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ORIGINAL ARTICLE

Synthesis and evaluation of in vitro bioactivity for polysubstituted *N*-arylpyrazole derivatives

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KEYWORDS

Thiophene;
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Bioactivity study

Abstract New polysubstituted *N*-arylpyrazole derivatives were synthesized from *N*1-arylsydnone with acetylene and boronic acid, including 2-thiophenyl, 3-thiophenyl, 2-benzo[*b*]thiophenyl, or dibenzothiophenyl-4-boronic acid, via 1,3-dipolar cycloaddition and Suzuki coupling reaction. Based on the growth inhibitory activity results against lung carcinoma (NCI-H226), nasopharyngeal (NPC-TW01), and T-cell leukemia (Jurkat) cancer cells, compounds **5d** and **7d** with dibenzothiophenyl bioisostere possessed the significant inhibitory activity for NPC-TW01 (32 μM and 16 μM) and NCI-H226 (16 μM and 8.9 μM), respectively.

1. Introduction

Pyrazole-containing compounds exhibit significant biological properties such as anti-cancer (Bandgar et al., 2010; Puthiyapurayil et al., 2012; Sun et al., 2013; Sangani et al., 2014), antihyperglycemic, analgesic, anti-inflammatory (Bandgar et al., 2010), antipyretic, antibacterial (Sangani et al., 2014), anticonvulsant, antidepressant, hypoglycemic, gastric secretion stimulatory, sedative-hypnotic activities (Stauffer et al., 2000, 2001; Baraldi et al., 2005; Singh et al., 2006). For example, several members of pyrazole-contained bioactive molecules were manufactured as the lead compounds or commercial drugs such as Ethiprol (Vidau et al., 2009), Fipronil (Vidau et al., 2009), Pyrazofurin (Olah et al., 1980), and Ribavirin (Manns et al., 2001). Recent investigation incorporated a pyrazole core in A_{2A} receptor antagonists, CB1 receptor antagonists, DNA intercalating agents, and estrogen receptor ligands (Baraldi et al., 2003; Pastorin et al., 2003; Lauria et al., 2008; Slee et al., 2008; Wang et al., 2008;

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Fustero et al., 2009; Kawashita and Hayashi, 2009; Romanelli and Autino, 2009). Thus, the modified pyrazole compounds have often offered the flexibility for design and construction of the structural analogs of biomedical interest and also are considered as the attractive targets for organic synthesis.

Heterocycles often seemed to be perfect bioisosteres; accordingly they can deliver equal or even better biological efficacy through their similarity in structural shape and electronic distribution (Sagara et al., 1995; Sharma et al., 2011). Based on the facts, the fused thiophene (Arroyo and Salas-Puig, 2001; Noguchi et al., 2004; Molvi et al., 2007; Ashalatha et al., 2007; Rai et al., 2008) and benzothiophene (Connor et al., 1992; Boschelli et al., 1994, 1995; Butera et al., 1995) moiety became the great potential subject of interest (Ashalatha et al., 2007; Rai et al., 2008; Roman, 2015; Sharma et al., 2011; Tu et al., 2014). For example, presently available active antiepileptic drugs (AEDs) such as tiagabine (Arroyo and Salas-Puig, 2001), etizolam (Polivka et al., 1984) and brotizolam (Noguchi et al., 2004) contain thiophene moiety in their structures. On the other hand, the fused benzothiophene derivatives have been shown to exert both anti-inflammatory and anti-HIV effects (Critchfield et al., 1997). PD144795, a new fused benzothiophene derivative, can block the adhesion of neutrophils to human umbilical vein endothelial cells and inhibit the expression of both E-selectin and ICAM-1 molecules (Carballo et al., 2002). Furthermore, the specific benzothiophene-substituted oxime ether strobilurins possessed the potent exhibition of fungicidal activities at a concentration of 0.39 mg/L compared to Enoxastrobin (Tu et al., 2014).

The goal of this work was to prepare a series of polysubstituted *N*-arylpyrazole derivatives utilizing thiophenyl and benzothiophene as bioisostere for investigating the attractive structural targets. Based on the structure–activity study and the biological assay, compounds **5d** and **7d** with dibenzothiophenyl moiety were indicated that they possessed the significant inhibitory activity for NPC-TW01 and NCI-H226 two cancer cells.

2. Experimental

2.1. Material and physical measurements

2.1.1. General procedure

All chemicals were reagent grade and used as purchased. All reactions were carried out under nitrogen atmosphere and monitored by TLC. Flash column chromatography was carried out on silica gel (230–400 mesh). Toluene and *p*-xylene were purchased from Merck Chemical Co., and Dichloromethane, chloroform, tetrahydrofuran, ethanol and methanol were purchased from Fluka & Aldrich. Phenylacetylene, diphenylacetylene, 2-thiopheneboronic acid, 3-thiopheneboronic acid, 2-benzothienylboronic acid and dibenzothiophene-4-boronic acid were purchased from Arcos Chemical Co., Potassium carbonate was purchased from TCI Chemical Co., Infrared (IR) spectra were measured on a Bomem Michelson Series FT-IR spectrometer. The wavenumbers reported are referenced to the polystyrene 1601 cm^{-1} absorption. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. UV–visible spectra were measured with a HP 8452A diode-array spectrophotometer. Photoluminescence (PL) spectra were obtained on a Perkin-Elmer fluorescence spectrophotometer (LS 55). Proton NMR spectra were obtained on a Bruker AC-300 (300 MHz) spectrometer by the use of DMSO-d_6 as the solvent. Carbon-13 NMR spectra were obtained on a Bruker AC-300 (75 MHz) spectrometer by using DMSO-d_6 as solvent. Carbon-13 chemical shifts are referenced to the center of the DMSO-d_6 sextet (δ 39.6 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz). Elemental analyses were carried out on a Heraeus CHN–O RAPID element analyzer.

2.2. Standard procedure for synthesis of pyrazoles (*2*, *3* and *4*) via 1,3-dipolar cyclization

A solution of 3-arylsydnone (**1a** or **1b**, 0.20 g, 1.0 equiv) in 4 mL *p*-xylene was added with acetylene, including phenylacetylene, diphenylacetylene, or 1-bromo-4-ethynylbenzene (1.05 equiv) and heated to reflux for 24 h. When the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove *p*-xylene. The residue solution was purified by column chromatography on silica gel (elution with 30:70 EtOAc/hexane) to give the corresponding pyrazoles **2**, **3**, and **4** in 71% (R_f = 0.28), 47% (R_f = 0.26), 74% (R_f = 0.26) isolated yields. Another minor regioisomer of compounds **2** and **4** was also obtained in 11% (R_f = 0.32) and 12% (R_f = 0.30) yields, respectively (Chang et al., 2006; Foster et al., 2011, 2012).

2.2.1. 1-(4-Bromophenyl)-3-phenyl-1H-pyrazole (*2*)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.78 (d, *J* = 2.4 Hz, 1H, ArH), 7.36 (t, *J* = 7.2 Hz, 1H, ArH), 7.44 (t, *J* = 7.5 Hz, 2H, ArH), 7.57 (d, *J* = 8.7 Hz, 2H, ArH), 7.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.90–7.92 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.4, 119.4, 120.3, 125.8, 127.8, 128.2, 128.7, 132.4, 132.8, 139.1, 153.2; FABMS *m*/*z* (%): 300 (M^+ + H, 100), 299 (M^+, 66) Anal. Calcd for $C_{15}H_{11}BrN_2$; C: 60.22; H: 3.71; N: 9.36; found: C: 60.21; H: 3.67; N: 9.39.

2.2.2. 1-(4-Bromophenyl)-3,4-diphenyl-1H-pyrazole (*3*)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33–7.35 (m, 8H, ArH), 7.57–7.61 (m, 4H, ArH), 7.69 (d, *J* = 8.8 Hz, 2H, ArH), 7.99 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz); δ 119.6, 120.2, 123.4, 126.4, 127.1, 128.1, 128.3, 128.4, 128.5, 128.7, 132.4, 132.5, 132.8, 138.9, 150.8; FABMS *m*/*z* (%): 376 (M^+ + H, 100), 375 (M^+, 72); Anal. Calcd for $C_{21}H_{15}BrN_2$; C: 67.21; H: 4.03; N: 7.47; found: C: 67.23; H: 4.02; N:7.45.

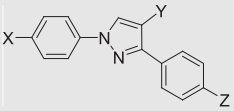
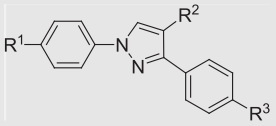
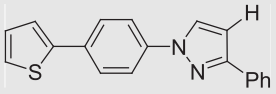
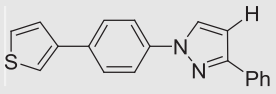
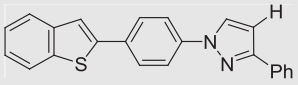
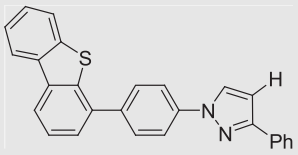
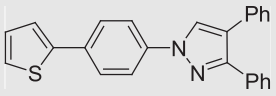
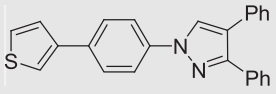
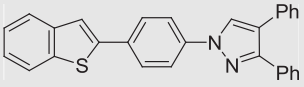
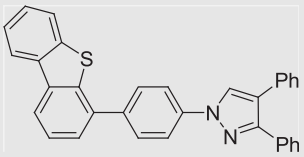
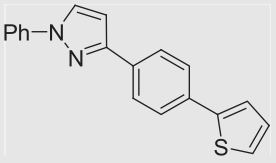
2.2.3. 3-(4-Bromophenyl)-1-phenyl-1H-pyrazole (*4*)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.73 (d, *J* = 2.9 Hz, 1H, ArH), 7.30 (t, *J* = 7.2 Hz, 1H, ArH), 7.47 (t, *J* = 7.5 Hz, 2H, ArH), 7.56 (d, *J* = 8.1 Hz, 2H, ArH), 7.74–7.81 (m, 4H, ArH), 7.93 (d, *J* = 3.0 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 104.9, 119.0, 121.9, 126.5, 127.3, 128.1, 129.4, 131.7, 132.0, 140.0, 151.7; FABMS *m*/*z* (%): 300 (M^+ + H, 100), 299 (M^+,36); Anal. Calcd for $C_{15}H_{11}BrN_2$; C: 60.22; H: 3.71; N: 9.36; found: C: 60.18; H: 3.72; N: 9.37.

2.3. Synthesis of fused-thiophenyl/phenylpyrazoles ***5a–d***, ***6a–d***, and ***7a–d*** via palladium(0)-catalyzed cross-coupling reaction

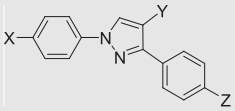
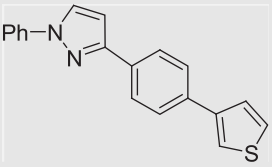
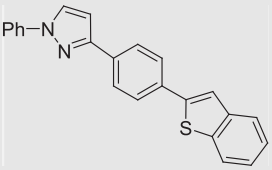
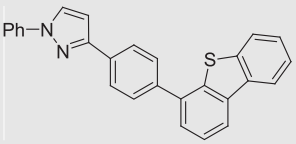
A mixture of 1,3-diaryl-1*H*-pyrazoles (**2**, **3**, or **4**, 5.46 mmol, 1.0 equiv), fused-thiophenyl-2-boronic acid (8.20 mmol, 2.0 equiv),

Table 1 The results of synthesis of polysubstituted *N*-arylpyrazole derivatives **5a–d**, **6a–d**, and **7a–d.**

Pyrazoles				Boronic acid $RB(OH)_2$	Polysubstituted *N*-arylpyrazole		
							
No.	X	Y	Z	R	No.		Yields (%)
2	Br	H	H	2-Thiophenyl	**5a**		82
2	Br	H	H	3-Thiophenyl	**5b**		85
2	Br	H	H	2-Benzo[*b*]thio-phenyl	**5c**		79
2	Br	H	H	Dibenzothio-phenyl	**5d**		81[a]
3	Br	Ph	H	2-Thiophenyl	**6a**		85
3	Br	Ph	H	3-Thiophenyl	**6b**		87
3	Br	Ph	H	2-Benzo[*b*]thio-phenyl	**6c**		76
3	Br	Ph	H	Dibenzothio-phenyl	**6d**		72
4	H	H	Br	2-Thiophenyl	**7a**		81

(*continued on next page*)

Table 1 (*continued*)

Pyrazoles				Boronic acid $RB(OH)_2$	Polysubstituted *N*-arylpyrazole		
							
4	H	H	Br	3-Thiophenyl	**7b**		88
4	H	H	Br	2-Benzo[*b*]thio-phenyl	**7c**		80
4	H	H	Br	Dibenzothio-phenyl	**7d**		83[a]

[a] Characterization data for compounds **5d** and **7d** were shown in reference (Roman G. 2015).

tetrakis(triphenylphosphine)palladium (0.199 mmol, 0.037 equiv), potassium carbonate (8 ml of 2 M aq. solution; 16.0 mmol, 3.0 equiv), and *p*-xylene/EtOH (40/20 ml) was heated under stirring to reflux for 24 h in nitrogen atmosphere. The mixture was concentrated, added with water (10 ml), and extracted with dichloromethane (3 × 50 ml). The combined organic solution was washed with a saturated aqueous sodium hydrogen carbonate solution (20 ml), brine (20 ml), dried with anhydrous magnesium sulfate and evaporated. The residue was purified by column chromatography (silica gel, elution with hexane), and fractions containing the product were collected and evaporated. Crystallization from ethyl acetate/EtOH afforded the corresponding fused thiophenyl/phenylpyrazole and benzothiophene/phenylpyrazole products **5a**–**d**, **6a**–**d**, and **7a**–**d**.

*2.3.1. 3-Phenyl-1-(4-(thiophen-2-yl)phenyl)-1H-pyrazole (**5a**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (d, J = 2.4 Hz, 1H, ArH), 7.09–7.12 (m, 1H, ArH), 7.30–7.38 (m, 3H, ArH), 7.45 (t, J = 7.5 Hz, 2H, ArH), 7. 7.70 (d, J = 8.4 Hz, 2H, ArH), 7.79 (d, J = 8.4 Hz, 2H, ArH), 7.93–7.98 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.2, 119.3, 123.2, 125.0, 125.9, 126.8, 127.9, 128.1, 128.6, 128.7, 132.5, 132.9, 139.2, 143.4, 153.0; FABMS *m*/*z* (%): 303 (M^+ + H, 100), 302 (M^+, 68); Anal. Calcd for $C_{19}H_{14}N_2S$; C: 75.47; H: 4.67; N: 9.26; found: C: 75.50; H: 4.65; N: 9.29.

*2.3.2. 3-Phenyl-1-(4-(thiophen-3-yl)phenyl)-1H-pyrazole (**5b**)*

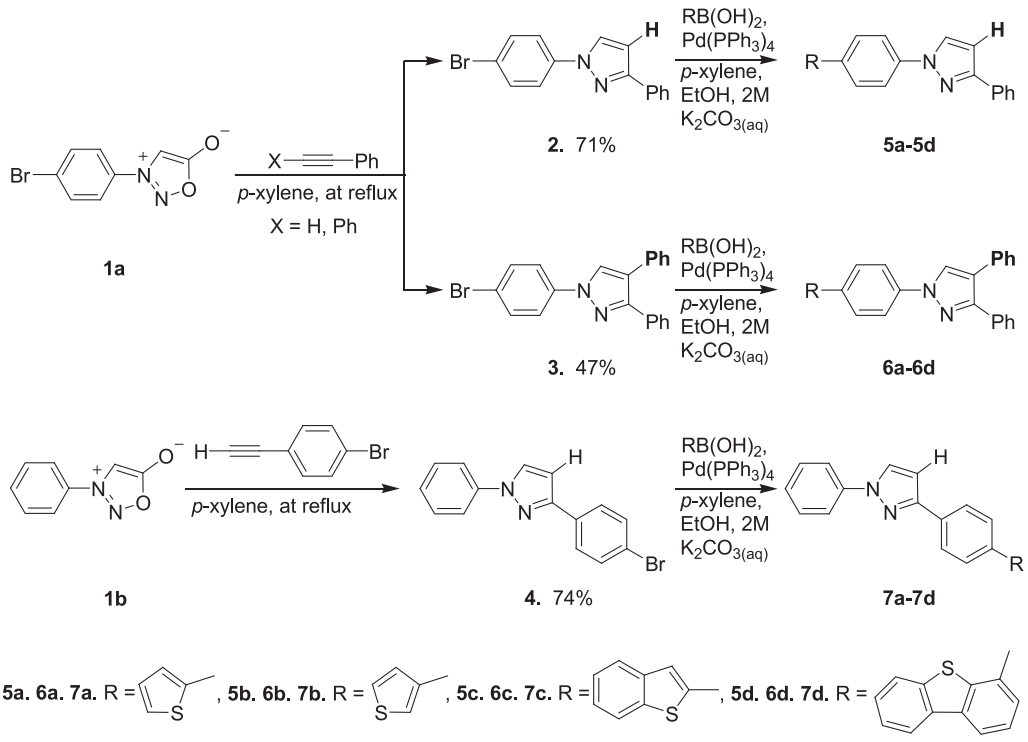
^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (d, J = 2.7 Hz, 1H, ArH), 7.35 (t, J = 7.2 Hz, 1H, ArH), 7.42–7.50 (m, 5H, ArH), 7.69 (d, J = 8.7 Hz, 2H, ArH), 7.80 (d, J = 8.7 Hz, 2H, ArH), 7.93–7.98 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.1, 119.3, 120.3, 125.9, 126.2, 126.5, 127.3, 127.9, 128.1, 128.7, 133.0, 134.0, 139.0, 141.3, 152.9; FABMS *m*/*z* (%): 303 (M^+ + H, 100), 302 (M^+, 71); Anal. Calcd for $C_{19}H_{14}N_2S$; C: 75.47; H: 4.67; N: 9.26; found: C: 75.43; H: 4.69; N: 9.23.

*2.3.3. 1-(4-(Benzo[b]thiophen-2-yl)phenyl)-3-phenyl-1H-pyrazole (**5c**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.81 (d, J = 2.4 Hz, 1H, ArH), 7.30–7.38 (m, 3H, ArH), 7.45 (t, J = 7.8 Hz, 2H, ArH), 7.58 (s, 1H, ArH), 7.78–7.86 (m, 6H, ArH), 7.93 (t, J = 6.8 Hz, 2H, ArH), 8.00 (d, J = 2.4 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.4, 119.2, 119.6, 122.3, 123.6, 124.5, 124.6, 125.9, 127.4, 127.9, 128.2, 128.7, 123.9, 139.5, 139.8, 140.7, 143.1, 153.2; FABMS *m*/*z* (%): 353 (M^+ + H, 100), 352 (M^+, 59); Anal. Calcd for $C_{23}H_{16}N_2S$; C: 78.38; H: 4.58; N: 7.95; found: C: 78.39; H: 4.61; N: 7.91.

*2.3.4. 1-(4-(Dibenzothiophen-4-yl)phenyl)-3-phenyl -1H-pyrazole (**5d**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.82 (d, J = 2.7 Hz, 1H, ArH), 7.38 (t, J = 7.2 Hz, 1H, ArH), 7.45–7.60 (m, 6H, ArH), 7.83–7.86 (m, 3H, ArH), 7.92–8.02 (m, 5H, ArH), 8.16–8.22 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.3, 119.2, 120.6, 121.7, 122.6, 124.4, 125.1, 125.9, 126.8, 126.9, 128.0, 128.1, 128.7, 129.3, 133.0, 135.7, 136.0, 136.3, 138.4, 138.5, 139.4,



Scheme 1

139.7, 153.1; FABMS *m/z* (%): 403 (M^+ + H, 100), 402 (M^+, 76); Anal. Calcd for $C_{27}H_{18}N_2S$; C: 80.57; H: 4.51; N: 6.96; found: C: 80.62; H: 4.56; N: 6.92.

*2.3.5. 3,4-Diphenyl-1-(4-(thiophen-2-yl)phenyl)-1H-pyrazole (**6a**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.11 (t, *J* = 4.2 Hz, 1H, ArH), 7.30–7.35 (m, 10H, ArH), 7.60–7.63 (m, 2H, ArH), 7.72 (d, *J* = 8.4 Hz, 2H, ArH), 7.81 (d, *J* = 8.7 Hz, 2H, ArH), 8.04 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 119.1, 123.1, 123.2, 125.0, 126.4, 126.8, 127.0, 128.0, 128.1, 128.3, 128.4, 128.5, 128.7, 132.6, 132.7, 133.0, 138.9, 143.4, 150.6; FABMS *m/z* (%): 379 (M^+ + H, 100), 378 (M^+, 47); Anal. Calcd for $C_{25}H_{18}N_2S$; C: 79.33; H: 4.79; N: 7.40; found: C: 79.35; H: 4.81; N: 7.45.

*2.3.6. 3,4-Diphenyl-1-(4-(thiophen-3-yl)phenyl)-1H-pyrazole (**6b**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30–7.38 (m, 8H, ArH), 7.42–7.44 (m, 2H, ArH), 7.49–7.51 (m, 1H, ArH), 7.60–7.64 (m, 2H, ArH), 7.71 (d, *J* = 8.4 Hz, 2H, ArH), 7.83 (d, *J* = 8.7 Hz, 2H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 119.2, 120.4, 123.0, 126.1, 126.4, 126.5, 127.0, 127.3, 127.9, 128.3, 128.4, 128.5, 128.7, 132.8, 133.0, 134.0, 138.7, 141.3, 150.5; FABMS *m/z* (%): 379 (M^+ + H, 100), 378 (M^+, 69); Anal. Calcd for $C_{25}H_{18}N_2S$; C: 79.33; H: 4.79; N: 7.40; found: C: 79.34; H: 4.81; N: 7.39.

*2.3.7. 1-(4-(Benzo[b]thiophen-2-yl)phenyl)-3,4-diphenyl-1H-pyrazole (**6c**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.32–7.39 (m. 10H, ArH), 7.58–7.62 (m, 3H, ArH), 7.78–7.92 (m, 6H, ArH), 8.06 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 119.1, 119.6, 122.3, 123.3, 123.6, 124.5, 124.6, 126.4, 127.0, 127.4, 128.0, 128.3, 128.4, 128.5, 128.7, 132.4, 132.7, 132.9, 139.4, 139.5, 140.7, 143.1, 150.8; FABMS *m/z* (%): 429 (M^+ + H, 100), 428 (M^+, 47); Anal. Calcd for $C_{29}H_{20}N_2S$; C: 81.28; H: 4.70; N: 6.54; found: C: 81.26; H: 4.72; N: 6.51.

*2.3.8. 1-(4-(Dibenzothiophen-4-yl)phenyl)-3,4-diphenyl -1H-pyrazole (**6d**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36–7.39 (m, 8H, ArH), 7.47–7.58 (m, 4H, ArH), 7.67 (t, *J* = 2.1 Hz, 2H, ArH), 7.84–7.88 (m, 3H, ArH), 7.95 (d, *J* = 8.7 Hz, 2H, ArH), 8.09 (s, 1H, ArH), 8.19–8.22 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 119.1, 120.6, 121.7, 122.6, 123.1, 124.4, 125.1, 126.6, 126.8, 126.9, 127.0, 128.0, 128.3, 128.4, 128.5, 128.7, 129.3, 132.7, 133.0, 135.7, 135.9, 136.3, 138.4, 138.6, 139.3, 139.4, 150.6; FABMS *m/z* (%): 479 (M^+ + H, 100), 478 (M^+, 41); Anal. Calcd for $C_{33}H_{22}N_2S$; C: 82.81; H: 4.63; N: 5.85; found: C: 82.82; H: 4.67; N: 5.88.

*2.3.9. 1-Phenyl-3-(4-(thiophen-2-yl)phenyl)-1H-pyrazole (**7a**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (d, *J* = 2.4 Hz, 1H, ArH), 7.09–7.12 (m, 1H, ArH), 7.28–7.33 (m, 2H, ArH), 7.37 (d, *J* = 3.6 Hz, 1H, ArH), 7.48 (t, *J* = 8.1 Hz, 2H, ArH), 7.68 (d, *J* = 8.4 Hz, 2H, ArH), 7.77 (d, *J* = 7.8 Hz, 2H, ArH), 7.92–7.97 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.0, 119.1, 123.1, 124.8, 126.1, 126.2, 126.4, 128.1, 129.4, 132.2, 134.0, 140.2, 144.2, 152.4, 154.1; FABMS *m/z* (%): 303 (M^+ + H, 100), 302 (M^+, 35); Anal. Calcd for $C_{19}H_{14}N_2S$; C: 75.47; H: 4.67; N: 9.26; found: C: 75.45; H: 4.69; N: 9.31.

*2.3.10. 1-Phenyl-3-(4-(thiophen-3-yl)phenyl)-1H-pyrazole (**7b**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.80 (d, *J* = 2.7 Hz, 1H, ArH), 7.30 (t, *J* = 7.2 Hz, 1H, ArH), 7.39–7.51 (m, 5H, ArH), 7.67

Table 2 The inhibitory activity of the polysubstituted *N*-arylpyrazole derivatives **5a**–**d**, **6a**–**d**, and **7a**–**d**.

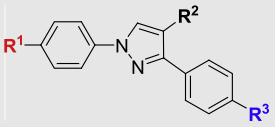
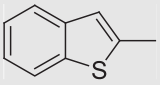
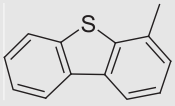
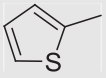
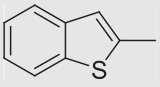
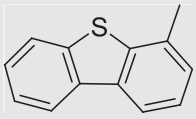
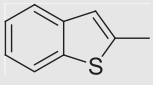
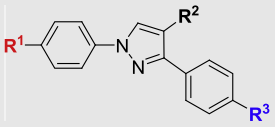
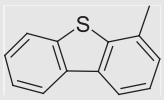
Compounds				GI_{50} (μM)[a,b]		
	R^1	**R^2**	**R^3**	NPC-TW01	NCI-H226	Jurkat
5a		H	Ph	> 50	> 50	> 50
5b		H	Ph	> 50	> 50	> 50
5c		H	Ph	34	> 50	> 50
5d		H	Ph	32	16	> 50
6a		Ph	Ph	43	32	> 50
6b		Ph	Ph	> 50	> 50	> 50
6c		Ph	Ph	> 50	> 50	> 50
6d		Ph	Ph	> 50	> 50	> 50
7a	Ph	H		> 50	> 50	> 50
7b	Ph	H		> 50	46	> 50
7c	Ph	H		> 50	> 50	> 50

Table 2 (*continued*)

Compounds				GI_{50} (μM)[a,b]		
	R^1	R^2	R^3	NPC-TW01	NCI-H226	Jurkat
7d	Ph	H		16	8.9	> 50
N′-(4-formyl-1,3-diphenyl-1*H*-pyrazol-5-yl)-*N*,*N*- dimethyl-methanimidamide				31.4	9.3	23.5

[a] NCI-H226: human lung carcinoma; NPC-TW01: human nasopharyngeal carcinoma; Jurkat: human T-cell leukemia.
[b] All tested compounds were dissolved in 100% DMSO at a concentration of 20 mM as the stock solution. Cells were cultured without or in the presence of the *N*-arylpyrazole derivatives at different concentrations for 72 h. Cell survival was determined by MTT assay. Drug molar concentration causing 50% cell growth inhibition (GI_{50}) was calculated. Each value represents the mean ± SD of three independent experiments.

(d, J = 8.1 Hz, 2H, ArH), 7.79 (d, J = 7.8 Hz, 2H, ArH), 7.95–7.97 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.0, 119.1, 120.2, 126.2, 126.3, 126.4, 126.6, 128.0, 129.4, 131.9, 135.4, 140.2, 142.0, 152.5, 154.3; FABMS *m*/*z* (%): 303 (M^+ + H, 100), 302 (M^+, 43); Anal. Calcd for $C_{19}H_{14}N_2S$; C: 75.47; H: 4.67; N: 9.26; found: C: 75.50; H: 4.871; N: 9.23.

2.3.11. 3-(4-(Benzo[b]thiophen-2-yl)phenyl)-1-phenyl-1H-pyrazole (7c)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.82 (d, J = 2.7 Hz, 1H, ArH), 7.29–7.39 (m, 3H, ArH), 7.49 (t, J = 7.8 Hz, 2H, ArH), 7.61 (s, 1H, ArH), 7.78–7.90 (m, 6H, ArH), 7.97–8.02 (m, 3H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.1, 119.1, 119.4, 122.3, 123.5, 124.3, 124.5, 124.9, 126.3, 126.5, 126.7, 128.1, 129.3, 129.5, 133.0, 139.5, 140.1, 152.6; FABMS *m*/*z* (%): 353 (M^+ + H, 100), 352 (M^+, 24); Anal. Calcd for $C_{23}H_{16}N_2S$; C: 78.38; H: 4.58; N: 7.95; found: C: 78.40; H: 4.59; N: 7.92.

2.3.12. 3-(4-(Dibenzothiophen-4-yl)phenyl)-1-phenyl-1H-pyrazole (7d)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.85 (d, J = 2.7 Hz, 1H, ArH), 7.32 (t, J = 7.5 Hz, 1H, ArH), 7.47–7.58 (m, 6H, ArH), 7.81–7.88 (m, 5H, ArH), 7.99 (d, J = 2.7 Hz, 1H, ArH), 8.09 (d, J = 8.4 Hz, 2H, ArH), 8.16–8.22 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.2, 119.0, 120.5, 121.7, 122.6, 124.3, 125.1, 126.2, 126.4, 126.7, 126.8, 128.1, 128.6, 129.4, 132.8, 135.8, 136.3, 136.7, 138.5, 139.6, 140.2, 152.5; FABMS *m*/*z* (%): 403 (M^+ + H, 100), 402 (M^+, 28); Anal. Calcd for $C_{27}H_{18}N_2S$; C: 80.57; H: 4.51; N: 6.96; found: C: 80.54; H: 4.56; N: 6.94.

2.4. Antiproliferative activity

2.4.1. Cell lines

Human non-small cell lung carcinoma (NCI-H226), T-cell leukemia (Jurkat) and nasopharyngeal carcinoma (NPC-TW01) were purchased from Bioresource Collection and Research Center (BCRC, Taiwan). All the tumor cell lines were maintained in either RPMI-1640 or Modified essential medium (MEM) supplied with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2/95% air in the presence of penicillin and streptomycin.

2.4.2. Anti-proliferative efficacy determination

Modified MTT method was used to determine the efficacy of anti-tumor activity and the GI_{50} value was calculated (Ref.). Briefly, logarithmic growth cells were seeded into 96-well plates and were subsequently treated with vehicle or various concentrations of tested compounds for 72 h. Two hours before the end of the incubation, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) at a final concentration of 5 μg/ml was added. Afterward, solubilization buffer (40% DMF and 20% SDS in H_2O) was added to wells to dissolve violet formazan precipitation for overnight at 37 °C. The absorbance at 570 nm was then detected by a microplate reader (Molecular Device, Sunnyvale, CA) and the GI_{50} value was calculated by linear regression analysis (Shia et al., 2011).

3. Result and discussion

3.1. Synthesis of fused thiophenyl/phenylpyrazoles and fused benzothiophene/phenylpyrazoles 5a–d, 6a–d, and 7a–d

Scheme 1 shows the synthetic routes for the fused thiophenyl/phenylpyrazole and benzothiophene/phenylpyrazole derivatives **5a–d**, **6a–d**, and **7a–d**. *N*1-Arylsydnones **1a** and **1b** were prepared following the procedure developed by our laboratory (Yeh et al., 1983). *N*1-Phenylsydnone **1a** and *N*1-(*p*-bromophenyl)sydnone **1b** were reacted with acetylenes including phenylacetylene, diphenylacetylene, and 1-bromo-4-ethynylbenzene to give the corresponding 1,3-diaryl-1*H*-pyrazoles **2–4** as light yellow solids in 47–74% yields (see Scheme 1, Dumitrascu et al., 2002). In the smooth and regioselective 1,3-dipolar cycli-

zation to synthesize compounds **2** and **4**, two regioisomers with pyrazolyl core can be individually accomplished (Chang et al., 2006), except for compound **3**. Identification of regioisomers of compounds **2** and **4** was made on the basis of its characteristic 1D ^{1}H NMR spectrum. Particular attention was given to the absorption of pyrazole proton. The ring proton (4-H) absorption of the 3-aryl-substituted isomers **2** and **4** moved 0.11–0.12 ppm upfield relative to the 3-H in the 4-aryl-substituted isomers (Chang et al., 2006). In all cases, the ratios of a mixture of pyrazole regioisomers (**2** or **4**) were obtained in ~7/1 and 3-aryl-substituted pyrazole isomers **2** and **4** were obtained as the major products in 71% and 74% yields, respectively (see Scheme 1). Another minor regioisomer of compounds **2** and **4** was also obtained in 11% and 12% yields, respectively (Chang et al., 2006; Foster et al., 2011, 2012). Following the previous literature, the substituted groups of alkynes were the significant factors for the regioselectivity in the cycloaddition reaction (Foster et al., 2011, 2012).

1-(*p*-Bromophenyl)-3-phenyl-1*H*-pyrazole **2**, 1-(*p*-bromophenyl)-3,4-diphenyl-1*H*-pyrazole **3**, and 1-phenyl-3-(*p*-bromophenyl)-1*H*-pyrazole **4** were individually presented in the palladium(0)-catalyzed cross-coupling reaction (Miyaura and Suzuki, 1995) with fused-heterocycle boronic acids, including 2-thiophenyl, 3-thiophenyl, 2-benzo[*b*]thiophenyl, or dibenzothiophenyl-4-boronic acid (Qian et al., 1999). The reaction mixture was accomplished by heating in a solution of *p*-xylene/EtOH/aqueous potassium carbonate system. After the reaction was completed, the normal work-up and purification were performed to give the corresponding polysubstituted *N*-arylpyrazole products **5a–d**, **6a–d**, and **7a–d** in 79–85%, 72–85%, and 80–88% yields (see Table 1, and Scheme 1).

3.2. Biological activity

Based on our previous literature reported data, 1,3-diphenyl-1*H*-pyrazole analogs showed the remarkable potential antitumor activity (Cheng et al., 2010; Wen et al., 2012; Huang et al., 2012). Therefore, a set of polysubstituted *N*-arylpyrazoles **5a–d**, **6a–d**, and **7a–d** compounds were synthesized to evaluate against a panel of human cancer cell lines in vitro, including lung carcinoma (NCI-H226), nasopharyngeal (NPC-TW01), and T-cell leukemia (Jurkat) cells. The GI_{50} value indicates the concentration of the compound that results in a 50% decrease in the cell growth relative to the vehicle. To test the substituted effect, the 1,5-disubstituted *N*-arylpyrazole compounds **5a–d** bearing with 2-thiophenyl, 3-thiophenyl, 2-benzo[*b*]thiophenyl, or dibenzothiophenyl at the *para*-position in *N*-1 phenyl of pyrazolic ring were preliminarily evaluated. Following the inhibitory results, 2-benzo[*b*]thiophenylpyrazole **5c** and 1-[3-(4-dibenzothienyl)phenyl]-4-phenyl-1*H*-pyrazole **5d** showed the better inhibitory activity against NCI-H226 cancer cell (~32–34 μM, see Table 2). Particularly, 1-[3-(4-dibenzothienyl)phenyl]-4-phenyl-1*H*-pyrazole **5d** also possessed significant inhibition for lung carcinoma cells (NCI-H226, ~16 μM, Table 2).

For the further SAR study, a series of trisubstituted *N*-arylpyrazoles **6a–d** with introducing a phenyl group at *C*-4 position on pyrazolic ring was used as the comparing model (see Table 2). Based on the biological activity data of compound **6a–d**, only compound **6a** possessed the negligible inhibitory activity against NPC-TW01 (43 μM) and NCI-H226 (32 μM, see Table 2). This experimental result indicated the gifting phenyl group at *C*-4 position on pyrazolic ring was not promoted the inhibitory activity.

Based on the inhibition result between compounds **5a–d** and compounds **6a–d**, we consequently introduced thiophenyl, benzothiophenyl and dibenzothiophenyl groups toward the *para*-position of *C*-3 phenyl moiety and removed *C*-4 phenyl group in the pyrazolic ring. Among others, compounds **7a–d** showed poor inhibitory potency against three cancer cell lines, except for dibenzothiophenyl/phenylpyrazole **7d.** Compound **7d** showed significant inhibition against lung carcinoma cell (NCI-H226, 16 μM, Table 2) and nasopharyngeal cell (NPC-TW01, 8.9 μM, see Table 2). As a result, dibenzothiophenyl group was regarded as the best active bioisostere to be introduced into the phenylpyrazole core molecule for the construction of the potent lead compounds. In the other hand, compounds **5d** and **7d** seemed to possess the significant inhibitory activity for NPC-TW01 and NCI-H226 two cancer cells, particularly for NCI-H226.

Following the data presented in Table 2 and our previous research results (Cheng et al., 2010; Wen et al., 2012; Huang et al., 2012), we could arrive at the conclusion that the activity of the tested compounds may be correlated to the variation and modifications of structure. For example, the substituents on the *C*-4 position of pyrazolic ring were not suitable for the antitumor activity comparing to other positions. In addition, change of substituents of *para*-phenyl on the *N*-1 or *C*-3 position of pyrazolic ring could also affect the activities of these compounds. A comparison of the *para*-substituents on the *N*-1 or *C*-3 phenyl demonstrated that dibenzothiophenyl group could dramatically improve anti-proliferative activity, particularly on the *C*-3 phenyl group. On the basis of our previous research, it was revealed that the class of 1*H*-pyrazol-5-yl-*N*,*N*-dimethylformamidines inhibitors possessed exclusively better anti-cancer inhibitory activity than 1,3-diphenyl-1*H*-pyrazoles, especially for NPC-TW01 and Jurkat (Cheng et al., 2010).

4. Conclusion

A series of polysubstituted *N*-arylpyrazole derivatives were synthesized from *N*1-arylsydnone with acetylene and boronic acid via 1,3-dipolar cycloaddition and Suzuki coupling reaction. 2-Thiophenyl, 3-thiophenyl, 2-benzo[*b*]thiophenyl, and dibenzothiophenyl groups were introduced into the *N*-arylpyrazole core as the bioisosteres. Following the structure–activity relationship study, 4-dibenzothiophenyl group was regarded as the best active bioisostere for the improvement of the inhibitory activity. Furthermore, compounds **5d** and 7d with dibenzothiophenyl moiety possessed the significant inhibitory activity for NPC-TW01 (32 μM and 16 μM) and NCI-H226 (16 μM and 8.9 μM) two cancer cells, respectively.

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