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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and biologic evaluation of some novel *N*-arylpyrazole derivatives as cytotoxic agents

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Abstract A novel series of *N*-arylpyrazole derivatives (**5a–5d**, **7a–7c**) has been designed and synthesized via aromatic substitution reaction of *N*-nonsubstituted pyrazoles with 4-fluoronitrobenzene in the presence of base. The structures of these compounds were established on the basis of elemental (C, H, and N) and spectral analysis (^{1}H NMR, ^{13}C NMR, HRMS, and FT-IR). All the compounds were tested for their cytotoxic activity in vitro against four human tumor cell lines: carcinoma (Bel-7402), nasopharyngeal carcinoma (KB), immature granulocyte leukemia (HL-60), and gastrocarcinoma (BGC-823) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The results showed that most of the obtained compounds exhibited promising cytotoxicity against tested carcinoma cell lines with low IC_{50} values. The bis-pyrazole derivative **7c**, bearing alkoxy group on the 5-position of phenyl ring, was the most effective one. It is inhibition of cell growth of Bel-7402 cells was 1.5-fold higher than that found for cisplatin. And, also mono-pyrazole derivatives **5a** and **5b**, decorated with trifluoromethyl group on the phenyl ring, displayed better cytotoxicity than that of cisplatin against Bel-7402 cell line.

Keywords Pyrazole · *N*-Arylpyrazole · Aromatic substitution · Cytotoxicity · Cancer cell lines

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Introduction

Pyrazoles represent a class of nitrogen containing five membered heterocyclic compounds of significant importance and are considered as extremely versatile building blocks in organic chemistry. Owing to their easy preparation, interesting chemical property and rich biologic activity, pyrazole, and its derivatives are widely used in pharmaceuticals, agrochemicals, food additives, dyes, materials, and cosmetic coloring agents (Katritzky *et al*., 1996; Schmidt and Dreger, 2011).

In addition, much interest has been given to the chemotherapeutic activity of *N*-arylpyrazole as antiparasitic (Rathelot *et al*., 2002), antifungal (Prakash *et al*., 2008; Sivaprasad *et al*., 2006), antibacterial (Finn *et al*., 2003), antiviral (Elgemeie *et al*., 2005), and antidiabetic (Bebernitz *et al*., 2001) agents. It has been also reported that some *N*-arylpyrazole derivatives have shown several biologic activities as seen in cyclooxygenase-2 (Bing and Lomnicka, 2002; Kurumbail *et al*., 1996; Habeeb *et al*., 2001; Chowdhury *et al*., 2009), p38 MAP kinase (Regan *et al*., 2002), estrogen receptor (Stauffer *et al*., 2000), and non-nucleoside HIV-1 reverse transcriptase inhibitory activity (Genin *et al*., 2000). Moreover, many *N*-arylpyrazole derivatives exhibited promising antiproliferative properties against several kinds of human tumor cell lines (Balbi *et al*., 2011; Farag *et al*., 2008; Insuasty *et al*., 2010; Joksović *et al*., 2010; Rostom *et al*., 2003). Therefore, the use of *N*-arylpyrazole motif is believed to be a powerful tool for novel compounds with potential pharmacological and biologic activities.

Much effort has been made to construct such an *N*-arylpyrazole structural unit. Important methods involve

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approaches based on the condensation of *N*-arylhydrazines with a variety of 1,3-carbonyl compounds or its related compounds (Chang *et al*., 2002; Giacomelli *et al*., 2003; Peruncheralathan *et al*., 2005) and the transition metal-catalyzed N-arylation of 1*H*-pyrazoles (Klapars *et al*., 2001; Son *et al*., 2004; Zhang *et al*., 2005). In addition, aromatic substitution of *N*-nonsubstituted pyrazoles with activated halogenated benzenes is also a powerful method for synthesis of *N*-arylpyrazoles (Wang *et al*., 2000; Bouabdallah *et al*., 2007). Based on the previous findings and with the aim of obtaining new compounds with antitumor activity, we synthesized a novel series of *N*-arylpyrazole derivatives (**5a–5d**, **7a–7c**) via aromatic substitution reaction of *N*-nonsubstituted pyrazoles with 4-fluronitrobenzene in the presence of base. And, the cytotoxic activity of the obtained compounds was evaluated by MTT assay against four different human tumor cell lines (Bel-7402, KB, HL-60, and BGC-823).

Experimental

Materials and methods

All chemicals and reagents were of analytical grade. 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 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, Bruker DMX300, and Bruker Avance DMX400 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 analyses were determined on an Elementar Vario EL III elemental analyzer. The progress of reactions was monitored by thin layer chromatography (TLC) analyses and TLC visualization was checked with UV lamp.

Synthesis

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

$POCl_3$ (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–1d** 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 on crushed ice. After decomposition of the excess Vilsmeyer reagent, a saturated solution of 4.3 g (30 mmol) $NaClO_4{\cdot}H_2O$ was added with stirring. The resulting nearly white crystalline deposit of the perchlorate salt **2a–2d** was filtered and washed with two 4 mL portions of water. This compound was used in the next step without further purification.

The perchlorate salt **2a–2d** (10 mmol) was added to a warm solution of 0.8 g (20 mmol) 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–3d**. Then, 1.5 mL hydrazine hydrate (80 %) was added at once with stirring, which effects dissolution of 3 and after 15–20 min stirring at room temperature the pyrazole compounds **4a–4d** deposits as light yellow solid. The mixture was left overnight and the precipitate was collected by filtration and oven-dried in vacuo.

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

Light yellow solid, yield 52 %; 145 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 13.11 (brs, 1H, NH), 8.37 (s, 1H, PzH), 8.05 (s, 1H, PzH), 7.84 (d, J = 7.8 Hz, 2H, Ar–$H_{3,5}$), 7.69 (d, J = 7.8 Hz, 2H, Ar–$H_{2,4}$). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 137.66, 137.15, 127.01, 126.11, 126.08, 125.88, 120.31. HRMS (ESI) *m/z* calcd. for $C_8H_8F_3N_2$: 213.0634 $[M+H]^+$, found: 213.0633.

4-(3,5-Bis(trifluoromethyl)phenyl)-1H-pyrazole ***4b***

Light yellow solid, yield 68 %; 150 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 13.18 (br s, 1H, NH), 8.45 (s, 2H, PzH), 8.31 (s, 2H, Ar–$H_{2,6}$), 7.84 (s, 1H, Ar–H_4); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 136.46, 131.47, 131.25, 125.69, 124.81, 123.00, 119.10, 119.02. HRMS (ESI) *m/z* calcd. for $C_{11}H_7F_6N_2$: 281.0507 $[M+H]^+$, found: 281.0509.

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

Orange solid, yield 63 %; 190 °C sublimation; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 13.24 (br s, 1H, NH), 8.45 (br s, 1H, PzH), 8.21 (d, J = 9.0 Hz, 2H, Ar–$H_{3,5}$), 8.13 (br s, 1H, PzH), 7.90 (d, J = 9.0 Hz, 2H, Ar–$H_{2,6}$); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 145.46, 140.64, 125.98, 124.70, 119.91. MS (ESI): 190.1 $[M+H]^+$ HRMS (ESI) *m/z* calcd. for $C_9H_8N_3O_2$: 190.0611 $[M+H]^+$, found: 190.0609.

4-(1H-Pyrazol-4-yl)pyridine ***4d***

Orange solid, yield 28 %. m.p. 195–198 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 13.19 (br s, 1H, NH), 8.49 (dd, $J_1 = 4.8$ Hz, $J_2 = 1.2$ Hz, 2H, Ar–H), 8.43 (brs, 1H, PzH), 8.12 (brs, 1H, PzH), 7.61 (dd, $J_1 = 4.8$ Hz, $J_2 = 1.2$ Hz, 2H, Ar–H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 150.46, 140.76, 120.12, 119.22. MS (ESI): 146.1 $[M+H]^+$. HRMS (ESI) *m/z* calcd. for $C_8H_8N_3$: 146.0712 $[M+H]^+$, found: 146.0710.

1,2-Bis(3,5-dimethyl-1*H*-pyrazol-4-yl)benzene (**6a**), 1,3-bis(3,5-dimethyl-1*H*-pyrazol-4-yl)benzene (**6b**) and 4,4′-(5-(octyloxy)-1,3-phenylene)bis(3,5-dimethyl-1*H*-pyrazole) (**6c**) were synthesized to the published methods (Ramirez *et al*., 1967; Li *et al*., 2006)from the corresponding aromatic dialdehyde: ortho-phthalaldehyde, isophthalaldehyde and 5-(octyloxy)isophthalaldehyde

1,2-Bis(3,5-dimethyl-1H-pyrazol-4-yl)benzene ***6a***

white solid, yield 21 %. m.p.>300 °C. ^{1}H NMR (d_6-DMSO, 300 MHz), δ: 7.28–7.31 (m, 2H), 7.19–7.22 (m, 2H), 1.74 (s, 12H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 134.09, 131.87, 126.91, 117.57, 13.46–11.67 (br s), 11.07–9.58 (br s). HRMS (ESI) *m/z* calcd. for $C_{16}H_{19}N_4$: 267.1604 $[M+H]^+$, found: 167.1600.

1,3-Bis(3,5-dimethyl-1H-pyrazol-4-yl)benzene ***6b***

White solid, yield 24 %. m.p. 288–289 °C. ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 12.29 (br s, 2H, –NH) 7.40 (t, $J = 7.5$ Hz, 1H, ArH-5), 7.14 (d, $J = 7.5$ Hz, 2H, ArH-4,6), 7.13 (s, 1H, ArH-2), 2.21 (s, 12H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 134.55, 129.42, 128.96, 126.48, 117.30, 11.81–12.26 (br s); MS (MALDI-TOF): 267.1 $[M+H]^+$; Anal. calcd. for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04. Found: C, 71.71; H, 6.92; N, 21.16.

4,4′-(5-(Octyloxy)-1,3-phenylene)bis(3,5-dimethyl-1H-pyrazole) ***6c***

White solid, yield 51 %. m.p. 167–168 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.75 (s, 3 H, ArH-2,4,6), 4.01 (t, $J = 6$ Hz, 2H), 2.37 (s, 12H, $-CH_3$), 1.82–1.80 (m, 2H), 1.48 (m, 2H), 1.29 (m, 8H), 0.88 (br s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.17, 141.94, 135.02, 122.77, 118.40, 113.32, 68.09, 31.83, 29.41, 29.37, 29.26, 26.67, 14.12, 11.82. MS (MALDI-TOF): 395.1 $[M+H]^+$. Anal. calcd. for $C_{24}H_{34}N_4O$: C, 73.06; H, 8.69; N, 14.20. Found: C, 72.87; H, 8.76; N, 13.96.

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

To a solution of compounds **4a–4d** (1.25 mmol) in dimethylsulfoxide (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 vacuo. The crude product was recrystallized from ethanol to give *N*-arylpyrazole derivative **5a–5d** in good to excellent yields.

1-(4-Nitrophenyl)-4-(4-(trifluoromethyl)phenyl)-1H-pyrazole ***5a***

Yellow solid, yield 97 %. m.p. 173–174 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.41 (s, 1H, PzH), 8.51 (s, 1H, PzH), 8.42 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 8.18 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 7.98 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.80 (d, $J = 8.2$ Hz, 2H, Ar–H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 145.51, 144.23, 141.27, 135.90, 127.07, 126.42, 126.34, 126.32, 125.97, 124.43, 118.99. IR (cm^{-1}): 3127, 1596, 1519, 1403, 1334, 1267, 1176, 1118, 1064, 954, 854, 750, 686, 597, 503. Anal. calcd. for $C_{16}H_{10}N_3O_2F{\cdot}0.25H_2O$: C, 56.89; H, 3.13; N, 12.44. Found: C, 56.81; H, 3.11; N, 12.10. HRMS (ESI) *m/z* calcd. for $C_{16}H_{11}F_3N_3O_2$: 334.0797 $[M+H]^+$, found: 334.0797.

4-(3,5-Bis(trifluoromethyl)phenyl)-1-(4-nitrophenyl)-1H-pyrazole ***5b***

Yellow solid, yield 98 %. m.p. >250 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.59 (s, 1H, PzH), 8.71 (s, 1H, PzH), 8.48 (s, 2H, Ar–H2′,6′), 8.44 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 8.16 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 7.97 (s, 1H, Ar–$H_{4'}$). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 145.63, 144.13, 141.43, 134.67, 131.61, 131.40, 127.79, 126.29, 126.01, 123.15, 122.94, 119.00. IR (cm^{-1}): 3118, 1596, 1521, 1336, 1288, 1180, 1128, 1033, 948, 893, 854, 794, 750, 701, 680. Anal. calcd. for $C_{17}H_9N_3O_2F_2{\cdot}0.5H_2O$: C, 49.77; H, 2.46; N, 10.24. Found: C, 49.53; H, 2.37; N, 9.81. HRMS (ESI) *m/z* calcd for $C_{17}H_{10}F_6N_3O_2$: 402.0671 $[M+H]^+$, found: 402.0676.

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

Red brown solid, yield 93 %. m.p. >250 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.50 (s, 1H, PzH), 8.57 (s, 1H, PzH), 8.44 (d, $J = 9.0$ Hz, 2H, Ar–H), 8.31 (d, $J = 9.0$ Hz,

2H, Ar–H), 8.19(d, $J = 9.0$ Hz, 2H, Ar–H), 8.04 (d, $J = 9.0$ Hz, 2H, Ar–H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 146.39, 145.66, 144.11, 141.53, 138.72, 127.83, 126.62, 125.99, 124.83, 123.90, 119.12. IR (cm^{-1}): 3133, 1592, 1513, 1405, 1344, 1268, 1211, 1108, 1031, 952, 852, 750, 684, 561. Anal. calcd. for $C_{15}H_{10}N_4O_4$: C, 58.07; H, 3.25; N, 18.06. Found: C, 57.93; H, 3.30; N, 17.78. HRMS (ESI) *m/z* calcd. for $C_{15}H_{11}N_4O_4$: 311.0774 $[M+H]^+$, found: 311.0779.

4-(1-(4-Nitrophenyl)-1H-pyrazol-4-yl)pyridine ***5d***

Yellow solid, yield: 82 %. m.p. 228–229 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 9.46 (s, 1H, PzH), 8.60 (d, $J = 5.1$ Hz, 2H, Pyridine), 8.55 (s, 1H, PzH), 8.43(d, $J = 9.0$ Hz, 2H, $ArNO_2$–H, 8.17(d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 7.74 (d, $J = 5.1$ Hz, 2H, Pyridine). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 150.70, 145.61, 144.12, 141.33, 139.08, 127.68, 125.97, 123.35, 120.39, 119.10. IR (cm^{-1}): 3067, 1594, 1517, 1407, 1334, 1275, 1222, 1110, 1035, 995, 961, 943, 854, 824, 748, 684, 526, 490. Anal. calcd. for $C_{14}H_{10}N_4O_2{\cdot}0.25H_2O$: C, 62.10; H, 3.91; N, 20.69. Found: C, 62.53; H, 3.72; N, 20.27. HRMS (ESI) *m/z* calcd. for $C_{14}H_{11}N_4O_2$: 267.0876 $[M+H]^+$, found: 267.0871.

General method for the synthesis of *N*-arylpyrazole compounds **7a**–**7c**

To a solution of compounds **6a**–**6c** (1.25 mmol) in dimethylsulfoxide (DMSO) (3.0 mL) solid potassium tert-butoxide (2.76 mmol) was added followed by the addition of 4-fluoronitrobenzene (2.62 mmol) in DMSO (1.0 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 vacuo. The residue was recrystallized from ethanol to give *N*-arylpyrazole derivative **7a**–**7c**.

1,2-Bis(3,5-dimethyl-1-(4-nitrophenyl)-pyrazol-4-yl)benzene ***7a***

Light yellow solid, yield 86 %. m.p. 117–119 °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 8.35 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 8.32 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 7.67 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 7.62 (d, $J = 9.0$ Hz, 2H, $ArNO_2$–H), 7.51 (m, 2H, Ar–H), 7.40 (m, 2H, Ar–H), 2.17 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.06 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 148.85, 148.83, 145.61, 145.56, 144.98, 144.92, 137.62, 137.48, 132.88, 132.85, 132.23, 128.22, 125.34, 125.32, 124.00, 123.95, 122.32, 122.28, 12.69, 12.64, 12.57, 12.51. IR (cm^{-1}): 3086, 2928, 1596, 1519, 1436, 1338, 1110, 1033, 1007, 854, 752, 692, 516; Anal. calcd. for $C_{28}H_{24}N_6O_4{\cdot}0.25H_2O$: C, 65.55; H, 4.81; N, 16.38. Found: C, 65.34; H, 4.78; N, 16.18. MS (ESI): 531.5 $[M+Na]^+$; 547.2 $[M+K]^+$. HRMS (ESI) *m/z* calcd. for $C_{28}H_{25}N_6O_4$: 509.1931 $[M+H]^+$, found: 509.1932.

1,3-Bis(3,5-dimethyl-1-(4-nitrophenyl)-pyrazol-4-yl)benzene ***7b***

Light yellow solid, yield 89 %. m.p. 235–236 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.39 (d, $J = 9.0$ Hz, 4H, $ArNO_2$–H), 7.91 (d, $J = 9.0$ Hz, 4H, $ArNO_2$–H), 7.60 (t, $J = 7.8$ Hz, 1H, Ar–H_5), 7.38 (dd, $J_1 = 7.8$ Hz, $J_2 = 1.5$ Hz, 2H, Ar–$H_{4,6}$), 7.36 (d, $J = 1.5$ Hz, 1H, Ar–H_2), 2.47 (s, 6H, CH_3), 2.33 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 149.18, 145.86, 144.93, 136.61, 133.29, 130.71, 128.92, 128.20, 124.80, 123.87, 122.86, 12.81, 12.60. IR (cm^{-1}): 3086, 2919, 1594, 1506, 1429, 1340, 1281, 1110, 1018, 854, 790, 750, 690, 509, 347; Anal. calcd. for $C_{28}H_{24}N_6O_4$: C, 66.13; H, 4.76; N, 16.53. Found: C, 66.25; H, 4.70; N, 16.52. HRMS (ESI) *m/z* calcd. for $C_{28}H_{25}N_6O_4$: 509.1931 $[M+H]^+$, found: 509.1935.

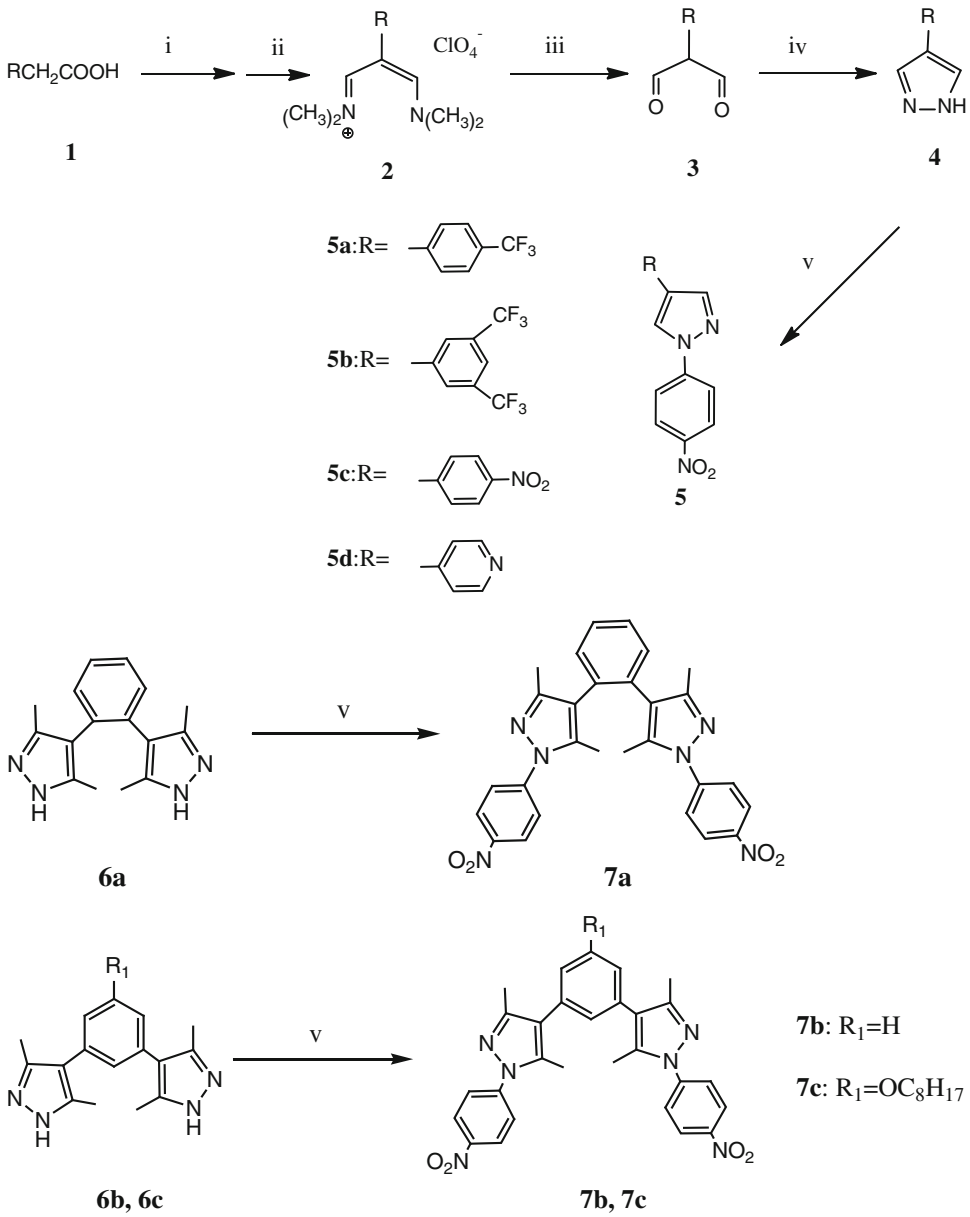
4,4′-(5-(Octyloxy)-1,3-phenylene)bis(3,5-dimethyl-1-(4-nitrophenyl)-pyrazole) ***7c***

Light yellow solid, yield 92 %. m.p. 165–166 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.39 (d, $J = 9.0$ Hz, 4H, $ArNO_2$–H), 7.90 (d, $J = 9.0$ Hz, 4H, $ArNO_2$–H), 6.91 (t, $J = 1.2$ Hz, 1H, Ar–H_2), 6.90 (d, $J = 1.2$ Hz, 2H, Ar–$H_{4,6}$), 4.07 (t, $J = 6.8$ Hz, 2H), 2.47 (s, 6H, CH_3), 2.32 (s, 6H, CH_3), 1.76 (m, 2H), 1.45 (m, 2H), 1.25–1.37 (m, 8H), 0.86 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 159.45, 149.18, 145.85, 144.94, 136.61, 134.40, 124.79, 123.85, 123.16, 122.92, 114.41, 68.28, 31.81, 29.38, 29.33, 29.24, 26.11, 22.65, 14.08, 12.84, 12.63. IR (cm^{-1}): 3095, 2919, 1594, 1506, 1428, 1340, 1193, 1110, 1018, 854, 750, 690; Anal. calcd. for $C_{36}H_{40}N_6O_5$: C, 67.91; H, 6.33; N, 13.20. Found: C, 67.90; H, 6.32; N, 13.20. HRMS (ESI) *m*/z calcd. for $C_{36}H_{41}N_6O_5$: 637.3132 $[M+H]^+$, found: 637.3131.

Determination of in vitro cytotoxic activity

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.



Scheme 1 Reagents and conditions: (*i*) DMF, $POCl_3$, 90–95 °C; (*ii*) $NaClO_4$; (*iii*) NaOH, 90 °C; (*iv*) $NH_2NH_2H_2O$, r.t.; (*v*) *p*-Fluoronitrobenzene, *t*-BuOK, DMSO, 72 °C, 2 h

Solutions

The compounds 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 (1983). Upon completion of the incubation for 48 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 protocol used to synthesize the title compounds is outlined in Scheme 1. The synthetic route to compounds **4a**–**4d** began with a Vilsmeyer–Haack-type reaction of substituted phenylacetic acid **1a**–**1d** with $POCl_3$/DMF to give **2a**–**2d**, which was isolated as its perchlorate salt. Hydrolysis of **2a**–**2d** followed by treatment of the resulting dialdehyde **3a**–**3d** (not isolated) with hydrazine furnished the pyrazole derivative **4a**–**4d** as light yellow solids (Lozan *et al*., 2007; Barton *et al*., 1980).

The synthesis of *N*-arylpyrazole derivatives **5a**–**5d** and **7a**–**7c** was performed by the literature method (Wang *et al*., 2000; Bouabdallah *et al*., 2007) 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 FT-IR, HRMS, ^{1}H NMR, ^{13}C NMR techniques, and elemental analysis.

Cytotoxicity

The in vitro cytotoxic activity of compounds **5a**–**5d** and **7a**–**7c** against Bel-7402, KB, HL-60, and BGC-823 was evaluated by MTT assay. The IC_{50} values were listed in Table 1 and the activities of Cisplatin were as a control. The results showed that all of the target compounds exhibited cytotoxicity against tested carcinoma cell lines with a lower IC_{50} value. The bis-pyrazole derivative **7c**, bearing alkoxy group on the 5-position of phenyl ring, was the most effective one. It's inhibition of cell growth of Bel-7402 cells was 1.5-fold higher than that found for cisplatin under the same experimental conditions. Among mono-pyrazole derivatives, compounds **5a** and **5b** also displayed better cytotoxicity than that of cisplatin against Bel-7402.

Table 1 The cytotoxicity of the compounds (**5a**–**5d** and **7a**–**7c**) against Bel-7402, KB, HL-60, and BGC-823

Compounds	IC_{50} (μM)			
	Bel-7402	KB	HL-60	BGC-823
5a	7.15	21.34	17.33	13.79
5b	7.39	11.99	11.40	18.52
5c	9.48	8.40	8.78	11.92
5d	23.17	19.16	17.80	20.75
7a	19.86	25.58	16.18	25.36
7b	11.75	10.07	10.62	11.92
7c	5.22	6.31	7.91	8.40
Cisplatin	8.12	2.65	2.29	6.48

Accordingly, for mono-pyrazole derivatives **5a–5d**, the structure–activity relationships are summarized as follows: (1) Phenyl substitution at 4-position of pyrazole ring makes the obtained compounds more toxic than pyridyl substitution. Compound **5a**–**5c** exhibited better activity than compound **5d** against Bel-7402, KB, HL-60, and BGC-823 cells. (2) The introduction of different electron withdrawing groups on the phenyl ring could obviously affect the cytotoxicity. Compounds **5a** and **5b** exhibited better activity than compound **5c** against Bel-7402 cells, but they showed weaker activity than compound **5c** against KB, HL-60, and BGC-823 cells. For bis-pyrazole derivatives **7a**–**7c**, the structure–activity relationships are summarized as follows: (1) *meta* bis-pyrazole derivatives are more toxic than *ortho* bis-pyrazole derivative. Compounds **7b** and **7c** exhibited better activity than compound **7a** against Bel-7402, KB, HL-60, and BGC-823 cells. (2) For *meta* bis-pyrazole derivatives, the alkoxy group on the 5-position of phenyl ring indeed influence its cytotoxicity. Compound **7c** decorated with octyloxy group on the 5-position of the phenyl ring showed better activity than compound **7b**.

Conclusions

A series of *N*-arylpyrazole derivatives was synthesized and their structures were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, elemental analyses, and mass spectrometry. The cytotoxicity of the obtained compounds against four human cancer cell lines was studied and showed a variable extent of IC_{50} values. The preliminary results have shown that modification of the substituents on phenyl ring with various electron-withdrawing substituents indeed influenced the cytotoxicity. Compounds **5a, 5b**, and **7c** exhibited better cytotoxicity than that of cisplatin against Bel-7402. It is important to point out that *meta* bis-pyrazole derivatives bearing octyloxy group on the 5-position of the phenyl ring is the most potent compound of the series against all cell lines. Further structure–activity studies are required to clearly elucidate the role of the substitution on the benzene ring.

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