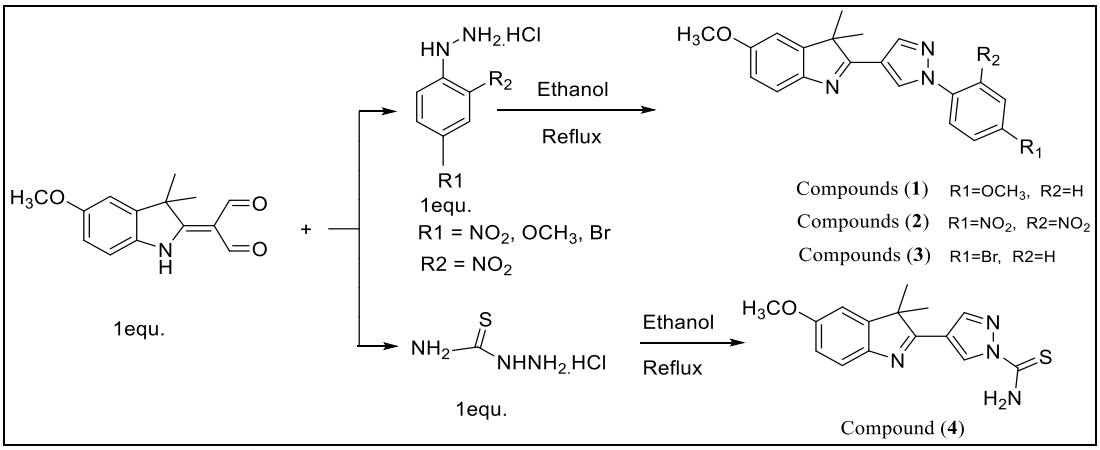


Figure. 1: Structures of some indol alkaloids compounds



Scheme 1: The synthetic pathway of new pyrazole derivatives (1 - 4)

MATERIAL AND METHODS:

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 Mohammadnejad [15]. 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 5-Methoxy-2-[1-(4-methoxy-phenyl)-1H-pyrazol 4-yl]-3,3- dimethyl-3*H*-indole (1)

A mixture solution of (0.2g, 1mmol) of 12-(5-methoxy-3,3-dimethyl-1,3-dihydro-indoll-2-ylidene) malonaldehyde (2) dissolved in 10 ml ethanol and (0.14g, 1mmol) of 4-methoxy-phenylhydrazine hydrochloride dissolved in 15ml ethanol was reflux at 78ºC for 10h in water a bath. The solvent concentered under reduced pressure, and the brown residue was filtered off, washed with hexane and dried in the oven at 78ºC. The purity of this compound determined by using TLC (4:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot on the polar area. Yield: (0,29g, 97%), m.p.183184oC. IR data in (cm-1): 3083.6, 2974.5, 1607.3, 1585.5, 1352.7, 1251, 1087.3, 1021.8, 738.18. ^{1}H-NMR (400 MHz, DMSO, δ in ppm): δ = 9.63 (s, 1H, pyrazole ring), 8.95 (s, 1H, pyrazole ring), 7.03-7.94 (7H, Ar-H), 3.84 (s, 6H, OCH_3) 1.70 (s, 6H, 2xCH_3). APT^{13}C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH_3 appeared at negative side (below baseline of the spectrum) δ = 142.14, 121.10, 117.04, 114.63, 113.99, 108.72 (carbon atoms of the aromatic and pyrazole ring), 55.72 & 55.45 (2xOCH_3), 24.42

($2xCH_3$). Whereas quaternary carbons, CH_2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ = 176.54, 159.17, 158.78, 145.72, 131.82, 112.32 (carbon atoms of the aromatic and pyrazole ring) and 52.73(CH_3-C-CH_3.

Synthesis of 2-[1-(2,4-Dinitro-phenyl)-1H-pyrazol-4-yl]-5methoxy-3,13-dimethyl-3*H*-indole (2)

A mixture solution of (0.15g, 6mmol) of 2-(5-methoxy-3,3- dimethyl-1,3-dihydro-indol-2-ylidene) malonaldehyde was dissolved in 10 ml ethanol and (0.12g, 6mmol) of 2,4-dinitrophenylhydrazine dissolved in 40ml ethanol, the mixture was left refluxing at 78ºC for 6h in water a bath. The solvent concentered under reduced pressure, and the red residue was filtered off, washed with hexane and dried in the oven at 78ºC. The purity of this compound determined by using a recrystallization process by hot water and TLC (4:1) hexane: ethyl acetate, with pre-coated silica gel, which gave one spot. Yield: (0.25g, 93%), m.p. 197-198 ºC. IR data in (cm^{-1}): 3192.7, 2931, 1607.3, 1581.8, 1545.5, 1520, 1323.6, 1258, 1138.2, 745.45. ^{1}H-NMR (400 MHz, DMSO, δ ppm): 9.26 (s, 1H, pyrazole ring), 8.89 (s, 1H, pyrazole ring), 6.87-8.68 (6H, Ar-H), 3.8 (s, 3H, OCH_3) 1.49 (s, 6H, $2xCH_3$). APT ^{13}C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH_3 appeared at negative side (below baseline of the spectrum) δ = 142.80, 129.42, 127.79, 126.07, 112.71, 107.70 (carbon atoms of the aromatic and pyrazole ring), 55.40 (OCH_3), 23.63 ($2xCH_3$) Whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above baseline of the spectrum) δ =174.95, 157.87, 148.41, 146.71, 145.57, 142.52, 135.34, 118.7 (carbon atoms of the aromatic and pyrazole ring) and 52.95 (CH_3-C-CH_3).

Synthesis of 2-[1-(4-Bromo-phenyl)-1H-pyrazol-4-yl]-5- methoxy-3,3- dimethyl-3H-indole (3):

A solution of (0.2g, 8mmol) of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in ethanol 10ml and (0.18g, 8mmol) of 4-bromo-phenylhydrazine hydrochloride was dissolved in ethanol 20ml. The mixture was refluxed in a water bath at 78ºC for 13h. Brown precipitate was formed, filtered off, washed with hexane and dried in oven at 78ºC. The purity of this compound was determined by using TLC (4:1) hexane: ethyl acetate, which gave one spot. Yield: (0,27g, 98%), m.p. 254-255ºC. IR data in (cm^{-1}): 3040, 2936 υ (CH aliphatic), 1607.3 υ (C=N), 1581.8 υ (N=N), 1538.2 and 1491 υ (C=C aromatic), 1352.7 υ (CH_3), 1280 υ (C-N), 1080 υ (C-O), 738.18 υ (C-H bending), and 541.82 υ(C-Br). ^{1}H NMR (400 MHz, DMSO, δ ppm): 9.32 (s, 1H, pyrazole ring), 8.53 (s, 1H, pyrazole ring), 6.89 -8.01 (7H, Ar-H), 3.79 (s, 3H, OCH3) 1.53 (s, 6H, 2xCH3). APT 13C NMR (100MHz, DMSO, *δ* in ppm): shown signals for CH and CH_3 appeared at negative side (below base line of the spectrum) *δ* = 141.33, 132.27, 120.86, 119.25, 112.95, 107.93 (carbon atoms of the aromatic and pyrazole ring), 55.49 (OCH_3), 23.88 ($2x\underline{C}H_3$) Whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above base line of the spectrum) *δ*=175.78, 158.02, 147.79, 138.14, 119.39, 116.71 (carbon atoms of the aromatic and pyrazole ring), and 52.86 (CH_3-$\underline{C}$-CH_3).

Synthesis of 4-(5-Methoxy-3,3-dimethyl-2,3-dihydro-1H-indol-2-yl)-pyrazole-1-carboxylic acid amide (4):

A mixture solution of (0.89g, 36mmol) of 2-(5-methoxy-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde was dissolved in 40 ml ethanol and (0.4g, 23mmol) of semi carbazide was dissolved in 15ml ethanol, the mixture was left refluxing at 78ºC for 24h in water a bath. The solvent was concentered under reduced pressure and the yellow residue was filtered off, washed with hexane and dried in oven at 78 ºC. The purity of this compound was determined by using TLC (4:1) hexane: ethyl acetate, with pre-coated silica gel, which gave one spot on nonpolar area. Yield: (0,87g, 98.8%), m.p. 265-266ºC. IR data in (cm^{-1}): 3440, 3258.2, 3069, 2887.3, 1691, 1589, 1534.5, 1327.3, 1283.6, 1181.8, 770. ^{1}H NMR (400 MHz, DMSO, *δ* ppm): 10.01 (s_{broad}, 1H, NH indole ring), 9.11 (s, 1H, pyrazole ring), 8,87 (s, 1H, pyrazole ring), 7.20 -7.96 (3H, Ar-$\underline{H}$), 7.04 (s, 2H, $C\underline{H}_2$), 6.56 (s_{broad}, 2H, NH_2), 3.82(s, 3H, $OC\underline{H}_3$), 1.68 (s, 6H, 2xCH3). APT ^{13}C NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH3 appeared at negative side (below base line of the spectrum) *δ* = 162.96, 116.02, 114.23, 109.03 (carbon atoms of the aromatic and pyrazole ring), 55.79 (OCH_3), 24.87 ($2x\underline{C}H_3$) Whereas quaternary carbons, CH_2 carbons and carbons deuterated DMSO solvent were observed at positive side (above base line of the spectrum) *δ* = 177.48, 159.35 ,157.58 , 144.59, 133.18, 108.84 (carbon atoms of the aromatic and pyrazole ring) and 52.50 for (CH_3-$\underline{C}$-CH_3).

Biological Part:

Cell line:

In this experiments the stable cell line of hela and RD cell was used cell line were obtained from the Department of Biology, Faculty of Medicine, Huazhong University in Wuhan - China. The cell line were grown on uncoated coverslips in a Dulbecco's Minimal Essential Medium (DMEM) with 10 fetal bovine serum (PAA), 2 µM glutamine (PAA), 100 µ/ml penicillin, and 100 *µ*g/ml streptomycin (China)[16]

Exposure to compound:

The first series of experiments, to investigate the action of compound on to the cell line, 24 and 48 h. a solution

containing 1 mg of compound in 1 ml of the medium or an original solution containing 5 mg of compound in 5 ml of medium was mixed with 3 ml of growth medium in each of the Petri dishes so that the final concentration of compound was 1 μg/ml, the second series of experiment the cell cultivated with extraction 1 μg/ml Each concentration was in two dishes[17]

RESULTS AND DISCUSSIONS:

Chemistry part:

IR Study:

The results of the IR spectra of the new synthesized compounds displayed stretching bands in range between 400 - 4000 cm^{-1}. Disappearance of absorption bands of NH_2-NH_2 and NH groups at 3200-3300 cm^{-1} which belonged to substituted hydrazine and phenyl hydrazine as well as absorption bands functional groups of aldehydes at 1700 cm^{-1} from a spectrum of the products that is confirmed of the formation of new compounds.

NMR Stud:

^{1}H-NMR and APT ^{13}C-NMR spectra were reported in (dimethyl sulfoxide) DMSO with chemical shifts in ppm and using TMS (tetramethylsilane) as standard. ^{1}H-NMR results of the new synthesized compounds showed disappearance signals of starting materials and appearance new signals of new synthesized compounds such as, disappearance signals two proton atoms of the carbonyl groups and appearance new signals of two proton atoms of pyrazole ring. As well as disappearance signals protons atoms of substituted phenylhydrazine and hydrazine. This is evidence to form of new synthesized compounds.

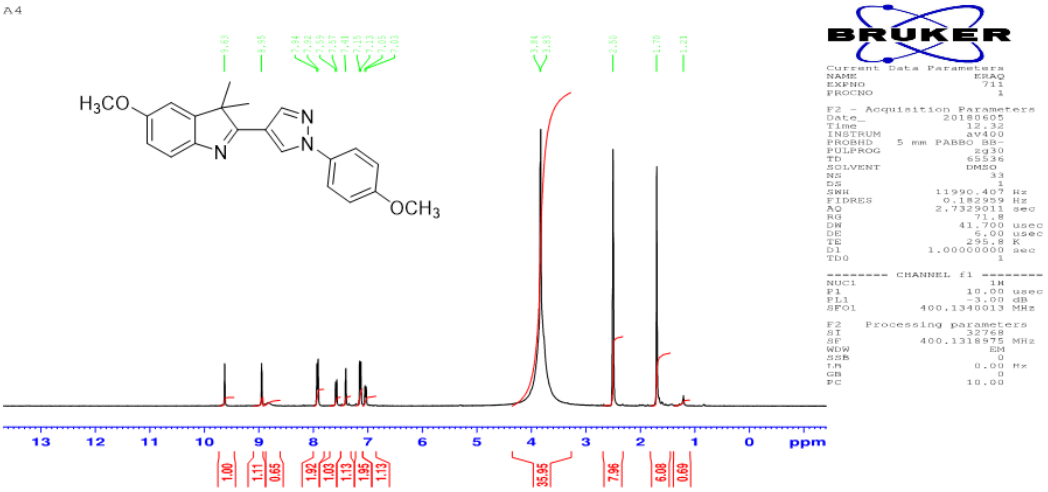


Figure 2: 1H-NMR of of 5-Methoxy-2-[1-(4-methoxy-phenyl)-1H-pyrazol 4-yl]-3,3-dimethyl-3H-indole (1)

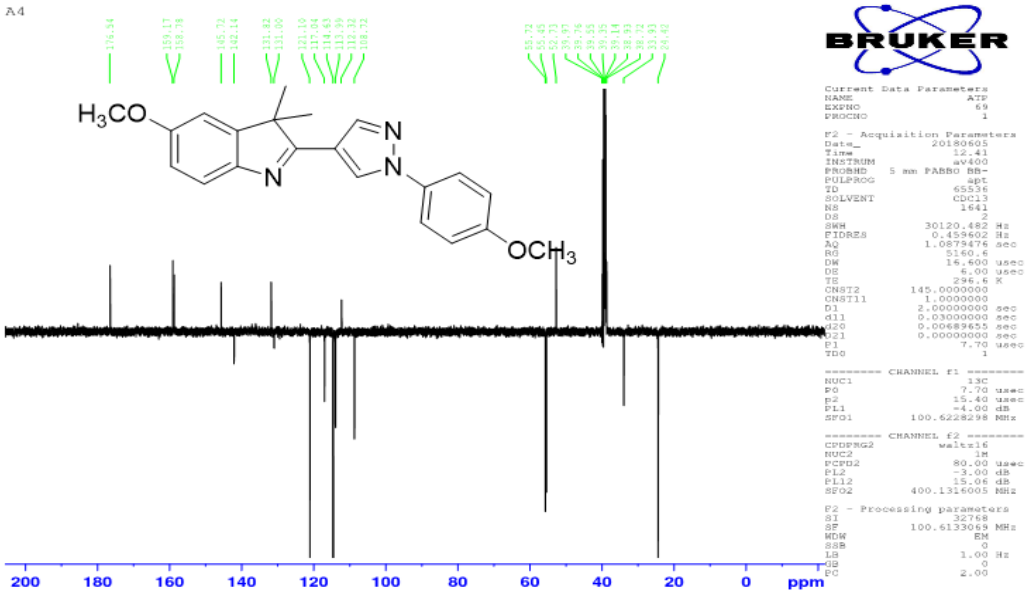


Figure 3: APT 13CNMR of 5-Methoxy-2-[1-(4-methoxy-phenyl)-1H-pyrazol 4-yl]-3,3-dimethyl-3H-indole (1)

^{1}H-NMR and APT ^{13}CNMR results of 5-Methoxy-2-[1-(4-methoxy-phenyl)-1H-pyrazol 4-yl]-3,3-dimethyl-3*H*-indole(**1**) figure 2 displayed single signals at 9.63 ppm and 8.95 ppm belonged to two protons of pyrazole ring[17,18]. Signals appeared in the region between 7.94-7.03 ppm were belonged to seven protons of an aromatic ring[19,20]. Also two single signals at 3.84 and 3.83 ppm was attributed to six protons of the two methoxy group (2x OCH_3)[21,22,23]. Finally single signal was appeared at 1.21 ppm belonged to six protons of two methyl groups (2x CH_3)[24,25].

APT ^{13}C NMR results were used to characterize this compound figure 3 was displayed eleven signals for carbon atoms of CH, OCH_3 and CH_3 in rang between (142. -124ppm) observed at a negative side (below of the spectrum) for (pyrazole ring, aromatic ring, OCH_3 and CH_3)[26,27,28]. As well as single eight signals were shown in range between 176-52 which assigned to the quaternary carbons of (pyrazole ring, and aromatic ring) which appeared at a positive side (above of the spectrum)[29,30,31]. All these results founded the ^{1}H-NMR and APT-^{13}C NMR spectrum matched well with the expected signals and are regular with the formation of this new compound.

The 1H NMR results of compounds (2, 3 and 4) are illustrated in table 1

Table (1): Chemical shift in ppm to1H NMR results for compounds (2, 3 and 4)

Com.No.	pyrazole ring	Ar-H	OCH3	2xCH3
1	9.26 and 8.89	8.68-6.87	3.80	1.49
3	9.32 and 8.53	6.89 -8.01	3.79	1.53
4	10.01 and 9.11	7.04 -7.96	3.82	1.68

Biological Part:

Hella cell line

Included exposure 24 hours

Table (2) showed that the compound an inhibitory Hella cancer cell line Included exposure 24 hours

48hours	24hours	Mg \ ml(0.2)
% inhibitory	% inhibitory	
f 0.24+ 5.40	f 0.64+1.48	Compound 1
e 0.81+18.61	d 0.81+7.86	Compound 2
b 0.33+34.90	b 0.33+ 24.89	Compound 3
a 0.33+47.73	a 0.33+31.28	Compound 4

This study showed that hella cell line was begun inhibitory with compound **1** at a concentration of 0.2. Mg/ml and increased in compound **2** concentrations became 7.86 % in 24hours then became 31.28% in A8 at 0.2 Mg/ml. There was significant difference at P≤ 0.05 between the compound included exposures 24 hours in Hella cell line.

Hella cell line:

Included exposure 48 hours

This study showed that hella cell line was begun inhibitory with compound **1** at a concentration of 0.2.Mg/ml 5.40 % and increased in compound **3** concentrations became 34.90 % in 24hours then became 47.73% in A8 at 0.2 Mg/ml. There was significant difference at P≤ 0.05 between the compound included exposures 24 hours in Hella cell line.

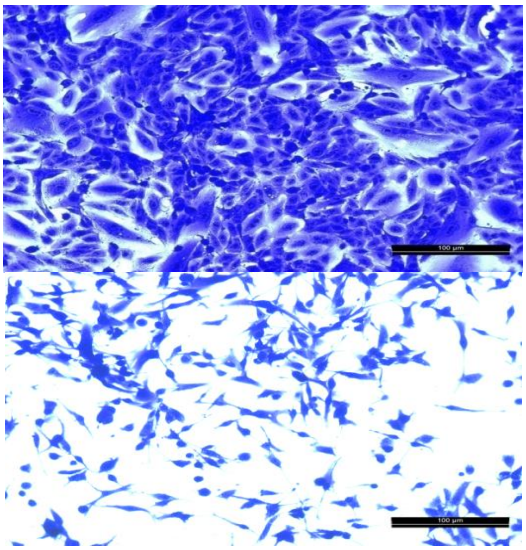


Figure. 4: Effect of the compound 1 in Hella cell line after 24 hour of exposure and control.

RD cell line:

Included exposure 24 hours

Table 3: showed that the compound an inhibitory RD cancer cell line Included exposure 24 hours

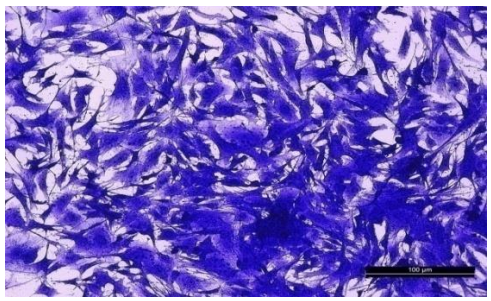
48hours	24hours	Mg \ ml(0.2)
% inhibitory	% inhibitory	
e 0.17+ 5.40	f 0.014+1.50	compound 1
d 0.36+10.48	e 0.33+8.98	compound 2
a 0.63+17.08	b 1.40+ 12.53	compound 3
A 0.63+21.35	A 0.33+ 17.53	compound 4

This study showed that RD cell line was begun inhibitory with compound **1** at a concentration of 0.2. Mg/ml and increased In compound **2** concentrations became 8.98 % in 24hours then became 17.53 % in A8 at 0.2 Mg/ml. There was significant difference at P≤ 0.05 between the compound included exposures 24 hours in Hella cell line.

RD cell line:

Included exposure 48 hours.

This study showed that hella cell line was began inhibitory with compound **1** at a concentration of 0.2.Mg/ml 5.40 % and increased In compound **2** concentrations became 10.48 % in 24hours then became 21.35% in A8 at 0.2 Mg/ml. There was significant difference at P≤ 0.05 between the compound included exposure 24 hours in RD cell line.



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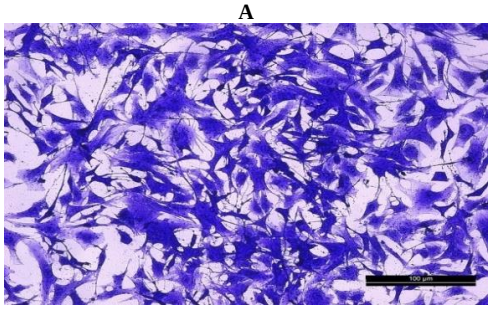

B

Figure. 4: Effect of the compound 1B in RD cell line after 24 hour of exposure and A control.

The results of this study reinforced the findings of many researchers in local studies on the activity of plant extracts on cancer cells. The pharmacological studies on *compound* showed that it possessed antioxidant, anticancer, antimicrobial, dermatological immunological, anti-inflammatory, antidiabetic, diuretic, inhibition of platelet aggregation, anti-leishmanial and many other activities. A local study reported that the crude proteins extracted from *compound inhibited* the proliferation of hella and RD cell line[32.]. Acytotoxic study was performed to assess the effect of compound **1**, compound **2**, compound **3**, compound **4** on the growth of human cervical carcinoma cells (HeLa cells) used 24 and 84 h that show all compound the ability to inhibit the cells growth of HeLa cells. This is because these extracts contain compounds that affect the physiological state of these cells, and contain compounds that stop the cycle of cancer cells (arrest cell cycle) at a certain stage and prevent of reproduction or contain compounds that stimulate cancer cells[33].apoptosis[The tumor cells are unique in their ability to invade cells Proliferation, as well as changes in their proteins and surface antigens, characterized by the permeability of its membranes and this feature facilitates the process of entering the materials into the cells irregular, which negatively affects those cells and makes it easier to respond to the anticancer to which they are exposed [34] as summarizing.

There are several studies that show the toxicity of compound **1** on cancer cells. The compound 1 showed cytotoxicity towards Hella cells line by stimulating the fragmentation of nuclear material and the intensification of chromatin[35,36]. The effect of the compound 1 on RD cancer cells line illustrates morphological programmed apoptosis, such as cell shrinkage, nuclear material intensification, and fragmentation of RD cancer cells[37,38].

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CONFLICT OF INTEREST:

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

CONCLUSION:

The newly indol derivatives bearing pyrazolin moiety wear successfully synthesized and characterized. The structures of synthesized compounds were identified by its H NMR, IR, and APT13C NMR data. The four synthesized compounds have been testing in vitro for their antitumor activity against the Hella cell and RD cell line. The results indicated that these compounds showed moderated to high anticancer activity after treatment with specified compounds at concentration 0.2Mg/ml for two different exposure times at 24 and 48 hours.

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