Synthesis and Antitumor Studies of Acylhydrazone and Hydrazone Derivatives Bearing 3-(1*H*-indol-3-yl)-1*H*-pyrazole Scaffold

DATONG ZHANG[1*], RONGRONG XU[1], SHOUDONG GUO[2]

[1] Shandong Provincial Key Laboratory of Fine Chemicals, School of Chemistry and Pharmaceutical Engineering, Qilu University of Technology, Jinan, Shandong 250353, PR China

[2] Key Laboratory of Atherosclerosis in Universities of Shandong Province, Institute of Atherosclerosis, Taishan Medical University, Taian 271000, PR China.

In our efforts to develop effective treatment agents for cancer, a series of acylhydrazone and hydrazone derivatives containing 3-(1H-indol-3-yl)-1H-pyrazole scaffold were synthesized, and their effects on the growth of A549, Ho-8910, KG-1 and HepG-2 cells were investigated. The results showed that most of the fifteen compounds had weak to moderate inhibitory effects against 4 tumor cell lines. The acylhydrazone derivative ***2e*** *with 4-F-Bn at position-1 of indole displayed the best activity against HepG-2 cells with* IC_{50} *of 10.97 μM, which was comparable to that of the reference drug 5-Fu. The hydrazone derivative* ***6c*** *showed the highest inhibitory activity against A549 and KG-1.*

Keywords: 3-(1H-indol-3-yl)-1H-pyrazole; acylhydrazone; hydrazone; synthesis; antitumor

One of the most dreaded diseases of mankind and the principal cause of mortality worldwide is cancer [1-3]. In the past several decades, the search for new anticancer agents continues to draw attention to the research community. Cancer is characterized by uncontrolled cellular growth and proliferation. Thus, inhibition of proliferative pathways is considered to be an effective strategy to treat cancer. Many pyrazole derivatives are known to exhibit a broad spectrum of biological properties such as anti-inflammatory [4], antibacterial [5, 6], antitumor [7-10] and anti-proliferative [11, 12] activity. Indole derivatives constitute an important class of therapeutic agents in medicinal chemistry including anticancer [13], antifungal [14], antioxidant [15] and antibacterial [16]. In our previous work, we reported the synthesis and antitumor activity study of a series of novel compounds bearing 3-(1*H*-indol-3-yl)-1*H*-pyrazole scaffold [17] and demonstrated that some of the analogs were potent and selective against different cancer cell lines. Recently metal ion chelation therapy has attracted much attention [18-20]. Ligands with metal ions, acylhydrazone and hydrazone moieties possess a variety of excellent coordination properties [21, 22]. Some compounds exhibited a wild range of valuable biological effects [23-26], especially as multidentate ligands to prevent proliferation of cancer cells. In view of these previous observations, combination of the 3-(1*H*-indol-3-yl)-1*H*-pyrazole scaffold with the acylhydrazone and hydrazone functionalities may enhance these activities. In continuation of our interest in the development of small molecules targeting cancer cells, we reported herein the synthesis and *in vitro* cytotoxic activity of some novel compounds against 4 tumor cell lines, A549, Ho-8910, KG-1 and HepG-2 (fig.1).

Experimental part

Materials and methods

All reagents were used as purchased from commercial suppliers without further purification unless otherwise noted. Thin layer chromatography (TLC) was performed on silica gel F_{254} plates with visualization by UV or iodine vapour. Melting points were determined by a WRS-1B digital melting-point apparatus and were uncorrected. ^{1}H NMR spectra were measured on Bruker AVANCE II 400 spectrometers with $CDCl_3$ or DMSO-d_6 as solvents and TMS as an internal standard. The high resolution mass spectra were obtained with an Agilent 6510 Q-TOF spectrometer. The IR spectra were recorded on a Bruker Vector FT-IR spectrophotometer as KBr pellets or thin films. Elemental analyses were performed on Vario EL III elemental analyzer and were within ± 0.4 % of the theoretical values.

General procedure for the synthesis of compound ***2a-2k***.

Compound **1a** (105 mg, 0.282 mmol) and 2,4-dichlorobenzaldehyde (54 mg, 0.309 mmol) were mixed in THF (50 mL), and the reaction mixture was refluxed for 5 h. Then the reaction mixture was cooled to ambient temperature and concentrated *in vacuo* to give the crude product which was purified by silica gel column chromatography to give compound **2a**. Compounds **2b-2k** were prepared analogously to compound **2a**.

*Benzyl-N'-(2,4-dichlorobenzylidene)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**2a**)*

Yellow solid, yield: 73%, m.p. 179-180°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84 (t, J = 7.2 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76~1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.18 (t, J = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.81 (s, 2H, $\mathbf{CH_2}$Ph), 7.12~7.34 (m, 7H, **ArH**),

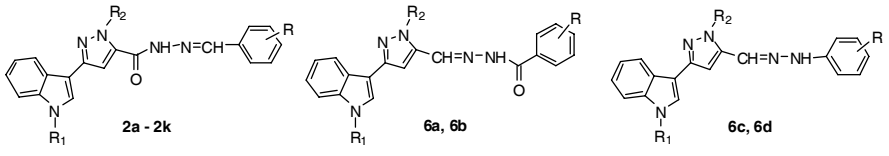


Fig. 1. Target compounds in present work

* email: dtzhang@qlu.edu.cn; Tel.: +86 531 89631208

7.39 (s, 1H, **ArH**), 7.53 (d, *J* = 8.0 Hz, 2H, **ArH**), 7.73 (d, *J* = 8.0 Hz, 1H, **ArH**), 7.81 (s, 1H, **ArH**), 8.01 (d, 2H, **ArH**), 8.12 (d, *J* = 7.8 Hz,1H, **ArH**), 8.77 (s, 1H, **N=CH**), 12.20 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3180, 3034, 2960, 2872, 1643, 1583, 1346, 806, 732. HRMS (ESI) calcd. for [M+H]$^+$ $C_{29}H_{25}Cl_2N_5O$: 530.1515, found: 530.1500. Anal. calcd. for $C_{29}H_{25}Cl_2N_5O$(530.44): C, 65.66; H, 4.75; N, 13.20%; found: C, 65.52; H, 4.86; N, 13.44%.

*N'-(2,4-dichlorobenzylidene)-1-(4-fluorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbo-hydrazide(**2b**)*

Yellow solid, yield: 74%, m.p. 208-209°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.73 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76~1.81 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.17 (t, *J* = 7.2 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.78 (s, 2H, $\mathbf{CH_2}$Ph), 7.12~7.22 (m, 4H, **ArH**), 7.33 (t, *J* = 7.2 Hz, 2H, **ArH**), 7.40 (s, 1H, **ArH**), 7.53 (d, *J* = 8.0 Hz, 2H, **ArH**), 7.73 (d, *J* = 8.0 Hz, 1H, **ArH**), 7.80 (s, 1H, **ArH**), 8.02 (d, 1H, **ArH**), 8.13 (d, *J* =8.0 Hz , 1H, **ArH**), 8.78 (s, 1H, N=**CH**), 12.21 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3057, 3035, 2960, 2870, 1641, 1583, 1346, 806, 734. HRMS (ESI) calcd. for [M+H]$^+$ $C_{29}H_{24}Cl_2FN_5O$: 548.1420, found: 548.1434. Anal. calcd. for $C_{29}H_{24}Cl_2FN_5O$ (548.43): C, 63.51; H, 4.41; N, 12.77%; found: C, 63.44; H, 4.51; N, 12.95%.

*N'-(2,4-dichlorobenzylidene)-1-(4-methoxybenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-car-bohydrazide (**2c**)*

Yellow solid, yield: 96%, m.p. 208-209°C. ^{1}H NMR (400 MHz, DMSO-d6) δ: 0.86 (t, *J* = 7.4 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76~1.81 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 3.69 (s, 3H, $\mathbf{OCH_3}$), 4.18 (t, *J* = 7.0 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.72 (s, 2H, $\mathbf{CH_2}$Ph), 6.87 (d, *J* = 8.4 Hz, 2H, **ArH**), 7.12~7.26 (m, 4H, **ArH**), 7.36 (s, 1H, **ArH**), 7.52 (d, *J* = 7.6 Hz, 1H, **ArH**), 7.66 (s, 1H, **ArH**), 7.70 (s, 1H, **ArH**), 8.02 (d, *J* = 8.4 Hz, 1H, **ArH**), 8.13 (d, *J* = 7.6 Hz, 1H, **ArH**), 8.77 (s, 1H, **N=CH**), 12.17 (s, 1H, **CONH**). IR (KBr, ν/cm^{-1}): 3228, 2958, 2872, 1658, 1583, 1251, 742. HRMS (ESI) calcd for [M+H]$^+$ $C_{30}H_{27}Cl_2N_5O$: 560.1620, found: 560.1625. Anal. calcd. for $C_{30}H_{27}Cl_2N_5O_2$ (560.47) C, 64.29; H, 4.86; N, 12.50%; found: C, 64.37; H, 4.98; N, 12.34%.

*N'-(2,4-dichlorobenzylidene)-1-propyl-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**2d**)*

Yellow solid, yield: 73%, m.p. 169-171°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.83~0.90 (m, 6H, $CH_2CH_2\mathbf{CH_3}$), 1.76~1.85 (m, 4H, $CH_2\mathbf{CH_2}CH_3$), 4.18 (t, *J* = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.51 (t, *J* = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 7.12~7.19 (m, 2H, **ArH**), 7.30 (s, 1H, **ArH**), 7.53 (t, *J* = 8.0 Hz, 2H, **ArH**), 7.76~7.78 (m, 2H, **ArH**), 8.02 (d, *J* = 8.4 Hz, 1H, **ArH**), 8.12 (d, *J* = 7.6 Hz, 1H, **ArH**), 8.78 (s, 1H, N=**CH**), 12.16 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3165, 3026, 2962, 2872, 1653, 1585, 1469, 740. HRMS (ESI) calcd. for [M+H]$^+$ $C_{25}H_{25}Cl_2N_5O$: 482.1515, found: 482.1508. Anal. calcd. for $C_{25}H_{25}Cl_2N_5O$ (482.40) C, 62.24; H, 5.22; N, 14.52%; found: C, 62.33; H, 5.26; N, 14.35%.

*N'-(2,4-dichlorobenzylidene)-3-[1-(4-fluorobenzyl)-1H-indol-3-yl]-1-propl-1H-pyrazole-5-carbo-hydrazide (**2e**)*

Yellow solid, yield: 63%, m.p. 204-205°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (t, *J* = 7.4 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.82~1.97 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.51 (t, *J* = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.46 (s, 2H, $\mathbf{CH_2}$Ph), 7.13~7.24 (m, 4H, **ArH**), 7.32 (t, *J* = 8.0 Hz, 3H, **ArH**), 7.45~7.55 (m, 2H, **ArH**), 7.73 (s, 1H, **ArH**), 7.94 (d, *J* = 7.6 Hz, 1H, **ArH**), 8.01 (s, 1H, **ArH**), 8.14 (d, *J* = 7.8 Hz, 1H, **ArH**), 8.78 (s, 1H, N=**CH**), 12.16 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3169, 3062, 2966, 2873, 1653, 1585, 1469, 748. HRMS (ESI) calcd. for [M+H]$^+$ $C_{29}H_{24}Cl_2FN_5O$: 548.1420, found: 548.1408. Anal. calcd. for $C_{29}H_{24}Cl_2FN_5O$ (548.44) C, 63.51; H, 4.41; N, 12.77%; found: C, 63.67; H, 4.51; N, 12.52%.

*3-(1-Benzyl-1H-indol-3-yl)-N'-(2,4-dichlorobenzylidene)-1-propyl-1H-pyrazole-5-carbohydrazide (**2f**)*

Yellow solid, yield: 81%, m.p. 207-208°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (t, *J* = 7.4 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.80~1.89 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.52 (t, *J* = 6.2 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.47 (s, 2H, $\mathbf{CH_2}$Ph), 7.12~7.32 (m, 8H, **ArH**), 7.46~7.55 (m, 2H, **ArH**), 7.74 (s, 1H, **ArH**), 7.85~7.95 (m, 1H, **ArH**), 7.97~8.03 (m, 1H, **ArH**), 8.13~8.15 (s, 1H, **ArH**), 8.78 (s, 1H, N=**CH**), 12.18 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3433, 3170, 2964, 1654, 1587, 1552, 1469, 1386, 1263, 744. HRMS (ESI) calcd. for [M+H]$^+$ $C_{29}H_{25}Cl_2N_5O$: 530.1515, found: 530.1510. Anal. calcd. for $C_{29}H_{25}Cl_2N_5O$ (530.45) C, 65.66; H, 4.75; N, 13.20%; found: C, 65.54; H, 4.83; N, 13.37%.

*N'-(2,4-dichlorobenzylidene)-3-[1-(4-methoxybenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-5-car-bohydrazide (**2g**)*

Yellow solid, yield: 63%, m.p. 204-205°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (t, *J* = 7.4 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.83~1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 3.69 (s, 3H, $\mathbf{OCH_3}$), 4.51 (t, *J* = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.37 (s, 2H, $\mathbf{CH_2}$Ph), 6.87 (d, 2H, **ArH**), 7.15 (t, *J* = 8.0 Hz, 5H, **ArH**), 7.25 (s, 2H, **ArH**), 7.54 (d, *J* = 7.8 Hz, 1H, **ArH**), 7.73 (s, 1H, **ArH**), 7.86 (s, 1H, **ArH**), 8.11 (s, 1H, **ArH**), 8.77 (s, 1H, N=**CH**), 12.15 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3184, 2964, 2933, 1654, 1583, 1510, 1450, 1342, 1247, 1168, 810, 732. HRMS (ESI) calcd. for [M+H]$^+$ $C_{30}H_{27}Cl_2N_5O_2$: 560.1620, found: 560.1614. Anal. calcd. for $C_{30}H_{27}Cl_2N_5O_2$ (560.47) C, 64.29; H, 4.86; N, 12.50%; found: C, 64.18; H, 4.94; N, 12.62%.

*N'-benzylidene-1-(4-fluorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**2h**)*

White solid, yield: 36%, m.p. 194-196°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.86 (t, *J* = 7.6 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76~1.83 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.19 (t, *J* = 8.0 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.78 (s, 2H, $\mathbf{CH_2}$Ph), 7.12~7.15 (m, 4H, **ArH**), 7.32~7.35 (m, 3H, **ArH**), 7.46~7.54 (m, 4H, **ArH**), 7.65~7.80 (m, 3H, **ArH**), 8.13 (d, *J* = 7.8 Hz, 1H, **ArH**), 8.43 (s, 1H, N=**CH**), 11.95 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3178, 3035, 2960, 1660, 1560, 1448, 1265, 1110, 740. HRMS (ESI) calcd. for [M+H]$^+$ $C_{29}H_{26}FN_5O$: 480.2200, found: 480.2196. Anal. calcd. for $C_{29}H_{26}FN_5O$ (479.55) C, 72.63; H, 5.46; N, 14.60%; found: C, 72.51; H, 5.58; N, 14.77%.

*1-(4-Fluorobenzyl)-N'-(4-methoxybenzylidene)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbo-hydrazide (**2i**)*

White solid, yield: 70%, m.p. 228-229°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (t, *J* = 7.6 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.77~1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 3.81 (s, 3H, $\mathbf{OCH_3}$), 4.19 (t, *J* = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.77 (s, 2H, $\mathbf{CH_2}$Ph), 7.03 (d, 2H, **ArH**), 7.13~7.22 (m, 4H, **ArH**), 7.31~7.35 (m, 3H, **ArH**), 7.52 (d, *J* = 7.8 Hz, 1H, **ArH**), 7.68 (d, 2H, **ArH**), 7.79 (s, 1H, **ArH**), 8.10 (t, *J* = 7.8 Hz, 1H, **ArH**), 8.36 (s, 1H, N=**CH**), 11.82 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3180, 3010, 2960, 2931, 1653, 1602, 1510, 1450, 1257, 744. HRMS (ESI) calcd. for [M+H]$^+$ $C_{30}H_{28}FN_5O_2$: 510.2305, found: 510.2321. Anal. calcd. for $C_{30}H_{28}FN_5O_2$ (509.57) C, 70.71; H, 5.54; N, 13.74%; found: C, 70.89; H, 5.68; N, 13.54%.

*1-(4-Fluorobenzyl)-N'-(4-fluorobenzylidene)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohy-drazide (**2j**)*

White solid, yield: 71%, m.p. 186-188°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.77~1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4. 19 (t, *J* = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 5.77 (s, 2H, $\mathbf{CH_2}$Ph), 7. 18 (d, 4H, **ArH**),

7.29~7.36 (m, 5H, **ArH**), 7.53 (d, 1H, **ArH**), 7.65~7.80 (m, 3H, **ArH**), 8.12 (d, 1H, **ArH**), 8.42 (s, 1H, N=**CH**), 11.96 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3186, 3043, 2958, 1662, 1508, 1450, 1232, 738. HRMS (ESI) calcd. for $[M+H]^+$ $C_{29}H_{25}F_2N_5O$: 498.2106, found: 498.2090. Anal. calcd. for $C_{29}H_{25}F_2N_5O$ (497.54) C, 70.01; H, 5.06; N, 14.08%; found: C, 69.82; H, 5.19; N, 14.18%.

*N'-(4-Chlorobenzylidene)-1-(4-fluorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohy-drazide (**2k**)*

White solid, yield: 84%, m.p. 224-225°C. ^{1}H NMR (400MHz, DMSO-d_6)δ: 0.84 (t, J = 7.6 Hz, 3H, CH_2CH_2**CH_3**),1.76~1.85 (m, 2H, CH_2**CH_2**CH_3), 4.19 (t, J =7.6 Hz, 2H, **CH_2**CH_2CH_3), 5.77 (s, 2H, **CH_2**Ph), 7.13~7.22 (m, 4H, **ArH**), 7.34 (q, 3H, **ArH**), 7.53 (q, 3H, **ArH**), 7.75 (q, 3H, **ArH**), 8.12 (d, 1H, **ArH**), 8.41 (s, 1H, N=**CH**), 12.01 (s, 1H, CO**NH**). IR (KBr, ν/cm^{-1}): 3174, 3030, 2962, 1658, 1562, 1450, 1265, 1226, 1116, 740. HRMS (ESI) calcd. for $[M+H]^+$ $C_{29}H_{25}ClFN_5O$: 514.1810, found: 514.1796. Anal. calcd. for $C_{29}H_{25}ClFN_5O$ (513.99) C, 67.77; H, 4.90; N, 13.63%; found: C, 67.89; H, 4.98; N, 13.46%.

*General procedure for the synthesis of compounds **6a-6d**.*

To a solution of Lithium Aluminum Hydride (405 mg, 10.67 mmol) in THF (50 mL), compound **3** (1.08 g, 2.67 mmol) was added slowly. The mixture was stirred at room temperature for 3 h. The filtrate was poured into water (50 mL) and extracted with EtOAc (3× 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product which was purified by silica gel with petroleum ether/ethyl acetate (1:1) to afford compound **4.**

Manganese dioxide (3.1 g, 35.50 mmol) was added to a solution of compound **4** (860 mg, 2.367 mmol) in dry CH_2Cl_2 (25 mL), and the reaction mixture was refluxed for 10 h. Then the reaction mixture was cooled to ambient temperature and concentrated *in vacuo* to give the crude product which was purified by silica gel with petroleum ether/ethyl acetate (5:1) to give compound **5**.

To a solution of compound **5** (70 mg, 0.194 mmol) in anhydrous EtOH (5 mL) was added benzoylhydrazine (34 mg, 0.25 mmol), and the reaction mixture was refluxed for 3 h. Then the reaction mixture was cooled to ambient temperature and concentrated *in vacuo* to give the crude product which was purified by silica gel with petroleum ether/ethyl acetate (4:1) to give compound **6a**. Compounds **6b-6d** were prepared analogously to compound **6a**.

*N'-[[1-(4-Fluorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazol-5-yl]methylene]benzohydrazide (**6a**)*

Light yellow solid, yield: 42.23%; m.p. 181.7-182.4°C. ^{1}H NMR (400MHz, DMSO-d_6) δ: 0.86 (t, J =7.2 Hz, 3H, CH_2CH_2**CH_3**), 1.78~1.85 (m, 2H, CH_2**CH_2**CH_3), 4.16 (t, J = 6.8 Hz, 2H, N**CH_2**C_2H_5), 5.75 (s, 2H, N**CH_2**Ph), 7.02 (s, 1H, **ArH**),7.10~7.20 (m, 4H, **ArH**), 7.48~7.63 (m, 6H, **ArH**), 7.87 (s, 1H, **ArH**), 7.93 (d, 2H, **ArH**), 8.18 (d, 1H, **ArH**), 8.52 (s, 1H, Ar**CH**=N), 11.96 (s, 1H, CO-**NH**). IR (KBr, ν/cm^{-1}): 3221, 3201, 3061, 2964, 2926, 2860, 1654, 1510, 1282, 1222, 736. HRMS (ESI) calcd. for $[M+H]^+$ $C_{29}H_{26}FN_5O$: 480.2200, found: 480.2199. Anal. calcd. for $C_{29}H_{26}FN_5O$ (479.55) C, 72.63; H, 5.46; N, 14.60%; found: C, 72.78; H, 5.60; N, 14.41%.

*4-Chloro-N'-[[1-(4-fluorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazol-5-yl]methylene]benzohydrazide (**6b**)*

Light yellow solid, yield: 76.06%; m.p. 195.5-196.1°C. ^{1}H NMR (400MHz, DMSO-d_6) δ: 0.87 (t, J = 7.2 Hz, 3H, CH_2CH_2**CH_3**), 1.76~1.85 (m, 2H, CH_2**CH_2**CH_3), 4.16 (t, J = 6.8 Hz, 2H, N**CH_2**C_2H_5), 5.75 (s, 2H, N**CH_2**Ph), 7.03(s,1H, **ArH**), 7.06~7.20 (m, 4H, **ArH**), 7.43~7.52 (m, 3H, **ArH**), 7.64 (t, J = 7.2 Hz, 2H, **ArH**), 7.87 (s, 1H, **ArH**), 7.96 (d, 2H, **ArH**), 8.18 (d, 1H, **ArH**), 8.51 (s, 1H, Ar**CH**=N), 12.02 (s, 1H, CO-**NH**). IR (KBr, ν/cm^{-1}): 3414, 3228, 3043, 2960, 2926, 1645, 1558, 1510, 1303, 742. HRMS (ESI) calcd. for $[M+H]^+$ $C_{29}H_{25}ClFN_5O$: 514.1810, found: 514.1803. Anal. calcd. for $C_{29}H_{25}ClFN_5O$ (513.99) C, 67.77; H, 4.90; N, 13.63%; found: C, 67.65; H, 4.99; N, 13.76%.

*3-[1-(4-Fluorobenzyl)-5-[(2-phenylhydrazono)methyl]-1H-pyrazol-3-yl]-1-propyl-1H-indole (**6c**)*

Light yellow solid, yield: 65%; m.p. 49-52.8vC. ^{1}H NMR (400MHz, DMSO-d_6) δ: 0.86 (t, J = 7.2 Hz, 3H, CH_2CH_2**CH_3**), 1.76~1.85 (m, 2H, CH_2**CH_2**CH_3), 4.16 (t, J = 6.8 Hz, 2H, N**CH_2**CH_2CH_3), 5.71 (s, 2H, N**CH_2**Ph), 6.74 (t, J = 7.2 Hz, 1H, **ArH**), 6.88 (s, 1H, **ArH**), 6.93 (d, 2H, **ArH**), 7.07~7.25 (m, 8H, **ArH**), 7.48 (s, 1H, **ArH**), 7.83 (s, 1H, **ArH**), 7.93 (s, 1H, **ArH**), 8.15 (d, 1H, Ar**CH**=N), 10.44 (s, 1H, Ar**NH**). IR (KBr, ν/cm^{-1}): 3251, 3051, 2962, 2929, 2873, 1687, 1598, 1510, 1220, 1157, 746. HRMS (ESI) calcd. for $[M+H]^+$ $C_{28}H_{26}FN_5$: 452.2251, found:452.2242. Anal. calcd. for $C_{28}H_{26}FN_5$ (451.54) C, 74.48; H, 5.80; N, 15.51%; found: C, 74.61; H, 5.92; N, 15.39%.

*3-[5-[2-(4-Chlorophenyl)hydrazono]methyl-1-(4-fluorobenzyl)-1H-pyrazol-3-yl]-1-propyl-1H-indole (**6d**)*

Light yellow solid, yield: 68.33%; m.p. 71-73°C. ^{1}H NMR (400MHz, DMSO-d_6) δ: 0.85 (t, J = 7.2 Hz, 3H, CH_2CH_2**CH_3**), 1.78~1.83 (m, 2H, CH_2**CH_2**CH_3), 4.16 (t, J = 6.4 Hz, 2H, N**CH_2**C_2H_5),

5.70 (s, 2H, N**CH_2**Ph), 6.85~6.94 (m, 3H, **ArH**), 7.07~7.33 (m, 8H, **ArH**), 7.49 (d, 1H, **ArH**), 7.83 (s, 1H, **ArH**), 7.93 (s, 1H, **ArH**), 8.15 (d, 1H, Ar**CH**=N), 10.57(s, 1H, **ArNH**). IR (KBr, ν/cm^{-1}): 3375, 3292, 3053, 2962, 2929, 2873, 1687, 1598, 1508, 1220, 746. HRMS (ESI) calcd. for $[M+H]^+$ $C_{28}H_{25}ClFN_5$: 486.1861, found:486.1867. Anal. calcd. for $C_{28}H_{25}ClFN_5$ (485.98) C, 69.20; H, 5.19; N, 14.41%; found: C, 69.33; H, 5.31; N, 14.29%.

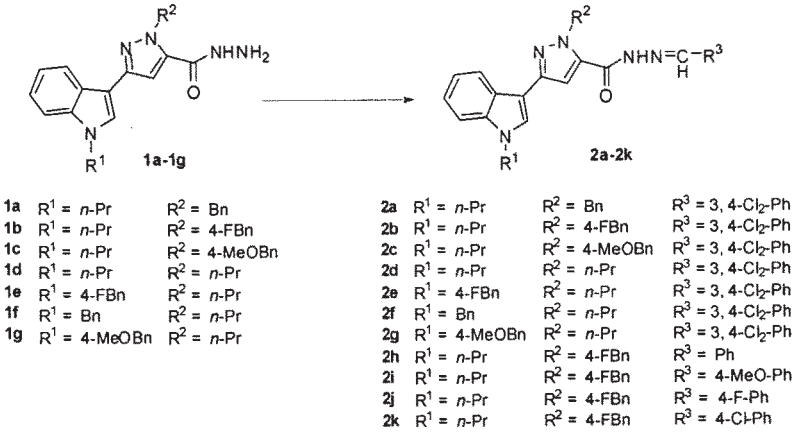
Antitumor activity

The 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was conducted for growth inhibition studies. All the title compounds were dissolved in DMSO and screened for preliminary anticancer activity against four different cell lines: HepG-2 (human hepatocellular carcinoma cell line), A-549 (human lung carcinoma cell line), Ho-8910 (human ovarian carcinoma cell line) and KG-1 (human leukemia cell line). Cells were incubated with the tested compounds at different concentrations for 72 h, and the concentrations that caused 50% of cell growth inhibition (their IC_{50} values) were determined. 5-Fluorouracil (5-Fu) was used as the reference drug.

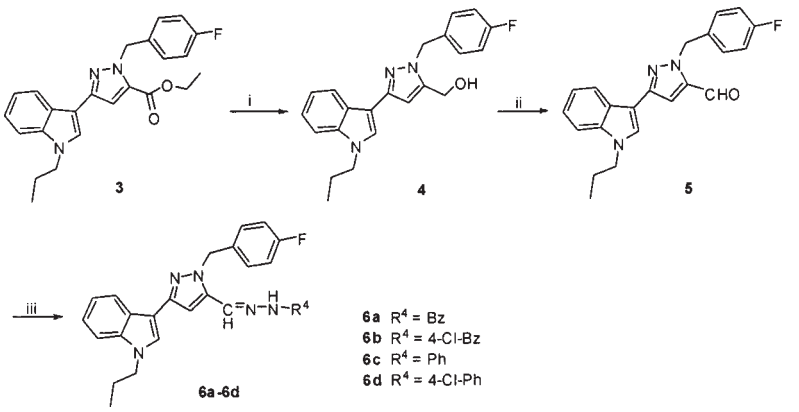
Results and discussions

Chemistry

The synthetic route of the target compounds is illustrated in Scheme 1 and 2. The general method of preparing **1a-1g** and **3** was based on the published literatures [17, 27]. Compounds **2a-2k** (scheme 1) were prepared *via* condensation of **1a-1g** with the corresponding aromatic aldehydes in yields ranging from 36% to 96%. The key intermediate **5** was obtained from **3** via the reduction and oxidation reaction. Compounds **6a-6d** (scheme 2) were finally synthesized by the condensation of **5** with the corresponding phenylhydrazine or benzoylhydrazine in yields ranging from 42% to 76%.



Scheme 1. Synthetic route to compounds **2a-2k**. Reagents and conditions: Aromatic aldehydes, EtOH, reflux



Scheme 2. Synthetic route to compounds **6a-6d**. Reagents and conditions: (i) $LiAlH_4$, THF, rt; (ii) MnO_2, CH_2Cl_2, rt; (iii) Phenylhydrazine or Benzoylhydrazine, EtOH, reflux

In order to investigate the role of the substitutents on the two N atoms of pyrazole and indole rings for cytotoxic activity, various disubstituted compounds were prepared. Various aromatic aldehydes were employed to condense with disubstituted 3-(1*H*-indol-3-yl)-1*H*-pyrazole -5-carbohydrazide to afford eleven acylhydrazone derivatives (**2a-2k**). Two pairs of chemical isomers (**2h/6a** and **2k/6b**) with reversed acylhydrazone were prepared to examine the influences of the acylhydrazone structure. Moreover, two hydrazone derivatives, **6c** and **6d,** were synthesized to evaluate the effects of the hydrazone structure.

The structures of the acylhydrazone and hydrazone derivatives were determined by IR, ^{1}H NMR, HRMS and elemental analysis. All of the compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. Thus, for example **2a**, obtained as yellow solid, gave a $[M+H]^+$ ion peak at *m/z* 530.1500 in the HRMS, which was in good accordance with the molecular formula $C_{29}H_{25}Cl_2N_5O$. The NH band in CONH was observed at 3215 cm^{-1} in IR spectrum. The C-Cl band was observed at 740 cm^{-1}. The ^{1}H NMR spectrum of **2a** indicated the chemical shift of the NH at δ = 12.20 ppm in the form of singlet. The N=CH signal appeared at δ = 8.77 ppm as singlet. The singlet signal appearing at δ = 5.81 ppm was consistent with methylene protons in benzyl group. The *n*-propyl protons had the chemical shifts of 0.84, 1.76~1.85 and 4.18 ppm in the higher field. IR spectrum of **6a** exhibited bands around 3220 cm^{-1} (NH). ^{1}H NMR spectrum of **6a** exhibited the the NH at δ = 11.96 ppm as singlet. The N=CH signal appeared at δ = 8.52 ppm as singlet. ^{1}H NMR spectrum of **6c** showed the characteristic singlet signals at 10.44 ppm and 8.15 ppm assignable to PhNH and N=CH respectively. The NH band was observed at 3251 cm^{-1} in IR spectrum of **6c**.

Antitumor activity

The antitumor activity of the synthesized compounds against 4 human cancer cells was evaluated *via* MTT cell proliferation assay (table 1). It can be seen from table 1 that most of the acylhydrazone and hydrazone derivatives revealed weak to moderate cytotoxic activity against HepG-2, Ho-8910, KG-1 and A-549 cells. Among compounds **2a-2k**, only **2c** showed weak activity against KG-1 with IC_{50} value of 68.74 μM. The hydrazone derivative **6c** exhibited better inhibition against KG-1 (IC_{50} = 40.71 μM). Compound **2a** and its counterpart **2f** had higher IC_{50} values against Ho-8910 than all the other compounds. Altering the substituents on the benzyl group, swapping the position of substituents adjacent to N-1 of pyrazole and N-1 of indole or introducing various aromatic aldehydes in the acylhydrazone moiety did not improve the activities against Ho-8910.

The hydrazone compound **6c** displayed the highest inhibition against A549 (IC_{50} = 46.44 μM). Compound **2k** (IC_{50} = 53.58μM) was more potent against A-549 than other acylhydrazone compounds. Compounds **2b** and **2c**, with 4-F-Bn or 4-MeO-Bn at position-1 of pyrazole displayed higher inhibition than compound **2a** with benzyl group on pyrazole N-1 atom against HepG-2 cells. Swapping the position of substituents adjacent to N-1 of pyrazole and N-1 of indole of **2a-2c** caused significantly increase in the activity against HepG-2. Thus, compound **2e**, the counterpart of **2b**, exhibited high growth inhibition against HepG-2 with the IC_{50} value of 10.97 μM, which was 7-fold more potent than that of **2b** and comparable to that of the reference drug. From the comparison of **2e**, **2f** and **2g**, it can be observed that F atom on the benzyl at the N-1 position of indole ring played an important role in the activity. The acylhydrazone **6b,** the chemical isomer of **2k**, showed moderate activity against HepG-2 (IC_{50} = 43.33 μM).

Table 1
ANTITUMOR ACTIVITIES OF COMPOUNDS **2a-2k** AND **6a-6d** (IC_{50}, μM)

Compd.	IC_{50}[a]			
	HepG-2	Ho-8910	A-549	KG-1
2a	--	62.39 ± 8.11	--	--
2b	76.81 ± 5.76	--	--	--
2c	94.53 ± 5.82	--	128.48 ± 7.46	68.74 ± 8.94
2d	79.63 ± 4.94	--	--	--
2e	**10.97 ± 1.13**	--	93.36 ± 4.42	--
2f	112.71 ± 8.32	75.09 ± 10.44	60.43 ± 3.59	--
2g	48.79 ± 1.39	--	129.32 ± 4.26	--
2h	72.94 ± 3.47	--	74.38 ± 3.59	--
2i	108.46 ± 13.02	--	113.68 ± 6.02	--
2j	--	--	--	--
2k	128.54 ± 9.05	105.68 ± 21.09	53.58 ± 7.39	--
6a	--	--	--	--
6b	43.33±0.45	99.22±0.81	--	97.28±18.69
6c	83.71±0.82	76.18±1.52	46.44±0.38	40.71±0.60
6d	77.72±15.87	144.04±1.75	102.21±8.64	124.49±16.44
5-Fu[b]	10.66 ± 0.98	26.29 ± 1.49	9.83 ± 0.42	32.10 ± 2.14

[a] Each experiment was independently performed three times and expressed as means ± SD. "--" means IC_{50} values > 150 μM.

[b] 5-Fu was used as reference drug.

Conclusions

Acylhydrazone and hydrazone functionalities have been successfully incorporated into position-5 on pyrazole ring of 3-(1*H*-indol-3-yl)-1*H*-pyrazole scaffold and fifteen novel compounds thus obtained were evaluated for their cytotoxic activity against 4 human cancer cell lines. The hydrazone derivative **6c** showed the highest inhibitory activity against A549 and KG-1. The acylhydrazone derivative **2e** (IC_{50} = 10.97 μM) with 4-F-benzyl group at position-1 of indole displayed potent activity against HepG-2 which was comparable to the reference drug.

Acknowledgments: This study was supported by the Self-innovation Project for Universities and Institutes of Jinan City (No. 201202035) and Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province (No. 21376125).

References

1.SEFFRIN, J.R., HILL, D., BURKART, W., MAGRATH, I., BADWE, R.A., NGOMA, T., MOHAR, A., GREY, N., CA Cancer J. Clin. **59**, nr 5, 2009, p. 282
2.SMITH, B.D., SMITH, G.L., HURRIA, A., HORTOBAGYI, G.N., BUCHHOLZ, T.A., J. Clin. Oncol. **27**, nr 17, 2009, p. 2758
3.JEMAL, A., SIEGEL, R., WARD, E., HAO, Y.P., XU, J.Q., THUN, M.J., CA Cancer J. Clin. **59**, nr 4, 2009, p. 225
4.COTTINEAU, B., TOTO, P., MAROT, C., PIPAUD, A., CHENAULT, J., Bioorg. Med. Chem. Lett. **12**, nr 16, 2002, p. 2105
5.LEE, K.Y., KIM, J.M., KIM, J.N., Tetrahedron lett. **44**, nr 35, 2003, p. 6737
6.BARBUCEANU, S., SARAMET, G., BANCESCU, G., DRAGHICI, C., APOSTOL, T., TARAN, L., ELENA DINU-PIRVU, C., Rev. Chim. (Bucharest), **64**, no 4, 2013, p. 355
7.LI, J., ZHAO, Y., ZHAO, X., YUAN, X., GONG, P., Arch. Pharm. Chem. Life Sci. **339**, nr 11, 2006, p. 593
8.XIA, Y., DONG, Z., ZHAO, B., GE, X., MENG, N., SHIN, D., MIAO, J., Bioorg. Med. Chem. **15**, nr 22, 2007, p. 6893
9.XIA, Y., FAN, C., ZHAO, B., ZHAO, J., SHIN, D., MIAO, J., Eur. J. Med. Chem. **43**, nr 11, 2008, p. 2347
10.PARK, H.J., LEE, K., PARK, S.J., AHN, B., LEE, J.C., CHO, H., LEE, K.I., Bioorg. Med. Chem. Lett. **15**, nr 13, 2005, p. 3307
11.SCHENONE, S., BRUNO, O., RANISE, A., BONDAVALLI, F., BRULLO, C., FOSSA, P., MOSTI, L., MENOZZI, G., GARRARO, F., NALDINI, A., BERNINI, C., Bioorg. Med. Chem. Lett. **14**, nr 10, 2004, p. 2511
12.DAIDONE, G., RAFFA, D., MAGGIO, B., RAIMONDI, M.V., PLSECIA, F., SCHILLACI, D., Eur. J. Med. Chem. **39**, nr 3, 2004, p. 219
13.CAO, R., GUAN, X., SHI, B., CHEN, Z., REN, Z., PENG, W, SONG, H., Eur. J. Med. Chem. **45**, nr 6, 2010, p. 2503
14.LEBOHO, T.C., MICHAEL, J.P., VAN OTTERLO, W.A.L., VAN VUUREN, S.F., DE, KONING, C.B., Bioorg. Med. Chem. Lett. **19**, nr 17, 2009, p. 4948
15.BASAVARAJAIAH, S.D.M., MRUTHYUNJAYASWAMY, B.H.M., Chem. Pharm. Bull. **57**, nr 6, 2009, p. 557
16.SUZEN, S., BUYUKBINGOL, E., IL Farmaco, **55**, nr 4, 2000, p. 246
17.ZHANG, D., WANG, G., ZHAO, G., XU, W., HUO, L., Eur. J. Med. Chem. **46**, nr 12, 2011, p. 5868
18.BERNHARDT, P.V., SHARPE, P.C., ISLAM, M., LOVEJOY, D.B., KALINOWSKI, D.S., RICHARDSON, D.R., J. Med. Chem. **52**, nr 2, 2009, p. 407
19. RICHARDSON, D.R., SHARPE, P.C., LOVEJOY, D.B., SENARATNE, D., KALINOWSKI, D.S., ISLAM, M., BERNHARDT, P.V., J. Med. Chem. **49**, nr 22, 2006, p. 6510
20.KALINOWSKI, D.S., YU, Y., SHARPE, P.C., ISLAM, M., LIAO, Y., LOVEJOY, D.B., KUMAR, N., BERNHARDT, P.V., RICHARDSON, D.R., J. Med. Chem. **50**, nr 15, 2007, p. 3716
21.OGATA, L., WATANABE, A., YUNOKUCHI, T., TOYOTA, A., Inorg. Chem. Commun. **11**, nr 2, 2008, p. 215
22.GUP, R., KIRKAN, B., Spectrochim. Acta, Part A. **64**, nr 3, 2006, p. 809
23.BERNHARDT, P.V., CALDWELL, L.M., CHASTON, T.B., CHIN, P., RICHARDSON, D.R., J. Biol. Inorg. Chem. **8**, nr 8, 2003, p. 866
24. RICHARDSON, D.R., BERNHARDT, P.V., J. Biol. Inorg. Chem. **4**, nr 3, 1999, p. 266
25. FRAGA, C.A.M., BARREIRO, E.J., Curr. Med. Chem. **13**, nr 2, 2006, p. 167
26. CUI, Z., LI, Y., LING, Y., HUANG, J., CUI, J., WANG, R., YANG, X., Eur. J. Med. Chem. **45**, nr 12, 2010, p. 5576
27. ZHANG, D., WANG, G., TAN, C., XU, W., PEI, Y., HUO, L., Arch. Pharm. Res. **34**, nr 3, 2011, p. 343

Manuscript received: 4.02.2014