Res Chem Intermed
DOI 10.1007/s11164-013-1051-1

Synthesis and biological evaluation of some novel *N*-arylpyrazole derivatives as cytotoxic agents

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Received: 25 August 2012 / Accepted: 4 January 2013

Abstract A series of novel *N*-arylpyrazole derivatives, **5a–5i**, were achieved from substituted phenylacetic acid via Vilsmeier–Haack reaction, hydrolysis, condensation, and aromatic substitution reaction. Their chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR, FTIR, HRMS, and elemental analysis. The newly synthesized compounds were tested for their in vitro cytotoxic activity against Bel-7402, KB, HL-60, and BGC-823 cell lines and found to possess moderate activity.

Keywords Synthesis · *N*-arylpyrazole · Cytotoxicity · Cancer cell lines

Introduction

Heterocyclic compounds have played very important roles in the development of medicines and pesticides in recent years and have become the new trend [1, 2]. Pyrazole derivatives in general are well-known nitrogen-containing heterocyclic compounds, and various procedures have been developed for their synthesis [3–5]. The chemistry of pyrazole derivatives has been the subject of much research due to their importance in various applications and their widespread potential biological and pharmacological activities such as antimicrobial [6, 7], anti-cancer [8, 9], anticonvulsant [10], antihistaminic [11], anti-inflammatory [12], antipyretic [13], anti-infective [14], anti-virus [15], anti-anxiety [16], pain relief [17], etc. [18, 19].

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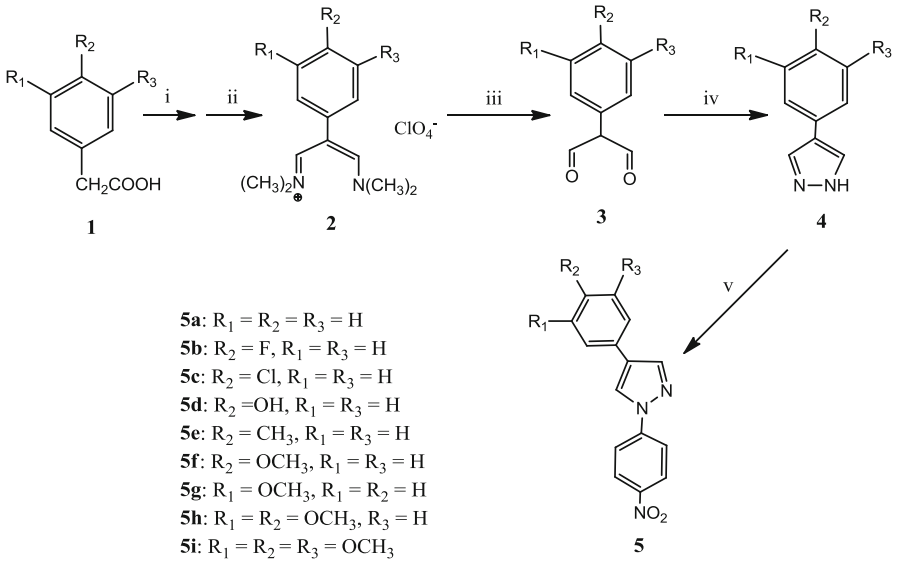
Published online: 26 January 2013



Scheme 1 Synthetic routine of the novel *N*-arylpyrazole derivatives **5a**–**5i**. Reagents and conditions: *1* DMF, $POCl_3$, 90–95 °C; *2* $NaClO_4$; *3* NaOH, 90 °C; *4* $NH_2NH_2{\cdot}H_2O$, r.t.; *5* *p*-Fluoronitrobenzene, *t*-BuOK, DMSO, 72 °C, 2 h

Among pyrazole derivatives, *N*-arylpyrazole structures are often encountered in pharmaceuticals and agrochemicals [20]. For example, *N*-arylpyrazoles have been found to be a main pharmacophore in many non-steroidal anti-inflammatory drugs such as the famous anti-inflammatory drugs Celecoxib and SC-558 [21, 22]. Recently, Melhaoui et al. synthesized two *N*-arylpyrazoles derivatives, 1-(4-nitrophenyl)-3, 5-dimethylpyrazole and 1,1′-bis(4-nitrophenyl)-5,5′-diisopropyl-3, 3′-bipyrazole, and investigated their cytotoxity against Hep cell line (human laryngeal carcinoma). The results showed that these two compounds showed good cytotoxic activity against the Hep cell line, with IC_{50}: 8.25 and 10.20 mg mL^{-1} [23]. In this paper, in order to find better anticancer compounds, we design and synthesize some new *N*-arylpyrazole derivatives by *N*-arylation reactions for the first time. The cytotoxicity was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Compounds **5d** and **5f** exhibit the best cytotoxicity among the nine compounds; moreover, their cytotoxicity is better than that of cisplatin against Bel-7402 cell line (Scheme 1).

Experimental

Chemistry

RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT, benzylpenicillin and streptomycin were from Sigma. Four different human carcinoma cell lines: Bel-7402 (liver carcinoma), KB (nasopharyngeal carcinoma),

HL-60 (immature granulocyte leukemia), and BGC-823 (gastrocarcinoma) were obtained from the American type culture collection. Mps were measured on a XT-4 microscopic melting-point spectrometer and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were obtained from solution in DMSO-d_6 with tetramethylsilane (TMS) as internal standard using a Bruker AVIII 600 NMR spectrometer. The IR spectra were recorded using KBr pellets and a Perkin-Elmer Model-683 spectrophotometer. The mass spectra were measured by LC–MS apparatus Agilent 1200-6310. High-resolution mass spectra (HRMS) were recorded on a Bruker Apex Ultra 7.0T FTMS mass spectrometer. Elemental analysis was determined on an Elementar Vario EL III elemental analyzer.

General method for the preparation of pyrazole compounds **4a**–**4i**

$POCl_3$ (30.0 mmol) was added dropwise to 11.3 mL of dry DMF at 5–10 °C with constant stirring. The mixture was stirred for an additional hour at room temperature. Then, substituted phenylacetic acid (10.0 mmol) **1a**–**1i** was added at once and the clear solution formed was stirred for 4 h at 90–95 °C and then at room temperature overnight. The resulting black mixture was poured onto crushed ice. After decomposition of the excess Vilsmeyer reagent, a saturated solution of 4.3 g $NaClO_4{\cdot}H_2O$ was added with stirring. The resulting nearly white crystalline deposit of the perchlorate salt **2a**–**2i** was filtered and washed with two 4-mL portions of water. This compound was used in the next step without further purification, in which 2.422 g of the perchlorate salt **2a**–**2i** were added to a warm solution of 1.0 g NaOH in 6 mL water, and the mixture was heated with stirring for 15 min (bath temperature 90 °C) until total dissolution of the organic salt was observed. The yellow-colored mixture was cooled to room temperature, diluted with 1.5 mL water, and 10 % HCl solution was added to pH 5, leading to precipitation of compound **3a**–**3i**. Then, 1.5 mL hydrazine hydrate (80 %) was added at once with stirring, which effects dissolution of **3** after 15–20 min stirring at room temperature. The pyrazole compounds **4a**–**4i** deposit as thin white needles. The mixture was left overnight and the precipitate was collected by filtration and oven-dried in vacuum.

*4-phenyl-1H-pyrazole (**4a**)*

White solid; yield: 56 %; 200 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.95 (brs, 1H, NH), 8.05 (brs, 2H, PzH), 7.60 (d, $J = 7.2$ Hz, 2H, Ar–$H_{2,6}$), 7.35 (t, $J = 7.2$ Hz, 2H, Ar–$H_{3,5}$), 7.18 (t, $J = 7.2$ Hz, 1H, Ar–H_4); MS (ESI): 145.0 $[M+H]^+$.

*4-(4-fluorophenyl)-1H-pyrazole (**4b**)*

White solid; yield: 68 %; 130 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.92 (brs, 1H, NH), 8.02 (brs, 2H, PzH), 7.62 (dd, $J_1 = 9.0$ Hz, $J_2 = 5.4$ Hz, 2H, Ar–$H_{2,6}$), 7.16 (t, $J = 9.0$ Hz, 2H, Ar–$H_{3,5}$); MS (ESI): 163.0 $[M+H]^+$.

*4-(4-chlorophenyl)-1H-pyrazole (**4c**)*

Light yellow solid; yield: 64 %; 165 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.10 (s, 2H, PzH), 7.64 (d, J = 8.4 Hz, 2H, Ar–$H_{2,6}$), 7.40 (d, J = 8.4 Hz, 2H, Ar–$H_{3,5}$); MS (ESI): 179.0 $[M+H]^+$.

*4-(1H-pyrazol-4-yl)phenol (**4d**)*

White solid; yield: 59 %; mp: 222–224 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.78 (brs, 1H, NH), 9.33 (brs, 1H, OH), 7.88 (brs, 2H, PzH), 7.39(d, J = 8.4 Hz, 2H, Ar–$H_{3,5}$), 6.75 (d, J = 8.4 Hz, 2H, Ar–$H_{2,6}$); MS (ESI): 161.0 $[M+H]^+$.

*4-(4-toyl)-1H-pyrazole (**4e**)*

Yellow solid; yield: 49 %; 160 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.91 (brs, 1H, NH), 8.01 (s, 2H, PzH), 7.49 (d, J = 8.4 Hz, 2H, Ar–$H_{2,6}$), 7.16 (d, J = 8.4 Hz, 2H, Ar–$H_{3,5}$), 2.29 (s, 3H, CH_3-H); MS (ESI): 159.1 $[M+H]^+$.

*4-(4-methoxyphenyl)-1H-pyrazole (**4f**)*

Light yellow solid; yield: 45 %; 180 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.87 (brs, 1H, NH), 7.96 (s, 2H, PzH), 7.52 (d, J = 8.4 Hz, 2H, Ar–$H_{2,6}$), 6.92 (d, J = 8.4 Hz, 2H, Ar–$H_{3,5}$); MS (ESI): 175.1 $[M+H]^+$.

*4-(3-methoxyphenyl)-1H-pyrazole (**4g**)*

Light yellow solid; yield: 48 %; mp: 140–141 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.94 (brs, 1H, NH), 8.21 (s, 1H, PzH), 7.93 (s, 1H, PzH), 7.26 (t, J = 7.8 Hz, 1H, Ar–H_5), 7.18 (d, J = 7.8 Hz, 1H, Ar–H_4), 7.17 (s, 1H, Ar–H_2), 6.76 (d, J = 7.8 Hz, 1H, Ar–H_6), 3.78 (s, 3H, –OCH_3); MS (ESI): 175.1 $[M+H]^+$.

*4-(3,4-dimethoxyphenyl)-1H-pyrazole (**4h**)*

Light yellow solid; yield: 55 %; mp: 160–161 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.85 (brs, 1H, NH), 8.12 (s, 1H, PzH), 7.17 (d, J = 1.8 Hz, 1H, PzH), 7.12 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz,1H, Ar–H_6), 6.92 (d, J = 8.4 Hz, 1H, Ar–H_5), 3.81 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3); MS (ESI): 205.1 $[M+H]^+$.

*4-(3,4,5-trimethoxyphenyl)-1H-pyrazole (**4i**)*

White solid; yield: 63 %; mp: 157–159 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 12.91 (brs, 1H, NH), 8.19 (s, 1H, PzH), 7.94 (s, 1H, PzH), 6.89 (s, 2H, Ar–$H_{2,6}$), 3.83 (s, 6H, 3,5-OCH_3), 3.65 (s, 3H, 4-OCH_3); MS (ESI): 235.1 $[M+H]^+$.

General method for the synthesis of *N*-arylpyrazole compounds **5a**–**5i**

To a solution of compounds **4a**–**4i** (1.25 mmol) in dimethylsulphoxide (DMSO) (1.5 mL) ,solid potassium tert-butoxide (1.38 mmol) was added followed by the addition of 4-fluoronitrobenzene (1.31 mmol) in DMSO (0.5 mL) through a syringe. The resulting mixture was heated to 72 °C and kept at this temperature for 2 h. Then, the mixture was cooled to room temperature and quenched with water (10 mL). The precipitate was collected by filtration and oven-dried in vacuum. The residue was recrystallizated by ethanol to give *N*-arylpyrazole derivative **5a**–**5i**.

*1-(4-nitrophenyl)-4-phenyl-1H-pyrazole (**5a**)*

Yellow solid; yield: 91 %; mp: 197–198 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.22(s, 1H, PzH), 8.40(d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.39 (s, 1H, PzH), 8.16 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 7.76 (d, J = 7.8 Hz, 2H, Ar–H), 7.44 (d, J = 7.8 Hz, 2H, Ar–H), 7.30 (t, J = 7.8 Hz, 1H, Ar–H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ:145.24, 144.41, 141.04, 131.62, 129.41, 127.54, 125.97, 125.93, 125.91, 125.82, 118.75; IR (KBr) ν: 3,081 ($\nu_{C-H,\ Pz}$), 1,593 ($\nu_{C=N}$), 1,519 (ν_{aNO2}), 1,340 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{15}H_{11}N_3$: C 67.92 H 4.18 N 15.84; Found C 68.01 H 4.16 N 15.81; HRMS (ESI) *m/z* calcd for $C_{15}H_{12}N_3O_2$: 266.0924 $[M+H]^+$, found: 266.0924.

*4-(4-fluorophenyl)-1-(4-nitrophenyl)-1H-pyrazole (**5b**)*

Golden yellow solid; yield: 95 %; mp: 240–241 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.22 (s, 1H, PzH), 8.41 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.38 (s, 1H, PzH), 8.15 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 7.80 (dd, J_1 = 9.0 Hz, J_2 = 5.4 Hz, 2H, ArF-H), 7.29 (t, J = 9.0 Hz, 2H, ArF-H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ:162.59, 160.97, 145.26, 144.37, 141.00, 128.20 128.18, 127.91 127.86, 125.95 125.80, 124.96, 118.74, 116.34 116.20; IR (KBr) ν: 3,081 ($\nu_{C-H,\ Pz}$), 1,592 ($\nu_{C=N}$), 1,502 (ν_{aNO2}), 1,330 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{15}H_{10}N_3F$: C 63.60 H 3.56 N 14.83; Found C 63.35 H 3.77 N 14.74; HRMS (ESI) *m/z* calcd for $C_{15}H_{11}N_3O_2F$ 284.0835 $[M+H]^+$, found: 284.0829.

*4-(4-chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazole (**5c**)*

Yellow solid; yield: 94 %; mp: 226–228 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.27 (s, 1H, PzH), 8.40 (m, 3H, PzH, $ArNO_2$-H), 8.14 (d, J = 8.4 Hz, 2H, $ArNO_2$-H), 7.78 (d, J = 7.8 Hz, 2H, Ar–H), 7.50 (d, J = 7.8 Hz, 2H, Ar–H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ:145.37, 144.32, 141.07, 131.93, 130.63, 129.42, 127.65, 126.22, 125.99, 124.73, 118.84; IR (KBr) ν: 3,116 ($\nu_{C-H,\ Pz}$), 1,596 ($\nu_{C=N}$), 1,515 (ν_{aNO2}), 1,328 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{15}H_{10}N_3O_2Cl$: C 60.11 H 3.36 N 14.02; Found C 59.52 H 3.46 N 13.43; HRMS (ESI) *m/z* calcd for $C_{15}H_{11}N_3O_2Cl$ 300.0534 $[M+H]^+$, found: 300.0539.

*4-(1-(4-nitrophenyl)-1H-pyrazol-4-yl)phenol (**5d**)*

Yellow solid; yield: 85 %; mp: >250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.53 (brs, 1H, OH), 9.06 (s, 1H, PzH), 8.40 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.14 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.26(s, 1H, PzH), 7.56 (d, J = 8.4 Hz, 2H, ArOH-H), 6.83 (d, J = 8.4 Hz, 2H, ArOH-H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 157.18, 145.01, 144.51, 140.77, 127.31, 126.21, 125.91, 124.47, 122.42, 118.50, 116.19; IR (KBr) ν: 3,350 (ν_{O-H}), 3,115 ($\nu_{C-H,\ Pz}$), 1,594 ($\nu_{C=N}$), 1,504 (ν_{aNO2}), 1,334 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{15}H_{11}N_3O_3$: C 64.05 H 3.94 N 14.94; Found C 64.26 H 3.97 N 14.90; HRMS (ESI) *m/z* calcd for $C_{15}H_{12}N_3O_3$ 282.0873 $[M+H]^+$, found: 282.0867.

*1-(4-nitrophenyl)-4-p-tolyl-1H-pyrazole (**5e**)*

Yellow solid; yield: 92 %; mp: 165–168 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.19 (s, 1H, PzH), 8.40 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.35 (s, 1H, PzH), 8.16 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 7.65 (d, J = 7.8 Hz, 2H, Ar–H), 7.25 (d, J = 7.8 Hz, 2H, Ar–H), 2.33 (s, 3H, $-CH_3$); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 145.12, 144.42, 140.92, 136.77, 129.93, 128.73, 125.89, 125.86, 125.3, 118.63, 21.22; IR (KBr) ν: 3,132 ($\nu_{C-H,\ Pz}$), 2,924 ($\nu_{C-H,\ CH3}$), 1,598 ($\nu_{C=N}$), 1,513 (ν_{aNO2}), 1,330 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{16}H_{13}N_3O_2$: C 68.81 H 4.69 N 15.05; Found C 67.98 H 4.70 N 14.59; HRMS (ESI) *m/z* calcd for $C_{16}H_{14}N_3O_2$ 280.1081 $[M+H]^+$, found: 280.1082.

*4-(4-methoxyphenyl)-1-(4-nitrophenyl)-1H-pyrazole (**5f**)*

Yellow solid; yield: 95 %; mp: 209–211 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.13 (s, 1H, PzH), 8.40 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.32 (s, 1H, PzH), 8.15 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 7.68 (d, J = 8.4 Hz, 2H, Ar–H), 7.01 (d, J = 8.4 Hz, 2H, Ar–H), 3.79 (s, 3H, $-OCH_3$); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 158.96, 145.11, 144.49, 140.89, 127.24, 125.95, 125.79, 124.93, 124.07, 118.69, 114.87, 55.63; IR (KBr) ν: 3,136 ($\nu_{C-H,\ Pz}$), 2,994, 2,827 ($\nu_{C-H,\ OCH3}$), 1,598 ($\nu_{C=N}$), 1,504 (ν_{aNO2}), 1,332 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{16}H_{13}N_3O_3 \cdot 0.5H_2O$: C 63.64 H 4.54 N 13.81; Found C 63.91 H 4.43 N 13.35; HRMS (ESI) *m/z* calcd for $C_{16}H_{14}N_3O_3$ 296.1030 $[M+H]^+$, found: 296.1033.

*4-(3-methoxyphenyl)-1-(4-nitrophenyl)-1H-pyrazole (**5g**)*

Yellow solid; yield: 94 %; mp: 191–193 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.25 (s, 1H, PzH), 8.40 (m, 3H, PzH, $ArNO_2$-H), 8.16 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 7.34 (m, 3H, Ar–$H_{2',4',6'}$), 6.86 (m, 1H, Ar–$H_{5'}$); ^{13}C NMR (DMSO-d_6, 150 MHz) δ:160.27, 145.20, 144.37, 141.14, 132.94, 130.44, 125.99, 125.90, 125.85, 118.69, 118.29, 113.12, 111.47, 55.61; IR (KBr) ν: 3,120 ($\nu_{C-H,\ Pz}$), 2,972, 2,841 ($\nu_{C-H,\ OCH3}$), 1,596 ($\nu_{C=N}$), 1,510 (ν_{aNO2}), 1,338 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{16}H_{13}N_3O_3 \cdot 0.5H_2O$: C 63.64 H 4.54 N 13.81; Found C 63.74 H 4.39 N 13.63; HRMS (ESI) *m/z* calcd for $C_{16}H_{14}N_3O_3$ 296.1030 $[M+H]^+$, found: 296.1030.

*4-(3,4-dimethoxyphenyl)-1-(4-nitrophenyl)-1H-pyrazole (**5h**)*

Yellow solid; yield: 93 %; mp: 141–143 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.16 (s, 1H, PzH), 8.41(d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.36 (s, 1H, PzH), 8.15 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 7.34 (d, J = 1.8 Hz, 1H, Ar–$H_{2'}$), 7.29 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H, Ar–$H_{6'}$), 7.01 (d, J = 8.4 Hz, 1H, Ar–$H_{2'}$), 3.86 (s, 3H, –OCH_3), 3.78 (s, 3H, –OCH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ:149.63, 148.59, 145.06, 144.47, 141.00, 126.10, 125.92, 125.04, 124.35, 118.53, 118.25, 112.70, 110.03, 56.12, 56.02; IR (KBr) ν: 3,123 ($\nu_{C-H,\ Pz}$), 2,933, 2,834 ($\nu_{C-H,\ OCH3}$), 1,596 ($\nu_{C=N}$), 1,504 (ν_{aNO2}), 1,334 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{17}H_{15}N_3O_4$: C 62.76 H 4.65 N 12.92; Found C 62.21 H 4.58 N 12.49; HRMS (ESI) m/z calcd for $C_{17}H_{16}N_3O_4$ 326.1135 $[M+H]^+$, found: 326.1135.

*1-(4-nitrophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrazole (**5i**)*

Yellow solid; yield: 95 %; mp: 198–199 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.38 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 8.23 (s, 1H, PzH), 8.04 (s, 1H, PzH), 7.96 (d, J = 9.0 Hz, 2H, $ArNO_2$-H), 7.29 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H, Ar–$H_{6'}$), 7.01 (d, J = 8.4 Hz, 1H, Ar–$H_{2',6'}$), 6.77 (s, 2H, Ar–$H_{2'}$), 3.96 (s, 6H, -OCH_3), 3.91 (s, 3H, -OCH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ:153.84, 145.19, 144,42, 141.27, 137.22, 127.23, 126.20, 125.98, 125.74, 118.63, 103.57, 60.57, 56.52; IR (KBr) ν: 3,122 ($\nu_{C-H,\ Pz}$), 2,937, 2,841 ($\nu_{C-H,\ OCH3}$), 1,592 ($\nu_{C=N}$), 1,511 (ν_{aNO2}), 1,332 (ν_{sNO2}) cm^{-1}; Anal. calcd for $C_{18}H_{17}N_3O_5 \cdot 0.5H_2O$: C 59.34 H 4.98 N 11.53; Found C 59.42 H 4.61 N 11.28; HRMS (ESI) m/z calcd for $C_{18}H_{18}N_3O_5$ 356.1241 $[M+H]^+$, found: 356.1238.

Cell culture

Four different human carcinoma cell lines: Bel-7402, KB, HL-60, and BGC-823 were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum, 100 units/mL of penicillin and 100 mg/mL of streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 5 % CO_2 in air.

Solutions

The obtained *N*-arylpyrazole derivatives, **5a**–**5i**, were dissolved in DMSO at a concentration of 5 mM as stock solution, and diluted in culture medium at concentrations of 1.0, 10, 100, and 500 mM as working-solution. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1 % (v/v) in all experiments.

Cytotoxicity analysis

The cells harvested from exponential phase were seeded equivalently into a 96-well plate, and then the compounds were added to the wells to achieve final concentrations. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. All experiments were

performed in quintuplicate. The MTT assay was performed as described by Mosmann [24]. Upon completion of the incubation for 44 h, stock MTT dye solution (20 mL, 5 mg/mL) was added to each well. After 4 h incubation, 2-propanol (100 mL) was added to solubilize the MTT formazan. The optical density (OD) of each well was measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} value was determined from plot of % viability against dose of compounds added.

Results and discussion

Chemistry

The synthetic route to compounds **4a–4i** began with a Vilsmeyer–Haack-type reaction of substituted phenylacetic acid **1a–1i** with $POCl_3$/DMF to give **2a–2i**, which was isolated as its perchlorate salt. Hydrolysis of **2a–2i** followed by treatment of the resulting dialdehyde **3a–3i** (not isolated) with hydrazine furnished the pyrazole derivative **4a–4i** as thin white needles [25]. The synthesis of *N*-arylpyrazole derivatives **5a–5i** was performed using the literature method [26] by the direct *N*-arylation of pyrazole derivatives with 4-fluoronitrobenzene using potassium tert-butoxide as a base in DMSO at 72 °C. Products of the reaction have been isolated, purified, and characterized by various spectral techniques such as FTIR, HRMS, ^{1}H NMR, ^{13}C NMR, and elemental analysis.

Pyrazole NH proton peaks in the ^{1}H NMR spectra of compounds **4a–4i**. characteristically appear around 12.78–12.95 ppm, which disappear in the ^{1}H NMR spectra of compounds **5a–5i**. Pair of ^{1}H doublets at ~8.14 and ~8.40 ppm strongly suggest *para*-disubstituted phenyl ring in the ^{1}H NMR spectra of compounds **5a–5i**. The IR spectra of compounds **5a–5i** showed characteristic absorption at 1,502–1,519 cm^{-1} and 1,328–1,340 cm^{-1}, attributable to the nitro group. From above, it is evident that the nitophenyl group has been successfully introduced into the pyrazole skeleton. Furthermore, the HRMS of compounds **5a–5i** all showed a protonated molecular ion peak $[M+H]^+$ in the positive mode. The mass spectrum of compound **5a** showed a molecular ion peak at $m/z = 266.0924$, corresponding to molecular formula $C_{15}H_{12}N_3O_2$. This was evidenced from HRMS analysis of the other compounds, **5b–5i**.

Cytotoxic activity

The in vitro cytotoxic activity of compounds **5a–5i** against Bel-7402, KB, HL-60, and BGC-823 was evaluated by MTT assay. The IC_{50} values are listed in Table 1 and the activities of Cisplatin were a control. It can be seen that most of the obtained compounds exhibited cytotoxic activity and were found to be more toxic against Bel-7402 than KB, HL-60, and BGC-823 cells. Compound **5f**, bearing the methoxy group on the 4′-position of the phenyl ring, was the most effective one. Its inhibition of cell growth of Bel-7402 cells was 1.6-fold higher than that found for Cisplatin under the same experimental conditions. Compound **5d**, with the hydroxyl group on the phenyl ring, was also more toxic to Bel-7402 cells than Cisplatin. The improved

Table 1 The cytotoxicity of the compounds **5a–5i** against Bel-7402, KB, HL-60, and BGC-823

Compounds	IC_{50} (μM)			
	Bel-7402	KB	HL-60	BGC-823
5a	11.67	12.40	15.62	10.30
5b	22.53	26.22	23.83	20.51
5c	10.55	12.58	28.54	15.19
5d	7.09	9.71	12.24	12.02
5e	9.59	11.24	26.71	15.14
5f	4.93	6.53	15.30	16.24
5g	13.19	20.62	13.04	26.88
5h	23.81	52.83	44.71	22.56
5i	12.24	21.54	22.94	21.24
Cisplatin	8.12	2.65	2.29	6.48

cytotoxic activity of **5d**, **5e**, and **5f** hinted that electron-donating groups on the benzene ring could enhance the cytotoxicity of *N*-arylpyrazole derivatives on comparing the activities with **5b** and **5c** with electron-withdrawing groups. The location of the substituent also had an important effect on cytotoxic activity. For example, compound **5f**, with the methoxy group at the 4′-position of the phenyl ring, showed stronger cytotoxic activity than compound **5g**, with the methoxy group on the 3′-position.

Conclusions

A series of *N*-arylpyrazole derivatives were designed and synthesized, and their cytotoxicity was evaluated in vitro. The results showed a number of the title compounds exhibiting potent activity against four human cancer cell lines. The compounds **5d** and **5f** exhibited better cytotoxicity than that of cisplatin against Bel-7402. The results suggest that the electronic parameters, location, and number of the methoxy group on the benzene ring had important effects on cytotoxic activity. These results may be helpful for the design of new antitumor agents.

Acknowledgments We gratefully thank the National Basic Research 973 Program (Grant no. 2010CB534913), the Pharmaceutical Joint Research Foundation of the Natural Science Foundation of Hebei Province and China Shiyao Pharmaceutical Group Co. Ltd. (B2011201174), Hebei Province Nature Science Fund for Distinguished Young Scholars (B2011201164), the Nature Science Fund of Hebei Province (B2011201135), the Key Basic Research Special Foundation of Science Technology Ministry of Hebei Province (11966412D) and Open Fund of Laboratory of Chemical Biology of Hebei Provincem (No. 09265631D-7).

References

1. A. Dömling, I. Ugi, Angew. Chem. Int. Ed. **39**, 3168 (2000)
2. A. Dömling, Chem. Rev. **106**, 17 (2006)

3. V.K. Aggarwal, J. de Vincente, R.V. Bonnert, J. Org. Chem. **68**, 5381 (2003)
4. P.A. Humphries, J.M. Finefield, Tetrahedron Lett. **47**, 2443 (2006)
5. S.M. Ahmed, K. Kobayashi, A. Mori, Org. Lett. **7**, 4487 (2005)
6. E.V. Pimerova, E.V. Voronina, Pharm. Chem. J. **35**, 18 (2001)
7. V.A. Chornous, M.K. Bratenko, M.V. Vovk, I.I. Sidorchuk, Pharm. Chem. J. **35**, 203 (2001)
8. S. Manfredini, R. Bazzanini, P.G. Baraldi, M. Guarneri, D. Simoni, M.E. Marongiu, A. Pani, E. Tramontano, P.L. Colla, J. Med. Chem. **35**, 917 (1992)
9. S. Manfredini, R. Bazzanini, P.G. Baraldi, M. Bonora, M. Marangoni, D. Simoni, A. Pani, F. Scintu, E. Pinna, L. Pisano, P.L. Colla, Anti-Cancer Drug Des. **11**, 193 (1996)
10. I. Bouabdallah, L.A. Barret, A. Zyad, A. Ramadan, I. Zidane, A. Melhaoui, Nat. Prod. Res. **11**, 1024 (2006)
11. V. Michon, C.H. Du Penhoat, F. Tombret, J.M. Gillardin, F. Lepagez, L. Berthon, Eur. J. Med. Chem. **30**, 147 (1995)
12. A.A. Bekhit, H. Ashour, M.A. Abdel, Y.S. Ghany, A.E. Bekhit, A.A. Baraka, Eur. J. Med. Chem. **43**, 456 (2008)
13. A.A.M. Eissa, N.A.H. Farag, G.A.H. Soliman, Bioorg. Med. Chem. **17**, 5059 (2009)
14. E.R. Orth, J. Pharm. Sci. **57**, 537 (1968)
15. S.L. Janus, A.Z. Magdif, B.P. Erik, N. Claus, Monatsh. Chem. **130**, 1167 (1999)
16. D.J. Wustrow, T. Capiris, R. Rubin, J.A. Knobelsdorf, H. Akunne, M.D. Davis, R. MacKenzie, T.A. Pugsley, K.T. Zoski, T.G. Heffner, L.D. Wise, Bioorg. Med. Chem. **8**, 2067 (1998)
17. L. Mosti, P. Fossa, F. Mattioli, M. Ghia, J. Heterocycl. Chem. **34**, 963 (1997)
18. K.L. Kees, J.J. Fitzgerald, K.E. Steiner, J.F. Mattes, B. Mihan, T. Tosi, D. Mondoro, M.L. McCaleb, J. Med. Chem. **39**, 3920 (1996)
19. G.R. Bebernitz, G. Argentieri, B. Battle, C. Brennan, B. Balkan, B.F. Burkey, M. Eckhardt, J. Gao, P. Kapa, R.J. Strohschein, H.F. Schuster, M. Wilson, D.D. Xu, J. Med. Chem. **44**, 2601 (2001)
20. J. Elguero, *Comprehensive Heterocyclic Chemistry* (Pergamon Press, Oxford, 1984)
21. R.J. Bing, M. Lomnicka, J. Am. Coll. Cardiol. **39**, 521 (2002)
22. R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stallings, Nature **384**, 644 (1996)
23. I. Bouabdallah, L. AitM'Barek, A. Zyad, A. Ramdani, I. Zidane, A. Melhaoui, Nat. Prod. Res. **21**, 298 (2007)
24. T. Mosmann, J. Immunol. Methods **65**, 55 (1983)
25. V. Lozan, P.Y. Solntsev, G. Leibeling, K.V. Domasevitch, B. Kersting, Eur. J. Inorg. Chem. **20**, 3217 (2007)
26. X.J. Wang, J. Tan, L. Zhang, Org. Lett. **2**, 3107 (2000)