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Design, synthesis and biological evaluation of some novel *N*-arylpyrazole derivatives bearing the sulfonamide moiety as cytotoxic agents

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Abstract A series of novel *N*-arylpyrazole derivatives (**4a–4l**) bearing the sulfonamide moiety were synthesized by the condensation reaction of 1,3-dicarbonyl compounds with 4-hydrazinylbenzenesulfonamide. The structures of the obtained compounds were established on the basis of elemental (C, H, and N) and spectral analysis (^{1}H NMR, ^{13}C NMR, ESIMS, and FT-IR). These compounds were tested for their in vitro cytotoxic activity against three human tumor cell lines: MCF-7, Hela, and A549. The results showed that most of the obtained compounds exhibited promising cytotoxicity against the tested cell lines with low IC_{50} values. The pyrazole derivative **4k**, bearing two methoxy groups on the 3-position and 4-position of the phenyl ring, was the most effective one. Its inhibition of cell growth of MCF-7 cells was better than that of celecoxib and cisplatin.

Keywords *N*-arylpyrazole · Sulfonamide · Anticancer · Cytotoxicity · Cancer cell lines

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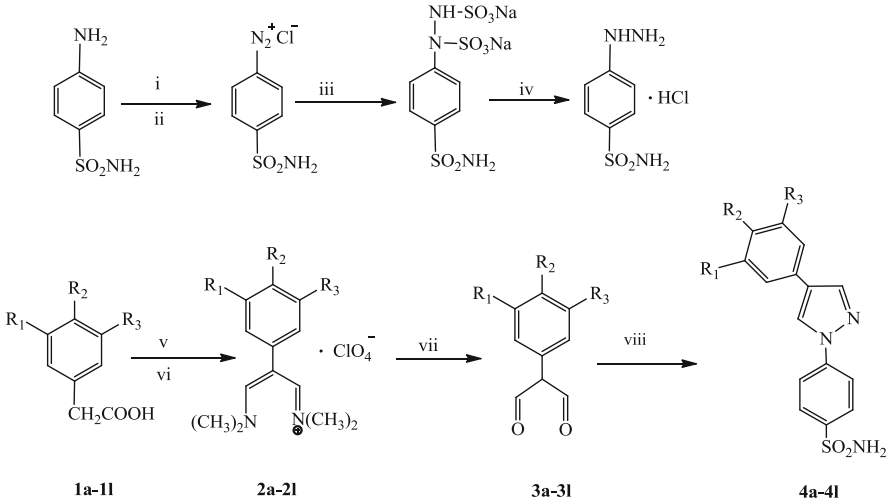
Introduction

Cancer is one of the most dreadful diseases in developed countries and many other parts of the world, currently remains the second leading cause of death and is expected to surpass heart disease as the leading cause in the next few years [1]. This serious major public health problem brings an enormous burden on society all over the world. Therefore, the discovery of selective, efficient, and safe drugs for cancer chemotherapy remains an urgent and high priority for medicinal research.

The presence of the pyrazole motif in commercial drugs or drug candidates has made this heterocycle a popular synthetic target for pharmaceutical and agrochemical sciences [2, 3]. Among the various pyrazoles, *N*-arylpyrazole derivatives play an important role in medicinal chemistry. Indeed, anti-inflammatory [4–7], antinociceptive [8], anti-obesity [9, 10], anti-allodynic [11], anticancer [12], and anti-HIV [13, 14] agents bearing the *N*-arylpyrazole moiety have been widely used as therapeutical drugs toward major therapeutical targets, such as cyclooxygenase (COX) [4–6], p38α MAP-kinase [7], σ_1 receptor (σ_1R) [8], cannabinoid hCB_1 and hCB_2 receptor [9, 10], COX-2/sEH [11], estrogen receptor [12] or HIV-reverse transcriptase [13, 14], respectively.

Sulfonamides constitute an important class of compounds which over the years have had different applications as pharmacological agents in the treatment of a wide variety of diseases [15, 16]. Indeed, they have also been reported to possess various types of biological properties such as anticancer [17], antibacterial [18], antimicrobial [19, 20], antimalarial [21], antihypertensive [22], antitumor [23], and anti-inflammatory activities [24–31], among others. Among these, the anticancer activity was found to take place through a variety of mechanisms such as disruption of the microtubule assembly, cell cycle arrest in the G1 phase, functional suppression of the transcriptional activator NF-Y, angiogenesis and carbonic anhydrase inhibition [15, 16]. The most prominent mechanism was the inhibition of carbonic anhydrase isozymes [32–35].

Moreover, a host of structurally novel *N*-arylpyrazole derivatives bearing the sulfonamide moiety have attracted considerable attention as they showed promising anticancer and antitumor properties, both in vitro and in vivo [36]. In fact, the most commonly used anti-inflammatory drug, celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfamide), possessing these structural features, displays important inhibitory capacity on not only cyclooxygenase-2 (COX-2) but also cancer-related human carbonic anhydrase IX (hCA IX) [37]. All these findings encouraged us to explore the synthesis of *N*-arylpyrazole derivatives bearing the sulfonamide moiety as potential cytotoxic agents. In this paper, novel *N*-arylpyrazole derivatives bearing the sulfonamide moiety were designed and synthesized by condensation reaction of 1,3-dicarbonyl compounds with 4-hydrazinylbenzenesulfonamide (Scheme 1). The cytotoxic activity of the obtained compounds **4a**–**4l** was evaluated in vitro against MCF-7, Hela, and A549 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and found to possess moderate activity.



Scheme 1 Reagents and condition: (i) H_2O, HCl, 80 °C; (ii) $NaNO_2$, below 0 °C; (iii) $NaHSO_3$, below 0 °C (iv) HCl, reflux; (v) DMF, $POCl_3$; (vi) $NaClO_4$; (vii) NaOH; (viii) 4-hydrazinylbenzenesulfonamide hydrochloride, ethanol, reflux. **4a**: $R_1 = R_2 = R_3 = H$; **4b**: $R_2 = CH_3$, $R_1 = R_3 = H$; **4c**: $R_2 = CF_3$, $R_1 = R_3 = H$; **4d**: $R_2 = NO_2$, $R_1 = R_3 = H$; **4e**: $R_2 = F$, $R_1 = R_3 = H$; **4f**: $R_2 = Cl$, $R_1 = R_3 = H$; **4g**: $R_2 = Br$, $R_1 = R_3 = H$; **4h**: $R_2 = OH$, $R_1 = R_3 = H$; **4i**: $R_2 = OCH_3$, $R_1 = R_3 = H$; **4j**: $R_1 = OCH_3$, $R_2 = R_3 = H$; **4k**: $R_1 = R_2 = OCH_3$, $R_3 = H$; **4l**: $R_1 = R_2 = R_3 = OCH_3$

Experimental

Materials and methods

RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT, benzylpenicillin and streptomycin were from Sigma. Three different human carcinoma cell lines, MCF-7, Hela and A549, were obtained from the American Type Culture Collection. Mps were measured on a XT-4 microscopic melting-point spectrometer and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVIII 600 NMR spectrometer. 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). Elemental analysis was determined on an Elementar Vario EL III elemental analyzer. 4-Hydrazinylbenzenesulfonamide hydrochloride was synthesized from 4-aminobenzenesulfonamide by diazotization and then reduction with sodium bisulfite in an overall yield of 44 % [38].

*General procedure for the preparation of dialdehyde compounds **3a–3l** [39–41]*

$POCl_3$ (15.0 mmol) was added dropwise to dry DMF (6 mL) at 5–10 °C with constant stirring. The mixture was stirred at room temperature for an additional hour. Then, substituted phenylacetic acid (4.95 mmol) **1a–1l** was added at once and the resulting clear solution was stirred for 4 h at 95 °C and then at room temperature overnight. The excess vilsmeyer reagent was decomposed with crushed ice, and

then a saturated solution of $NaClO_4H_2O$ 2.21 g (15 mmol) was added with stirring. The resulting nearly white crystalline deposit of the perchlorate salt **2a–2l** was filtered and washed with two 2.3-mL portions of water. This compound was used in the next step without further purification.

The perchlorate salt **2a–2l** (7.5 mmol) was added to a warm solution of 600 mg (15 mmol) NaOH in 5 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. Then, the reaction mixture was gradually cooled to room temperature and diluted with ice-water, acidified by dilute hydrochloric acid, and extracted with CH_2Cl_2. The organic phase was dried over anhydrous Na_2SO_4, filtrated, and then concentrated to give 1,3-dialdehydes compound as an oil-like crude product.

*General procedure for compounds **4a–4l***

4-Hydrazinylbenzenesulfonamide hydrochloride (201 mg, 0.9 mmol) was added to the stirred solution of aryl malondialdehyde (1 mmol) in 15 mL ethanol. The mixture was heated to reflux for 4 h. Then, the reaction mixture was cooled to room temperature and the precipitate was filtrated, dried, and recrystallized using ethyl alcohol as solvent.

*4-(4-Phenyl-1H-pyrazol-1-yl)benzenesulfonamide **4a*** Pink solid; yield 71.7 %; m.p. 258.1–259.2 °C; IR (KBr, cm^{-1}): 3326, 3126, 3068, 1598, 1510, 1404, 1336, 1155, 1099, 1037, 958, 906, 867, 829, 757, 691, 615, 544, 503; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.14 (s, 1H, pyrazole C_3-H), 8.33 (s, 1H, pyrazole C_5-H), 8.10 (d, $J = 9.0$ Hz, 2H, Ar), 7.97 (d, $J = 9.0$ Hz, 2H, Ar), 7.75 (dd, $J_1 = 7.8$ Hz, $J_2 = 1.2$ Hz, 2H, Ar), 7.45 (s, 2H, SO_2NH_2), 7.44 (t, $J = 7.8$ Hz, 2H, Ar), 7.29 (tt, $J_1 = 7.4$ Hz, $J_2 = 1.2$ Hz, 1H, Ar); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 141.56, 141.25, 139.60, 131.25, 128.96, 127.36, 126.94, 125.38, 124.85, 124.82, 118.14; MS(ESI): 300.9 $[M + H]^+$ 322.3 $[M + Na]^+$. Anal. calcd for $C_{15}H_{13}N_3O_2S$: C 60.19 H 4.38 N 14.04 Found: C 60.18 H 4.25 N 13.69. H_2O.

*4-(4-(p-Tolyl)-1H-pyrazol-1-yl)benzenesulfonamide **4b*** Light yellow solid; yield 81.4 %; m.p. 263.8–264.3 °C; IR (KBr, cm^{-1}): 3350, 3255, 1598, 1504, 1429, 1402, 1307, 1199, 1162, 1099, 954, 908, 876, 822, 752, 524, 449; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.08 (s, 1H, pyrazole C_3-H), 8.28 (s, 1H, pyrazole C_5-H), 8.08 (d, $J = 9.0$ Hz, 2H, Ar), 7.96 (d, $J = 9.0$ Hz, 2H, Ar), 7.64 (d, $J = 8.4$ Hz, 2H, Ar), 7.44 (s, 2H, SO_2NH_2), 7.24 (d, $J = 8.4$ Hz, 2H, Ar), 2.33 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 141.58, 141.15, 139.50, 136.19, 129.50, 128.37, 127.34, 125.30, 124.85, 124.42, 118.06, 20.68; MS(ESI): 336.7 $[M + Na]^+$. Anal. calcd for $C_{16}H_{15}N_3O_2S$: C 61.32 H 4.82 N13.41, Found: C 61.73 H 4.28 N 13.33.

*4-(4-(4-(Trifluoromethyl)phenyl)-1H-pyrazol-1-yl)benzenesulfonamide **4c*** Yellow solid; yield 62.0 %; m.p. 267.2–268.0 °C; IR (KBr, cm^{-1}): 3340, 3134, 3076, 1596, 1517, 1405, 1336, 1157, 1122, 1064, 1016, 960, 902, 841, 760, 694, 622, 543, 493; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.31 (s, 1H, pyrazole C_3-H), 8.45 (s, 1H,

pyrazole C_5-H), 8.11 (d, $J = 8.4$ Hz, 2H, Ar), 7.98 (d, $J = 8.4$ Hz, 2H, Ar), 7.97 (d, $J = 8.4$ Hz, 2H, Ar), 7.80 (d, $J = 8.4$ Hz, 2H, Ar), 7.45 (s, 2H, SO_2NH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 141.55, 141.38, 139.86, 135.53, 127.37, 127.01 (q, $^2J_{CF} = 33$ Hz), 125.97, 125.83, 125.79, 124.28 (q, $^1J_{CF} = 270$ Hz), 123.38, 118.34; MS(ESI): 390.3 $[M + Na]^+$. Anal. Calcd for $C_{16}H_{12}F_3N_3O_2S$: C 52.31 H 3.29 N11.44, Found: C 52.28 H 3.04 N 11.26.

4-(4-(4-Nitrophenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4d*** Gold solid; yield 70.0 %; m.p. 262.0–263.0 °C; IR (KBr, cm^{-1}): 3315, 3154, 1594, 1508, 1402, 1338, 1271, 1166, 1108, 903, 856, 833, 754, 620, 551, 441; ^{1}H NMR (600 MHz, DMSO-d6) δ: 9.44 (s, 1H, pyrazole C_3-H), 8.52 (s, 1H, pyrazole C_5-H), 8.30 (d, $J = 8.4$ Hz, 2H, Ar), 8.12 (d, $J = 9.0$ Hz, 2H, Ar), 8.04 (d, $J = 9.0$ Hz, 2H, Ar), 7.99 (d, $J = 8.4$ Hz, 2H, Ar), 7.48 (s, 2H, SO_2NH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 145.70, 141.74, 141.24, 140.18, 138.40, 127.39, 126.78, 126.04, 124.32, 122.85, 118.46; MS(ESI): 367.9 $[M + Na]^+$. Anal. Calcd for $C_{15}H_{12}N_4O_4S{\cdot}0.25$-$CH_3CH_2OH$: C 52.32 H 3.51 N16.27, Found: C 52.35 H 3.80 N 15.76.

4-(4-(4-Fluorophenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4e*** Light yellow solid; yield 87.9 %; m.p. 251.0–251.7 °C; IR (KBr, cm^{-1}): 3326, 3131, 1596, 1502, 1402, 1337, 1230, 1159, 957, 831, 770, 598, 542, 517; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.13 (s, 1H, pyrazole C_3-H), 8.31 (s, 1H, pyrazole C_5-H), 8.08 (d, $J = 9.0$ Hz, 2H, Ar), 7.97 (d, $J = 9.0$ Hz, 2H, Ar), 7.78 (dd, $J_1 = 9.0$ Hz, $J_2 = 5.4$ Hz, 2H, Ar), 7.45 (s, 2H, SO_2NH_2), 7.28 (t, $J = 9.0$ Hz, 2H, Ar); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 161.16 (d, $^1J_{CF} = 243.0$ Hz), 141.52, 141.28, 139.54, 127.84 (d, $^4J_{CF} = 3.0$ Hz), 127.36, 127.31 (d, $^3J_{CF} = 9.0$ Hz), 124.80, 123.90, 118.14, 115.76 (d, $^2J_{CF} = 22.5$ Hz); MS(ESI): 340.7 $[M + Na]^+$. Anal. Calcd for $C_{15}H_{12}FN_3O_2S$: C 56.77 H 3.81 N13.24, Found: C 57.11 H 3.52 N 13.12.

4-(4-(4-Chlorophenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4f*** Light yellow solid; yield 67.6 %; m.p. 254.0–255.5 °C; IR (KBr, cm^{-1}): 3384, 3336, 3268, 3120, 1593, 1487, 1402, 1323, 1155, 1097, 951, 831, 756, 662, 627, 540, 513, 442; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.18 (s, 1H, pyrazole C_3-H), 8.35 (s, 1H, pyrazole C_5-H), 8.08 (d, $J = 8.4$ Hz, 2H, Ar), 7.97 (d, $J = 8.4$ Hz, 2H, Ar), 7.78 (d, $J = 7.8$ Hz, 2H, Ar), 7.50 (d, $J = 7.8$ Hz, 2H, Ar), 7.45 (s, 2H, SO_2NH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 141.46, 141.37, 139.62, 131.25, 130.26, 128.89, 127.36, 127.08, 125.16, 123.65, 118.20; MS(ESI): 357.4 $[M + Na]^+$. Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C 53.98 H 3.62 N12.59, Found: C 54.16 H 3.29 N 12.45.

4-(4-(4-Bromophenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4g*** Brownish yellow solid; yield 59.0 %; m.p. 276.4–276.8 °C; IR (KBr, cm^{-1}): 3338, 3247, 1596, 1510, 1403, 1336, 1267, 1159, 1103, 1068, 954, 823, 660, 539, 505, 416; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.19 (s, 1H, pyrazole C_3-H), 8.35 (s, 1H, pyrazole C_5-H), 8.08 (d, $J = 9.0$ Hz, 2H, Ar), 7.97 (d, $J = 9.0$ Hz, 2H, Ar), 7.72 (d, $J = 8.4$ Hz, 2H, Ar), 7.63 (d, $J = 8.4$ Hz, 2H, Ar), 7.44 (s, 2H, SO_2NH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 141.45, 141.38, 139.59, 131.79, 130.63, 127.40, 127.36, 125.17,

123.68, 119.68, 118.21; ESI–MS *m/z*: 400.1 $[M + Na]^+$. Anal. Calcd for $C_{15}H_{12}BrN_3O_2S$: C 47.63 H 3.20 N11.11, Found: C 48.00 H 2.93 N 10.99.

4-(4-(4-Hydroxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4h*** Brownish yellow solid; yield 58.1 %; m.p. 301.8–304 °C; IR (KBr, cm^{-1}): 3321, 3127, 1597, 1504, 1402, 1344, 1298, 1267, 1199, 1149, 1097, 1027, 952, 910, 831, 787, 750, 661, 607, 548, 445; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.57 (s, 1H, pyrazole C_3-H), 8.97 (s, 1H,pyrazole C_5-H), 8.18 (s, 1H, OH), 8.07 (d, $J = 9.0$ Hz, 2H, Ar), 7.95 (d, $J = 9.0$ Hz, 2H, Ar), 7.54 (d, $J = 8.4$ Hz, 2H, Ar), 7.44 (s, 2H, SO_2NH_2), 6.83 (d, $J = 8.4$ Hz, 2H, Ar); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 156.43, 141.64, 140.96, 139.27, 127.34, 126.71, 125.08, 123.62, 122.15, 117.91, 115.72; MS(ESI): 338.6 $[M + Na]^+$.

Anal. Calcd for $C_{15}H_{13}N_3O_3S$: C 57.13 H 4.16 N13.33, Found: C 56.99 H 4.26 N13.23.

4-(4-(4-Methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4i*** Yellowish green solid; yield 75.3 %; m.p. 237.8–241.3 °C; IR (KBr, cm^{-1}): 3370, 3264, 1601, 1571, 1402, 1338, 1261, 1157, 1097, 1029, 952, 914, 825, 790, 752, 661, 605, 543, 457; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.03 (s, 1H, pyrazole C_3-H), 8.25 (s, 1H, pyrazole C_5-H), 8.08 (d, $J = 9.0$ Hz, 2H, Ar), 7.96 (d, $J = 9.0$ Hz, 2H, Ar), 7.67 (d, $J = 8.4$ Hz, 2H, Ar), 7.44 (s, 2H, SO_2NH_2), 7.00 (d, $J = 8.4$ Hz, 2H, Ar), 3.79 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 158.29, 141.61, 141.07, 139.37, 127.34, 126.68, 124.69, 123.96, 123.75, 117.99, 114.34, 55.08; MS(ESI): 352.6 $[M + Na]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C 58.35 H 4.59 N 12.76, Found: C 58.62 H 4.18 N 12.63.

4-(4-(3-Methoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4j*** Brown solid; yield 82.6 %; m.p. 221.8–222.2 °C; IR (KBr, cm^{-1}): 3316, 3068, 1594, 1506, 1466, 1405, 1344, 1226, 1166, 1103, 1029, 960, 896, 831, 786, 692, 619, 548, 459; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.15 (s, 1H, pyrazole C_3-H), 8.34 (s, 1H, pyrazole C_5-H), 8.09 (d, $J = 8.4$ Hz, 2H, Ar), 7.97 (d, $J = 8.4$ Hz, 2H, Ar), 7.44 (s, 2H, SO_2NH_2), 7.35 (d, $J = 7.2$ Hz, 1H, Ar), 7.33 (s, 2H, Ar), 6.85 (d, $J = 7.2$ Hz, 1H, Ar), 3.83 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 159.75, 141.54, 141.25, 139,74, 132,63, 130.05, 127.35, 125.05, 124.77, 118.12, 117.76, 112.56, 110.79, 55.09; MS(ESI): 352.6 $[M + Na]^+$. Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C 58.35 H 4.59 N 12.76, Found: C 59.08 H 4.24 N 12.70.

4-(4-(3,4-Dimethoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4k*** Chartreuse solid; yield 90.8 %; m.p. 256.5–258.6 °C; IR (KBr, cm^{-1}): 3321, 3150, 3072, 2991, 1596, 1506, 1404, 1342, 1247, 1162, 1101, 1025, 962, 900, 839, 796, 754, 665, 613, 547, 449; ^{1}H NMR (600 MHz, DMSO-d_6) δ: 9.06 (s, 1H, pyrazole C_3-H), 8.28 (s, 1H, pyrazole C_5-H), 8.07 (d, $J = 8.4$ Hz, 2H, Ar), 7.96 (d, $J = 8.4$ Hz, 2H, Ar), 7.43 (s, 2H, SO_2NH_2), 7.33 (d, $J = 1.8$ Hz, 1H, Ar), 7.27 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.8$ Hz, 2H, Ar), 7.00 (d, $J = 8.4$ Hz, 1H, Ar), 3.86 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 149.06, 147.89, 141.61, 141.05,

139.52, 127.34, 124.99, 124.16, 124.07, 117.94, 117.66, 112.18, 109.36, 55.57, 55.49; MS(ESI): 382.7 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{17}N_3O_4S$: C 56.81 H 4.77 N 11.96, Found: C 57.38 H 4.41 N 11.55.

4-(4-(3,4,5-Trimethoxyphenyl)-1H-pyrazol-1-yl)benzenesulfonamide ***4l*** Pale yellow solid; yield 89.1 %; m.p. 229.1–232.5 °C; IR (KBr, cm^{-1}): 3311, 3049, 1597, 1504, 1403, 1363, 1333, 1247, 1159, 1126, 1004, 960, 908, 831, 777, 626, 543, 451; ^1H NMR (600 MHz, DMSO-d_6) δ: 9.13 (s, 1H, pyrazole C_3-H), 8.35 (s, 1H, pyrazole C_5-H), 8.07 (d, $J = 9.0$ Hz, 2H, Ar), 7.97 (d, $J = 9.0$ Hz, 2H, Ar), 7.43 (s, 2H, SO_2NH_2), 7.06 (s, 2H, Ar), 3.87 (s, 6H, 2 × OCH_3), 3.68 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.30, 141.55, 141.17, 139.79, 136.48, 127.35, 127.01, 125.09, 124.83, 118.00, 102.90, 60.09, 55.98; MS(ESI): 390.1 $[M + H]^+$ 428.3 $[M + K]^+$. Anal. Calcd for $C_{18}H_{19}N_3O_5S$: C 55.52 H 4.92 N 10.79, Found: C 56.07 H 4.62 N 10.77.

Cytotoxic evaluation in vitro

Cell culture

Three different human carcinoma cell lines, MCF-7, Hela and A549, 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 compounds **4a–4l** 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 solutions. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1 % (v/v) in all experiments.

Cytotoxicity analysis

The cells harvested from the 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 [42]. Upon completion of the incubation for 48 h, stock MTT dye solution (20 μL, 5 mg/mL) was added to each well. After 4 h incubation, 2-propanol (100 μL) 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 (**4a**–**4l**) is outlined in Scheme 1. 4-Hydrazinylbenzenesulfonamide hydrochloride was synthesized from 4-aminobenzenesulfonamide by diazotization and then reduction with sodium bisulfite in an overall yield of 44 %. **2a**–**2l**, obtained as its perchlorate salt, were prepared by Vilsmeyer–Haack-type reaction of substituted phenylacetic acid **1a**–**1l** treated with $POCl_3$/DMF. Hydrolysis of **2a–2l** with sodium hydroxide aqueous solution gave the key intermediates **3a–3l**. The cyclization to pyrazole derivatives bearing a benzenesulfonamide moiety was afforded by the condensation of appropriate dialdehyde compounds **3a–3l** with 4-hydrazinobenzenesulfonamide hydrochloride in ethanol in 59–90 % yield.

The structures of **4a–4l** were determined using various spectral techniques such as ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectrometry, and elemental analysis. Spectral data IR, ^{1}H NMR, ^{13}C NMR and MS of compounds were found in full agreement with the proposed structure. In ^{1}H NMR spectra of these *N*-arylpyrazole derivatives, two "parts" corresponded to the benzenesulfonamide moiety and the substituted aromatic part of molecule were observed. The double doublets peaks at $\sim$8.08 and $\sim$7.97 ppm strongly suggest the phenyl ring of benzenesulfonamide. Another pair of ^{1}H doublets can be assigned to the *para*-substituted phenyl ring for most of the obtained compounds. Two one-proton singlet peaks at 9.02–9.57 and 8.24–8.97 can be assigned to the pyrazole ring protons. In addition, the signal for SO_2NH_2 was also observed as a two-proton singlet at δ 7.43–7.48. In the ^{13}C NMR spectra, aromatic carbon resonated between δ 102.9 and 161.16, while the C=N carbon resonated around δ 141.05–141.38. The IR spectra of these pyrazole derivatives displayed two absorption bands, at 3370–3311 and 3255–3126 cm^{-1}, indicative of the NH_2 group, in addition to the strong bands for SO_2N moiety at 1344–1333 and 1166–1155 cm^{-1}, and for C=N at 1601–1593 cm^{-1}. The structures of the above compounds (**4a**–**4l**) were further supported by their mass spectrometry and elemental analyses. The ESIMS of the sodium adduct of the obtained compounds **4a**–**4l** yielded molecular ion peaks $[M + Na]^+$ in the positive mode. Elemental analysis (C, H and N) data were within $\pm$0.4 % of the theoretical values.

Cytotoxicity

The in vitro cytotoxic activity of compounds **4a**–**4l** against MCF-7, Hela and A549 was evaluated by MTT assay. The IC_{50} values are listed in Table 1 and the activities of celecoxib and cisplatin were used as control. The results showed that all of the obtained compounds exhibited cytotoxicity against the tested carcinoma cell lines with a lower IC_{50} value. Compound **4k**, bearing the two methoxy groups on the 3-position and 4-position of the phenyl ring, was the most effective one. Its inhibition of cell growth of MCF-7 cells was 1.2-fold higher than that found for celecoxib under the same experimental conditions. Compound **4j** with the methoxy

Table 1 The cytotoxicity of the compounds (**4a–4l**) against MCF-7, Hela and A549 cells

Compounds	IC_{50} (μM)		
	MCF-7	Hela	A549
4a	14.46	19.30	25.52
4b	13.25	20.96	35.00
4c	27.26	28.72	52.62
4d	28.85	29.02	15.97
4e	31.09	25.99	10.04
4f	28.51	25.82	12.01
4g	17.75	24.22	32.12
4h	19.23	22.72	25.79
4i	30.98	24.86	58.94
4j	10.89	19.36	37.54
4k	10.40	16.95	24.48
4l	13.54	22.74	12.02
Celecoxib	12.34	7.76	21.84
Cisplatin	11.06	8.61	8.53

group on the phenyl ring was also more toxic to MCF-7 cells than celecoxib. The improved cytotoxic activity of **4b**, **4j**, **4k** and **4l** toward MCF-7 cells hinted that electron-donating groups on the benzene ring could enhance the cytotoxicity of *N*-arylpyrazole derivatives on comparing the activities with **4c**, **4d**, **4e**, **4f** and **4g** with electron-withdrawing groups. The position of the electron-donating group seems to have a great effect on cytotoxic activity. Comparing compounds **4j**, **4k**, **4l** and **4i**, it could be concluded that the substituted C'-3 position is essential for cytotoxicity against the MCF-7 cell line.

Conclusions

In this study, a series of novel *N*-arylpyrazole derivatives bearing the sulfonamide moiety were designed and synthesized, and their in vitro cytotoxicity was evaluated. The results showed that a number of the obtained compounds exhibited potent activity against three human cancer cell lines. Compounds **4j** and **4k** exhibited better cytotoxicity than that of celecoxib and cisplatin against MCF-7. The preliminary results show that the location and number of the methoxy group on the benzene ring had important effects on cytotoxic activity. These results provided a novel access to *N*-arylpyrazole derivatives bearing the sulfonamide moiety from simple commercially available substituted phenylacetic acid. In addition, the carbonic anhydrase (CA) IX activity of the obtained compounds needs to be further studied.

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