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Synthesis and Characterization of Novel Pyrazole Derivatives from 4-Florophenylhydrazine and Study Their Cytotoxicity as Anti-Cancer Agent

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Abstract

Series of new pyrazole derivatives have been successfully synthesized, their purity confirmed by thin-layer chromatography and the chemical structures identified by some spectroscopic techniques like 1H-NMR, APT 13C-NMR and FT-IR. Two synthetic precursors were synthesized: The first compound was 5-Fluoro-2,3,3-trimethyli-3H-indole (1) and the second compound was 2-(5-Fluoro-3,3-idimethyl-1,3dihydro-indoli-2-ylidene)-malonaldehyde (2). The target compounds were obtained from the reaction of compound (2) with different substituted phenylhydrazine hydrochloride. The toxicity of the new synthesized compounds was tested against breast cancer cell lines (MCF-7) and results showed that some cancer cells were killed.

Keywords: Heterocyclic, pyrazole derivatives and breast cancer cell lines

1. Note

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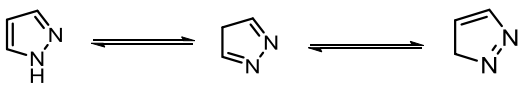
2. Introduction

Many categories of heterocyclic systems have attracted and are still attracting attention due to their vital importance in drug, insecticides, herbicides and anti-corrosive agents' industry and due to their significant biological activities [1-5]. The pyrazoles are an interesting class of heterocyclic compounds as important building blocks in organic, bio-organic, pharmaceutical and drug chemistry [6]. of these compounds is 3,5-dimethyli–1H–pyrazolei4-chloro-1-(2-chloroethyl) -2,3 –dimethyli-1H –iPyrazole and N-Chloroethyl-3,5-dimethylipyrazole-4-carbaldehyde was carried out by the formylation of at C-4 position of 4-chloro-1-(2-chloroethyl)-3.5dimethyl-1H-pyrazoleiby Vilsmeier Haack reaction [7].. The structures of these products were established from elemental analysis, FT-IR, and 1H NMR spectra [8]. Pyrazoles are aromatic molecules due to their planar conjugated ring structures with six aromatic delocalized π-electrons. Therefore, many important properties of these molecules were analyzed by comparing them with the properties of benzene derivatives. Like other nitrogen involving heterocycles, different tautomeric structures can be written for pyrazoles. Unsubstituted pyrazole can be represented inithree tautomeric forms [9] Scheme 1.



Scheme 1: Tautomeric forms of substituted pyrazole

pyrazole-3- carboxylic acids and pyrazolo[1,5-c] quinazoline-2-carboxylates are nicotinic acid receptor agonists, bis(benzo[g]indazol-3-carboxamides) exhibit antiproliferative activity against various cancer cell lines. In addition, S.K Sahu et al. synthesized a series of pyrazole derivative and screened for their analgesic activity [10]. The objectives of this study are synthesizing a series of new pyrazole derivatives, identify their purity by TLC sheet and their chemical structures by various spectroscopic techniques like

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nuclear magnetic resonance spectroscopy 1H, APT 13C-NMR and Fourier Transform infrared spectroscopy (FT-IR), as well as determining their physical properties and evaluate the biological activity to inhibitory against breast cancer cell lines (MCF-7)

3. Experimental Section

Materials

All substances and solvents used in this research wereipurchased from Aldrich and Fluka companies and they were used as received without additional purification. The (4-fluoro-phenyl)-hydrazine hydrochloride was synthesized by known literature procedure [11]. The purity of the synthesized compounds was checked by TLC sheet, and the melting points determined by the open capillary melting point device.

Instruments

IR spectra were recorded on a Perkin-Elmer Spectrum version 10.02 by using a KBr disc ,1H and APT 13C-NMR spectra were measured on a Bruker 400 MHz spectrometer in Jordan, College of Science, University of Science and Technology, Irbid city. The purity of the new compounds was checked by using silica gel sheets, and the spots were detected by using a fluorescence analysis cabinet model CM10

Synthesis of compounds (1 and 2)

5-Fluoro-2,3,3-trimethyl-3H-indole (1), was synthesized by amount of 4-fluoro-phenylhydrazine hydrochloride (2g, 0.12 mole) and methyl isopropyl ketone (1.58 g, 0.18 moles) were dissolved in (35 ml) of glacial acetic acid, then the mixture was refluxed in an oil path at 117 ºC for 20 h. Then the reaction was quenched by addition of ice distilled water, a red oily product was obtained which was neutralized with aqueous 25% NaOH, then it was extracted with ethyl acetate and water three times (3×25 ml). The organic layer dried over Na2SO4, and the solvent was removed by vacuum pumping to afford the oily red liquid of indolenine. (1). Yield: (1.99g, 91%) 1H-NMR (400MHz, DMSO, δ in ppm): 6.71-7.57 (m, 3H, Ar-H), 2.17 (s, 3H, CH3), 1.40 (s, 6H, 2xCH3).

2-(5-Fluoro-3,3-dimethyli-1,3-dihydro-indol-2-ylidene)-malonaldehyde (2), was synthesized from N, N-dimethyl formamide (DMF) and phosphoryl chloride, (POCl3) (3 ml, 0.30 mol) which is added dropwise with stirring at 7 °C for 10 minutes. Then a solution of (1.99 g, 0.10 mol) indolenine (1) in DMF (5 ml) was added dropwise for 10 minutes at 7 °C. The reaction mixture was stirred in the ice bath for 1 h, and then was reflux for 3 h, at 85- 90 ºC. The resulting solution was poured on ice distilled water and was neutralized with aqueous 25% NaOH. The resulted product was a brown precipitate that was filtered off, washed with hot distilled water and dried in the oven at 78oC and finally recrystallized from ethanol to give pure product of 2-(5-Fluoro-3,3-dimethyl-1,3dihydro-indol-2-ylidene)-malonaldehyde (2). The purity of this compound was determined by using TLC (3:1) hexane: ethyl acetate as eluent, with pre-coated silica gel, which gave one spot on the polar area. Yield: (2 g, 81%), m.p. 178-180 °C. FT-IR data (cm-1): 3201, 3042, 2983, 2854, 1657.4, 1616.3, 1534, 1469.4, 1372, 1275.5, 1178, 814. 1H-NMR (400MHz, DMSO, δ in ppm): δ = 13,13 (s, 1H, NH indole ring), 9.75 (s, 2H, CHO) 7.12-7.62 (m, 3H, Ar-H), and 1.67 (s, 6H, 2xCH3).

Synthesis of compounds (3-8)

5-Fluoro-2-[1-(4-methoxy-phenyl)-1H-pyrazol-4-yl]-3,3-dimethyl-3H-indole (3), was synthesized using amount of (0.2g , 0.008moles) of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (2) was dissolved in 10 ml ethanol and the same mole ratio (0.14g) of 4-methoxy-phenylhydrazine hydrochloride, (0.21 g) of 2,4-dinitrophenylhydrazine, (0.20 g) of 4-choro-phenylhydrazine hydrochloride, (0.09 g) of 4-trifluoromethoxy phenylhydrazine hydrochloride, (0.16 g) of 4-Bromo-phenylhydrazine hydrochloride and (0.13 g) of 4-floro-phenylhydrazine hydrochloride dissolved in (25 ml) ethanol, the mixtures were left refluxing at 78 oC for 12 h in the water a bath. The solvent evaporated under the reduced pressure, and the residues were filtered off, washed with hexane and dried in the oven. The purity of these compounds determined by using TLC (3:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot: Yield: (0,28 g, 96%), m.p.240-242 oC. FT-IR (cm-1): 3065, 2942, 2836, 2443, 1880.7, 1587, 1349, 1243, 1172, 1075, and 743. i1H-NMR (400 MHz, DMSO, δ ppm): 10.31 (s, 1H, pyrazole ring), 9.60 (s, 1H, pyrazole ring), 7.95-8.77 (m, 7H, Ar-H), 4.66 (s. 3H, OCH3), and 1.67 (s, 6H, 2xCH3). APT 13C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH3 appeared at negative side (below baseline of the spectrum) δ = 142.18, 121.46, 119.05, 115.54, 115.30, and 115.12. (Carbon atoms of the aromatic and pyrazole ring), 24.51 (2xCH3). Whereas quaternary carbons, CH2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ= 179.10, 162.73, 160.31, 159.11, 130.65 (carbon atoms of the aromatic and pyrazole ring) and 53.71 (CH3C-CH3).

The other new compounds (4-8) were synthesized in the same synthetic method as descripted for compound (3)

2-[1-(2,4-Dinitro-phenyl)-1H-ipyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3H-indole (4). Yield: (0.27 g, 90%), m.p. 198-199 oC FT-IR (cm-1): 3259, 2977,

1642, 1607, 1540,1487,1381, 1331, 1272, 1181, and 737. 1H-NMR (400iMHz, DMSO, δ ppm): 10.35 (s, 1H, pyrazole ring), 9.56 (s, 1H, pyrazole ring), 8.12-8.96 (m, 6H, Ar-H), 1.73 (s, 6H, 2xCH3).

2-[1-(4-Chloro-phenyl)-1H-pyrazol-4-yli]-5-Fluoro- 3,3-dimethyl-3H-indole (5). Yield:(0,25 g,86%),m.p. 223-224 oC. FT-IR (cm-1): 3077, 2983, 2425,1883, 1607, 1584, 1352, 1260, 1087, 826,743 and 552. 1H-NMR (400 MHz, DMSO, δ ppm): 9.50 (s, 1H, pyrazole ring), 8.74 (s, 1H, pyrazole ring), 7.25-8.06 (m, 7H, Ar-H), 1.62 (s, 6H, 2x CH3). APT 13C-NMR (100MHz, DMSO, δin ppm): shown signals for CH and CH3 appeared at negative side (below baseline of the spectrum) δ= 138.03, 129.93, 119.73, 115.97, 115.25, 115.01 (carbon atoms of the aromatic and pyrazole ring), 24.24 (2xCH3). Whereas quaternary carbons, CH2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ = 178.73, 162.57, 160.15, 148.25, 138.03, 130.22, 115.97 (carbon atoms of the aromatic and pyrazole ring) and 53.77 (CH3-C-CH3).

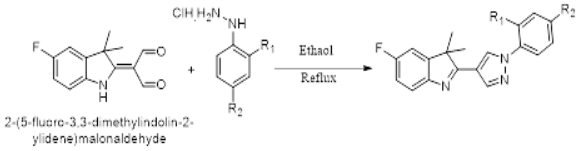
5-Fluoro-3,3-dimethyl-2-[1-(4-trifluoro methoxy-phenyl)-1H-pyrazol-4-yl]-3H-indole (6). Yield: (0,16g, 96%), m.p.225-226 oC. FT-IR (cm-1): 3065, 2977, 2290, 1895, 1592, 1475, 1357, 1260, 1166, 1075, and 743. 1H-NMR (400 MHz, DMSO, δ ppm): 1H-NMR (400 MHz, DMSO, δ ppm): 10.66 (s, 1H, pyrazole ring), 9.89 (s, 1H, pyrazole ring), 8.30-9.20 (m,7H, Ar-H), 1.85 (s, 6H, 2x CH3) APT13C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH3 appeared at negative side (below baseline of the spectrum) δ= 143.32, 131.45, 122.77, 121.72, 119.20, 115.57, 115.33 (carbon atoms of the aromatic and pyrazole ring), 24.37 (2xCH3). Whereas quaternary carbons, CH2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ = 178.90, 162.82, 160.40, 147.71, 147.57, 137.49, 115.57 (carbon atoms of the aromatic and pyrazole ring) and 53.76(CH3-C-CH3).

2-[1-(4-Bromo-phenyl)-1H-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3H-indole (7). Yield: (0,23g, 95%), m.p. 245-246 oC. FT-IR (cm-1): 3065, 3030, 2325, 1901, 1598, 1354, 1269, 1072, 743, and 685. 1H-NMR (400 MHz, DMSO, δ ppm): 10.52 (s, 1H, pyrazole ring), 9.78 (s, 1H, pyrazole ring), 8.12-9.84 (m,7H, Ar-H), 1.67 (s, 6H, 2xCH3). APT13C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH3 appeared at negative side (below baseline of the spectrum) δ = 132.81, 121.59, 118.93, 115.31, 110.68, 110.68 (carbon atoms of the aromatic and pyrazole ring), 24.26 (2xCH3). Whereas quaternary carbons, CH2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ=178.78 ,139.12 (carbon atoms of the aromatic and pyrazole ring) and 53.69 (CH3-C-CH3).

2-[1-(4-Fluoro-phenyl)-1H-pyrazol-4-yl]-5-Fluoro-3,3-dimethyl-3H-indole (8). Yield: (0,19 g, 95%), m.p. 200-202 °C. FT-IR (cm-1): 3083, 2971, 2360, 1895, 1587, 1331, 1231, 1184, 1081, and 740. 1H-NMR (400 MHz, DMSO, δ ppm): 10.42 (s, 1H, pyrazole ring), 9.69 (s, 1H, pyrazole ring), 8.08-8.91 (m,7H, Ar-H),1.66 (s, 6H, 2xCH3). APT13C-NMR (100MHz, DMSO, δ in ppm): shown signals for CH and CH3 appeared at negative side (below baseline of the spectrum) δ = 141.75, 121.34, 118.53, 116.21,115.98, 114.77, 114.53 (carbon atoms of the aromatic and pyrazole ring), 23.66 (2xCH3).Whereas quaternary carbons, CH2 carbons and carbons deuterated, DMSO solvent were observed at positive side (above the baseline of the spectrum) δ= 178.26, 162.01, 159.59, 147.15, 130.30, 115.98 (carbon atoms of the aromatic and pyrazole ring), and 53.02 (CH3-C-CH3).

4. Results and Discussion

A series of new pyrazole derivatives have been synthesized via condensation reaction of 2-(5- Fluoro-3,3-dimethyl-1,3-dihydro-indol-2ylidene)-malonaldehyde (2) with various substituted phenyl hydrazine hydrochloride in the ratio (1:1) according to the synthetic pathway, as shown in the figure (1):



R1=NO_2, R2=NO_2 (**3**) R1=H, R2=OCH_3 (**4**)
R1=H, R2=OCF_3 (**5**)
R1=H, R2=Cl (**6**) R1=H, R2=F (**7**)
R1=H, R2=Br (**6**

Figure 1: The synthetic pathway of the synthesized compounds (3-8)

2-(5-Fluoro-3,3-dimethyl-1,3-dihydro-indol-2-ylidene)-malonaldehyde (2) was synthesized by two steps as illustrated in reaction equation figure 2. The first step using Fischer Indole Synthesis via reaction of 4-fluoro phenylhydrazine hydrochloride with methyl isopropyl ketone in glacial acetic acid to produce 5-fluoro-2,3,3-trimethyl-3H-indole (1) in a good yield. The second step was using Vilsmeier Haack reaction by reaction of compound (1) with phosphoryl chloride (POCI3) in the presence of N, N-dimethyl formamide (DMF) to produce compound (2) in a good yield.

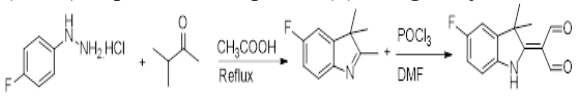


Figure 2: The synthetic pathway of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydroindol- 2-ylidene)-malonaldehyde (2)

All the newly synthesized compounds are colored, stable in air and their chemical structures have been confirmed by some spectroscopic techniques: TLC, FT-IR, 1H-NMR and APT 13C-NMR, as well as their purity were tested by TLC sheet.

FT-IR Study

The results of the FT-IR for the newly synthesized compounds displayed absorption bands inithe range 400-4000 cm-1. The FT-IR spectra of compound (2) figure 3 displayed the following absorption bands at 3201 cm-1 for (N-H) group [12], at 3042 cm-1 for asymmetic streching (C-H aromatic ring) [13], at 2983 cm-1 was assigned to symmetic stretching (C-H aromatic ring) [14], at 1657 cm-1 belonged to (C=O) [15], at 1616 cm-1 belongedito (C=C). as well as absorption bands were appeared at 1372 cm-1 was belonged to bending vibration of CH3 group, at 1275 cm-1 which attributed to (C-N), at 1178 cm-1 for (C-O), at 814 cm-1 for (C-F) and finally absorption band at 732 cm-1 for (C-H bending).

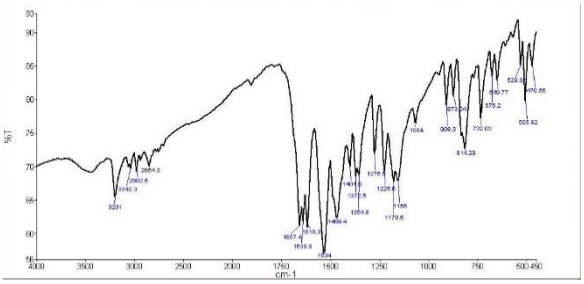


Figure 3: The FT-IR spectra of the compound (2)

FT-IR spectra of 5-Fluoro-2-[1-(4-methoxy-phenyl)-1Hpyrazol-4-yl]-3,3-dimethyl-3H-indole (3) figure 4 displayed absorption bands, absorption at 3065 cm-1 for asymmetic streching (C-H aromatic ring), at 2942 cm-1 was assigned to symmetic stretching(C-H aromatic ring), at 2443 cm-1 and at 1880cm-1 belonged to (C=N) overtones of pyrazole ring [16] as well as the stretching frequency at 1587 cm-1was referred to (C=C) group [17], at 1349 cm-1 was belonged to bending vibration of CH3 group [18], at 1243 cm-1 which attributed to (C-N) [19], at 1172 cm-1 for (C-O) [20], at 1075 cm-1 which attributed to (C-F). Finally, a sharpiband at 743 cm-1 attributed to bending vibrations (out-of-plane) (C-H) [21].

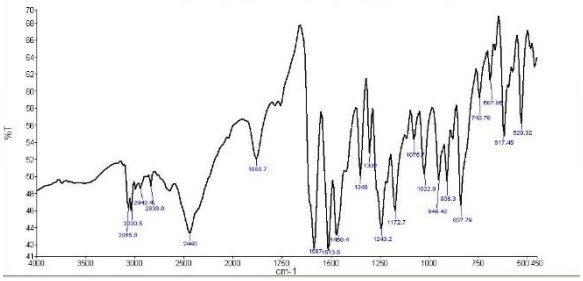


Figure 4: The FT-IR spectra of the compound. (3)

The FT- IRispectra of the other compounds (2-8) are listed in Table 1

Table 1: FT- IR spectra for the compounds (2-8)

Com. No.	N-H	C-H Asym	C-H Sym	overtones (C=N) of pyrazole	C=O	C=C	CH_3	C-N	C-O	Others
2	3201	3042	2983	-	1657	1534	1372	1275	1178	-
3	-	3065	2942	2443, 1880	-	1587	1349	1243	1172	-
4	-	3259	2977	2352, 1871	-	1540	1331	1272	-	1381, 1487 (N-O)
5	-	3077	2983	2425, 1883	-	1584	1352	1260	-	826 (C-Cl)
6	-	3065	2977	2290, 1895	-	1592	1357	1260	1166	1075 (C-F)
7	-	3065	2980	2325. 1901	-	1598	1354	1269	-	685 (C-Br)
8	-	3083	2971	2360, 1895	-	1587	1331	1231	-	1081 (C-F)

NMR Study

1H-NMR and APT13C-NMR spectra were reported in (deuterated dimethyl sulfoxide) DMSOd6 with chemical shifts (δ) in ppm. 1H-NMR results of the new starting materials (5-Fluoro-2,3,3-trimethyl-3H-indole (1) and 2-(5-Fluoro-3,3-idimethyl-1,3-dihydroindol-2-ylidene)-malonaldehyde (2) were confirmed the cyclation of 5-Fluoro-2,3,3-trimethyl-3H-indole (1) and formation of 2-(5-Fluoro-3,3-dimethyl-1,3-dihydroindol-2-ylidene)-malonaldehyde (2) through the new signals on their spectrums. As well as 1H-NMR results of the newly synthesized compounds (3-8) showed disappearance signals of starting materials and appearance of newisignals of new synthesized compounds, Such as the disappeared of two protons atoms from two carbonyl groups and proton atoms of amines of substituted phenylhydrazine hydrochloride, as well as

appearance of new signals of two protons atoms of pyrazole ring. All new signals which appearance on spectrums of new compounds and disappearance signals of starting materials are a good an evidence to form newly synthesized compounds.

1H-NMR results of 2-(5-Fluoro-3,3-idimethyl-1,3-dihydro-indol-2ylidene)-malonaldehyde (2), figure 5 displayed a single signal at 13,13 ppm which belonged to one proton atom of indole ring (NH) [22]. A long single signal at 9.75 ppm which belonged to two protons of two aldehyde groups (2 x CHO) [23]. Signals were appeared in the region between (7.12-7.62) ppm which belonged to three proton atoms of aromatic ring . Also a single signal at 1.67 ppm belonged to six protons of two methyl groups (2 x CH3) [24].

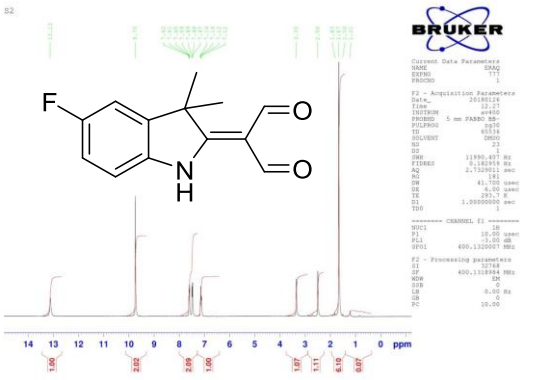


Figure 5: 1H NMR spectrum of 2-(5-fluoro-3,3-dimethylindolin-2 ylidene)malonaldehyde (2)

1H-NMR and APT 13C-NMR results of 5-Fluoro-2-[1-(4-methoxy-phenyl)-1Hpyrazol-4-yl]-3,3-dimethyl-3H-indole (3) figure 6 displayed a single signal at 10.31 and 9.60 ppm belonged to the two proton atoms of the pyrazole ring [25]. As well as signals was appeared in the region between (7.95-8.77) ppm which belonged to seven proton atoms of an aromatic ring for this compound [26]. Also a single signal at 4.66 ppm attributed to three protons of methoxy group OCH3[27]. Finally, a single signal at 1.67 ppm belonged to six protons of two methyl groups CH3 [28].

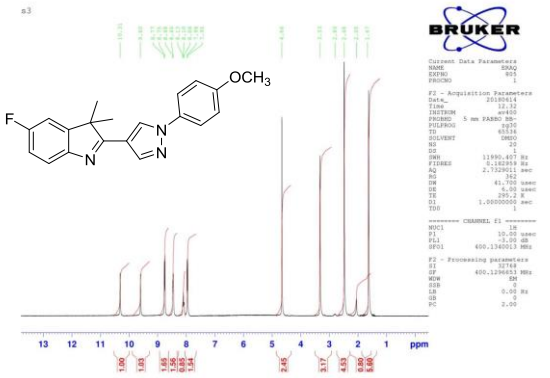


Figure 6: 1H NMR spectrum of 5-fluoro-2-(1-(4-methoxyphenyl)-1H-pyrazol-4-yl)-3,3-dimethyl-3H-indolei (3)

APT 13C-NMR spectra was used to characterize and supported 1H-NMR results of compound (3) as shown in figure 7 which was displayed signals for CH and CH3 at a negative side (below of the spectrum) (110.54- 132.61) ppm for (pyrazole and Ar-CH) [29], 55.97 ppm assigned to carbon atom of methoxy group OCH3 and 24.51ppm for the two methyl groups (2 x CH3) [30]. While the quaternary carbons, and carbon atoms of DMSO solvent which appeared at a positive side (above of the spectrum) (176.10-142.18)ppm for (pyrazole and Ar-H)(110-111) [31 and 32] and 55.97ppm for CH3-C-CH3 [33]. All 1H-NMR and APT13C-NMR results were matched well with the expected signals and was regular with the formation of this new synthesized compound.

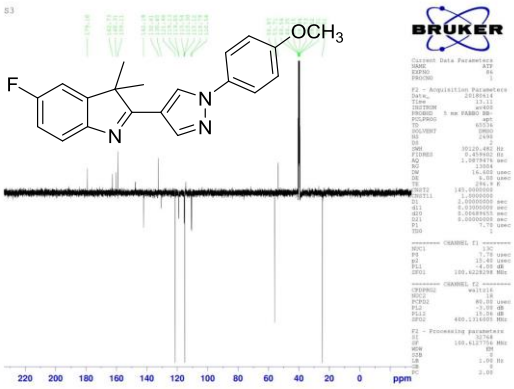


Figure 7: APT 13C NMR spectrum of 5-fluoro-2-(1-(4-methoxyphenyl)-1H-pyrazol-4-yl)-3,3-dimethyl-3H-indolei

The 1H-NMR results of the other compounds (1 and 4-8) were listediin the table 2

Table 2: the chemicalishift in ppm to 1H NMR results for compounds (1 and 4-8)

Com.No.	Chemical shift of pyrazole ring in ppm	Chemical shift of aromatic ring in ppm	Chemical shift of methyl group in ppm	Chemical shift of two methyl group inppm
١	-	٦,٧١-٧,٥٧	٢,١٧	١,٤٠
٤	١٠,٣٥ and ٩,٥٦	٨,١٢-٨,٩٦	-	١,٧٣
٥	٩,٥٠ and ٨,٧٤	٧,٢٥-٨,٠٦	-	١,٦٢
٦	١٠,٦٦ and ٩,٨٩	٨,٣٠-٩,٢٠	-	١,٨٥
٧	١٠,٥٢ and ٩,٧٨	٨,١٢-٩,٣٥	-	١,٦٧
٨	١٠,٤٢ and ٩,٦٩	٨,٠٨-٨,٩١	-	١,٦٦

Cytotoxicity assay

Prepare the concentrations of the six new indole Schiff bases compounds: these compounds were air-stable for extended periods and soluble in dimethylformamide and DMSO. For this reason, we added the amount of DMSO and then added RPMI medium slightly to prepare the concentrationsi (25, 50 and 100 µg/ml).

Cytotoxicity toward AMJ13 cell line

Cancer cell line AMJ13 were seeded as 2*104 cells / well in 96 well plats and after 24 h. when the cells become confluent monolayer, they were exposed to the compound's concentrations at 25, 50 and 100 µg/ml and incubatediat 37°C for 48 h, then stained with crystal violate dye and calculated the inhibition rate (%) for each compound. The cytotoxicity of (2) showed a good cytotoxic inhibition rate after 48 h. of

exposure to AMJ13 cancer cell line at concentrations 25, 50 and 100 µg/ml were 63.6, 76.60 and 75.90 % respectively without any significant correlations between them. Concentration 50 µg/ml represents the ideal concentration prepared from compound (2) that inhibited 50.3% of the AMJ13 cell line after 48 h. as presented below in figure 8. Cytotoxic activity results of compound (3) and itsiconcentrations 25, 50 and 100 µg/ml were illustrated in figure 6 that showed their dependence on concentration at 48h. The inhibition rates were 63.9, 74.00 and 81.50 % for 48 h inhibition rates were 63.9, 74.00 and 81.50 % for 48 h.

Figure 8: AMJ13 cell line treated with compound (3) concentrations (25, 50 and 100) µg/ml for 48ihours

Cytotoxic activity of compound (5)

The results of this compound and its concentrations are shown in figure 9. The concentration 25 and 50 pg/ml has 43.9 and 50.40 % inhibition rates respectively after 48h to AMJ13 cell exposure. While the concentration 100 µg/ml has 63.90 % inhibition rates respectively after 48h to AMJ13 cell exposure.

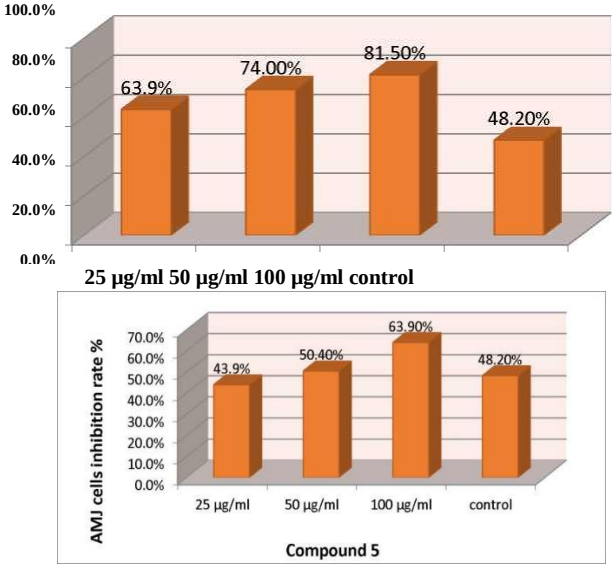


Figure 9: AMJ13 cell line treated with compound (5) concentrations (25, 50 and 100) µg/ml for

Cytotoxic activityiof compound (6)

The results of this compound and its concentrations determined that the higher concentrations represented the ideal concentrations prepared from this compound. This compound showed a higher cytotoxic inhibition rate after 48 h of exposure to AMJ13 cancer cell line at concentrations 25, 50 and 100 pg/ml were 66.8, 60.70, 44.10% respectively. The concentration of 100 µg/ml of this compound showed the highest inhibition rate among the rest of the tested compounds.

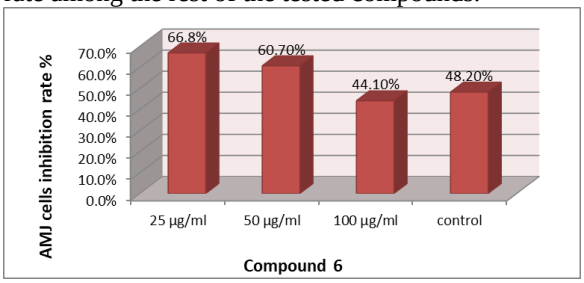


Figure (10): AMJ13 cell line treated with compound (6) concentrations (25, 50 and 100) µg/ml for 48 hours

Cytotoxic activity of compound (7)

The results of compound (7) and its concentrations 25, 50 and 100 µg/ml were illustrated in figure 10 that showed their dependence on concentration at 48h. The inhibition rates were 22.8, 29.40 and 58.00 % for the concentrations 25, 50 and 100 µg/ml respectively.

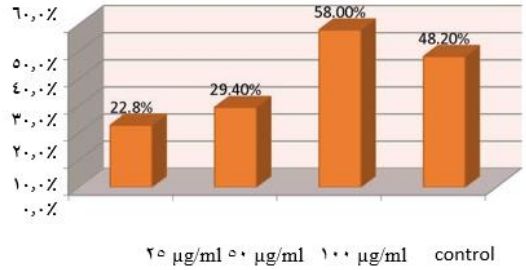


Figure 11: AMJ13 cell line treated with compound (7) concentrations (25, 50 and 100) µg/ml for 48 hours

Cytotoxic activity of compound (8)

The inhibition of this compound and its dilutions was showed in figure 9. The concentrations 25, 50 and 100 µg/ml in 48 h. of exposure time that inhibited AMJ13 cell lines growth with inhibition rates 31.5, 42.90 and 18.00%The inhibition of this compound and its dilutions was showed in figure (11). The concentrations 25, 50 and 100 µg/ml in 48 h. of exposure time that inhibited AMJ13 cell lines growth with inhibition rates 31.5, 42.90 and 18.00%.

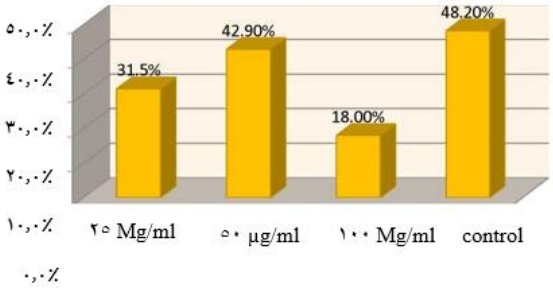


Figure 12: AMJ13 cell line treated with compound (8) concentrations (25, 50 and 100) µg/ml for 48 hours

Biological efficacy conclusions

The best compound is 5-Fluoro-2-[1-(4-methoxy-phenyl)-1H- pyrazole-4- yl]-3,3-dimethyl-3H-indole (3) which gives the highest inhibitory compared with other compounds; that showed their dependence on concentration at 48h. The inhibition rates were 63.9, 74.00 and 81.50 % for 48 h. The compound 2-[1-(4-Fluoro-phenyl)-1H-pyrazol-4-yl]-5 Fluoro- 3,3-dimethyl-3H-indole (8) showed less inhibitory percent among all other compounds, The concentrations 25, 50 and 100 µg/ml in 48 h. of exposure time that inhibited AMJ13 cell lines growth with inhibition rates 31.5, 42.90 and 18.00%.

5. Conclusion

Series of new pyrazole derivatives have been synthesized from reaction of new synthesized compound 2-(5-Fluoro-3,3- dimethyl-1,3dihydro-indol-2-ylidene)-malonaldehyde compound (2) with substituted phenyl hydrazine hydrochloride. The chemical structures of newly synthesized compounds have been characterized and confirmed by some spectroscopic techniques such as, (FT-IR, 1H, and APT13C-NMR). The cytotoxic activity revealed that 5-Fluoro-2-[1-(4-methoxy-phenyl)-1H- pyrazole-4-yl]-3,3-dimethyl-3H-indole (3) is the best compound which gives the highest inhibitory compared with other compounds and 2-[1-(4-Fluoro-phenyl)-1H-pyrazol-4-yl]-5 Fluoro- 3,3-dimethyl-3H-indole (8) showed less inhibitory percent among all other compounds,

Conflict of Interests

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

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