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Synthesis, Reactions and Antitumor Activity of Certain 1,3-diphenylpyrazole-4-carboxaldehyde Derivatives

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IN continuation of our interest in synthesis of novel heterocycles with anticipated biological activity especially the antitumor activity.In this paper we have discussed synthesis and reaction of 1,3-diphenylpyrazol-4-carboxaldehyde **4** with acetophenone derivatives **1a–d**, active methylene compounds, hydrazines and aniline derivatives to yield the expected derivatives **5a–d**. Also, a series of penta-substituted pyridine derivatives **15a-e** have been synthesized by one-pot three component cyclocondensation reaction of 1,3-diphenylpyrazole-4-carboxaldehyde 1, malononitrile and thiol derivatives **13a–e** in presence of triethylamine as a catalyst. Also, 2-(1,3-diphenyl-1H-pyrazol-4-yl)-3-(aryl)thiazolidine-4-one **20a-e** has been synthesized from N-((1,3-diphenyl-1H-pyrazol-4-yl)methylene-aniline derivatives **11a-e** and thioglycolic acid. Some of the synthesized derivatives were screened for their antitumor activity. All the newly synthesized compounds have been characterized by means of elemental analyses, IR, ^{1}HNMR, ^{13}CNMR, MS and in some cases by comparison with the known properties of compound or by comparison with samples prepared by reported unambiguous routs.

Keywords: 1,3-diphenylpyrazol-4-carboxaldehyde, Vilsmeier-Haack reaction, 3,5-Pyridinedicarbonitrile, Antitumor activity.

Introduction

The pyrazole ring is found in numerous pharmaceutically active compounds. This is mainly due to the ease of preparation and the important biological activity. Many pyrazole derivatives have been reported to possess diverse pharmacological activities such as antimicrobial [1–6], anti-inflammatory [7–10], anti-viral [11, 12], antidiabetic [13], analgesic [14] and antiparasitic properties [15].

Pyrazole showed promising anticancer effects [16–22]. In search for better antitumor treatment, a large number of pyrazole derivatives were synthesized and tested over the years, the use of this powerful pharmacophore is very popular and modern [23–25]. On the other hand, pyridine derivatives have occupied a unique position in medicinal chemistry, besides many naturally occurring pyridines. Several synthetic derivatives show interesting biological activities for example, 2-amino-3-cyanopyridines have antibacterial, antimicrobial, antifungal and cardiotonic activities [26, 27]. In connection with these findings and our interest in the synthesis of fused nitrogen heterocyclic compounds with expected antitumor activity, we have described the synthesis of 4-substituted pyrazoles utilizing 4-formyl pyrazoles as starting materials.

Experimental

General

All melting points were determined using Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 383 spectrometer (KBr). ^{1}H NMR spectra were recorded on Bruker AC 200F 300 MHz spectrometer using TMS as an internal reference. ^{13}C NMR spectra were measured on a Varian spectrophotometer at 300 MHz, using DMSO-d_6 or $CDCl_3$ as solvent. The Electron Impact mass spectra were obtained at 70 eV using Shimadzu QP-2010 Plus mass spectrometer. The reactions were monitored by thin-layer chromatography (TLC) on silica gel F254 aluminum sheets (Merck), and spots were visualized by UV lamp at 254–365 nm. All cell culture material was obtained from Cambrex Bio Science (Copenhagen, Denmark). All chemicals were from Sigma/ Aldrich, USA, except mentioned. Human breast adenocarcinoma cell line (MCF-7) and human hepatocarcinoma cell line (Hep-G2), were purchased from ATCC, USA

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Chemistry

*Synthesis of 1,3-diphenylpyrazole-4-carboxaldehyde **4*** [28]

To (0.01 mole) of acetophenonephenyl hydrazine, (0.01 mole) of Vilsmerier reagent (14.6 mL DMF and 19.10 mL $POCl_3$) was added dropwise with stirring for one hour. The reaction mixture was refluxed for six hours at 70-80°C, then hydrolyzed on ice/water mixture, and neutralized by 5% NaOH solution till pH 4, the solid formed was filtered, washed with water, dried and crystalized from isopropanol to give compound **4** as yellow white powder in (80%) yield, m.p. = 142–143°C, IR (KBr, cm^{-1}): 3125, 3062 (CH_{ar}), 1673 (C=O), 163, 1599, 1526 (C=N), 1511 ($C=C_{\text{aromatic system}}$); ^{1}H NMR($CDCl_3$): δ (ppm) = 10.09 (s, 1H, CHO), 8.57 s, 1H, $H_{pyrazole}$), 7.87 – 7.29 (m, 10H, H_{ar}), 7.53–7.39 (m, 10H, H_{ar});Anal. Calcd for $C_{16}H_{12}N_2O$ (248.29): C, 77.40; H, 4.87; N, 11.28. Found: C, 77.25; H, 4.72; N, 11.13.

*General procedure for synthesis of 1-aryl-3-(1,3-diphenyl-1H-pyrazol-4-yl)prop-2-en-1-*one ***5a–d*** [28, 29]

To a solution of 1,3-diphenyl-1H-pyrazol-4-carboxaldehyde **4** (2.48 g, 0.01 mole), aryl methyl ketone **1a–d** (0.01 mole) in ethanol (30 mL), a pellet of KOH was added. The reaction mixture was stirred at room temperature for overnight. The yellow solid precipitated was separated by filtration and recrystallized from (1 : 1) EtOH/DMF mixture to give α-β-unsaturated compounds **5a–d.**

*(E)-3-(1,3-Diphenyl-1H-pyrazol-4-yl)-1-phenylprop-2-en-1-one **5a***

Yield 82 %; mp124–126 °C; IR (KBr, cm^{-1}): 1668 (C=O), 1627 (C=N), 1606–1482 ($C=C_{ar}$);^{1}H NMR($CDCl_3$): δ (ppm) = 8.57 (s, 1H, $H_{pyrazole}$), 8.38–7.29 (m, 17H, H_{ar} and CH=CH); ^{13}C NMR ($CDCl_3$): δ (ppm): 190.51 (C=O), 154.26, 139.85, 138.65, 135.78, 133.05, 132.74, 129.97, 129.68, 129.17, 129.12, 128.97, 128.55, 127.66, 127.21, 121.96, 119.79, 118.72 (HC=CH, C=N, C_{ar}); Anal. Calcd for $C_{24}H_{18}N_2O$ (350.42): C, 82.26; H, 5.18; N, 7.99. Found: C, 82.35; H, 5.10; N, 7.80.

*(E)-3-(1,3-Diphenyl-1H-pyrazol-4-yl)-1-(p-tolyl)prop-2-en-1-one **5b***

Yield 75 %; mp 150–152 °C; IR (KBr, cm^{-1}): 3126, 3056 (CH_{ar}), 1673 (C=O), 1660 (C=N), 1597, 1530, 1510 ($C=C_{ar}$); Anal. Calcd for $C_{25}H_{20}N_2O$ (364.45): C, 82.39; H, 5.53; N, 7.69. Found: C, 82.50; H, 5.40; N, 7.70.

*(E)-1-(4-Chlorophenyl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)prop-2-en-1-one **5c***

Yield 85 %; mp 160–162 °C; ^{1}H NMR($CDCl_3$): δ (ppm) = 8.38 (s, 1H, $H_{pyrazole}$), 7.96–7.29 (m, 16H, H_{ar}); ^{13}C NMR ($CDCl_3$): δ (ppm): 186.01 (C=O), 153.27, 138.74, 138.40, 135.90, 135.20, 131.65, 129.09, 128.92, 128.22, 128.17, 126.67, 126.27, 120.24, 118.75, 117.53 (HC=CH, C=N, C_{ar}); Anal. Calcd for $C_{24}H_{17}ClN_2O$ (384.86): C, 74.90; H, 4.45; N, 7.28. Found: C, 75.00; H, 4.40; N, 7.30.

*(E)-1-(4-Bromophenyl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)prop-2-en-1-one **5d***

Yield 77 %; mp 180–182 °C; Anal. Calcd for $C_{24}H_{17}BrN_2O$ (429.32): C, 67.14; H, 3.99; N, 6.53. Found: C, 67.25; H, 3.80; N, 6.50.

*2-((1,3-Diphenyl-1H-pyrazol-4-yl)methylene)-malononitrile **6*** [30]

A mixture of malononitrile (0.66, 0.01 mole), 1,3-diphenyl pyrazole-4-carboxaldehyde 4 (2.48 g, 0.01 mole) in ethanol (20 mL) containing few drops of piperidine was refluxed for two hours. After cooling the formed solid was filtered, dried and recrystallized from ethanol to give compound **6** in 85% yield, m.p. 190–191°C, IR (KBr, cm^{-1}): 3068 (CH_{ar}), 2200 (CN), 1590, 1536, 1512($C=C_{ar}$); ^{1}HNMR ($CDCl_3$): δ (ppm) = 9.08(s, 1H, –HC=), 8.57(s, 1H, $H_{pyrazole}$), 8.337.87–7.46 (m, 11H, H_{ar}); Anal. Calcd for $C_{19}H_{12}N_4$ (296.33): C, 77.01; H, 4.08; N, 18.91. Found: C, 76.85; H, 4.16; N, 19.

*Ethyl 2-cyano-3-(1,3-diphenyl-1H-pyrazol-4-yl) acrylate **7*** [28]

A mixture of 1,3-diphenylpyrazol-4-carboxaldeyde **4** (2.48 g, 0.01 mole), ethyl cyanoacetate (0.135 g, 0.012 mole) in ethanol containing few drops of piperidine were refluxed for 3 hours. The precipitated solid was filtered off and recrystallized from ethanol to give compound **7** as a pale yellow powder, yield 78%, m.p. 200–202°C, IR (KBr, cm^{-1}): 3068 (CH_{ar}), 2200 (CN), 1590, 1536, 1512($C=C_{ar}$); ^{1}H NMR ($CDCl_3$): δ (ppm) = 9.17(s, 1H, –HC=), 8.57(s, 1H, $H_{pyrazole}$), 7.88–7.43 (m, 10H, H_{ar}), 4.36 (q, J=7.2,2H, OCH_2), 1.40 (t, J=7.1, 3H, CH_3); Anal. Calcd for $C_{21}H_{17}N_3O_2$ (343.39): C, 73.45; H, 4.99; N, 12.24. Found: C, 73.50; H, 5.10; N, 12.12.

*(E)-2-cyano-N'-((1,3-diphenyl-1H-pyrazol-4-yl) methylene)acetohydrazide **8*** [30]

A mixture of 1,3-diphenylpyrazol-4-carboxaldehyde **4** (2.48 g, 0.01 mole) and cyanoacetic hydrazide (1.00 g, 0.01 mole) in 25 mL ethanol was stirred at room temperature for 5 hours. The precipitate was collected and

recrystallized from ethyl acetate, yield 80%, m.p. 190-192°C. IR (KBr, cm^{-1}): 3225 (NH), 3062 (CH_{ar}), 2980($CH_{aliphatic}$), 2218 (CN), 1668 (C=O), 1600, 1586, 1512, 1486 (aromatic ring). Anal. Calcd for $C_{19}H_{15}N_5O$ (329.36): C, 69.29; H, 4.59; N, 21.26. Found: C, 69.15; H, 4.52; N, 21.21.

4-((2-Methylhydrazono)-methyl)-1,3-diphenyl-1H-pyrazole ***9***

A mixture of 1,3-diphenylpyrazol-4-carboxaldehyde **4** (2. 48 g, 0.01 mole) and methyl hydrazine (0.01 mole) in 20 mL methanol containing few drops of acetic acid were refluxed for 3 hours. After cooling the formed solid was filtered, dried and crystalized from methanol to give compound 9, yield 77%, m.p. 240–242°C. Anal. Calcd for $C_{17}H_{16}N_4$ (276.34): C, 73.89; H, 5.84; N, 20.27. Found: C, 73.75; H, 5.80; N, 20.10.

General procedure for synthesis of 4-((2-arylhydrazono)-methyl)-1,3-diphenyl-1H-pyrazole ***10 a-e***

A mixture of 1,3-diphenylpyrazol-4-carboxaldehyde **4** (2.48 g, 0.01 mole) and aryl hydrazine derivatives (0.01 mole) in 20 mL methanol containing few drops of acetic acid were refluxed for 3 hours. After cooling the formed solid was filtered, dried and recrystallized from methanol to give derivatives**10 a-e**.

1,3-Diphenyl-4-((2-phenylhydrazineylidene) methyl)-1H-pyrazole ***10 a*** [31]

Yield 70%, m.p. 220–222°C. Anal. Calcd for $C_{22}H_{18}N_4$ (338.41): C, 78.08; H, 5.36; N, 16.56. Found: C, 78.1; H, 5.15; N, 16.42.

4-((2-(4-Bromophenyl)hydrazineylidene) methyl)-1,3-diphenyl-1H-pyrazole ***10 b***

Yield 72%, m.p. 200–202°C. Anal. Calcd for $C_{22}H_{17}BrN_4$ (416.06): C, 63.32; H, 4.11; N, 13.43. Found: C, 63.40; H, 4.20; N, 13.38.

4-((2-(4-Nitrophenyl)hydrazineylidene) methyl)-1,3-diphenyl-1H-pyrazole ***10 c***

Yield 65%, m.p. 212–214°C. Anal. Calcd for $C_{22}H_{17}N_5O_2$ (383.41): C, 68.92; H, 4.47; N, 18.27. Found: C, 68.71; H, 4.31; N, 18.15.

4-((2-(2,4-Dinitrophenyl)hydrazineylidene) methyl)-1,3-diphenyl-1H-pyrazole ***10 d***

Yield 85%, m.p. 271–273°C. Anal. Calcd for $C_{22}H_{16}N_6O_4$ (428.41): C, 61.68; H, 3.76; N, 19.62. Found: C, 61.62; H, 3.74; N, 19.58.

(E)-1,3-Diphenyl-4-((2-(2,4,6-trichlorophenyl)hydrazineylidene)methyl)-1H-pyrazole ***10 e***

Yield 65%, m.p. 165–167°C. Anal. Calcd for $C_{22}H_{15}Cl_3N_4$(441.74): C, 59.82; H, 3.42; N, 12.68. Found: C, 59.75; H, 3.55; N, 12.60.

General procedure for synthesis ofN-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)aniline derivatives ***11a-f***.

A solution of 1,3-diphenylpyrazole-4-carboxaldehyde **4** (2.48 g, 0.01 mole), aniline derivatives (0.01 mole), acetic acid (1 mL) and methanol (30mL) was refluxed for one hour (some yellow crystals formed under reflux condition). After cooling the reaction mixture was poured into crushed ice, the yellow product was filtered and recrystallized from ethyl acetate to give derivatives **11a-f.**

1-(1,3-Diphenyl-1H-pyrazol-4-yl)-N-phenylmethanimine ***11a***

Yield 65%, m.p. 150–152°C.Anal. Calcd for $C_{22}H_{17}N_3$ (323.40): C, 81.71; H, 5.30; N, 12.99. Found: C, 81.75; H, 5.22; N, 13.10.

1-(1,3-Diphenyl-1H-pyrazol-4-yl)-N-(p-tolyl) methanimine ***11b***

Yield 82%, m.p. 150–152°C. IR (KBr, cm^{-1}): 3060 (CH_{ar}), 2913 ($CH_{aliphatic}$) 1622 (CH=N), 1595, 1542, 1503, 1450 (Aromatic rings); ^{1}H NMR($CDCl_3$): δ (ppm) = 8.69 (s, 1H, CH=N), 8.56 (s.1H, $H_{pyrazole}$), 7.89 – 7.11 (m, 14H, Ar-H), 2.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm): 154.36, 152.66, 150.16, 139.97, 136.06, 132.75, 130.23, 130.07, 129.96, 129.71, 129.39, 129.27, 129.18, 129.09, 127.83, 127.62, 121.22, 120.81, 119.79 (aromatic carbon and HC=N-), 21.39 (CH_3). Anal. Calcd for $C_{23}H_{19}N_3$ (337.43): C, 81.87; H, 5.68; N, 12.45. Found: C, 81.90; H, 5.57; N, 12.50.

1-(1,3-Diphenyl-1H-pyrazol-4-yl)-N-(4-nitrophenyl)methanimine ***11c***

Yield 60%, m.p. 142–144°C. Anal. Calcd for $C_{22}H_{16}N_4O$ (368.40): C, 71.73; H, 4.38; N, 15.21. Found: C, 71.60; H, 4.40; N, 15.00.

N-(4-Bromophenyl)-1-(1,3-diphenyl-1H-pyrazol-4-yl)methanimine ***11d***

Yield 58%, m.p. 168–170°C. Anal. Calcd for $C_{22}H_{16}BrN_3$ (402.30): C, 65.68; H, 4.01; N, 10.45. Found: C, 65.70; H, 4.15; N, 10.30.

1-(1,3-Diphenyl-1H-pyrazol-4-yl)-N-(3-methoxyphenyl)methanimine ***11e***

Yield 71%, m.p. 270–272°C. Anal. Calcd for $C_{23}H_{19}N_3O$ (353.43): C, 78.16; H, 5.42; N, 11.89. Found: C, 78.20; H, 5.32; N, 11.90.

2-(((1,3-Diphenyl-1H-pyrazol-4-yl) methylene)amino)aniline ***11f***

Yield 62%, m.p. 280-282°C. Anal. Calcd for $C_{22}H_{18}N_4$ (338.41): C, 78.08; H, 5.36; N, 16.56. Found: C, 78.10; H, 5.45; N, 16.40.

General procedure for synthesis of2-amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(arylthio or alkylthio) pyridine-3,5-dicarbonitrile ***15a-e***

Method A:

A mixture of 1,3-diphenylpyrazole-4-carboxaldehyde 4 (2.48 g, 0.01 mole), malononitrile (1.32 g, 0.02 mole) and thiol derivatives 13a–e (0.01 mole); namely: thiophenol, *o*-aminothiophenol, *p*-aminothiophenol, thioethanol, 2-hydroxythioethanol)were refluxed in ethanol (20 mL) containing three drops of triethylamine for 3 hours then the product formed was filtered, dried and recrystallized from ethanol to give **15a-e**

Method B:

A mixture of compound **6** (2.96 g, 0.01 mole), malononitrile (0.66 g, 0.01 mole) and thiol derivativess l3a-e (0.01 mole) were refluxed in ethanol (20 mL) containing three drops of triethylamine for 3 hours, then the product formed was filtered off, dried and recrystallized from ethanol to give 15a-e.

2-Amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(phenylthio)pyridine-3,5-dicarbonitrile ***15a***

Yield 57%, m.p. 240–242°C, IR (KBr, cm^{-1}): 3432, 3367, 3311 (NH_2), 3052 (CH_{ar}), 2218 (CN) 1626 (C=N); ^{1}H NMR(DMSO-d6): δ (ppm) = 8.28 (s, 1H, Pyrazole), 7.84–7.41 (m, 15H, H_{ar}),5.41 (br, 2H, NH_2); ^{13}C NMR (DMSO-d6): δ (ppm): 159.61, 152.12, 139.73, 136.18, 132.46, 130.38, 130.21, 129.97, 129.72, 129.59, 129.18, 128.17, 127.92, 127.48, 120.17, 114.25, 8861, 87.00 (C_{ar}, 2CN). Anal. Calcd for $C_{28}H_{18}N_6S$ (470.55): C, 71.47; H, 3.86; N, 17.86. Found: C, 71.50; H, 3.77; N, 17.90.

2-Amino-6-((2-aminophenyl)thio)-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarbonitrile ***15b***

Yield 55%, m.p. 250–251°C. IR (KBr, cm^{-1}): 3400-3300 ($2NH_2$), 3059 (CH_{ar}), 2223 (CN), 1627 (C=N), 1596, 1530,1503, 1479 (aromatic rings); ^{1}H NMR(DMSO-d6): δ (ppm) = 9.17 (s, 1H, NH_2), 8.21 (s, 1H, $H_{pyrazole}$), 8.06 (d, 1H, J= 8.2, 1H, H_{ar}), 7.87 (d, J=8.3, 3H, H_{ar}, NH_2), 7.70–7.87 (m, 2H, H_{ar}), 3.43–7.60 (m, 13H, Har);^{13}C NMR (DMSO-d6): δ (ppm):162.95, 156.14 , 154.11, 139.48, 138.54, 135.06, 131.70, 130.07, 129.62, 129.43, 128.75, 128.35, 127.21, 126.21, 123.93, 121.91, 120.30, 117.59, 115.96, 103.94 (C_{ar}, 2CN). Anal. Calcd for $C_{28}H_{19}N_7S$ (485.57): C, 69.26; H, 3.94; N, 20.19. Found: C, 69.30; H, 4.00; N, 20.25.

2-Amino-6-((4-aminophenyl)thio)-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarbonitrile ***15c***

Yield 60%, m.p. 260–262°C, IR (KBr, cm^{-1}): 3470, 3337, 3233 ($2NH_2$), 2212 (CN), 1626 (C=N);^{1}H NMR(DMSO-d6): δ (ppm) =7.97 (s, 1H, $H_{pyrazole}$), 7.95 (d, J= 7.8Hz, 2H, H_{ar}), 7.74 (br, 2H, NH_2), 7.18 (d, J=8.7Hz, 2H, H_{ar}), 6.62 (d, J= 8.7Hz, 2H, H_{ar}), 5.59 (s, 2H, NH_2). Anal. Calcd for $C_{28}H_{19}N_7S$ (485.57): C, 69.26; H, 3.94; N, 20.19. Found: C, 69.29; H, 4.00; N, 20.25.

2-Amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(ethylthio)pyridine-3,5-dicarbonitrile ***15d***

Yield 62%, m.p. 250–252°C. IR (KBr, cm^{-1}): 3446, 3361, 3176 (NH_2), 2214 (CN), 1632 (C=N); ^{1}H NMR($CDCl_3$): δ (ppm) = 8.25 (s, 1H, $H_{pyrazole}$), 7.81 (d, J=7.8Hz,2H, H_{ar}), 7.52 (s, 4H, H_{ar}), 7.40 (s, 4H, H_{ar}), 5.60 (s, 2H, NH_2), 3.22 (q, *J=7.3* Hz, 2H,CH_2), 1.41 (t, *J*= 7.3Hz, 3H, CH); ^{13}C NMR ($CDCl_3$): δ (ppm): 169.87, 159.69, 152.03, 150.39, 139.72, 132.52, 130.00, 129.18, 128.13, 127.91, 120.10, 115.51, 115.24, 114.39, 97.63, 87.51 (Car, 2CN), 25.54(CH_2), 14.45 (CH_3). Anal. Calcd for $C_{24}H_{18}N_6S$ (422.51): C, 68.23; H, 4.29; N, 19.89. Found: C, 68.12; H, 4.23; N, 19.81.

2-Amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-((2-hydroxyethyl)thio)pyridine-3,5-dicarbonitrile ***15e***

Yield 48%, m.p. 200-203°C. Anal. Calcd for $C_{24}H_{18}N_6OS$ (438.51): C, 65.74; H, 4.14; N, 19.17. Found: C, 65.69; H, 4.12; N, 19.12.

Synthesis of 2-amino-4-(1,3-diphenyl-1H-pyrazol-4yl)-6-hydroxypyridine-3,5-dicarbonitrile ***16***.

2-Amino-4-(1,3-diphenyl-*1H*-pyrazol-4-yl)-6-(phenylthio)-pyridine-3,5-dicarbnonitrile **15a** (0.47 g, 0.001 mole) was refluxed in ethanolic NaOH 30% (10 mL) at 120°C with stirring for 5 hours, the reaction was then cooled, the solid separated was filtered off, dried and recrystallized from acetic acid to give compound **16** as colorless crystals in 70% yield, m.p.: 236°C.

IR (KBr, cm^{-1}): 3489, 3440, 3367, 3168(NH_2, OH), 2226 (CN), 1636 (C=N). Anal. Calcd for $C_{22}H_{14}N_6O$ (378.40): C, 69.83; H, 3.73; N, 22.21. Found: C, 69.81; H, 3.68; N, 22.15.

*Synthesis of 3,6-diamino-4-(1,3-diphenylpyrazol-4-yl)pyrazolo[3,4-b]pyridine-5-carbontrile **17**.*

A solution of 2-amino-4-(1,3-diphenyl-*1H*-pyrazol-4-yl)-6-(phenylthio)pyridine-3,5-dicarbonitrile **15a** (4.70 g, 0.01 mole) or 2-amino-6-chloro-4-(1,3,-diphenyl-*1H*-pyrazol-4-yl)-pyridine-3,5-dicarbnonitrile **18** (47.70 g, 0.01 mole) with hydrazine hydrate (1.00 g, 0.02 mole) were refluxed in presence ofn-butanol at 120°C for 30 min.The yellow solid formed on hot was collected by filtration, washed with alcohol and recrystallized from ethanol to give 3,6-diamino-4-(1,3-diphenylpyrazol-4-yl)pyrazolo[3,4-*b*] pyridine-5-carbonitrile **17** in 65% yield, m.p. 280°C. IR (KBr, cm^{-1}): 3468, 3382, 3362, 3185 (NH, NH_2), 2220 (CN), 1632 (C=N), 1598, 1508, 1492 (aromatic rings); ^{1}H NMR(DMSO-d6): δ (ppm) = 8.56 (s, 1H, $H_{pyrazole}$), 7.81 (d, J= 8.4Hz, 2H, H_{ar}), 7.48–7.60 (m, 8H, H_{ar}), 6.83 (S, 2H, NH_2, pyridine), 4.36 (s, 2H, NH_2, pyrazole). Anal. Calcd for $C_{22}H_{16}N_8$ (392.43): C, 67.34; H, 4.11; N, 28.55. Found: C, 67.40; H, 4.21; N, 28.37.

*Synthesis of 2-amino-6-choloro-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarbonitrile **18***

A mixtures of 2-amino-6-hydroxy-4-(1,3-diphenyl-*1H*-pyrazol-4-yl)pyridine-3,5-dicarbonitrile **16** (3.92 g, 0.01 mole), PCl_5 (2.0 g) and $POCl_3$ (l0 mL) was heated on water bath for 7 hours, the resulting solution was added dropwise onto crushed ice. The solid product obtained was filtered off, washed several times with water, dried and recrystallized from ethanol to give 2-amino- 6-chloro-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarbonitrIe **18** in 62% yield, m.p. > 300°C.

Anal. Calcd for $C_{22}H_{13}ClN_6$ (396.84): C, 66.59; H, 3.30; N, 21.18. Found: C, 66.52; H, 3.28; N, 21.12.

*Synthesis of 3,6-diamino-1-methyl-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyrazolo[3,4-b] pyridine-5-carbonitrile **19**.*

A mixture of 2-amino-4-(1,3-diphenyl-*1H*-pyrazol-4-y1)-6-(phenylthio)-pyridine-3,5-dicarbonitrle **15a** (4.70 g, 0.01 mole) or 2-amino-6-chloro-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarbonitrile 18 (4.70 g, 0.01 mole) and methyl hydrazine (10 mL) was heated with stirring under reflux at 150°C for 3 hours the orange yellow solid formed was separated by filtration, washed with alcohol and recrystallized from ethyl acetate or acetic acid to give the expected product **19** in 75% yield m.p.300°C.

Anal. Calcd for $C_{23}H_{18}N_8$ (406.45): C, 67.97; H, 4.46; N, 27.57. Found: C, 68.00; H, 4.42; N, 27.50.

*Synthesis of 3-aryl-2-(1,3-diphenyl-1H-pyrazol-4-yl)thiazolidine-4-one **20a-e*** [32]

A mixture of 1,3-diphenyl-4-(aryliminomethylene)pyrazole (0.01 mole) and mercaptoacetic acid (1.10 g, 0.012 mole) in dry benzene (80 mL) was refluxed for 15 hours using Dean-Stark separator (to separate the aqueous benzene layer from time to time). Then, evaporate all the solvent, washed the oil residue with petroleum ether 40-60 or ether and recrystallize the yellow solid from methylene chloride or ethyl acetate to give derivatives **20a–e**.

*2-(1,3-Diphenyl-1H-pyrazol-4-yl)-3-phenylthiazolidin-4-one **20a***

Yield 50%, m.p. 177–179°C; ^{1}H NMR($CDCl_3$): δ (ppm) = 8.02 (s, 1H, $H_{pyrazole}$), 7.07–7.70 (m, 15H, H_{ar}), 6.38 (s, 1H, CH), 3.91 (d, *J=1.3Hz,* 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ (ppm): 170.87 (C=O), 152.15, 139.92, 137.67, 132.57, 129.65, 129.51, 129.10, 128.95, 128.86, 127.44, 127.40, 127.34, 125.62, 120.99, 119.49 (aromatic carbons), 57.47 (CH), 33.93 (CH_2).Anal. Calcd for $C_{24}H_{19}N_3OS$ (397.50): C, 72.52; H, 4.82; N, 10.57. Found: C, 72.48; H, 4.78; N, 10.47.

*2-(1,3-Diphenyl-1H-pyrazol-4-yl)-3-(p-tolyl) thiazolidin-4-one **20b***

Yield 50%, m.p. 183–185°C. ^{1}H NMR($CDCl_3$): δ (ppm) = 8.10 (s, 1H, $H_{pyrazole}$), 6.90–7.83 (m, 14H, H_{ar}), 6.40 (s, 1H, CH), 3.6 (d, *J=1.3* Hz, 2H, CH_2), 2.33 (s, 3H, CH_3). Anal. Calcd for $C_{25}H_{21}N_3OS$ (411.52): C, 72.97; H, 5.14; N, 10.21. Found: C, 72.80; H, 5.20; N, 10.10.

*2-(1,3-Diphenyl-1H-pyrazol-4-yl)-3-(4-nitrophenyl)thiazolidin-4-one **20c***

Yield 35%, m.p. 175–177°C. MS (m/z): 444 [M^++2, 24%], 443 [M^++1, 37%], 442 [M^+, 37%], 61 [base peak, 100%]. Anal. Calcd for $C_{24}H_{18}N_4O_3S$ (442.49): C, 65.15; H, 4.10; N, 12.66. Found: C, 65.20; H, 4.00; N, 12.70.

*3-(4-Bromophenyl)-2-(1,3-diphenyl-1H-pyrazol-4-yl)thiazolidin-4-one **20d***

Yield 35%, m.p. 165–167°C. IR (KBr, cm^{-1}): 3050 (CH_{ar}), 2921 ($CH_{aliphatic}$) 1710 (C=O),1651(C=N), 1617, 1594, 1537, 1484(Aromatic rings); ^{1}H NMR($CDCl_3$): δ (ppm) = 8.08 (s, 1H, $H_{pyrazole}$), 7.10–7.70 (m, 14H, H_{ar}), 6.41 (s, 1H, CH), 3.88 (d, *J=1.3Hz,* 2H, CH_2). Anal. Calcd for $C_{24}H_{18}BrN_3OS$ (476.39): C,

60.51; H, 3.81; N, 8.82. Found: C, 60.38; H, 4.00; N, 8.70.

2-(1,3-Diphenyl-1H-pyrazol-4-yl)-3-(4-methoxyphenyl)thiazolidin-4-one ***20e***

Yield 38%, m.p. 187–189°C. Anal. Calcd for $C_{25}H_{21}N_3O_2S$ (427.52): C, 70.24; H, 4.95; N, 9.83. Found: C, 70.21; H, 4.86; N, 9.79.

Antitumor activity

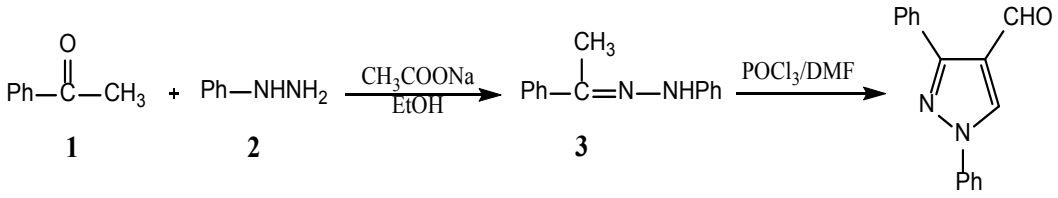
Human breast adenocarcinoma cell line (MCF-7) and human hepatocarcinoma cell line (Hep-G2) were used to evaluate the cytotoxic effect of the tested drugs. Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium), which was supplemented with 10% fetal bovine serum (FBS), 2 mML-glutamine, containing 100 units/mL penicillin G sodium, 100 units/mL streptomycin sulphate, and 250 ng/mL amphotericin B. Cells were maintained at sub-confluency at 37ºC in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested drugs were dissolved in dimethyl sulphoxide (DMSO), and then diluted thousand times in the assay. Cytotoxicity of tested drugs was measured against MCF-7 and Hep-G2 cells using the MTT Cell Viability Assay. MTT (3-[4,5-dimethylthiazole-2- yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which are largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm [33]. MTT solution was prepared at concentration of 5mg/mL in 0.9%NaCl and acidified isopropanol was prepared by dissolving 0.04 N HCl in absolute isopropanol. Cells (0.5X105 cells/ well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20µL of different concentrations of the tested drugs for 48 h at 37º C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 µL MTT solution / well were added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µL of acidified isopropanol/well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability. Percentage of relative viability was calculated using the following equation:

[Absorbance of treated cells/ Absorbance of control cells)] X 100

Then the half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve.

Results and Discussion

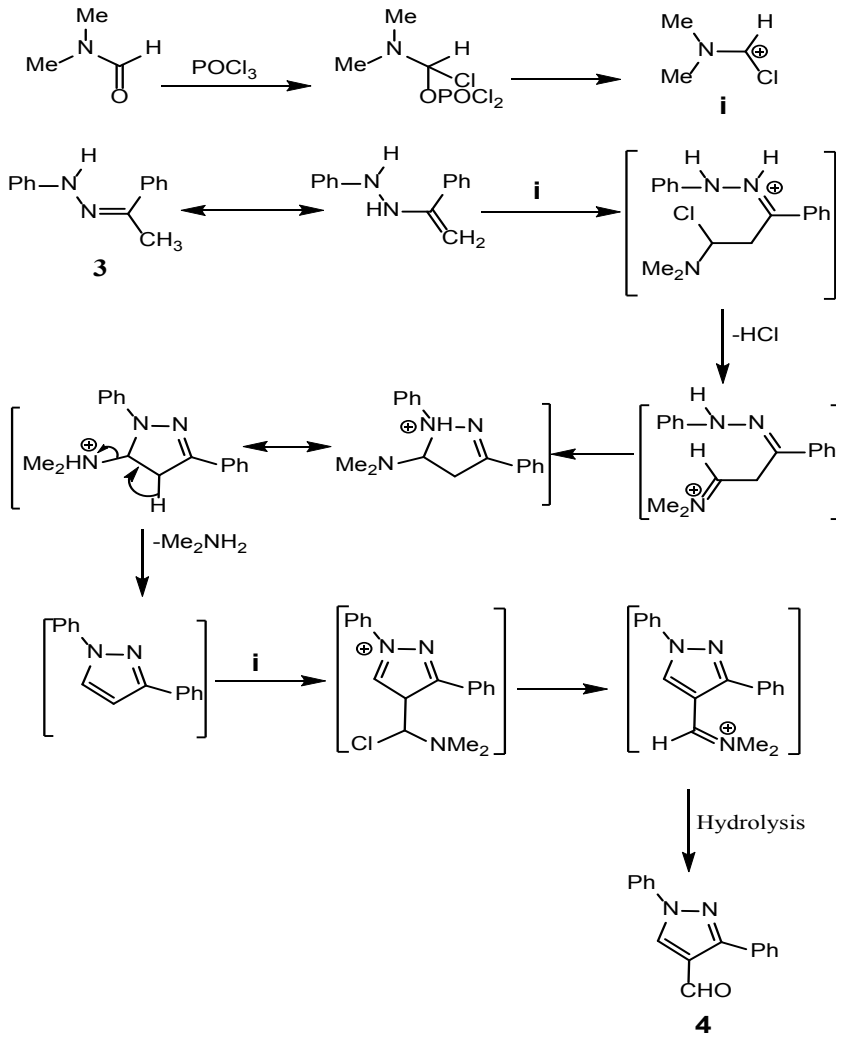
1,3-diphenylpyrazole-4-carboxaldehyde **4** was prepared by reaction of acetophenone **1** with phenyl hydrazine **2** and sodium acetate in ethanol; subsequent reaction of benzyl hydrazone3 under Vilsmeier-Haack conditions afforded 1,3-diphenylpyrazole-4-carboxaldehyde **4**. The structure of 1,3-diphenylpyrazole-4-carboxaldehyde **4** was confirmed through comparison of its physical data with reported data [28, 29].



1 **2** **3** **4**

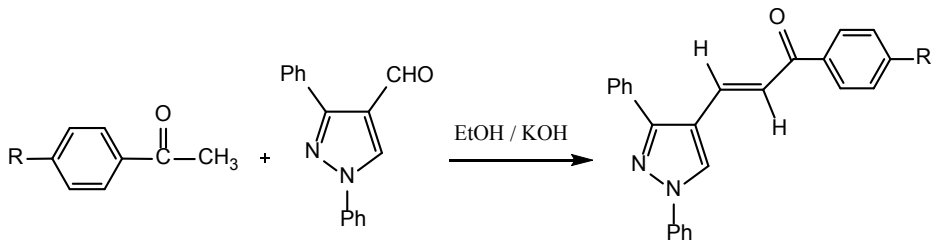
Scheme 1

A proposed mechanism for the formation of compound **4** is outlined in the following scheme [36]



Propsed mechanism for the formation of 4- formylpyrazoles 4

The Claisen-Schcmidt condensation of 1,3-diphenyl-pyrazole-4-carboxaldehyde **4** with acetophenone derivatives **1a-d** afforded the corresponding pyrazolicchalcones **5a-d**.



1a-d
a, R= H
b, R= CH_3
c, R= Cl
d, R=Br

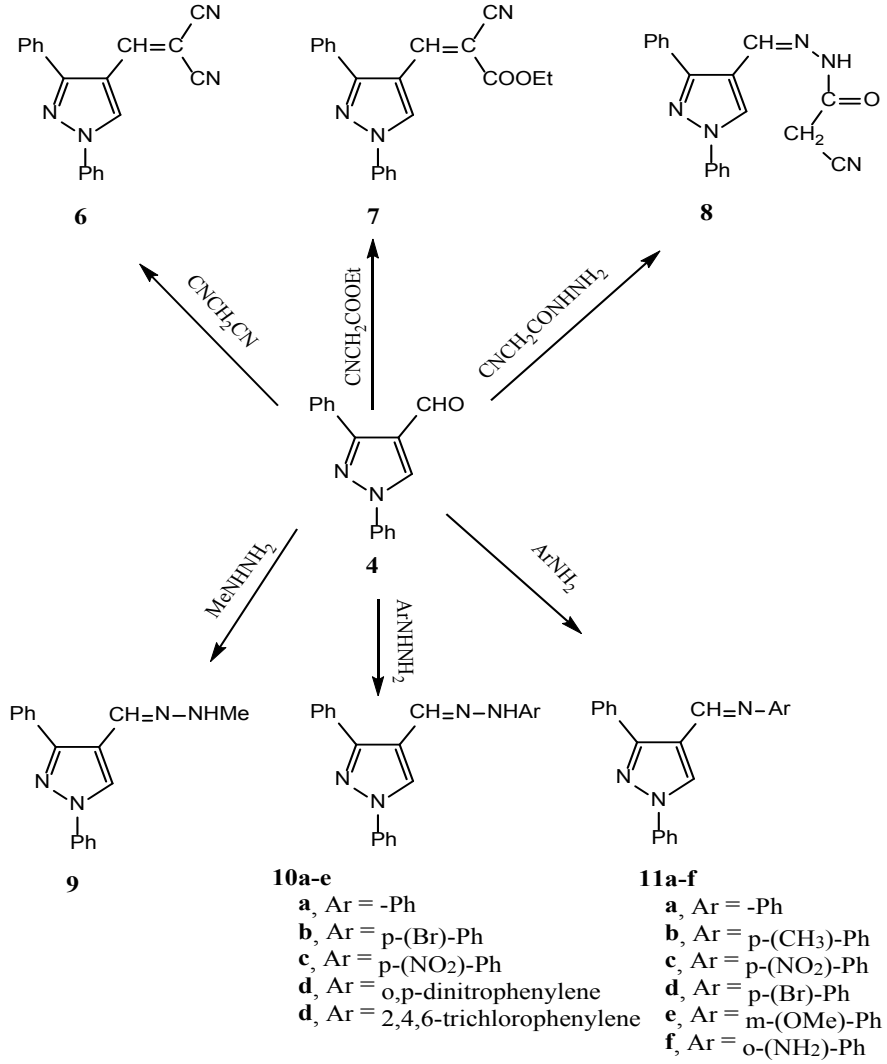
4

5a-d
a, R= H
b, R= CH_3
c, R= Cl
d, R=Br

Scheme 2.

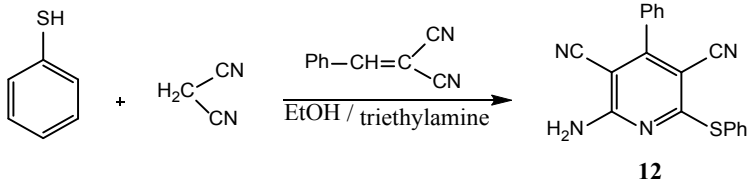
The structure of α,β-unsaturated compounds **5a-d** were established on the basis of their elemental analysis, spectral data, and comparison of its physical data with reported data [37]. The IR spectrum showed absorption bands at 1668–1627 cm^{-1} due to carbonyl group, C=N group and 1606–1482 cm^{-1} due to aromatic ring. ^1H NMR spectrum of **5a** in $CDCl_3$ showed signals at 7.29–8.38 (m, 17H, H_{ar}, CH=CH), 8.57 (s, 1H, $H_{pyrazole}$). The IR spectrum of **5b** showed absorption bands at 3126, 3056 cm^{-1} due to CH aromatic, 1673 cm^{-1} due to carbonyl group, 1660 cm^{-1} due to C=N group, 1597, 1530, 1510 cm^{-1} due to aromatic ring. The ^1H NMR spectrum of **5c** in $CDCl_3$ showed signals at 7.29–7.96 (m, 16H, H_{ar}), 8.38 (s, 1H, $H_{pyrazole}$).

The condensation reaction of 1,3-diphenylpyrazole-4-carboxaldehyde **4** with active methylene compounds namely: malononitrile, ethyl cyanoacetate, and cyanoacetic acid hydrazide afforded the expected compounds **6–8** respectively as shown in Scheme 3. While treatment of **4** with hydrazines namely: methyl hydrazine, phenyl hydrazine, p-bromophenylhydrazines, p-nitrophenyl hydrazine, o,p-dinitrophenyl hydrazine, 2,4,6-trichlorophenyl hydrazine afforded the expected hydrazones **9,10a-e** respectively. Also, the condensation reaction of 1,3-diphenylpyrazol-4-carboxaldehyde **4** with aniline derivatives namely; aniline, p-methylaniline, p-nitroaniline, p-bromoaniline, m-methoxyaniline, o-phenylenediamine, 4-nitro-m-phenylenediamine, in refluxing methanol containing traces of acetic acid afforded the expected base compounds **11a-g** respectively as shown in Scheme 3.



Scheme 3

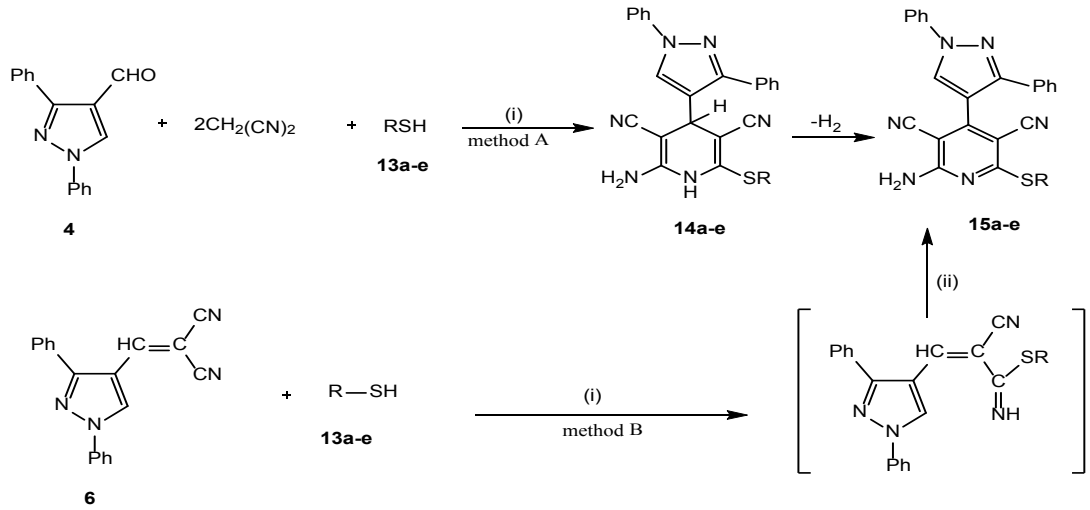
In the ^{1}H NMR spectra of these Schiff bases, the pyrazole H-5 and the azomethine proton resonate as singlets at 8.56 and 8.69 respectively. The structure of compounds **6, 11a-g** were confirmed by elemental analysis and spectral data. The IR spectrum of 2-(l,3-diphenyl-1H-pyrazol-4-yl)methylene) malononitrile **6** showed absorption bands at 3068 cm^{-1} due to CH aromatic, 2200 cm^{-1} due to cyano group and 1590, 1536, 1512 cm^{-1}due to aromatic rings. The ^{1}H NMR spectrum of **6** in $CDCl_3$ showed signals at δ 7.46–7.87 (m, 11H, H_{ar}) and 9.08 (s, 1H, -CH=) ppm. The ^{1}HNMR spectrum of **7** in **$CDCl_3$** showed signals at 1.40 (t, J=7.1, 3H, CH_3), 4.36 (q, J=7.1, 2H,OCH_3), 7.43–7.88 (m, 10H, H_{ar}), 8.33 (s, 1H, $H_{pyrazole}$) 9.17 (s, 1 H, -HC=)ppm. The IR spectrum of (E)-2-cyano-N'(l,3-diphenyl-1H-pyrazol-4-yl methylene) acetohydrazide **8** showed absorption bands at 3225 cm^{-1} due to NH group, 3062 cm^{-1} due to CH aromatic, 2980 cm^{-1} due to CH aliphatic, 2218 cm^{-1} due to cyano group, 1668 cm^{-1} due to carbonyl group and 1600, 1586, 1512,1486 cm^{-1} due to aromatic rings. The IR spectrum of **11b** showed absorption bands at 3060 cm^{-1} due to CH aromatic, 2913 cm^{-1} due to CH aliphatic, 1622 due to C=N group and 1595, 1542, 1503, 1450 cm^{-1} due to aromatic rings. The ^{1}H NMR spectrum of 11b in $CDCl_3$ showed signals at 2.39 (s, 3H, CH_3), 7.11–7.89 (m, 14H, H_{ar}), 8.56 (s, 1H, $H_{pyrazole}$) and 8.69 (s, 1H, HC=N-) ppm.It has been reported [38] that the condensation of benzylidenemlononitrile with benzenethiol and malonoitrile was carried out in ethanol containing triethylamine at reflux temperature afforded 2-amino-4-phenyl-3,5-dicyano-6-thoiphenylpyridine **12** [39].



12

Scheme 4

A series of pentasubstituted pyridine derivatives **15a-e** has been synthesized by one-pot three component cyclocondensation reaction of 1,3-diphenylpyrazole-4-carboxaldehyde **4**, malonoitrile and thiol **13a-e** in the presence of triethylamine as catalyst (method A). The previous mixture in refluxing ethanol gives moderate to good yield 35–69% in a short experimental time (Scheme 5). On the other hand, 2-(1,3-diphenyl-1Hpyrazol-4-yl)-methylene)malononitrile **6**, malononitrile and thiol 13a-e was carried out in ethanol containing triethylamine to yield **15a-e** (method B) as shown in Scheme 5.



4 **14a-e** **15a-e**

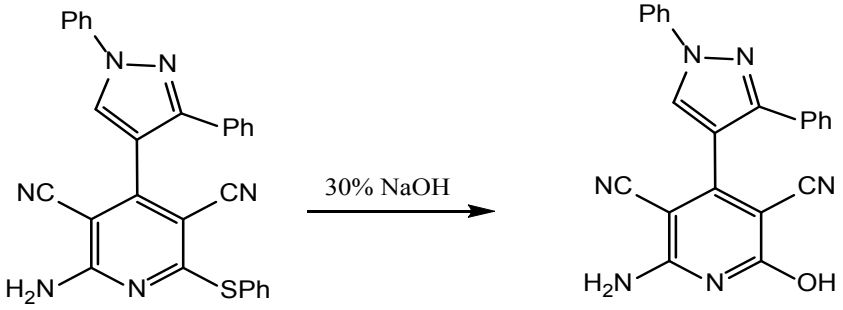
6

Reagents and conditions:
(i) Et_3N, EtOH, reflux 90 min
(ii) $CH_2(CN)_2$, **15a**, R=Ph, **b**, R= o-(NH_2)Ph, **c**, R= p-(NH_2)Ph, **d**, R=CH_2CH_3 **e**, R=CH_2CH_2OH

Scheme 5.

The structures of all the new synthesized compounds were established by ^{1}HNMRand elemental analysis. The IR spectra of **15a** showed absorption bands at 3432, 3367 and 3311 cm^{-1} for amino group, 3052 cm^{-1} due to CH aromatic, 2218 cm^{-1} due to cyano group and 1626 cm^{-1} due to C=N bonds. The ^{1}H NMR spectrum of 2-amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-(phenyl thio)pyridine-3,5-dicarbonitrile **15a** in **$CDCl_3$** exhibited bands at δ 5.41 (br, 2H, NH_2), 7.41–7.84 (m, 15H, H_{ar}), 8.28 (s, 1H, $H_{pyrazole}$) ppm. The IR spectrum of 2-amino-6-(2-amino-phenylthio)-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarbonitrle **15b** showed absorption bands at 3400–3300 cm^{-1} (broad) due to two amino groups, 3059 cm^{-1} due to CH aromatic 2223 cm^{-1} due to cyano group, 1627 cm^{-1} due to C=N group, and 1596, 1530, 1503, 1479 cm^{-1} due to aromatic rings. The ^{1}H NMR spectrum of compound **15b** in DMSO-d_6 exhibited bands at 3.43–7.60 (m, 13H, H_{ar}), 7.70–7.87 (m, 2H, H_{ar}), 7.87 (d, J=8.3,3H, H_{ar} + NH_2), 8.06 (d, 1H, J= 8.2, 1H, H_{ar}), 8.21 (s, 1H, $H_{pyrazole}$), 9.17 (s, 1H, NH_2) ppm. The ^{1}H NMR spectrum of 2-amino-6-(4- aminophenylthio)-4-(1,3-diphenyl-1H-pyrazol-4-yl)pyridine-3,5-dicarbonitrile **15c** in DMSO-d_6 showed bands at δ5.59 (s, 2H, NH_2), 6.62 (d, J= 8.7Hz, 2H, H_{ar}), 7.18 (d, J=8.7Hz, 2H, H_{ar}), 7.74 (br, 2H, NH_2), 7.95 (d, J= 7.8Hz, 2H, H_{ar}), 7.97 (s, 1H, $H_{pyrazole}$) ppm. The IR spectrum of 15c showed absorption bands at 3470, 3337, 3233 cm^{-1} due to amino groups, 2212 cm^{-1} due to cyano groups and 1626 cm^{-1} due to C=N group. The ^{1}H NMR spectrum of 2-amino-6-ethylthio-4-(l,3-diphenyl-1H-pyrazol-4-yl)pyridine- 3,5-dicarbonitrile **15d** in $CDCl_3$ showed bands at δ 1.41 (t, J= 7.3Hz, 3H, CH) 3.22 (q, J=7.3 Hz, 2H,CH_2), 5.60 (s, 2H, NH_2), 7.40 (s, 4H, H_{ar}), 7.52 (s, 4H, H_{ar}), 7.81 (d, J=7.8Hz,2H, H_{ar}), 8.25 (s, 1H, $H_{pyrazole}$) ppm. The IR spectrum of **15d** showed absorption bands at 3446. 3361, 3176 cm^{-1}due to NH_2 group, 2214 cm^{-1} due to two cyano groups, 1632 cm^{-1} due to C=N groups.

Hydrolysis of 2-amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)- 6-(phenylthio)pyridine-3,5-dicarbonitrile **15a** with NaOH (30%) under reflux condition for two hours afforded 2-amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6- hydroxypyridine-3,5-dicarbonitrile **16**.



15a **16**

Scheme 6.

The structure of compound **16** was confirmed by elemental analysis and spectral data. The IR spectrum of compound **16** showed absorption bands at 3489, 3440, 3367, 3168 cm^{-1} due to NH_2 and OH groups, 2222 cm^{-1} due to cyano groups and 1636 cm^{-1} due to C=N group.

Synthesis of 3,6-diamino-4-(1,3-diphenyl pyrazol-4-yl)pyrazolo[3,4-b]pyridine-5-carbonitrile **17** in good yield was reported via the fusion of 2-amino-4-(1,3-diphenyl-1H-pyrazolo-4-yl)-6-(phenylthio)pyridine-3,5-dicarbonitrile **15a** or 2-amino-6-(2-amino phenylthio)-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarbonitrile **15b** with hydrazine hydrate. On the other hand, reaction of 2-amino-6-chloro-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyridine-3,5-dicarbontrile **18** with hydrazine hydrate in n-butanol at reflux temperature gave the same compound **17**. Similarly, treatment of compounds **15a** or **15b** with methyl hydrazine gave 3,6-diamino-l-methyl-4-(1,3-diphenyl-1H-pyrazol-4-yl) pyrazolo[3,4-b]pyridine-5-carbontirle **19** as shown in Scheme 7.

The chemical structure of compounds **17** and **19** was confirmed by elemental analysis and spectral data. The IR spectrum of **17** showed absorption bands at 3468, 3382, 3362, 3185 cm^{-1} characteristic for NH and NH_2 groups, 2220cm^{-1} due to cyano group,1632 cm^{-1} due to C=N group, 1598, 1508, 1492 cm^{-1} due to aromatic rings. The ^{1}H NMR spectrum of **17** in DMSO-d6 showed signals at δ. 4.36 (s, 2H, $NH_{2pyrazole}$), 6.83 (s,2H, NH_2pyridine), 7.48–7.60 (m, 8H, H_{ar}), 7.81 (d, J= 8.4Hz, 2H, H_{ar}), 8.56 (s, 1H, $H_{pyrazole}$) ppm.

The reaction of N-((1,3-diphenyl-1H-pyrazol-4- yl) methylene-aniline derivatives **11a-e** with thioglycolic acid in non-polar solvent at reflux temperature gave 2-(1,3-diphenyl-1H-pyrazol-4-yl)-3-(aryl)-thiozolidine-4-one **20a–e** in low yield 20–38%.

11a-e
a, X = H
b, X = CH_3
c, X = NO_2
d, X = Br
e, X = OMe

20a-e
a, X = H
b, X = CH_3
c, X = NO_2
d, X = Br
e, X = OMe

Scheme 8.

Structure of all the synthesized compounds, were established by element analysis and spectral data. The IR spectra of **20d** showed absorption bands at 3050 cm^{-1} due to CH aromatic, 2921 cm^{-1} due to CH aliphatic, 1710 cm^{-1} due to carbonyl group, 1651 cm^{-1} due to C=N group, 1617, 1594, 1537, 1484 cm^{-1} due to aromatic rings. The ^{1}HNMR of 2-(1,3-diphenyl-1H-pyrazol-4-yl)-3-phenylthiazolidin-4-one **20a** in **$CDCl_3$** exhibited signals at δ 53.91 (d, J=1.3Hz, 2H, CH_2,) 6.38 (s, 1H, CH) 7.07–7.70 (m, 15H, H_{ar}), 8.02 (s, 1H, $H_{pyrazole}$).The ^{1}HNMR spectrum of 2-(1,3-diphenyl-1H-pyrazol-4-yl)-3-p-tolylthiazolidin-4-one **20b** in $CDCl_3$ exhibited signals at δ 2.33 (s, 3H, CH_3), 3.6 (d, J=1.3 Hz, 2H, CH_2), 6.40 (s, 1H,CH), 6.90–7.83 (m, 14H, H_{ar}), 8.10 (s, 1H, $H_{pyrazole}$) ppm. The mass spectrum of 2-(1,3-diphenyl-1H -pyrazol-4-yl)-3-(4-nitrophenyl)thiazolidin-4-one 20c showed molecular ion peak at m/e 444 [M^++2, 24%],443 [M^++1,37%], 442 [M^+, 37%] and base peak at 61 [100%]. The ^{1}H NMR spectrum of 3-(4-bromophenyl)-2-(1,3-diphenyl-1H-pyrazol-4-yl)-thiazolidin-4-one **20d** in **$CDCl_3$** showed signals at δ 3.88 (d, J=1.3Hz, 2H, CH_2), 6.41 (s, 1H, CH), 7.10–7.70 (m, 14H, H_{ar}), 8.08 (s, 1H, $H_{pyrazole}$) ppm.

Antitumor activity

Some of the new prepared compounds were screened for antitumor activity. Using MTT assay, the effect of each compound on the proliferation of MCF-7 and Hep-G2 cells were studied after 48 h of incubation. As shown in the figures, the treatment of MCF-7 cells as well as Hep-G2 with some drugs does not show any valuable cytotoxic effect against MCF-7 or HepG2 as shown in Fig. 1 and 2 respectively concluded from their IC_{so} values >1000 µg/mL while the treatment with the other drugs showed increase in the proliferation of the cells.

TABLE 1. Drugs that show some sort of cytotoxicity

S/N	Drug	Calculated IC_{50} µg/mL
1	**5b**	638.655
2	**10c**	452.5519
3	**10d**	4426.147
4	**15a**	1464.463
5	**15b**	8025.00
6	**20a**	926.7225

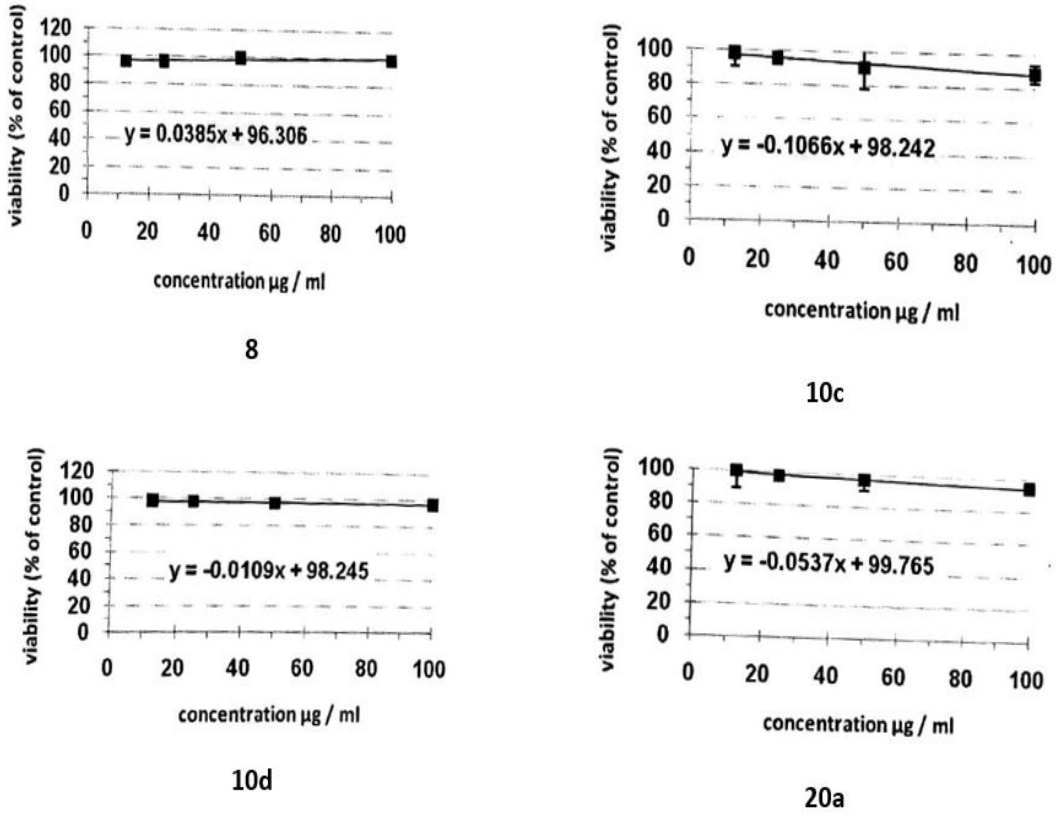


Fig. 1. Cytotoxic effect of the samples against MCF-7cells using MTT assay (n=4), data expressed as mean value of cell viability (% of control) ±S.D.

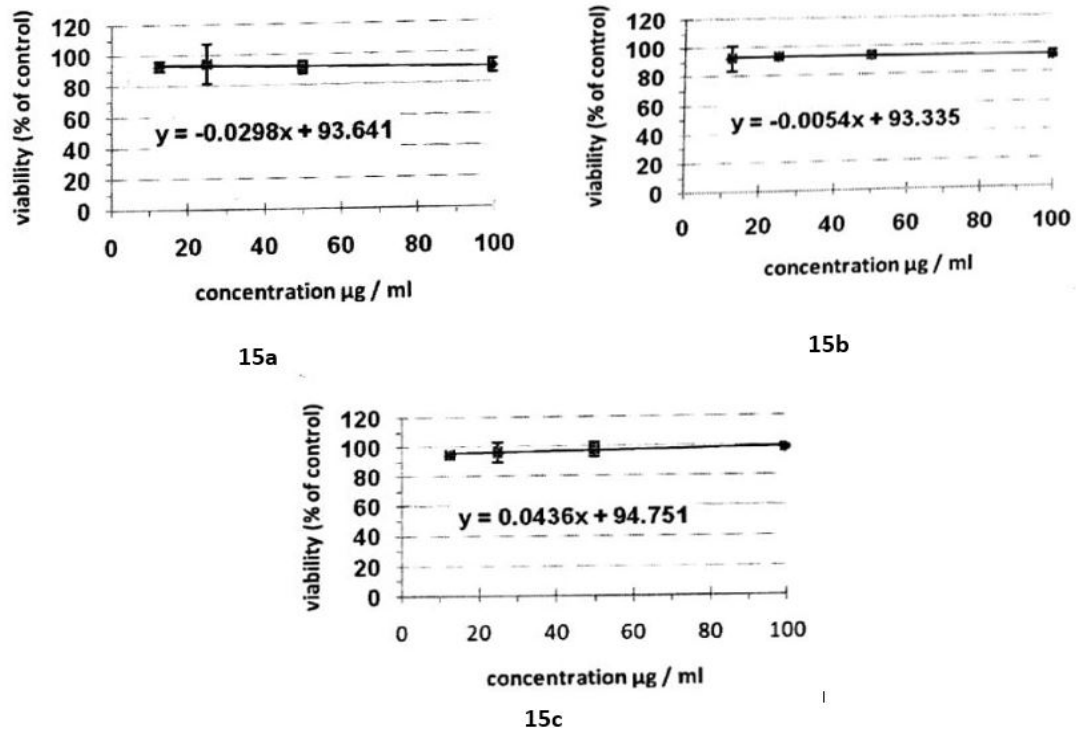


Fig. 2. Cytotoxic effect of the samples against Hep-G2 cells using MTT assay (n=4), data expressed as mean value of cell viability (% of control) ±S.D.

References

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تحضير وتفاعلات وتقييم النشاط المضاد للأورام السرطانية لبعض مشتقات ١ و٣- ثنائي فينيل بيرازول - ٤ - كاربوكس ألديهيد الجديدة

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تأكيداً على اهتمامنا المستمر بتحضير مركبات حلقية غير متجانسة ذات النشاط البيولوجي المتوقع، تم في هذه الدراسة تحضير بعض مشتقات البيرازول الجديدة المحتوية على نواة البيريدين من خلال تفاعل «فيلزماير- هاك» والذي يُعد إحدى الطرق الشائعة لتحضير مشتقات -4 فورميل بيرازول. تم إجراء مسح بيولوجي لبعض المركبات الجديدة كمضادات للأورام السرطانية، وأظهرت بعض المركبات نشاطاً ملحوظاً مقارنةً بالأدوية المرجعية. تم إثبات التركيب الكيميائي لجميع المركبات الجديدة عن طريق التحليل العنصري للكربون والهيدروجين والنيتروجين وطيف الأشعة تحت الحمراء وكذلك الرنين النووي المغناطيسي لنواة ذرة الهيدروجين إضافةً إلى تحليل طيف الكتلة لجميع المركبات.