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RESEARCH ARTICLE

## Design, synthesis, and *in vitro* antiproliferative and kinase inhibitory effects of pyrimidinylpyrazole derivatives terminating with arylsulfonamido or cyclic sulfamide substituents

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### Abstract

A novel series of substituted pyrimidine compounds bearing *N*-phenylpyrazole and terminating with aryl and cyclic sulfonamido moiety were designed, synthesized, and evaluated *in vitro* as antiproliferative agents against a panel of 53 cell lines of different tissues at the NCI. Among them, compound **1d** with *p*-chlorobenzenesulfonamido terminal moiety, ethylene spacer, and 4-chloro-3-methoxyphenyl ring at position 3 of the pyrazole nucleus showed the highest mean percentage inhibition value over the whole cancer cell line panel at 10  $\mu$ M concentration. It showed broad-spectrum antiproliferative activity over many cell lines of different cancer types. For instance, compound **1d** inhibited the growth of HL-60 (TB), SR leukemia, and T-47D and MCF-7 breast cancer cell line by 135.92%, 119.44%, 95.32%, and 82.03% at 10  $\mu$ M, respectively. And it inhibited the growth of COLO 205 colon, HT29 colon, BT-549 breast, and ACHN renal cancer cell lines by more than 80% at the same test concentration. However, testing compound **1d** upon determining its IC<sub>50</sub> against the most sensitive cell lines showed to good extent selectivity against HT29 colon cancer cell line than HL-60 leukemia and MRC-5 lung fibroblasts (normal cells). Compound **1d** was further tested against 12 kinases of different kinase families, and the highest inhibitory effect was exerted against RAF1, V600E-B-RAF, and V600K-B-RAF kinases.

### Introduction

Cancer is considered the second leading cause of death after cardiovascular diseases all over the world<sup>1,2</sup>. It is a collection of serious diseases caused by uncontrolled growth of cells invading surrounding tissues and spread to other parts of the body. In the statistics according to new report from the World Health Organization's International Agency for Cancer Research (IARC) suggests that the incidence of cancer worldwide will grow by 75%, nearly doubling in some of the developing countries<sup>3</sup>. Those increases will add much more burden on the poorly developed health-care systems in such countries. That is why intensive research should be done in this field for getting more potent drugs with less toxic side effects.

Many research articles have recently reported the potential antiproliferative activity of sulfonamide and cyclic sulfonamide moieties against a variety of cancer cell lines<sup>4–10</sup>.

### Keywords

Antiproliferative activity, arylsulfonamide, 1,3,4-triarylpyrazole, RAF kinases

### History

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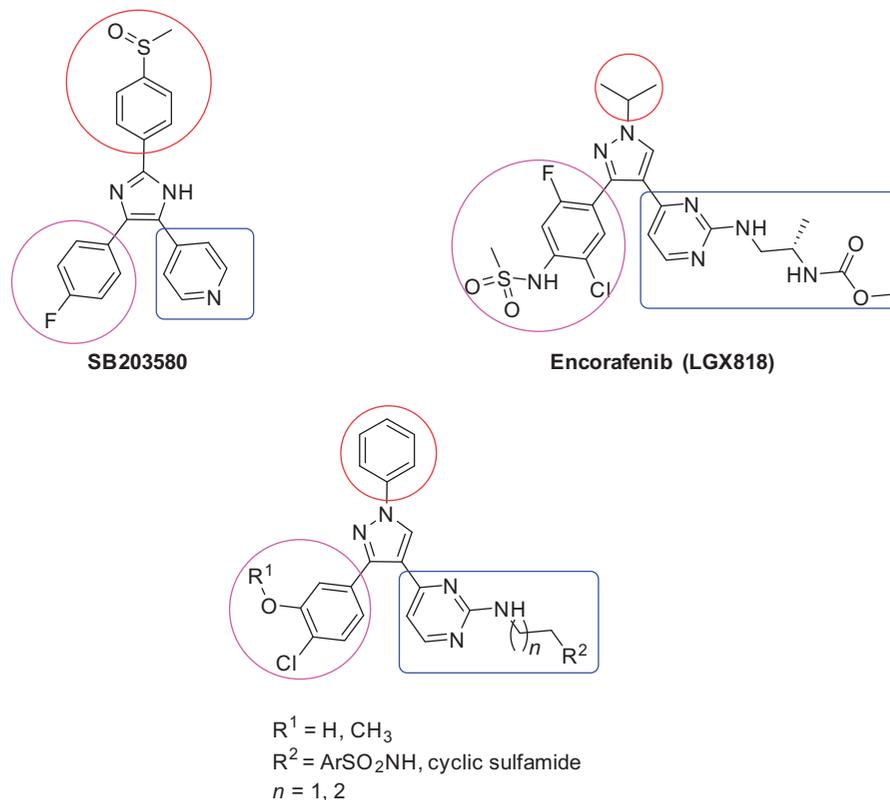
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In addition, much attention has been paid to the chemistry and biological activities of 1,3,4-triarylpyrazole scaffold<sup>11–15</sup>. In the present study, a new series of 1,3,4-triarylpyrazole derivatives containing terminal arylsulfonamido or cyclic sulfamide moieties were designed with similarity to SB203580 and Encorafenib (LGX818) (Figure 1). SB203580 was reported to bind to P38/MAP kinase via hydrophobic and hydrogen bonding interactions of the fluorophenyl ring with a hydrophobic region, hydrogen bonding of the pyridyl and imidazole rings with the kinase hinge region, and the methylsulfinylphenyl ring with the phosphate binding region<sup>16,17</sup>. There is another hydrophobic region below the pyridyl ring which was not occupied by SB203580<sup>16</sup>. That's why the terminal chain on the pyridyl ring was important. Our target compounds were also designed similar to Encorafenib through ligand-based design approach. The *N*-phenyl ring on the pyrazole ring mimics the *N*-isopropyl group of Encorafenib and estimated to undergo similar interaction. The two aryl rings at positions 3 and 4 of the pyrazole ring mimic the two aryl rings at the same positions on the Encorafenib structure. And the side chain on the pyridyl ring mimics that of Encorafenib with replacement of the terminal methoxycarbonyl moiety of Encorafenib with arylsulfonyl or cyclic sulfamide moiety.

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Figure 1. Structures of SB203580, Encorafenib (LGX818), and rationale design to the target compounds **1a–l** and **2a–l**.



In the present study, we report synthesis of a new series of 1,3,4-triarylpyrazole scaffold having amino pyrimidine moiety similar to that part present in compound B which have been reported as an important fragment presented in many antiproliferative agents<sup>18–20</sup> instead of pyridine and the aminopyrimidine was substituted with ethylene or propylenediamino aryl or cyclic sulfonamide derivatives to test their anticancer activities as illustrated in (Figure 1). Their *in vitro* antiproliferative activities against NCI-60 cancer cell line panel are reported.

## Experimental

### Chemistry

#### General

The target compounds were purified by column chromatography using silica gel (0.040–0.063 mm, 230–400 mesh) and technical grade solvents. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Bruker Avance 400 or 300 spectrometer (Bruker Bioscience, Billerica, MA) using tetramethylsilane as an internal standard. IR analysis (KBr discs, New Jersey, NJ) was carried out using a Bruker FT-IR instrument (Bruker Bioscience, Billerica, MA). Mass spectra (MS) were determined by LC-MS analysis using the following system: Waters 2998 photodiode array detector, Waters 3100 mass detector, Waters SFO system fluidics organizer, Waters 2545 binary gradient module, Waters reagent manager, Waters 2767 sample manager, Sunfire™ C18 column (4.6 × 50 mm, 5 μm particle size) (Waters, UK); solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) in water; solvent B: 0.035% TFA in CH<sub>3</sub>OH; flow rate = 3.0 mL/min. Solvents and liquid reagents were transferred using hypodermic syringes. Purity percentage of all the target compounds were determined by LC-MS and found to be >95%. The high-resolution mass spectrometry was done on positive mode with capillary: 3.1 KV, desolvation gas: 800 L/h, and extraction cone 4.0. Chromatography was performed on

ACQUITY UPLC BEH C18 (1.6 μm, 2.1 × 100 mm) (Waters, Milford, MI) at 25 °C using acetonitrile and water (containing 0.1% formic acid as mobile phase in gradient elution mode at a flow rate of 0.4 mL/min, and injection volume: 5 μL. The data acquisition was under the control of Mass Hunter workstation software. Melting points were obtained on a Walden Precision Apparatus Electrothermal 9300 apparatus (Barnstead, UK) and are uncorrected. Solvents and liquid reagents were transferred using hypodermic syringes. All solvents and reagents were commercially available and used without further purification.

Synthesis of the ethylenediamine and propylenediamine sulfonamides side chain compounds **3a–l** was carried out through a pathway as previously reported<sup>19</sup>.

#### Chloro-2-methoxy-4-methylbenzene (5)

To a solution of 2-chloro-5-methylphenol (**4**, 21.5 g, 150 mmol), dimethyl sulfate (20.8 g, 165 mmol) in acetone (250 mL), anhydrous K<sub>2</sub>CO<sub>3</sub> (51.8 g, 375 mmol) was added. The reaction mixture was heated under reflux for 3 h. The mixture was filtered, and the filtrate was evaporated under reduced pressure. Water (150 mL) was added to the residue, and the resulting mixture was carefully extracted with ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and filtered. The organic solvent was evaporated under reduced pressure, and the residue was used in the next step without further purification. MS *m/z*: 157.5 [M<sup>+</sup>+H].

#### Chloro-3-methoxybenzoic acid (6)

A stirred mixture of compound **5** (11.17 g, 71 mmol), potassium permanganate (35.0 g, 221 mmol), pyridine (36 mL), and water (107 mL) was heated at 50 °C for 24 h. The mixture was then stirred at room temperature for 13 h. The mixture was filtered and MnO<sub>2</sub> was suspended in hot water and again filtered off. The

combined aqueous filtrates were washed with ethyl acetate (3 × 75 mL), and then acidified with 2 N H<sub>2</sub>SO<sub>4</sub>. The precipitate was filtered off, washed with water, and dried to give the title compound (11.98 g, 90%). mp: 215–216 °C (lit. mp: 217–219 °C<sup>21</sup>).

#### Methyl 4-chloro-3-methoxybenzoate (7)

To a solution of the acid **6** (1.0 g, 5.4 mmol) in MeOH (40 mL) at 0 °C, acetyl chloride (1.9 mL, 28.1 mmol) was added dropwise at this temperature and the reaction mixture was then stirred at room temperature for 15 h. After evaporation of the organic solvent, the residue was purified by flash column chromatography (silica gel, hexane–ethyl acetate 5:1 v/v) to give **4** (0.91 g, 85%) as a crystalline solid. mp: 49–50 °C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.62–7.55 (m, 3H), 3.94 (s, 3H), 3.88 (s, 3H).

#### 1-(4-Chloro-3-methoxyphenyl)-2-(2-(methylthio)pyrimidin-4-yl)ethan-1-one (8)

To a solution of compound **7** (1.0 g, 5.0 mmol) and, 4-methyl-2-(methylthio)pyrimidine (0.78 g, 5.6 mmol) in THF (5 mL) in a cooled bath at –78 °C, LHMDs (3.7 mL, 1.0 M solution in THF, 19.9 mmol) was slowly added to maintain the temperature at –78 °C. The resulting mixture was stirred overnight at room temperature. After disappearance of the starting material, the mixture was quenched with saturated aqueous NH<sub>4</sub>Cl. Ethyl acetate (20 mL) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layer extracts were washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The organic solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (silica gel, hexane ethyl acetate 1:1 v/v then switching to hexane–ethyl acetate 1:5 v/v) to yield compound **6** (0.58 g, 45%). mp: 85–88 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.24–8.23 (m, 1H), 7.65–7.60 (m, 1H), 7.36–7.21 (m, 4H), 6.95–6.93 (m, 1H), 6.84–6.82 (m, 1H), 3.95 (s, 5H), 2.65 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 194.4, 157.2, 156.4, 155.4, 155.1, 135.8, 134.9, 130.3, 118.8, 112.8, 109.5, 56.3, 47.9, 14.1.

#### 4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-2-(methylthio)pyrimidine (9)

Compound **8** (1.1 g, 3.8 mmol) was added to DMF-DMA (5.14 mL, 38.2 mmol) and the mixture was stirred at 80 °C for 3 h. The resulting solution was concentrated to dryness to furnish oil which was used in the next step without purification. To a portion of the oil from the previous step (0.137 g, 0.457 mmol) in EtOH (3 mL) was added phenylhydrazine (98 mg, 0.9 mmol). The reaction mixture was stirred overnight at room temperature. Water (5 mL) was added to the reaction mixture and the organics were extracted with ethyl acetate (3 × 5 mL). The combined organic layer extracts were washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexane–ethyl acetate 1:1 v/v then switching to hexane–ethyl acetate 1:5 v/v) to yield compound **9** (1.18 g, 81%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.30 (s, 1H), 8.26 (d, 1H, *J* = 5.2 Hz), 7.36–7.21 (m, 5H), 6.82–6.80 (m, 2H), 6.71 (d, 1H, *J* = 6.4 Hz), 3.68 (s, 3H), 2.34 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.5, 158.9, 156.9, 155.1, 141.2, 140.6, 139.1, 130.6, 129.4, 128.9, 128.0, 125.3, 123.7, 123.3, 120.0, 114.1, 112.5, 56.2, 13.7.

#### 4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-2-(methylsulfonyl)pyrimidine (10)

To a solution of compound **9** (2.4 g, 6 mmol) in methanol (250 mL), a solution of oxone (12.3 g, 18 mmol) in water (50 mL)

was added. The mixture was stirred at room temperature for 16 h. The organic solvent was evaporated under reduced pressure and the remaining aqueous solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and the organic layer was separated. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 25 mL) and the combined organic layer extracts were washed with brine, dried over anhydrous MgSO<sub>4</sub>, and filtered. The organic solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography. 2.5 g of the title compound was obtained, yield 98%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.49 (s, 1H), 8.39 (d, 1H, *J* = 5.2 Hz), 7.46–7.29 (m, 5H), 6.92–6.85 (m, 2H), 6.79 (d, 1H, *J* = 6.0 Hz), 3.71 (s, 3H), 2.89 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 173.5, 159.1, 157.1, 156.1, 141.4, 140.8, 139.1, 130.7, 129.6, 128.9, 128.1, 125.3, 123.7, 123.3, 120.0, 114.1, 112.5, 56.5, 43.1.

#### General procedure for preparation of the target compounds 1a–l

A mixture of mesyl compound **10** (0.2 g, 0.5 mmol), amino side chain compound **3a–l** (1.2 mmol), and diisopropylethylamine (0.29 mL, 1.7 mmol) in DMSO (5 mL) was stirred at 80 °C for 16 h. The mixture was cooled to room temperature, quenched with water (20 mL), then extracted with ethyl acetate (3 × 20 mL). The combined organic layer extracts were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, appropriate ratio of hexane–ethyl acetate) to obtain the pure target compounds.

#### *N*-(2-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide (1a)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.21 (s, 1H), 8.02 (d, 1H, *J* = 5.2 Hz), 7.76 (d, 2H, *J* = 8.0 Hz), 7.51–7.21 (m, 9H), 6.81 (d, 1H, *J* = 8.0 Hz), 6.76 (s, 1H), 6.35 (brs, 1H), 5.85 (t, 1H, *J* = 6.4 Hz), 3.66 (s, 3H), 3.32 (brs, 2H), 3.05 (brs, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 162.3, 159.7, 157.4, 155.0, 141.0, 140.5, 140.0, 139.2, 132.4, 129.0, 128.9, 128.0, 126.9, 125.3, 120.5, 114.2, 107.7, 56.3, 43.6, 40.9.

#### *N*-(2-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzenesulfonamide (1b)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.21 (s, 1H), 8.03 (d, 1H, *J* = 5.2 Hz), 7.65 (d, 2H, *J* = 8.0 Hz), 7.33–7.20 (m, 8H), 6.81 (dd, 1H, *J* = 8.0, 1.2 Hz) 6.76 (d, 1H, *J* = 1.2 Hz), 6.35 (brs, 1H), 5.77 (t, 1H, *J* = 6.4 Hz), 3.67 (s, 3H), 3.31 (brs, 2H), 3.05 (brs, 2H), 2.37 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 162.3, 159.7, 157.8, 155.0, 143.2, 141.0, 140.5, 139.2, 130.5, 129.8, 129.6, 128.9, 128.0, 126.9, 125.3, 123.4, 120.5, 114.2, 107.7, 56.2, 43.6, 40.8, 21.5; LC-MS (*m/z*): 574.8 (M<sup>+</sup>).

#### *N*-(2-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-4-methoxybenzenesulfonamide (1c)

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.21 (s, 1H), 8.03 (d, 1H, *J* = 5.2 Hz), 7.70 (dd, 2H, *J* = 9.2, 2.0 Hz), 7.34–7.21 (m, 6H), 6.90–6.87 (m, 2H), 6.82 (dd, 1H, *J* = 8.4, 2.0 Hz), 6.35 (brs, 1H), 5.77 (t, 1H, *J* = 6.4 Hz), 3.81 (s, 3H), 3.67 (s, 3H), 3.33 (brs, 2H), 3.05 (brs, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 162.7, 162.3, 159.7, 157.8, 155.0, 141.0, 140.5, 139.3, 130.5, 129.8, 129.1, 128.9, 128.0, 125.3, 123.4, 120.5, 114.2, 114.1, 107.7, 56.2, 55.6, 43.6, 40.8.

**4-Chloro-N-(2-((4-(3-(4-chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide (1d)**

IR (KBr disc,  $\text{cm}^{-1}$ ): 3378, 3264, 3088, 2931, 2855, 1577, 1499;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.36 (brs, 1H), 8.09 (d, 1H,  $J = 5.2$  Hz), 7.76 (d, 3H,  $J = 8.0$  Hz), 7.62 (d, 2H,  $J = 8.4$  Hz), 7.41–7.28 (m, 6H), 6.82 (d, 1H,  $J = 1.2$  Hz), 6.92–6.86 (m, 2H), 6.47 (brs, 1H), 5.76 (s, 1H), 3.67 (s, 3H), 3.07 (brs, 2H), 2.70 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.3, 161.1, 159.7, 157.8, 154.7, 141.3, 141.1, 140.5, 139.7, 139.6, 137.7, 130.4, 129.7, 129.4, 128.9, 128.5, 126.1, 124.0, 120.8, 115.5, 107.9, 56.7, 55.4, 42.2; LC-MS ( $m/z$ ): 594.8 ( $\text{M}^+$ ).

**N-(2-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-3-fluorobenzenesulfonamide (1e)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.22 (s, 1H), 8.02 (d, 1H,  $J = 5.2$  Hz), 7.54 (d, 2H,  $J = 8.0$  Hz), 7.43–7.18 (m, 9H), 6.82 (d, 1H,  $J = 8.0$  Hz), 6.77 (s, 1H), 6.38 (brs, 1H), 5.87 (t, 1H,  $J = 6.4$  Hz), 3.67 (s, 3H), 3.31 (brs, 2H), 3.05 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.6, 162.3, 161.1, 159.7, 157.8, 155.0, 142.1, 141.1, 140.5, 139.2, 130.8, 130.4, 129.7, 128.9, 128.0, 126.9, 125.3, 123.4, 122.0, 120.3, 119.7, 119.5, 114.3, 114.1, 107.8, 56.2, 43.8, 40.8.

**N-(3-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (1f)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.19 (s, 1H), 8.06 (d, 1H,  $J = 5.2$  Hz), 7.68 (brs, 2H), 7.51–7.24 (m, 9H), 6.85–6.80 (m, 2H), 6.40 (brs, 1H), 5.85 (t, 1H,  $J = 6.4$  Hz), 3.67 (s, 3H), 3.20 (brs, 2H), 2.89 (brs, 2H), 1.58 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.5, 159.8, 157.9, 154.9, 141.3, 140.3, 140.2, 139.3, 132.3, 130.3, 129.6, 128.9, 128.1, 126.8, 125.6, 123.3, 120.5, 114.6, 107.6, 56.3, 39.9, 37.3, 30.1.

**N-(3-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-4-methylbenzenesulfonamide (1g)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.19 (s, 1H), 8.06 (d, 1H,  $J = 5.2$  Hz), 7.71 (d, 2H,  $J = 8.0$  Hz), 7.37–7.25 (m, 8H), 6.85 (dd, 1H,  $J = 8.0, 1.2$  Hz), 6.72 (d, 1H,  $J = 1.2$  Hz), 6.40 (brs, 1H), 5.67 (t, 1H,  $J = 6.4$  Hz), 3.66 (s, 3H), 3.08 (brs, 2H), 2.89 (brs, 2H), 2.34 (s, 3H), 1.58 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.2, 159.8, 157.9, 154.9, 143.1, 141.2, 140.4, 139.1, 130.4, 129.8, 129.6, 128.9, 128.0, 126.9, 125.3, 123.4, 120.5, 114.2, 107.7, 56.2, 43.6, 40.8, 30.3, 21.5.

**N-(3-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-4-methoxybenzenesulfonamide (1h)**

IR (KBr disc,  $\text{cm}^{-1}$ ): 3380, 3275, 3091, 2928, 1580, 1500;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.20 (s, 1H), 8.08 (d, 1H,  $J = 5.2$  Hz), 7.72 (dd, 2H,  $J = 9.2, 2.0$  Hz), 7.33–7.19 (m, 6H), 6.92–6.82 (m, 3H), 6.41 (brs, 1H), 5.67 (t, 1H,  $J = 6.4$  Hz), 3.80 (s, 3H), 3.65 (s, 3H), 3.08 (brs, 2H), 2.88 (brs, 2H), 1.58 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.5, 162.1, 159.8, 157.7, 155.0, 141.1, 140.4, 139.2, 130.5, 129.8, 129.1, 128.9, 128.0, 125.3, 123.4, 120.5, 114.2, 114.1, 107.7, 56.3, 55.7, 39.6, 37.3, 29.2; LC-MS ( $m/z$ ): 604.9 ( $\text{M}^+$ ).

**4-Chloro-N-(3-((4-(3-(4-chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (1i)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.34 (brs, 1H), 8.11 (d, 1H,  $J = 4.8$  Hz), 7.76 (d, 3H,  $J = 8.4$  Hz), 7.66 (d, 1H,  $J = 8.4$  Hz),

7.41–7.28 (m, 6H), 6.82 (s, 1H), 6.92–6.86 (m, 2H), 6.55 (brs, 1H), 5.76 (s, 1H), 3.66 (s, 3H), 2.88 (brs, 2H), 2.72 (brs, 2H), 1.45 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.6, 161.1, 159.7, 157.8, 154.7, 141.3, 141.1, 140.5, 139.8, 139.6, 137.7, 130.4, 129.7, 129.4, 128.9, 128.5, 126.1, 124.0, 120.8, 115.5, 107.9, 56.7, 55.2, 42.0, 29.5.

**N-(3-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-3-fluorobenzenesulfonamide (1j)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.21 (s, 1H), 8.08 (d, 1H,  $J = 5.2$  Hz), 7.45–7.16 (m, 9H), 6.82–6.80 (m, 2H), 6.40 (brs, 1H), 5.47 (t, 1H,  $J = 6.4$  Hz), 3.68 (s, 3H), 3.20 (brs, 2H), 2.89 (brs, 2H), 1.59 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.8, 162.4, 161.0, 159.8, 157.4, 155.0, 142.1, 141.1, 140.5, 139.2, 130.8, 130.4, 129.7, 128.9, 128.0, 126.9, 125.3, 123.4, 122.0, 120.3, 119.7, 119.5, 114.3, 114.1, 107.8, 56.2, 39.8, 37.4, 30.3.

**2-(2-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-5-ethyl-1,2,5-thiadiazolidine 1,1-dioxide (1k)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.18 (s, 1H), 8.06 (brs, 1H), 7.28–7.14 (m, 6H), 6.76 (dd, 1H,  $J = 8.0, 2.0$  Hz), 6.68 (d, 1H,  $J = 2.0$  Hz), 6.38 (brs, 1H), 5.62 (t, 1H,  $J = 6.0$  Hz), 3.61 (s, 3H), 3.46 (brs, 2H), 3.38–3.21 (m, 4H), 3.12 (brs, 2H), 3.00 (m, 2H), 1.18 (t, 3H,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  161.3, 159.9, 157.4, 155.0, 141.0, 140.6, 130.5, 129.8, 128.9, 128.0, 125.3, 123.5, 120.7, 114.1, 107.7, 56.2, 47.1, 46.1, 45.1, 42.6, 39.3, 13.0.

**2-(3-((4-(3-(4-Chloro-3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-5-ethyl-1,2,5-thiadiazolidine 1,1-dioxide (1l)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.24 (s, 1H), 8.09 (d, 1H,  $J = 5.2$  Hz), 7.36–7.21 (m, 6H), 6.81 (dd, 1H,  $J = 8.4, 2.0$  Hz), 6.76 (d, 1H,  $J = 2.0$  Hz), 6.33 (brs, 1H), 5.31 (t, 1H,  $J = 6.0$  Hz), 3.68 (s, 3H), 3.40 (brs, 2H), 3.30 (brs, 4H), 3.14–3.08 (m, 4H), 1.85 (brs, 2H), 1.26 (t, 3H,  $J = 7.6$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.4, 159.6, 157.9, 154.9, 140.8, 140.6, 139.4, 130.4, 129.9, 128.9, 128.0, 127.9, 125.3, 123.5, 120.9, 114.2, 107.5, 56.2, 45.5, 45.0, 42.5, 38.7, 27.5, 12.9.

**General procedure for preparation of the target compounds 2a–l**

A solution of the appropriate methoxy compound **1a–l** (0.2 mmol) in anhydrous dichloromethane (3 mL) was cooled to  $-78^\circ\text{C}$ , and  $\text{BBr}_3$  (1M in DCM solution, 3 mL, 2.1 mmol) was added dropwise thereto under nitrogen over a period of 10 min. The reaction mixture was stirred at the same temperature for 1 h, then the temperature was allowed to reach room temperature and kept overnight at same temperature. 10%  $\text{Na}_2\text{CO}_3$  aqueous solution (15 mL) and ethyl acetate (15 mL) were added, and the alkaline aqueous layer was extracted with ethyl acetate ( $3 \times 20$  mL). The combined organic layer extracts were washed with brine, separated, and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The organic solvent was evaporated under reduced pressure, and the residue was purified by column chromatography to obtain the purified title compounds.

**N-(2-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide (2a)**

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.15 (s, 1H), 7.85 (d, 1H,  $J = 5.2$  Hz), 7.75 (d, 2H,  $J = 7.2$  Hz), 7.47–7.15 (m, 9H), 6.85 (d, 1H,  $J = 1.6$  Hz), 6.59 (dd, 1H,  $J = 8.4, 5.0$  Hz), 6.35 (brs, 1H),

5.74 (t, 1H,  $J = 6$  Hz), 3.26 (brs, 2H), 2.99 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  161.8, 159.9, 157.2, 152.4, 141.1, 140.4, 139.8, 139.0, 132.5, 130.0, 129.8, 128.9, 128.0, 126.9, 125.2, 122.8, 121.7, 120.3, 118.8, 107.7, 43.2, 40.9.

*N*-(2-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzenesulfonamide (**2b**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.20 (s, 1H), 8.04 (d, 1H,  $J = 5.2$  Hz), 7.71 (d, 2H,  $J = 8.0$  Hz), 7.29–7.19 (m, 8H), 7.08 (s, 1H), 6.56 (dd, 2H,  $J = 8.0, 2.0$  Hz), 6.26 (brs, 2H), 3.34 (brs, 2H), 3.01 (brs, 2H), 2.32 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.6, 159.7, 158.9, 152.2, 143.3, 140.9, 138.8, 136.7, 129.9, 129.7, 128.3, 127.1, 125.3, 122.7, 121.9, 119.6, 118.9, 107.5, 41.1, 30.9, 21.5.

*N*-(2-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-4-hydroxybenzenesulfonamide (**2c**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.21 (s, 1H), 8.03 (d, 1H,  $J = 5.2$  Hz), 7.70 (dd, 2H,  $J = 9.2, 2.0$  Hz), 7.34–7.21 (m, 6H), 6.90–6.87 (m, 2H), 6.82 (dd, 1H,  $J = 8.4, 2.0$  Hz), 6.35 (brs, 1H), 5.77 (t, 1H,  $J = 6.4$  Hz), 3.35 (brs, 2H), 3.09 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.9, 162.4, 159.8, 157.9, 154.9, 141.4, 140.7, 139.8, 130.5, 129.8, 129.4, 128.9, 128.0, 125.3, 123.4, 120.5, 114.2, 114.2, 107.7, 41.6, 30.9.

4-Chloro-*N*-(2-((4-(3-(4-chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide (**2d**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.15 (brs, 1H), 7.85 (d, 1H,  $J = 5.2$  Hz), 7.65 (d, 2H,  $J = 8.8$  Hz), 7.32–7.15 (m, 8H), 6.85 (d, 1H,  $J = 1.6$  Hz), 6.60 (d, 1H,  $J = 8.0$  Hz), 6.36 (brs, 1H), 5.79 (t, 1H,  $J = 6.0$  Hz), 3.23 (brs, 2H), 2.97 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  161.1, 159.9, 157.1, 152.5, 141.2, 140.4, 138.9, 138.8, 138.3, 130.0, 129.8, 129.3, 128.9, 128.4, 125.5, 122.8, 121.8, 120.2, 118.8, 107.6, 43.1, 40.8.

*N*-(2-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-3-fluorobenzenesulfonamide (**2e**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.25 (s, 1H), 8.09 (d, 1H,  $J = 5.2$  Hz), 7.58 (d, 2H,  $J = 8.0$  Hz), 7.73–7.28 (m, 9H), 6.92 (d, 1H,  $J = 8.0$  Hz), 6.83 (s, 1H), 6.57 (brs, 1H), 5.96 (t, 1H,  $J = 6.4$  Hz), 3.35 (brs, 2H), 3.09 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  163.9, 162.8, 161.5, 159.9, 157.7, 155.6, 142.4, 141.3, 140.7, 139.7, 131.3, 130.0, 129.8, 128.8, 128.3, 126.9, 125.3, 123.4, 122.0, 120.3, 119.7, 119.5, 114.3, 114.2, 107.5, 41.2, 30.8.

*N*-(3-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (**2f**)

IR (KBr disc,  $\text{cm}^{-1}$ ): 3366, 3269, 3071, 2955, 2853, 1577, 1499;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.17 (s, 1H), 7.89 (d, 1H,  $J = 5.2$  Hz), 7.81 (d, 2H,  $J = 7.2$  Hz), 7.51–7.18 (m, 9H), 6.87 (d, 1H,  $J = 1.6$  Hz), 6.47 (dd, 1H,  $J = 8.4, 5.0$  Hz), 6.35 (brs, 1H), 5.74 (t, 1H,  $J = 6.0$  Hz), 3.26 (brs, 2H), 2.99 (brs, 2H), 1.84 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  161.9, 159.8, 157.4, 152.6, 141.4, 140.5, 139.7, 138.8, 132.6, 130.0, 129.8, 128.9, 128.0, 126.9, 125.2, 122.8, 121.7, 120.3, 118.8, 107.7, 43.2, 40.9, 29.5; LC-MS ( $m/z$ ): 559.9 ( $\text{M}^+$ ).

*N*-(3-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-4-methylbenzenesulfonamide (**2g**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.17 (s, 1H), 8.06 (d, 1H,  $J = 5.2$  Hz), 7.61 (d, 2H,  $J = 7.2$  Hz), 7.31–7.21 (m, 10H), 6.97

(s, 1H), 6.73 (d, 2H,  $J = 8.0$  Hz), 6.38 (brs, 1H), 3.28 (brs, 2H), 2.92 (brs, 2H), 2.38 (s, 3H), 1.62 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.6, 159.7, 158.9, 153.4, 142.9, 141.2, 140.5, 139.5, 138.1, 130.6, 129.4, 128.4, 128.0, 125.7, 122.5, 120.9, 118.8, 106.5, 43.3, 41.0, 29.5, 21.4.

*N*-(3-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-4-hydroxybenzenesulfonamide (**2h**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.22 (s, 1H), 8.11 (d, 1H,  $J = 5.2$  Hz), 7.82 (dd, 2H,  $J = 9.2, 2.0$  Hz), 7.36–7.21 (m, 6H), 6.96–6.88 (m, 3H), 6.41 (brs, 1H), 5.67 (t, 1H,  $J = 6.4$  Hz), 3.12 (brs, 2H), 2.92 (brs, 2H), 1.68 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.5, 162.1, 159.8, 157.7, 155.0, 141.1, 140.4, 139.2, 130.5, 129.8, 129.1, 128.9, 128.0, 125.3, 123.4, 120.5, 114.2, 114.1, 107.7, 37.4, 35.3, 29.2.

4-Chloro-*N*-(3-((4-(3-(4-chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (**2i**)

$^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$  8.36 (brs, 1H), 8.14 (d, 1H,  $J = 5.2$  Hz), 7.79 (d, 3H,  $J = 8.0$  Hz), 7.70 (d, 1H,  $J = 8.4$  Hz), 7.47–7.32 (m, 6H), 6.89 (s, 1H), 6.98–6.89 (m, 2H), 6.65 (brs, 1H), 5.86 (s, 1H), 2.95 (brs, 2H), 2.80 (brs, 2H), 1.61 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ )  $\delta$  162.6, 161.1, 159.9, 157.1, 153.4, 141.2, 140.6, 139.9, 139.6, 137.6, 129.8, 129.4, 128.9, 128.4, 128.1, 125.7, 122.5, 121.8, 120.9, 118.8, 107.6, 41.1, 31.2, 29.5.

*N*-(3-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-3-fluorobenzenesulfonamide (**2j**)

$^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$  8.22 (s, 1H), 8.13 (d, 1H,  $J = 5.2$  Hz), 7.75–7.29 (m, 11H), 7.00–6.72 (m, 3H), 6.51 (brs, 1H), 3.21 (brs, 2H), 2.81 (brs, 2H), 1.55 (brs, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ )  $\delta$  163.5, 162.6, 161.0, 159.2, 158.5, 153.4, 143.1, 141.2, 140.6, 139.6, 132.1, 130.5, 129.4, 128.4, 125.7, 123.2, 122.5, 120.9, 120.0, 119.8, 118.8, 114.1, 113.8, 106.6, 41.1, 38.3, 29.6.

2-(2-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)ethyl)-5-ethyl-1,2,5-thiadiazolidine 1,1-dioxide (**2k**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.21 (s, 1H), 8.06 (d, 1H,  $J = 5.2$  Hz), 7.32–7.18 (m, 6H), 6.77 (dd, 1H,  $J = 8.0, 2.0$  Hz), 6.72 (d, 1H,  $J = 2.0$  Hz), 6.38 (brs, 1H), 5.35 (t, 1H,  $J = 6.0$  Hz), 3.48 (brs, 2H), 3.34 (brs, 2H), 3.13–3.05 (m, 4H), 1.87 (brs, 2H), 1.31 (t, 3H,  $J = 7.2$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.5, 159.8, 157.7, 155.1, 141.1, 140.5, 139.5, 130.5, 129.8, 128.9, 128.0, 127.9, 125.3, 123.6, 120.9, 114.2, 107.7, 42.5, 41.9, 41.3, 36.5, 12.4.

2-(3-((4-(3-(4-Chloro-3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)propyl)-5-ethyl-1,2,5-thiadiazolidine 1,1-dioxide (**2l**)

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.25 (s, 1H), 8.11 (d, 1H,  $J = 5.2$  Hz), 7.46–7.23 (m, 6H), 6.84 (dd, 1H,  $J = 8.0, 2.0$  Hz), 6.86 (d, 1H,  $J = 2.0$  Hz), 6.36 (brs, 1H), 5.47 (t, 1H,  $J = 6.0$  Hz), 3.43 (brs, 2H), 3.35 (brs, 4H), 3.18–3.12 (m, 4H), 1.89 (brs, 2H), 1.37 (t, 3H,  $J = 7.6$  Hz);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  162.6, 159.8, 157.7, 155.0, 141.2, 140.2, 139.5, 130.5, 129.8, 128.9, 128.0, 127.9, 125.3, 123.7, 120.9, 114.2, 107.5, 42.5, 42.0, 41.5, 36.6, 25.4, 12.5.

### Cancer cell line screening at the NCI

Screening against the cancer cell lines was carried out at the National Cancer Institute (NCI), Bethesda, MD, applying the standard protocol of the NCI (DTP Human Tumor Cell Line Screen Process: <http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html>). Single-dose protocol: compounds submitted to the NCI 60 Cell screen are tested initially at a single high dose ( $10^{-5}$  M) in the full panel of 53 cancer cell line. The single-dose data will be reported as a mean graph of the percentage growth of treated cells and will be similar in appearance to mean graphs from the 5-dose assay. The number reported for the single-dose assay is growth relative to the no-drug control and to the time zero number of cells. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). The percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

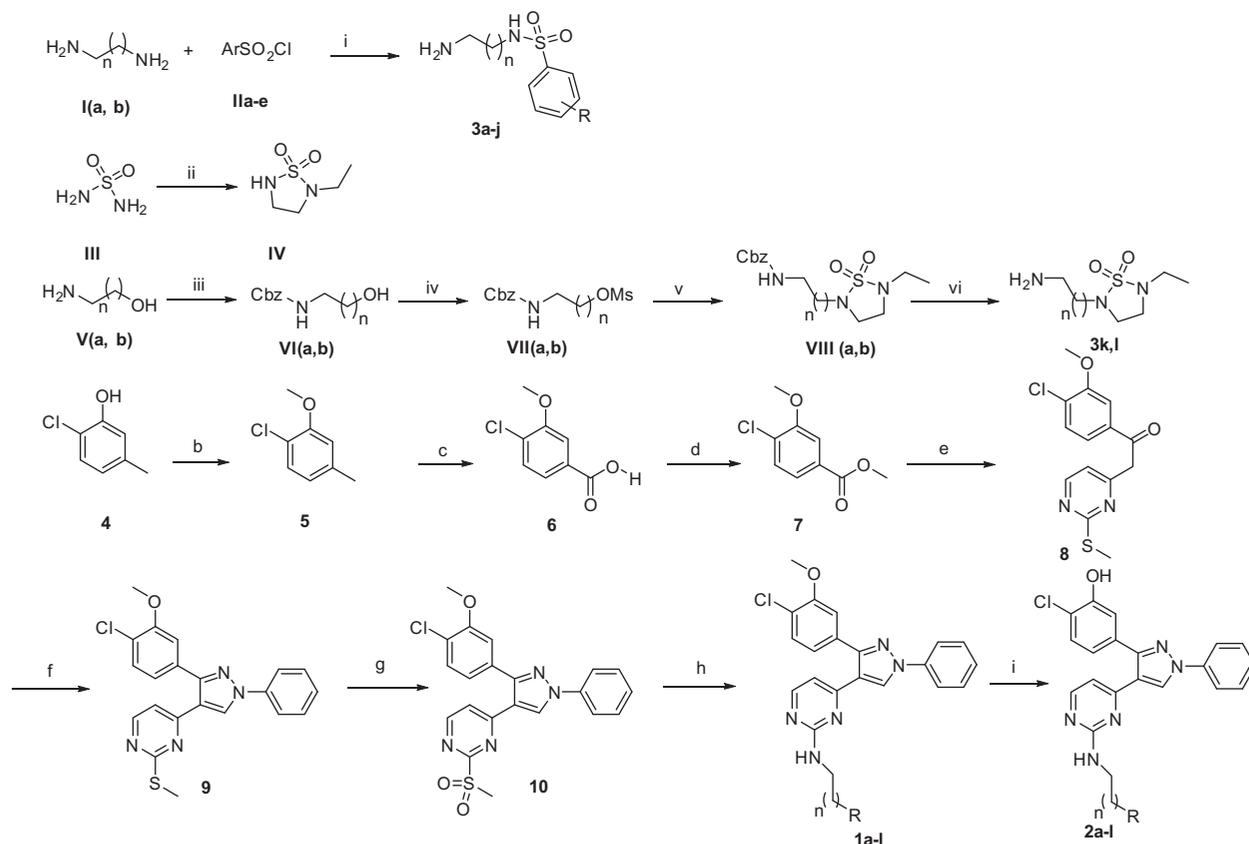
- $[(Ti - Tz)/(C - Tz)] \times 100$  for concentrations for which  $Ti \geq Tz$
- $[(Ti - Tz)/Tz] \times 100$  for concentrations for which  $Ti < Tz$ .

### MTT cell viability assay

#### Materials and methods

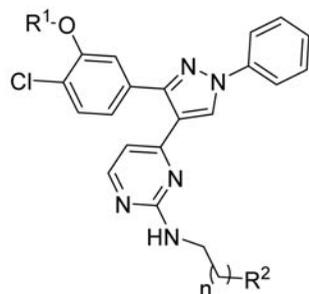
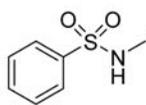
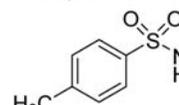
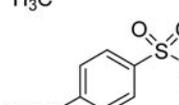
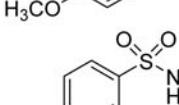
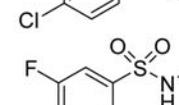
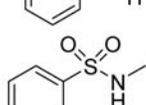
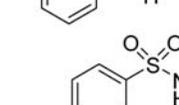
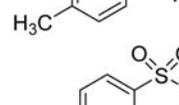
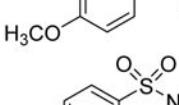
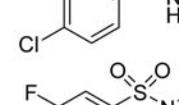
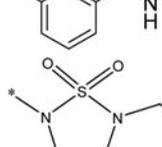
Compound **1d** was submitted to Reaction Biology Corporation (<http://www.reactionbiology.com>) for MTT assay. The targeted compound was dissolved in DMSO at 10 mM stock. Control compound was purchased from Sigma-Aldrich (Saint Louis, MO).

CellTiter 96<sup>®</sup> Non-Radioactive Cell Proliferation Assay (MTT) reagent was purchased from Promega (Madison, WI). HL-60, HT29 and MRC-5 cell lines were purchased from American Type Culture Collection (Manassas, VA). HL-60 cells were grown in IMDM medium, HT-29 in McCoy's 5 $\alpha$  medium, and MRC-5 cells in EMEM medium. 100  $\mu$ g/mL penicillin, 100  $\mu$ g/mL streptomycin, and 10% fetal bovine serum were added to all of the culture media. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air. Compound **1d** and the reference compound staurosporine were diluted with DMSO in 10-dose and 3-fold serial dilutions. 225 nL of **1d** or 25 nL of staurosporine was delivered from the source plate to the wells of a 384-well cell culture plate by Echo 550 Liquid Handler. 25  $\mu$ L of culture medium containing 2000 of cells was added to the wells of the 384-well culture plate. The plate was incubated at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air for 72 h. 5  $\mu$ L of MTT Dye Solution was added to each well. The plate was incubated at 37 °C in a CO<sub>2</sub> incubator for 6 h. After incubation, the formazan was produced in the cells. 25  $\mu$ L of Solubilization/Stop Solution was added to each well and the plate was incubated for another 1 h at 37 °C in a CO<sub>2</sub> incubator to dissolve the formazan crystals. The absorbance of each sample was measured at 590 nm using Envision 2104 Multilabel Reader (PerkinElmer, Santa Clara, CA). The cell viability was determined based on the quantification of the color intensity in each culture well. The IC<sub>50</sub> curves were plotted and the IC<sub>50</sub> values were calculated using the GraphPad Prism 4 program based on a sigmoidal dose–response equation.



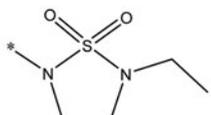
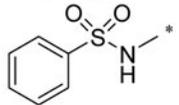
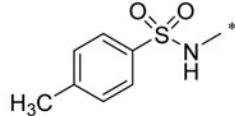
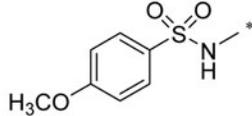
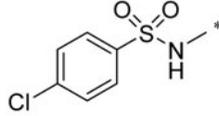
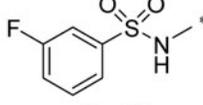
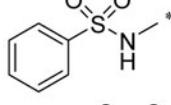
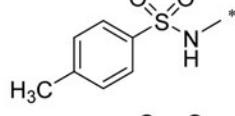
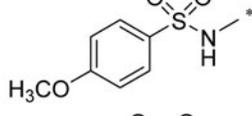
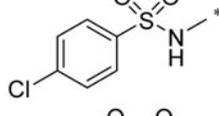
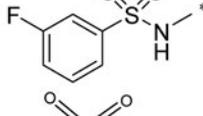
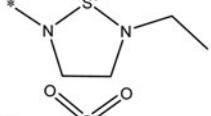
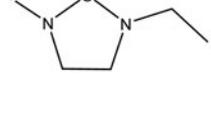
Scheme 1. Reagents and conditions: (a) (i) triethylamine, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, RT, overnight; (ii) *N*-ethyl ethylenediamine, C<sub>5</sub>H<sub>5</sub>N, reflux, 3 h; (iii) benzoyloxycarbonyl chloride, TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; (iv) methanesulfonyl chloride, TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; (v) **IV**, NaH (60% dispersion in mineral oil), DMF; (vi) H<sub>2</sub>/Pd-C, MeOH, RT, 1 h; (b) (CH<sub>3</sub>)<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, acetone, reflux, 3 h; (c) KMnO<sub>4</sub>, C<sub>5</sub>H<sub>5</sub>N, H<sub>2</sub>O, 50 °C, 24 h, then RT, 13 h; (d) acetyl chloride, CH<sub>3</sub>OH, RT, 15 h; (e) 4-methyl-2-(methylthio)pyrimidine, LiHMDS, THF, –78 °C, 2 h then RT, overnight; (f) (i) DMF-DMA, 80 °C, 3 h; (ii) phenylhydrazine, C<sub>2</sub>H<sub>5</sub>OH, RT, overnight; (g) oxone, MeOH, H<sub>2</sub>O, RT, 16 h; (h) **3a–l**, diisopropylethylamine, DMSO, 80 °C, 16 h; (i) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, –78 °C, 1 h; RT, overnight.

Table 1. Structures of the target compounds **1a-l** and **2a-l**, their yield percentage, and melting points.

Compound No.	R <sup>1</sup>	R <sup>2</sup>	n	Yield%	Melting point (°C)
 <b>1a-l, 2a-l</b>					
<b>1a</b>	CH <sub>3</sub>		1	66	153-5
<b>1b</b>	CH <sub>3</sub>		1	57	160-2
<b>1c</b>	CH <sub>3</sub>		1	50	154-6
<b>1d</b>	CH <sub>3</sub>		1	51	157-9
<b>1e</b>	CH <sub>3</sub>		1	63	160-2
<b>1f</b>	CH <sub>3</sub>		2	65	161-3
<b>1g</b>	CH <sub>3</sub>		2	69	163-5
<b>1h</b>	CH <sub>3</sub>		2	59	177-9
<b>1i</b>	CH <sub>3</sub>		2	52	142-4
<b>1j</b>	CH <sub>3</sub>		2	45	172-4
<b>1k</b>	CH <sub>3</sub>		1	57	167-9

(continued)

Table 1. Continued

<b>1l</b>	CH <sub>3</sub>		2	60	171-3
<b>2a</b>	H		1	38	162-4
<b>2b</b>	H		1	45	167-9
<b>2c</b>	H		1	35	169-71
<b>2d</b>	H		1	32	139-41
<b>2e</b>	H		1	39	135-7
<b>2f</b>	H		2	29	133-5
<b>2g</b>	H		2	34	136-8
<b>2h</b>	H		2	41	146-8
<b>2i</b>	H		2	45	154-6
<b>2j</b>	H		2	25	143-5
<b>2k</b>	H		1	23	145-7
<b>2l</b>	H		2	26	141-3

\*Means the rest of the attached compound.

Table 2. Mean percentage growth of the 53 cancer cell line panel after treatment with the tested target compounds (10  $\mu$ M concentration).

Compound No.	Mean percentage growth
<b>1a</b>	90.00
<b>1d</b>	<b>33.29</b>
<b>1h</b>	<b>68.88</b>
<b>1i</b>	88.81
<b>1j</b>	84.29
<b>1k</b>	94.93
<b>1l</b>	84.76
<b>2f</b>	<b>67.91</b>
<b>2i</b>	74.12
<b>2k</b>	99.38
<b>2l</b>	96.07

Bold figures indicate the most active compounds.

### *In vitro* kinase screening

Reaction Biology Corp. Kinase HotSpot<sup>SM</sup> service (<http://www.reactionbiology.com>) was used for screening of compound **1d**. Assay protocol: in a final reaction volume of 25  $\mu$ L, kinase (5–10 mU) is incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.66 mg/mL relevant substrate, and 10 mM magnesium acetate and [ $\gamma$ -<sup>33</sup>P-ATP] (specific activity approx. 500 cpm/pmol, concentration 1  $\mu$ M). The reaction is initiated by the addition of the Mg-ATP mix. After incubation for 40 min at room temperature, the reaction is stopped by the addition of 5  $\mu$ L of a 3% phosphoric acid solution. 10  $\mu$ L of the reaction is then spotted onto a P30 filtermat and washed thrice for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

## Results and discussion

### Chemistry

Synthesis of the target designed compounds **1a–l** and **2a–l** was achieved through the pathway shown in Scheme 1. Methylation of the phenolic hydroxyl group of 2-chloro-5-methylphenol (**4**) using dimethyl sulfate gave 1-chloro-2-methoxy-4-methylbenzene (**5**)<sup>22</sup>. Oxidation of the methyl group of **5** using KMnO<sub>4</sub> produced 4-chloro-3-methoxybenzoic acid (**6**)<sup>21</sup>. Esterification of the carboxylic acid **6** using methanol in the presence of acetyl chloride produced the corresponding methyl ester **7**. 4-Methyl-2-(methylthio)pyrimidine was prepared by methylation of 4-methylpyrimidine-2-thiol with iodomethane in the presence of aqueous NaOH. Upon reacting the ester with the pyrimidine methyl thioether in the presence of lithium bis(trimethylsilyl)amide (LiHMDS), the ketide intermediate **8** was formed. Reaction of compound **8** with dimethylformamide dimethyl acetal (DMF-DMA) followed by subsequent treatment with phenylhydrazine yielded the *N*-phenylpyrazole intermediate **9**<sup>23</sup>. Oxidation of the methylsulfide moiety of compound **9** using oxone<sup>19</sup> produced the corresponding mesyl compound **10**. Heating compound **10** with the appropriate amino reagents **3a–l** in the presence of diisopropylethylamine led to displacement of the mesyl group of compound **10** by the amino group and formation of the target compounds **1a–l**. Demethylation of the methoxy group of **1a–l** using BBr<sub>3</sub> afforded the corresponding hydroxyl derivatives **2a–l**. Structures of the target compounds, their yield percentages, and melting points are illustrated in Table 1.

### *In vitro* antiproliferative activity against NCI-60 cancer cell line panel (single dose)

Structures of the target synthesized compounds were submitted to National Cancer Institute (NCI, Bethesda, MD), and the 11

Table 3. IC<sub>50</sub> ( $\mu$ M) results exerted by the compound **1d** and staurosporine against HL-60 leukemia cell line, HT29 colon cancer cell line, and MRC-5 lung fibroblasts.

Cell line	IC <sub>50</sub> <sup>a</sup> ( $\mu$ M) compound <b>1d</b>	IC <sub>50</sub> (nM) Staurosporine
HL-60 leukemia cell line	28.87	81.90
HT29 colon cancer cell line	13.37 (2.77) <sup>b</sup>	186.0 (0.015) <sup>b</sup>
MRC-5 lung fibroblasts (normal cells)	37.02	2.8

<sup>a</sup>IC<sub>50</sub> value means the concentration required to inhibit 50% of the cell growth.

<sup>b</sup>Selectivity index calculated by dividing the IC<sub>50</sub> values against fibroblasts by IC<sub>50</sub> values against HT29 colon cancer cell line.

derivatives shown in Table 2 were selected on the basis of degree of structural variation technique for testing their antiproliferative activity. The selected compounds were subjected to an *in vitro* anticancer assay against a full panel of 53 cancer cell lines of nine different cancer types; leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancers. The compounds were tested at a single-dose concentration of 10  $\mu$ M, and the percentages of growth inhibition over the 53 tested cell lines were determined. The mean percentage growth of the NCI-60 cancer cell line panel after treatment with each of the tested compound is illustrated in Table 2.

Upon investigating the effect of linker length on antiproliferative activity, it was found that compound **1d** with ethylene linker was more active than the corresponding propylene derivative **1i**. The ethylene spacer might be optimal for appropriate fitting at the receptor site. Regarding the terminal moieties (arylsulfonamido and the cyclic sulfamide present in the terminal moieties), it was found that compounds **1a**, **d**, **h–j**, **2f**, and **2i** possessing arylsulfonamido showed higher antiproliferative activity than cyclic ones **1k**, **1l**, **2k**, and **2l**. This may be attributed to that increased hydrophobicity by the aromatic moieties might enhance the compound penetration through the cell membrane to inside the cell and/or stronger affinity at the receptor site due to stronger hydrophobic interactions. Any or both of these effects might lead to stronger antiproliferative activity. This assumption could be supported by the slightly higher antiproliferative activity of the methoxy compounds **1k** and **1l** than the corresponding hydroxyl analogs **2k** and **2l**. The results also showed that the compounds with substituted arylsulfonamide moieties **1d** and **1h–j** were more active than those with unsubstituted phenyl ring **1a**. The increased bulkiness and lipophilicity induced by those substituents might lead to enhanced activity due to more appropriate fitting at the receptor site and/or more penetration to inside the cancer cells.

The percentage inhibition values of the most active compounds over the most sensitive cell lines are summarized in Table 3. SR leukemia, MCF-7, and T-47D breast cancer cell lines were the most sensitive cell lines to this series of compounds. The most active compounds against SR leukemia were **1d**, **2f**, and **1h** with percentage inhibitions of 119.44%, 58.02, and 56.01%, respectively. MCF-7 and T-47D breast cancer cell line were also sensitive to the most potent compounds. Figure 2 illustrates the percentage inhibition values of the tested target compounds against SR leukemia, MCF-7, and T-47D breast cancer cell line. It is obvious from the figure that compounds **1d** then **2f** are the most active among the 10 tested compounds against those three cell lines.

Among all the tested derivatives, compound **1d** was the most active. It exerted broad-spectrum antiproliferative activity against different cell lines of different cancer types. It exerted the highest activities against HL-60 (TB) and SR leukemia cell lines.

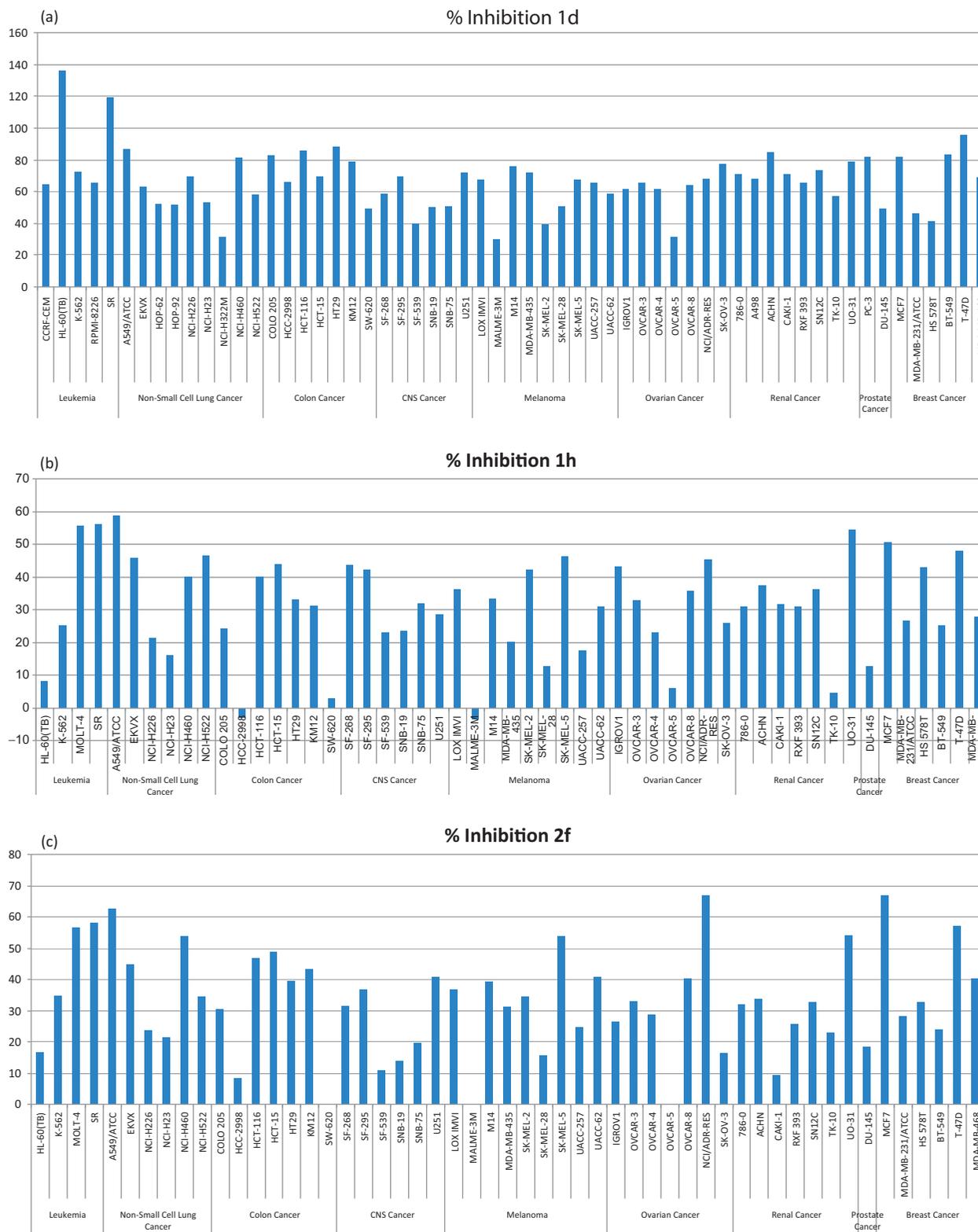


Figure 2. Percentage inhibition expressed by compounds **1d** (a), **1h** (b), and **2f** (c) at 10  $\mu$ M concentration over all cell lines of the NCI cancer cell line panel of nine different cancer types. The inhibition percentages are calculated by subtracting the growth percentages from 100.

Compounds **1d**, **1h**, and **2f** showed the highest mean inhibitions. The percentages of inhibition of these three compounds over each tested cell line of the NCI-60 panel at 10  $\mu$ M concentration are depicted in Figure 2. The three compounds exerted broad-spectrum antiproliferative activities against different cell lines of different cancer types. In general, compound **1d**

was significantly more active than compounds **1h** and **2f**. The *p*-chlorophenylsulfonamido terminal moiety of compound **1d** was more optimal than *p*-methoxyphenylsulfonamido and phenylsulfonamido moieties of compounds **1h** and **2f**, respectively. As mentioned previously, it might enhance the penetration into inside the cells and/or increase the affinity at the receptor site. The

Table 4. Percentage inhibition and IC<sub>50</sub> (μM) results exerted by the most potent compounds over the selected kinases.

Kinase	Percentage inhibition at 10 μM concentration	IC <sub>50</sub> (μM)
ABL1	7.31% ± 0.38%	ND
ABL1 (T315I)	-2.96% ± 0.60%	ND
FLT3	17.01% ± 0.42%	ND
V600E-B-RAF	74.47% ± 0.28%	3.57
RAF1	78.04% ± 0.32%	2.47
ALK5	7.90% ± 0.63%	ND
B-RAF (wild type)	6.26% ± 0.23%	ND
V600K-B-RAF	72.46% ± 0.37%	ND
EGFR	-3.57% ± 0.25%	ND
ERK2/MAPK1	-7.68% ± 1.96%	ND
JNK3	-3.07% ± 0.25%	ND
p38α/MAPK14	-26.57% ± 5.96%	ND

ethylene spacer of compound **1d** seems to be more optimal for appropriate fitting at the receptor site than the propylene linker of compounds **1h** and **2f**. And the methoxy group on the aryl ring at position 3 of the pyrazole ring could be more favorable than hydroxyl due to hydrophobicity, steric, and electronic differences between them.

#### MTT assay

Compound **1d** was further tested at Reaction Biology Corporation to identify its IC<sub>50</sub> against HL-60 (TB) leukemia and HT-29 colon cancer against which our compound showed the most promising growth inhibition percentage and also were available in reaction biology cell line screening panels. It was also tested against MRC-5 lung fibroblasts normal cell line in order to investigate the selectivity index. The IC<sub>50</sub> values are illustrated in Table 3. The compound showed higher potency against HT29 colon cancer cell line than HL-60 leukemia cell line, with good selectivity index toward cancer cells than normal cells. On the other hand, staurosporine, the reference standard drug, was much more potent against normal fibroblasts than cancer cells. So, compound **1d** has the merit of higher safety than staurosporine.

#### In vitro kinase screening

In order to investigate the probable mechanism of action of compound **1d** at molecular level, it was selected to be tested at a single-dose concentration of 10 μM over 12 kinases; from which V600E-B-RAF and RAF1 which are over-expressed in HT29 cancer cell line<sup>24-26</sup> and others belong to different kinase families to investigate the selectivity. As illustrated in Table 4, compound **1d** showed better inhibitory effect over three kinases at 10 μM. It exerted 78.04%, 74.47%, and 72.46% inhibitions against RAF1, V600E-B-RAF, and V600K-B-RAF kinases, respectively. But it demonstrated low activity against the other tested kinases. So, it was further tested in a 10-dose testing mode in order to determine its IC<sub>50</sub> values over RAF1 and V600E mutated B-RAF. The IC<sub>50</sub> values were 2.47 μM and 3.57 μM, respectively. As mentioned previously, we can deduce the selectivity of this compound against RAF1 and V600E-B-RAF kinases, and the corresponding cell line possessing the over-expressed kinases V600E-B-RAF and RAF1 as postulated (Table 4) which helps us deduce that this compound might be working, at least in part, through inhibition of RAF kinases as antiproliferative agent. V600K-B-RAF kinase has been reported more in melanoma than in colon cancer<sup>27</sup>. The moderate activity of compound **1d** against the melanoma subpanel might be partially due to V600K-B-RAF inhibition.

#### Conclusion

In this study, a novel series of pyrimidinylpyrazoles possessing terminal arylsulfonamido or cyclic sulfamide moiety separated by ethyl or propyl spacer was synthesized. The target compounds were tested for *in vitro* antiproliferative activities over NCI-60 cancer cell line panel of nine different cancer types. Among them, compound **1d** possessing *p*-chlorobenzenesulfonamido terminal moiety, ethylene linker, and 4-chloro-3-methoxyphenyl ring at position 3 of the pyrazole nucleus showed the highest activity. So these moieties together may comprise potential pharmacophoric hits for this series of compounds. Compound **1d** exerted broad-spectrum antiproliferative activities over different cell lines of different cancer types. The inhibition percentage values of compound **1d** over HL-60 (TB) and SR leukemia, HT29 colon, and T-47D and MCF-7 breast cancer cell lines were 135.92%, 119.44%, 88.66%, 95.32%, and 82.03%, respectively. SR leukemia, MCF-7, and T-47D breast cancer cell lines were found to be highly sensitive to the most active compounds. Compound **1d** gave 13.37 μM IC<sub>50</sub> value upon testing against HT29 colon cancer cell line, but it showed lower potency against HL-60 leukemia and MRC-5 lung fibroblasts. It showed higher selectivity toward HT29 colon cancer cell line than the normal fibroblasts, compared to staurosporine. So despite of inferior potency than staurosporine against HT29 colon cancer cell line, it is much safer for the normal cells. Upon testing the compound against kinases, it showed selectivity against RAF1 and V600E-B-RAF kinases which are over-expressed in HT29 colon cancer cell line from which we can deduce that this compound might express its antiproliferative effect, at least in part, through inhibition of RAF kinases. Therefore, compound **1d** could be utilized as a lead compound for future development of active anticancer agents inhibiting RAF kinases. Further modifications of this series of compounds in order to improve their anticancer activity are currently in progress.

#### Declaration of interest

The authors have declared no conflict of interest.

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Supplementary material available online