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Synthesis and Evaluation of 5-Chloro-2-Methoxy-*N*-(4-Sulphamoylphenyl) Benzamide Derivatives as Anti-cancer Agents

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Abstract

Sulphonamides embrace a sublime class of drugs with various biological activities. Since the discovery of E7010 in 1992, several sulphonamides as anti-cancer drug candidates have been identified. Herein, a new series of 5-chloro-2-methoxy-*N*-(4-sulphamoylphenyl)benzamide derivatives was synthesised, and their anti-proliferative activity was evaluated against human ovarian cancer (A2780) and colon cancer (HCT-116) cell lines. As one of the most potent anti-proliferative agents, compound 4j was further tested against a panel of cancer cell lines revealing that human pancreatic carcinoma (MIA PaCa-2) displayed the highest sensitivity. Cellular mechanistic studies on MIA PaCa-2 cells showed that 4j arrested the G2/M cell cycle and induced apoptosis.

4j
GI_{50} = 1.9 μM
in MIA PaCa-2 pancreatic carcinoma
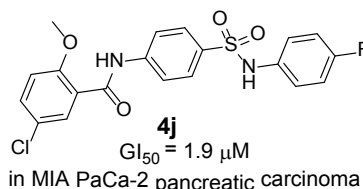
Keywords: Anti-proliferation; Apoptosis; Structure-activity relationship; Anti-cancer agent; Drug discovery

Introduction

Sulphonamide bioactive compounds have been developed as anti-bacteria (e.g. sulphathiazole [1]), diuretics (*e.g.*furosemide [2]), anti-diabetics (e.g. glibenclamide [3,4]), carbonic anhydrase inhibitors (e.g. acetazolamide [5,6]), anti-HIV (e.g. amprenavir [7]), and anti-cancer agents (e.g. E7010 [8]). There is considerable interest in extending and diversifying the sulphonamide framework to further explore therapeutic potentials [9].

Since the discovery of E7010 in 1992 [8], several classes of sulphonamide derivatives have been reported as potential anti-cancer drug candidates. Those compounds showed different cellular mechanisms such as inhibition of microtubule assembly [10], inhibition of transcription factor NF-Y and matrix metalloproteinase (MMP) [11], and carbonic anhydrase inhibition [12,13]. A series of patents presented novel sulphonamide derivatives targeting protein kinases including vascular endothelial growth factors, platelet-derived growth factors, and c-kit proteins [14,15].

On the other hand, various *N*-(4-sulphamoylphenyl)benzamide containing compounds have demonstrated a range of pharmacological activities including, anti-bacterial [16], inhibition of glucose stimulated insulin release [17], sirtuin-2 deacetylase [18] and viral integrase [19], anti-HIV [20] and other activities that associated with the inhibition of metalloprotease endothelin-converting enzyme and carbonic anhydrase [21-23]. However the anti-cancer potential of the *N*-(4-sulphamoylphenyl)benzamide derivatives has not been fully explored. The reconnaissance of the usefulness and versatility of sulphonamides coupled with the N-(*4*-sulphamoylphenyl)benzamide scaffold may lead to novel and potent anti-cancer agents. As the different aryl sulphonamides have been shown to act as anti-tumour agents through different mechanisms [24], we prepared a series of sulphonamide derivatives with an *N*-(4-sulphamoylphenyl)benzamide core and evaluated the anti-cancer activity of these compounds.

Materials and Methods

Chemistry

All materials, reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, Merck, GL Biochem, Combi-block or Ajax Finechem, and were used as received. ¹H and ¹³C NMR spectra were recorded at 298K on a Bruker AVANCE III 500 spectrometer (¹H at 500.16 MHz and ¹³C NMR at 125.76 MHz; Faellanden, Switzerland), and were processed using the Bruker Topspin 3.2 software. ¹H and ¹³C NMR spectra are referenced to ¹H signals of residual nondeuterated solvents and ¹³C signals of the deuterated solvents respectively. ¹H NMR signals are reported with chemical shift values δ (ppm), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad), relative integral, coupling constants *J* (Hz) and assignments. Mass spectra were recorded on an AB SCIEX TripleTOF° 5600 mass spectrometer, and ionisation of all samples was carried out using ESI. Melting points were determined on an Electrothermal IA

9100 digital melting point apparatus or a Stuart SMP10 melting point apparatus, and are uncorrected. The purity of compounds used for biological evaluation was determined by analytic RP-HPLC which was carried out on a Shimadzu Prominence UFLC system (Ultra Fast Liquid Chromatograph, Kyoto, Japan) equipped with a CBM-20A communications bus module, a DGU-20A5R degassing unit, an LC-20AD liquid chromatograph pump, an SIL-20AHT auto-sampler, an SPD-M20A photo diode array detector, a CTO-20A column oven and a Phenomenex Kinetex 5u C18 100A 250 mm × 4.60 mm column. Method A (gradient 5% to 95% CH_3OH containing 0.1% FA over 7 min at a flow rate of 1 mL/min, followed by 95% CH_3OH containing 0.1% FA over 13 min) and method B (gradient 5% to 95% CH_3CN containing 0.1% FA over 7 min at a flow rate of 1 mL/min, followed by 95% CH_3CN containing 0.1% FA over 13 min) were used for analytic RP-HPLC. Data acquired from analytic RP-HPLC were processed using LabSolutions Analysis Data System. Analytic TLC was performed on Merck silica gel 60 F254 pre-coated aluminium plates (0.2 mm) and visualised under UV light (254 nm). Column chromatography was carried out using a fritted solid loader packed with GRACE Davison DAVISIL˙ silica gel 60 Å (40-63 μm) on a Biotage FlashMaster Personal⁺ flash chromatography system.

5-Chloro-2-methoxy-*N*-phenylbenzamide (2): To a solution of 5-chloro-2-methoxybenzoic acid (1, 10.0 g, 53.6 mmol) in dichloromethane (DCM, 100 mL) at room temperature was added triethylamine (7.5 mL, 53.8 mmol) slowly, followed by ethylchloroformate (5.10 mL, 53.8 mmol). The reaction mixture was stirred at room temperature for 1 h, and aniline (4.90 mL, 53.8 mmol) was added. The reaction mixture was stirred at room temperature for further 2 h, and distilled water (100 mL) was added. The organic layer was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was recrystallised from MeOH to give **2** as prism crystals (13.2 g, 94%). ¹H-NMR (DMSO-d_6) δ3.92 (s, 3H, CH_3), 7.09 (t, 1H, *J*=7.4 Hz, Ar-H), 7.13 (dd, 1H, *J*=8.2 and 1.8 Hz, Ar-H), 7.27 (d, 1H, *J*=1.8 Hz, Ar-H), 7.34 (t, 2H, *J*=7.9 Hz, Ar-H), 7.63 (d, 1H, *J*=8.2 Hz, Ar-H), 7.72 (d, 2H, *J*=7.7 Hz, Ar-H), 10.13 (br, s, 1H, NH). MS (ESI) *m/z* [M+H]⁺ calcd. for $C_{14}H_{13}ClNO_2^+$ 262.0629 found 262.0578.

4-(5-Chloro-2-methoxybenzamido)benzenesulphonyl chloride (3): 5-chloro-2-methoxy-*N*-phenylbenzamide (2, 10 g, 38 mmol) was treated with chlorosulphonic acid (50 mL) on an ice bath with continuous stirring, then removed from the ice bath and stirring was continued at room temperature for 12 hours. The reaction mixture was added on ice slowly to afford white precipitate. The precipitate was filtered and washed with distilled water and recrystallised from DCM to afford white needle crystals (12 g, 87%). ¹H NMR (CDCl₃, 500 MHz) δ4.11 (s, 3H, CH_3), 7.02 (d, 1H, *J*=8.8 Hz, Ar-H), 7.50 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.91 (d, 2H, *J*=8.9 Hz, 2 Ar-H), 8.02 (d, 2H, *J*=8.9 Hz, 2 Ar-H), 8.24 (d, 1H, *J*=2.7 Hz, Ar-H), 10.11 (br s, 1H, NH). MS (ESI) *m/z* [M+H]⁺ calcd. for $C_{14}H_{12}Cl_2NO_4S^+$ 359.9858 found 359.9597

General synthetic procedure of 5-chloro-2-methoxy-*N*-(4 sulphamoylphenyl)benzamide derivatives (4a-t): To a solution of 4-(5-chloro-2-methoxybenzamido)benzenesulphonyl chloride (3, 0.25 g, 0.69 mmol) in tetrahydrofuran (THF) (10 mL) and sodium carbonate (0.73 g, 0.69 mmol) in water (5 mL) was added appropriate amine (1.05 mmol). The mixture was stirred for 24 hours at room temperature. The tetrahydrofuran was evaporated under vacuum, followed by acidification using 1N HCl. The precipitate formed was washed with water and purified by Biotage˙ Flash Master Personal⁺ flash chromatography (silica gel, petroleum benzene ramping to petroleum benzene:ethyl acetate=60:40 unless otherwise stated) to give the desired compound.

5-chloro-2-methoxy-*N*-(4-(*N*-phenylsulphamoyl)phenyl) benzamide (4a): White crystalline solid, yield: 75%. mp: 202-204°C. ¹H-NMR (DMSO-d_6): δ3.85 (s, 3H, CH_3), 7.02 (t, 1H, *J*=7.4 Hz, Ar-H), 7.09 (d, 2H, *J*=7.6 Hz, Ar-H), 7.20 (d, 1H, *J*=8.8 Hz, Ar-H), 7.23 (t, 2H, *J*=7.4 Hz, Ar-H), 7.55 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.57 (d, 2H, *J*=8.8 Hz, Ar-H), 7.72 (d, 2H, *J*=8.8 Hz, Ar-H), 7.83 (d, 2H, *J*=8.8 Hz, Ar-H), 10.19 (br s, 1H, NH), 10.54 (br s, 1H, NH). ¹³C-NMR (CDCl₃): δ56.9, 113.3, 120.1, 122.1, 122.6, 125.8, 127.6, 128.8, 129.6, 132.5, 133.6, 134.0, 136.5, 142.5, 155.8, 162.3. MS (ESI) *m/z* [M-H]⁻ calcd. for $C_{20}H_{16}ClN_2O_4S^-$ 415.0525 found 415.0665. Anal. RP-HPLC Method A: *t*R 12.02 min, purity>99%; Method B: *t*R 11.53 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-(*N*-(m-tolyl)sulphamoyl)phenyl) benzamide (4b): White crystalline solid, yield: 72%. mp: 201-203°C. 1H NMR ¹H-NMR (CDCl₃): δ2.52 (s, 3H, CH_3), 4.30 (s, 3H, CH_3), 6.82 (br s, 1H, NH), 7.11 (d, 1H, *J*=8.3 Hz, Ar-H), 7.17 (d, 2H, *J*=8.7 Hz, Ar-H), 7.23 (d, 1H, *J*=8.9 Hz, Ar-H), 7.35 (t, 1H, *J*=7.7 Hz, Ar-H), 7.71 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.95 (d, 1H, *J*=2.7 Hz, Ar-H), 7.97 (d, 2H, *J*=8.7 Hz, Ar-H), 8.0 (d, 2H, *J*=8.7 Hz, Ar-H), 8.46 (d, 1H, *J*=2.7 Hz, Ar-H), 10.17 (br s, 1H, NH). ¹³C-NMR (CDCl₃): δ21.5, 57.0, 113.3, 118.8, 120.9, 122.5, 122.6, 126.5, 127.6, 128.8, 129.3, 132.5, 133.6, 134.1, 136.4, 139.6, 142.4, 155.9, 162.4. MS (ESI) *m/z* [M-H]⁻ calcd. for $C_{21}H_{18}ClN_2O_4S^-$ 429.0681 found 429.0827. Anal. RP-HPLC Method A: *t*R 12.26 min, purity>97%; Method B: *t*R 11.80 min, purity>97%.

5-chloro-2-methoxy-*N*-(4-(*N*-(p-tolyl)sulphamoyl)phenyl) benzamide (4c): White powder, yield: 72%. mp: 140-142°C. ¹H-NMR (DMSO-d_6): δ2.18 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 6.97 (d, 2H, *J*=8.5 Hz, Ar-H), 7.03 (d, 2H, *J*=8.5 Hz, Ar-H), 7.20 (d, 1H, *J*=8.8 Hz, Ar-H), 7.55 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.57 (d, 1H, *J*=2.7 Hz, Ar-H), 7.69 (d, 2H, *J*=8.8 Hz, Ar-H), 7.82 (d, 2H, *J*=8.8 Hz, Ar-H), 10.03 (br s, 1H, NH), 10.54 (br s, 1H, NH). ¹³C-NMR (DMSO-d_6): δ20.3, 56.3, 114.0, 119.4, 120.6, 124.2, 126.7, 127.9, 128.7, 129.6, 131.5, 133.3, 133.7, 135.1, 142.6, 155.3, 163.9. MS (ESI) *m/z* [M-H]⁻ calcd. for $C_{21}H_{18}ClN_2O_4S^-$ 429.0754 found 429.0772. Anal. RP-HPLC Method A: *t*R 12.17 min, purity>99%; Method B: *t*R 11.80 min, purity>99%.

5-chloro-*N*-(4-(*N*-(2-ethylphenyl)sulphamoyl)phenyl)-2-methoxybenzamide (4d): White crystalline solid, yield: 45%. mp: 189-191°C. ¹H-NMR (DMSO-d_6): δ1.18 (t, 3H, *J*=7.6 Hz, CH3), 2.58 (q, 2H, *J*=7.6 Hz, CH2), 4.06 (s, 3H, CH3), 6.50 (br s, 1H, NH), 6.99 (d, 1H, *J*=8.2 Hz, Ar-H), 7.15 (t, 1H, *J*=7.8 Hz, Ar-H), 7.24 (d, 1H, *J*=8.9 Hz, Ar-H), 7.29 (t, 1H, *J*=7.9 Hz, Ar-H), 7.71 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.79 (d, 1H, *J*=7.9 Hz, Ar-H) 7.94 (d, 2H, *J*=8.8 Hz, Ar-H), 8.00 (d, 2H, *J*=8.8 Hz, Ar-H), 8.47 (d, 1H, *J*=2.7 Hz, Ar-H),10.15 (br s, 1H, NH). ¹H-NMR (DMSO-d_6): δ15.4, 28.2, 56.8, 111.0, 113.5, 120.0, 121.6, 122.9, 126.0, 126.5, 128.1, 129.0 132.3, 133.8, 133.9, 134.5, 142.6, 149.8, 156.3, 162.4. MS (ESI) *m/z* [M-H]⁻ calcd. for $C_{22}H_{20}ClN_2O_4S^-$ 443.0838 found 443.1058. Anal. RP-HPLC Method A: *t*R 12.42 min, purity>99%; Method B: *t*R 12.11 min, purity>99%.

5-chloro-*N*-(4-(*N*-(4-ethylphenyl)sulphamoyl)phenyl)-2-methoxybenzamide (4e): White crystalline solid, yield: 64%. mp: 181-183°C. ¹H-NMR (DMSO-d_6): δ1.10 (t, 3H, *J*=7.6 Hz, CH3), 2.48 (q, 2H, *J*=7.6 Hz, CH2), 3.85 (s, 3H, CH_3) 6.99 (d, 2H, *J*=8.5 Hz, Ar-H), 7.06 (d, 2H, *J*=8.5 Hz, Ar-H), 7.20 (d, 1H, *J*=8.8 Hz, Ar-H), 7.55 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.57 (d, 1H, *J*=2.7 Hz, Ar-H), 7.71 (d, 2H, *J*=8.8 Hz, Ar-H), 7.83 (d, 2H, *J*=8.8 Hz, Ar-H), 10.07 (br s, 1H, NH), 10.55 (br s, 1H, NH). ¹³C-NMR (DMSO-d_6): δ15.5, 27.5, 56.4, 114.1, 119.4, 120.6, 124.3, 126.7, 128, 128.5, 128.8, 131.6, 133.9, 135.4, 139.6, 142.6, 155.3, 163.9. MS (ESI) *m/z* [M-H]⁻ calcd. for $C_{22}H_{20}ClN_2O_4S^-$ 443.0838 found 443.0850. Anal. RP-HPLC Method A: *t*R 12.41 min, purity>99%; Method B: *t*R 12.11 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-(*N*-(2-(trifluoromethyl)phenyl) sulphamoyl)phenyl)benzamide (4f): White powder, yield: 46%. mp: 170-172°C. ^{1}H-NMR (DMSO-d_6): δ3.84 (s, 3H, CH$_3$), 7.20 (d, 1H, *J*=8.8 Hz, Ar-H), 7.36-7.38 (m, 3H, Ar-H), 7.48 (t, 1H, *J*=8.0 Hz, Ar-H), 7.55 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.57 (d, 1H, *J*=2.7 Hz, Ar-H), 7.76 (d, 2H, *J*=8.8 Hz, Ar-H), 7.86 (d, 2H, *J*=8.8 Hz, Ar-H), 10.58 (br s, 1H, NH), 10.69 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ56.4, 114.1, 115.4, 119.6, 120.2, 123.0, 124.9, 126.7, 128.0, 128.7, 129.7, 130.0, 130.7, 131.6, 133.3, 139.0, 142.9, 155.3, 163.9. MS (ESI) *m/z* [M-H]$^-$ calcd. for C$_{21}$H$_{15}$ClF$_3$N$_2$O$_4$S$^-$ 483.0399 found 483.0507. Anal. RP-HPLC Method A: *t*R 12.35 min, purity>99%; Method B: *t*R 12.07 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-(*N*-(4-(trifluoromethyl)phenyl) sulphamoyl)phenyl)benzamide (4g): White powder, yield: 49%. mp: 173-175°C. ^{1}H-NMR (DMSO-d_6): δ3.84 (s, 3H, CH$_3$), 7.20 (d, 1H, *J*=8.8 Hz, Ar-H), 7.28 (d, 2H, *J*=8.8 Hz, Ar-H), 7.55 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.57 (d, 1H, *J*=2.7 Hz, Ar-H), 7.60 (d, 2H, *J*=8.8 Hz, Ar-H), 7.80 (d, 2H, *J*=8.8 Hz, Ar-H), 7.87 (d, 2H, *J*=8.8 Hz, Ar-H), 10.59 (br s, 1H, NH), 10.83 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6): 56.8, 114.5, 119.1, 120.0, 123.6, 124.6, 125.8, 127.0, 127.1, 128.5, 129.1, 132.0, 143.4, 155.7, 164.4. MS (ESI) *m/z* [M-H]$^-$ calcd. for C$_{21}$H$_{15}$ClF$_3$N$_2$O$_4$S$^-$ 483.0399 found 483.0594. Anal. RP-HPLC Method A: *t*R 12.46 min, purity>99%; Method B: *t*R 12.11 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-(*N*-(2-methoxyphenyl)sulphamoyl) phenyl)benzamide (4h): White powder, yield: 83%. mp: 173-175°C. ^{1}H-NMR (CDCl$_3$): δ3.91 (s, 3H, CH$_3$), 4.30 (s, 3H, CH$_3$), 6.99 (d, 1H, *J*=8.2 Hz, Ar-H), 7.15 (t, 1H, *J*=7.8 Hz, Ar-H), 7.24 (d, 1H, *J*=8.9 Hz, Ar-H), 7.26 (br s, 1H, NH), 7.29 (t, 1H, *J*=7.9 Hz, Ar-H), 7.71 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.79 (d, 1H, *J*=7.9 Hz, Ar-H) 7.94 (d, 2H, *J*=8.8 Hz, Ar-H), 8.00 (d, 2H, *J*=8.8 Hz, Ar-H), 8.47 (d, 1H, *J*=2.7 Hz, Ar-H),10.15 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ55.9, 57.0, 110.8, 113.4, 119.9, 121.3, 121.5, 122.7, 125.7, 126.1, 127.6, 128.8, 132.5, 133.7, 134.3, 142.3, 149.8, 155.9, 162.4. MS (ESI) *m/z* [M-H]$^-$ calcd. for C$_{21}$H$_{18}$ClN$_2$O$_5$S$^-$ 445.0630 found 445.0874. Anal. RP-HPLC Method A: *t*R 11.99 min, purity>99%; Method B: *t*R 11.72 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-(*N*-(4-methoxyphenyl)sulphamoyl) phenyl)benzamide (4i): White crystalline solid, yield: 62%. mp: 217-219°C. ^{1}H-NMR (DMSO-d_6): δ3.67 (s, 3H, CH$_3$), 3.85 (s, 3H, CH$_3$), 6.80 (d, 2H, *J*=9.0 Hz, Ar-H), 6.98 (d, 2H, *J*=9.0 Hz, Ar-H), 7.20 (d, 1H, *J*=8.5 Hz, Ar-H), 7.55 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.58 (d, 1H, *J*=2.7 Hz, Ar-H), 7.64 (d, 2H, *J*=8.8 Hz, Ar-H), 7.82 (d, 2H, *J*=8.8 Hz, Ar-H), 9.83 (br s, 1H, NH), 10.54 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ55.2, 56.3, 114.1, 114.3, 119.3, 123.4, 124.2, 126.7, 127.9, 128.7, 130.2, 131.5, 133.7, 142.5, 155.3, 156.5, 163.9. MS (ESI) *m/z* [M-H]$^-$ calcd. for C$_{21}$H$_{18}$ClN$_2$O$_5$S$^-$ 445.0630 found 445.0757. Anal. RP-HPLC Method A: *t*R 11.85min, purity>98%; Method B: *t*R 11.43 min, purity>98%.

5-chloro-*N*-(4-(*N*-(4-fluorophenyl)sulphamoyl)phenyl)-2-methoxybenzamide (4j): White powder, yield: 45%. mp: 197-199°C. ^{1}H-NMR (DMSO-d_6) δ3.85 (s, 3H, CH$_3$), 7.08 (d, 4H, *J*=6.6 Hz, Ar-H), 7.20 (d, 1H, *J*=8.8 Hz, Ar-H), 7.55 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.53 (d, 1H, *J*=2.7 Hz, Ar-H), 7.67 (d, 2H, *J*=8.8 Hz, Ar-H), 7.67 (d, 2H, *J*=8.8 Hz, Ar-H), 10.15 (br s, 1H, NH), 10.56 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ56.4, 114.1, 115.9, 119.4, 122.8, 124.3, 124.3, 126.7, 127.9, 128.8, 131.6, 133.5, 134.1, 142.7, 155.3, 164.0. MS (ESI) *m/z* [M-H]$^-$ calcd. for C$_{20}$H$_{15}$ClFN$_2$O$_4$S$^-$ 433.0431 found 433.0430. Anal. RP-HPLC Method A: *t*R 12.02 min, purity>96%; Method B: *t*R 11.59 min, purity>98%.

5-chloro-*N*-(4-(*N*-(5-fluoro-2-methylphenyl)sulphamoyl) phenyl)-2-methoxybenzamide (4K): White powder, yield: 52%. mp:

185-187°C. ^{1}H-NMR (CDCl$_3$): δ2.03 (s, 3H, CH$_3$), 4.09 (s, 3H, CH$_3$), 6.27 (br s, 1H, NH), 6.84 (d, 1H, *J*=9.4 Hz, Ar-H), 6.87 (d, 1H, *J*=2.8 Hz, Ar-H), 7.01 (d, 1H, *J*=8.9 Hz, Ar-H) 7.21-7.24 (m, 1H, Ar-H), 7.49 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.69 (d, 2H, *J*=8.8 Hz, Ar-H), 7.77 (d, 2H, *J*=8.8 Hz, Ar-H), 8.25 (d, 1H, *J*=2.7 Hz, Ar-H), 10.00 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ18.1, 57.0, 113.4, 113.9, 117.5, 120.1, 122.6, 127.6, 127.9 128.0, 128.7, 130.2, 132.5, 133.7, 134.4, 135.8, 155.9, 160.2, 162.4. MS (ESI) *m/z* [M-H]$^-$ calcd.C$_{21}$H$_{17}$ClFN$_2$O$_4$S$^-$ 447.0587 found 447.0736. Anal. RP-HPLC Method A: *t*R 12.17 min, purity>99%; Method B: *t*R 11.83 min, purity>99%.

***N*-(4-(*N*-benzylsulphamoyl)phenyl)-5-chloro-2-methoxybenzamide (4l):** White powder, yield: 62%. mp: 172-174°C. ^{1}H-NMR (CDCl$_3$): δ4.34 (s, 3H, CH$_3$), 4.40 (d, 2H, *J*=6.2 Hz, CH$_2$), 4.85 (t, 1H, *J*=7.4 Hz, NH), 7.27 (d, 1H, *J*=8.9 Hz, Ar-H), 7.46 (d, 2H, *J*=8.2 Hz, Ar-H), 7.52 (t, 1H, *J*=7.4 Hz, Ar-H), 7.54 (t, 2H, *J*=7.4 Hz, Ar-H), 7.74 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 8.07 (d, 2H, *J*=8.8 Hz, Ar-H), 8.13 (d, 2H, *J*=8.8 Hz, Ar-H), 8.51 (d, 1H, *J*=2.7 Hz, Ar-H), 10.23 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ47.5, 56.9, 113.3, 120.4, 122.7, 127.6, 128.1, 128.2, 128.7, 128.9, 132.5, 133.6, 134.9, 136.3, 142.3, 155.9, 162.4. MS (ESI) *m/z* [M-H]$^-$ calcd. for C$_{21}$H$_{18}$ClN$_2$O$_4$S$^-$ 429.0681 found 429.0740. Anal. RP-HPLC Method A: *t*R 12.07 min, purity>99%; Method B: *t*R 11.64 min, purity>99%.

5-chloro-*N*-(4-(*N*-isopropylsulphamoyl)phenyl)-2-methoxybenzamide (4m): White powder, yield: 93%. mp: 185-187°C. ^{1}H-NMR (CDCl$_3$): δ1.09 (d, *J*=6.6 Hz, 6H, 2 CH$_3$), 3.44-3.51 (m, 1H, CH), 4.09 (s, 3H, CH$_3$), 6.52 (br d, 1H, *J*=7.3 Hz, NH), 6.99 (d, 1H, *J*=8.9 Hz, Ar-H), 7.47 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.80 (d, 2H, *J*=8.8 Hz, Ar-H), 7.87 (d, 2H, *J*=8.8 Hz, Ar-H), 8.24 (d, 1H, *J*=2.7 Hz, Ar-H), 9.96 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ24.1, 46.3, 57.0, 113.4, 120.3, 122.7, 127.6, 128.5, 132.5, 133.7, 136.3, 142.1, 156.0, 162.4. MS (ESI) *m/z* [M+H]$^+$ calcd. for C$_{17}$H$_{20}$ClN$_2$O$_4$S$^+$ 383.0827 found 383.0748. Anal. RP-HPLC Method A: *t*R 11.77 min, purity>99%; Method B: *t*R 11.28 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-(*N*-propylsulphamoyl)phenyl) benzamide (4n): White powder, yield: 92%. mp: 182-184°C. ^{1}H-NMR (CDCl$_3$): δ0.9 (t, *J*=7.4 Hz, 3H, CH$_3$), 1.50-1.60 (m, 2H, CH$_2$), 2.95 (q, *J*=6.7 Hz, 2H, CH$_2$), 4.11 (s, 3H, CH$_3$), 4.34-4.36 (br m, 1H, NH), 7.03 (d, 1H, *J*=8.9 Hz, Ar-H), 7.50 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.83 (d, 2H, *J*=8.9 Hz, Ar-H), 7.87 (d, 2H, *J*=8.9 Hz, Ar-H), 8.27 (d, 1H, *J*=2.7 Hz, Ar-H), 9.98 (br s, 1H, NH).^{13}C-NMR (CDCl$_3$): δ11.3, 23.2, 45.2, 57.0, 113.4, 120.4, 122.9, 127.7, 128.6, 132.6, 133.7, 135.2, 142.1, 155.9, 162.4. MS (ESI) *m/z* [M+H]$^+$ calcd. for C$_{17}$H$_{20}$ClN$_2$O$_4$S$^+$ 383.0827 found 383.0553. Anal. RP-HPLC Method A: *t*R 12.81 min, purity>99%; Method B: *t*R 11.34 min, purity>99%.

5-chloro-*N*-(4-(*N*-(3-(dimethylamino)propyl)sulphamoyl) phenyl)-2-methoxybenzamide (4o): White crystalline solid, yield: 46%. mp: 224-226°C. ^{1}H-NMR (DMSO-d_6): δ1.76-1.81 (m, 2H, CH$_2$), 2.70 (s, 6H, 2CH$_3$), 2.78 (q, *J*=6.7 Hz, 2H, CH$_2$), 3.02 (t, *J*=6.7 Hz, 2H, CH$_2$), 3.87 (s, 3 H, CH$_3$), 7.23 (d, 1H, *J*=8.9 Hz, Ar-H), 7.57 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.60 (d, 1H, *J*=8.9 Hz, Ar-H), 7.78 (d, 2H, *J*=8.9 Hz, Ar-H), 7.92 (d, 2H, *J*=8.9 Hz, Ar-H), 10.14 (br s, 1H, NH), 10.60 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ24.2, 42.1, 54.3, 56.4, 114.1, 119.6, 124.3, 126.2, 126.7, 127.8, 128.7, 131.6, 134.5, 142.4, 155.3, 163.9. MS (ESI) *m/z* [M+H]$^+$ calcd. for C$_{19}$H$_{25}$ClN$_3$O$_4$S$^+$ 426.1249 found 426.1424. Anal. RP-HPLC Method A: *t*R 10.01 min, purity>95%; Method B: *t*R 8.61 min, purity>95%.

5-chloro-*N*-(4-(*N*-cyclohexylsulphamoyl)phenyl)-2-methoxybenzamide (4p): White powder, yield: 56%. mp: 170-172°C. ^{1}H-NMR (CDCl$_3$): δ1.05-1.24 (m, 6H, 3CH$_2$), 1.48-1.50 (m, 1H, CH), 1.60-1.63 (m, 2H, CH$_2$), 1.73-1.75 (m, 2H, CH$_2$), 4.06 (s, 3H, CH$_3$), 4.83

(br d, 1H, *J*=7.2 Hz, NH), 6.98 (d, 1H, *J*=8.9 Hz, Ar-H), 7.43 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.78 (d, 2H, *J*=8.8 Hz, Ar-H), 7.84 (d, 2H, *J*=8.8 Hz, Ar-H), 8.19 (d, 1H, *J*=2.7 Hz, Ar-H), 9.96 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ24.8, 25.3, 34.1, 52.8, 57.0, 113.4, 120.27, 122.7, 127.4, 128.3, 132.3, 133.5, 136.6, 141.9, 155.9, 162.4. MS (ESI) *m/z* [M+H]$^+$ calcd. for C$_{20}$H$_{24}$ClN$_2$O$_4$S$^+$ 423.1140 found 423.1053. Anal. RP-HPLC Method A: *t*R 12.33 min, purity>99%; Method B: *t*R 12.01 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-(morpholinosulphonyl)phenyl) benzamide (4q): White crystalline solid, yield: 78%. mp: 238-240°C. ^{1}H-NMR (CDCl$_3$): δ3.02 (t, *J*=9.2 Hz, 4H, 2CH$_2$), 3.77 (t, *J*=9.2 Hz, 4H, 2CH$_2$), 4.11 (s, 3H, CH$_3$), 7.03 (d, 1H, *J*=8.8 Hz, Ar-H), 7.50 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.78 (d, 2H, *J*=8.8 Hz, Ar-H), 7.87 (d, 2H, *J*=8.8 Hz, Ar-H), 8.26 (d, 1H, *J*=2.7 Hz, Ar-H), 10.00 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ46.2, 57.1, 66.3, 113.4, 120.4, 122.7, 127.6, 129.4, 130.2, 132.5, 133.7, 142.6, 156.0, 162.5. MS (ESI) *m/z* [M+H]$^+$ calcd. C$_{18}$H$_{20}$ClN$_2$O$_5$S$^+$ 411.0776 found 411.0483. Anal. RP-HPLC Method A: *t*R 11.69 min, purity>99%; Method B: *t*R 11.21 min, purity>99%.

5-chloro-*N*-(4-((4-hydroxypiperidin-1-yl)sulponyl)phenyl)-2-methoxybenzamide (4r): White crystalline solid, yield: 56%. mp: 220-222°C. ^{1}H-NMR (CDCl$_3$): δ1.05-1.12 (m, 1H, CH), 1.40-1.49 (m, 1H, CH), 1.69-1.72 (m, 2H, CH$_2$), 2.09-2.13 (m, 1H, CH), 2.29-2.33 (m, 1H, CH), 3.27-3.29 (m, 1H, CH), 3.39-3.41 (m, 1H, CH), 3.50-3.55 (m, 1H, CH), 3.87 (s, 3H, CH$_3$), 4.99 (d, 1H, *J*=4.65 Hz, OH), 7.22 (d, 1H, *J*=8.9 Hz, Ar-H), 7.57 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.60 (d, 1H, *J*=2.7 Hz, Ar-H), 7.71 (d, 2H, *J*=8.7 Hz, Ar-H), 7.96 (d, 2H, *J*=8.7 Hz, Ar-H), 10.63 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ22.3, 32.0, 45.9, 52.7, 56.4, 65.0, 114.1, 119.6, 124.3, 126.9, 128.7, 128.8, 129.8, 131.6, 143.0, 155.4, 164.1. MS (ESI) *m/z* [M+H]$^+$ calcd. for C$_{19}$H$_{22}$ClN$_2$O$_5$S$^+$ 425.0932 found 425.0949. Anal. RP-HPLC Method A: *t*R 11.83 min, purity>99%; Method B: *t*R 10.32 min, purity>99%.

5-chloro-2-methoxy-*N*-(4-((4-methylpiperazin-1-yl)sulphonyl) phenyl)benzamide (4s): White powder, yield: 66%, mp: 180-182°C. ^{1}H-NMR (CDCl$_3$): δ2.40 (s, 3H, CH$_3$), 2.63 (br s, 4H, 2CH$_2$), 3.18 (br s, 4H, 2CH$_2$), 4.19 (s, 3H, CH$_3$), 7.10 (d, 1H, *J*=8.9 Hz, Ar-H), 7.57 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.83 (d, 2H, *J*=8.8 Hz, Ar-H), 7.92 (d, 2H, *J*=8.8 Hz, Ar-H), 8.33 (d, 1H, *J*=2.7 Hz, Ar-H), 10.06 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ45.8, 46.0, 54.2, 57.1, 113.4, 120.2, 122.7, 127.6, 129.3, 130.4, 132.5, 133.7, 142.5, 155.9, 162.4. MS (ESI) *m/z* [M+H]$^+$ calcd. for C$_{19}$H$_{23}$ClN$_3$O$_4$S$^+$ 424.1092 found 424.0984. Anal. RP-HPLC Method A: *t*R 10.10 min, purity>99%; Method B: *t*R 8.6 min, purity>99%.

_N_-(4-((4-acetylpiperazin-1-yl)sulphonyl)phenyl)-5-chloro-2-methoxybenzamide (4t): White powder, yield: 52%. mp: 170-172°C. ^{1}H-NMR (CDCl$_3$): δ2.04 (s, 3H, CH$_3$), 3.00 (t, 2H, *J*=9.7, CH$_2$), 3.04 (t, 2H, *J*=9.5, CH$_2$), 3.56 (t, 2H, *J*=9.7, CH$_2$), 3.71 (t, 2H, *J*=9.5, CH$_2$), 4.10 (s, 3H, CH$_3$), 7.02 (d, 1H, *J*=8.8 Hz, Ar-H), 7.49 (dd, 1H, *J*=8.8 and 2.7 Hz, Ar-H), 7.74 (d, 2H, *J*=8.7 Hz, Ar-H), 7.86 (d, 2H, *J*=8.7 Hz, Ar-H), 8.24 (d, 1H, *J*=2.7 Hz, Ar-H), 10.00 (br s, 1H, NH). ^{13}C-NMR (CDCl$_3$): δ21.4, 40.8, 45.8, 46.0, 46.3, 57.0, 113.4, 120.4, 122.6, 127.6, 129.2, 130.1, 132.4, 133.7, 142.7, 155.9, 162.5, 169.1. MS (ESI) *m/z* [M+H]$^+$ calcd. for C$_{20}$H$_{23}$ClN$_3$O$_5$S$^+$ 452.1041 found 452.0593. Anal. RP-HPLC Method A: *t*R 11.37 min, purity>99%; Method B: *t*R 10.54 min, purity>99%.

Biology

Cell culture: Human pancreatic epithelioid carcinoma cell line (PANC-1), pancreatic adenocarcinoma cell line (PANC 10.05) and pancreatic carcinoma cell line (Mia PaCa-2) were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Epithelial ovarian cancer cell line (A2780) was purchased from the European Collection of Cell Culture (ECACC). Biphenotypic B myelomonocytic leukaemia cell line (MV-4-11), acute myeloblastic leukaemia cell line (Kasumi-1), acute myeloid leukaemia cell line (PL-21), acute myelogenous leukaemia cell line (KG-1) and histiocytic lymphoma cell line (U-937) were kindly provided by Prof. R. D'Andrea (University of South Australia). Epithelial colon cancer cell line (HCT-116) was obtained from the cell bank at the Centre for Drug Discovery and Development, University of South Australia. The cell lines were cultured in RPMI-1640 with 10% fetal bovine serum (FBS).

Cell viability assay: The cell viability experiments of suspension cell lines *i.e.* MV-4-11, Kasumi-1, PL-21, KG-1 and U-937 were performed with resazurin (Sigma-Aldrich) assay as previously described [25]. Cells were seeded into 96-well plates and incubated at 37°C, 5% CO$_2$ overnight. Each compound was diluted from a 2 or 10 mM stock solution to prepare a five-fold dilution series in 100 μL of cell medium, added to cells (in triplicates), and incubated at 37°C, 5% CO$_2$ for 72 h. Resazurin (Sigma-Aldrich) was made up as a stock of 0.1 mg/mL in cell medium and filter-sterilised. The resazurin solution was added at 20 μL/well and incubated in the dark at 37°C, 5% CO$_2$ for 4 h. The plate was left at room temperature for 10-15 min, and absorbance was measured at 585 nm using an EnVision multi-label plate reader (PerkinElmer, Buckinghamshire, UK).

On the other hand, the cell viability experiments of non-suspension cell lines *i.e.* A2780, HCT-116, PANC-1, PANC 10.05 and Mia PaCa-2 were carried out with MTT (Sigma-Aldrich) assays as described previously [26]. In short, cells were seeded into 96-well plates according to doubling time and incubated overnight at 37°C. Test compounds were made up in DMSO, and a 3-fold dilution series was prepared in 100 μL of cell medium, added to cells (in triplicates), and incubated for 72 or 96 h at 37°C. MTT was made up as a stock of 5 mg/mL in cell medium, and the solution was filter-sterilised. Medium was removed from cells followed by a wash with 200 μL/well of PBS. MTT solution was then added at 20 μL/well and incubated in the dark at 37°C for 4 h. MTT solution was removed and cells were again washed with 200 μL of PBS. MTT dye was solubilised with 200 μL/well of DMSO with agitation. Absorbance was read at 540 nm. Compound concentrations required to inhibit 50% of cell growth (GI$_{50}$) were calculated using non-linear regression analysis.

Cell cycle and apoptosis detection: The cell cycle experiment and apoptosis detection for MiaPaCa-2 cells were tested with flow cytometry, as described previously [25]. Briefly, the MiaPaCa-2 cells were seeded at 8×10^4 and incubated overnight at 37°C, 5% CO$_2$ before treatment. After treatment with the compounds, cells were trypsinised and collected for staining. For cell cycle experiments, collected cells were fixed with 70% ethanol on ice for 15 min and centrifuged again at 300 g for 5 min to recollect the cells. The collected pellets were incubated with propidium iodide (PI) staining solution (50 μg/mL PI, 0.1 mg/mL RNase A, 0.05% Triton X-100) at room temperature for 1 h and analysed by Gallios flow cytometry with FACS (Beckman Coulter). The apoptosis detections were performed with annexin-V/PI assay. The treated cell pellets were collected and stained with annexin-V FITC/PI commercial kit (Becton Dickinson) following the supplier's protocol. The samples were analysed by fluorescence-activated cell sorting (FACS) with Gallios flow cytometry (Beckman Coulter) within 1 h after staining. The data were analysed using Kaluza v1.2 (Beckman Coulter).

Results and Discussion

Chemistry

The synthetic route to 5-chloro-2-methoxy-*N*-(4-sulphamoylphenyl)benzamide derivatives 4a-4t is outlined in Scheme 1. 5-Chloro-2-methoxybenzoic acid 1 was reacted with

aniline using ethylchloroformate as coupling reagent in the presence of triethylamine in dichloromethane (DCM) to give 5-chloro-2-methoxy-*N*-phenylbenzamide 2 in a yield of 94%. Subsequently, the chlorosulphonation of amide 2 was achieved by reacting with chlorosulphonic acid affording 4-(5-chloro-2-methoxybenzamido) benzenesulphonyl chloride 3 in a yield of 87%. Finally, sulphonyl chloride 3 was coupled with appropriate amines in the presence of sodium carbonate in a mixture of tetrahydrofuran (THF) and water to yield the desired 5-chloro-2-methoxy-*N*-(4-sulphamoylphenyl) benzamide derivatives 4a-4t; in moderate to excellent yields (45-93%).

Structure-activity relationship analysis

The anti-proliferative activity of these sulphonamide derivatives was evaluated with A2780 and HCT-116 cell lines using MTT assay. Both cell lines are frequently used as model systems for exploration of cancer pathways and for innovation of new therapeutic approaches [27]. Moreover, they are commonly used in the assessment of the anti-proliferative activity of many sulphonamide compounds [27-29]. The GI_{50} values are summarised in Table 1. E7010, a known anti-cancer sulphonamide, was used as a positive control in the assays.

In general, ovarian cancer A2780 cells seemed more sensitive to compounds with aromatic sulphonamide substitutions, while HCT-116 cells were more sensitive to compounds with aliphatic sulphonamide groups. To put this in perspective, for compounds with aromatic sulphonamides, the nature and position of the substituents of R had a tangible effect on the cellular activity. Compound 4j, *i.e.* R=NH-(*p*-F)Ph, exhibited the most potent anti-proliferative activity with GI_{50} values of 29.1 and 39.3 μM in A2780 and HCT-116 cells, respectively, suggesting the importance of the *para*-fluorine substituent for cellular activity. Further introduction of an additional methyl group at the *ortho* position resulting in 4k not only reduced the activity against A2780 but also abolished the activity against HCT-116 cells. In general, all substitutions gave less active derivatives when compared to 4a, 4j and 4b against A2780. However, the effects of the substituents on cellular potency were not clear. For example, 4d, *i.e.* R=NH-(*o*-Et)Ph, was more cytotoxic than 4e, *i.e.* R=NH-(*p*-Et)Ph, to A2780 cells. In contrast, 4f (R=NH-(*o*-CF₃)Ph) was less potent than 4g, *i.e.* R=NH-(*p*-CF₃)Ph). Also, the *ortho* substitution led to the similar activities, although *ortho*-OCH₃ substituted derivative (4h) gave rise to the least potent compound against A2780 cells among all compounds with aromatic sulphonamide groups. In addition, spacing the phenyl ring with one methylene group

halved the anti-proliferative activity against A2780 cells and abrogated the activity against HCT-116 cells (i.e. 4a versus 4l).

For the aliphatic sulphonamide analogues, 4m (R=NH-*i*Pr) was more active than 4n (R=NH-*n*Pr), suggesting the importance of the branching structure for the activity. 4o, *i.e.* R=NH(CH₂)₃N(CH₃)₂, exhibited the most potent anti-proliferative activity among all aliphatic sulphonamides.

Moreover, compounds 4q (R=1-morpholinyl) and 4r (R=*p*-hydroxypipridinyl) exerted similar activity against A2780 cells, but 4q was more active in HCT-116 cells. Similarly, the derivatives containing *p*-methylpiperazinyl (4s) or *p*-acetylpiperazinyl group (4t) had a similar effect on A2780 cells but the former was more active against HCT-116 cells. Noticeably, the derivatives containing an aliphatic chain were more potent compared to their cyclic counterparts. Most compounds were more cytotoxic to A2780 cells than HCT-116 cells except 4o and 4q.
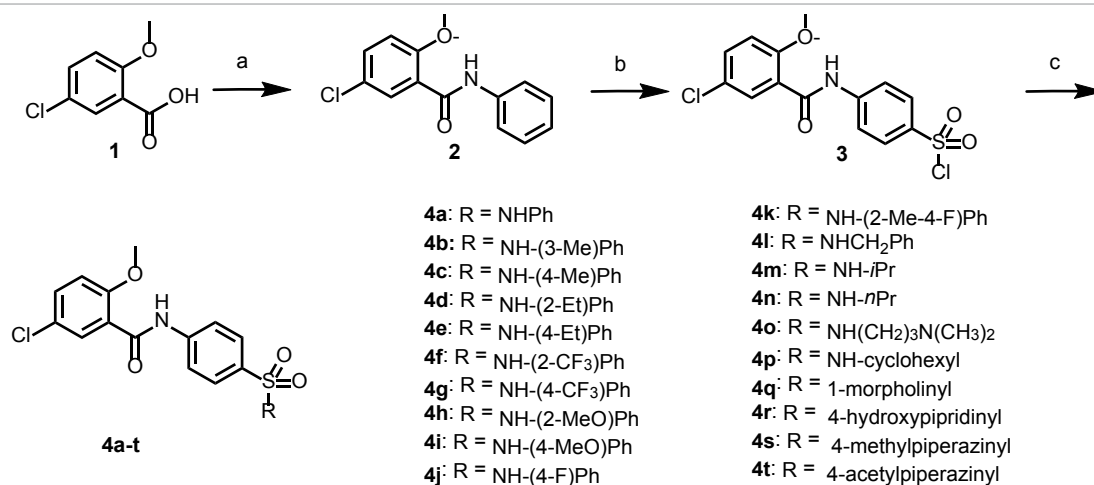
Cell-type sensitivity

As one of the most biological active compounds, 4j was selected for further evaluation of anti-proliferative activity against a panel of eight human tumour cell lines, including leukaemia MV-4-11, Kasumi-1, PL-21, KG-1, U-937 and pancreatic cancer PANC-1, PANC 10.05 and MIA PaCa-2, to investigate its cell-type selectivity. The results are summarised in Table 2.
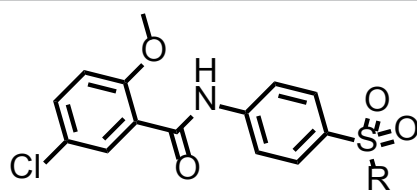
MIA PaCa-2 cell line was shown to be most sensitive towards the treatment of 4j (GI_{50}=1.9 μM), whilst PANC 10.05 was the least sensitive (GI_{50}=83.4 μM). Despite the fact that 4j exhibited a high activity in MIA PaCa-2 over other two pancreatic cell lines (*i.e.* PANC-1 and PANC 10.05) the compound showed little difference in the leukaemia cells tested (*i.e.* MV-4-11, Kasumi-1, PL-21, KG-1 and U-937), giving GI_{50} values ranging from 22.7 to 37.5 μM. These findings agree with the previous studies that different aryl sulphonamides had variable cell-type specificity [30-36], presumably due to their different cellular mechanisms of action [24].

Cellular mechanism of action

We next investigated the cellular mode of action of 4j in MIA PaCa-2 cells. E7010 served as a positive control. To evaluate whether the anti-proliferative effect of 4j is a consequence of cell cycle effects, MIA PaCa-2 cells were exposed to each compound at the concentration of 5x or $10 \times GI_{50}$ μM for a period of 24 hours, and the cell cycle effects were

4a: R = NHPh
4b: R = NH-(3-Me)Ph
4c: R = NH-(4-Me)Ph
4d: R = NH-(2-Et)Ph
4e: R = NH-(4-Et)Ph
4f: R = NH-(2-CF₃)Ph
4g: R = NH-(4-CF₃)Ph
4h: R = NH-(2-MeO)Ph
4i: R = NH-(4-MeO)Ph
4j: R = NH-(4-F)Ph

4k: R = NH-(2-Me-4-F)Ph
4l: R = NHCH₂Ph
4m: R = NH-*i*Pr
4n: R = NH-*n*Pr
4o: R = NH(CH₂)₃N(CH₃)₂
4p: R = NH-cyclohexyl
4q: R = 1-morpholinyl
4r: R = 4-hydroxypipridinyl
4s: R = 4-methylpiperazinyl
4t: R = 4-acetylpiperazinyl

Scheme 1: Synthesis of 5-chloro-2-methoxy-*N*-(4-sulphamoylphenyl) benzamide derivatives (4a-t). Reagents and conditions: (a) aniline, triethylamine, ethylchloroformate, DCM, rt, 3 h, 94%; (b) chlorosulphonic acid, neat, rt, 12 h, 87%; (c) appropriate amine, sodium carbonate, THF/H₂O (2:1), rt, 12 h, 45-93%.

Citation: Abdelaziz AM, Yu M, Li P, Zhong L, Singab ANB, et al. (2015) Synthesis and Evaluation of 5-Chloro-2-Methoxy-*N*-(4-Sulphamoylphenyl) Benzamide Derivatives as Anti-cancer Agents. Med chem 5: 253-260. doi:10.4172/2161-0444.1000272

Compounds	R	Cytotoxicity GI$_{50}$ (µM)*	
		A2780	HCT-116
4a	NHPh	38.7 ± 3.7	48.5 ± 3.0
4b	NH-(*m*-Me)Ph	31.8 ± 5.9	>100
4c	NH-(*p*-Me)Ph	44.6 ± 3.1	>100
4d	NH-(*o*-Et)Ph	42.8 ± 4.8	>100
4e	NH-(*p*-Et)Ph	52.1 ± 27.3	>100
4f	NH-(*o*-CF$_3$)Ph	45.0 ± 1.3	>100
4g	NH-(*p*-CF$_3$)Ph	40.6 ± 4.5	>100
4h	NH-(*o*-MeO)Ph	63.5 ± 6.2	76.8 ± 4.7
4i	NH-(*p*-MeO)Ph	46.7 ± 4.6	58.1 ± 5.6
4j	NH-(*p*-F)Ph	29.1 ± 3.4	39.3 ± 2.9
4k	NH-(*o*-Me-4-F)Ph	42.9 ± 5.8	>100
4l	NHCH$_2$Ph	73.9 ± 5.5	>100
4m	NH-*i*Pr	34.7 ± 2.4	59.2 ± 6.9
4n	NH-*n*Pr	55.5 ± 16.7	69.7 ± 5.2
4o	NH(CH$_2$)$_3$N(CH$_3$)$_2$	31.7 ± 2.5	24.7 ± 3.4
4p	NH-cyclohexyl	37.6 ± 8.8	50.1 ± 4.9
4q	1-morpholinyl	73.8 ± 6.7	56.4 ± 5.8
4r	*p*-hydroxypipridinyl	68.7 ± 22.3	96.6 ± 10.7
4s	*p*-methylpiperazinyl	45.9 ± 10.7	61.6 ± 8.5
4t	*p*-acetylpiperazinyl	48.9 ± 8.2	>100
E7010	-	40.2 ± 2.1	64.4 ± 4.3

Table 1: The structures and anti-proliferative activity of **4a-t**.

Human cell line		Cytotoxicity
Origin	Designation	GI$_{50}$ µM ± S.D.
Biphenotypic B myelomonocytic leukaemia	MV-4-11	29.2 ± 7.4*
Acute myeloblastic leukaemia	Kasumi-1	37.5 ± 5.0*
Acute myeloid leukaemia	PL-21	24.0 ± 11.4*
Acute myelogenous leukaemia	KG-1	26.4 ± 10.1*
Histiocytic lymphoma	U-937	22.7 ± 9.9*
Pancreatic epithelioid carcinoma	PANC-1	47.0 ± 26.9**
Pancreatic adenocarcinoma	PANC 10.05	83.4 ± 31.6**
Pancreatic carcinoma	MIA PaCa-2	1.9 ± 0.2**

*GI$_{50}$ values were determined by 72 h resazurin assay. **GI$_{50}$ values were determined by 72 h MTT assay. Data given are the mean ± standard deviation derived from at least two replicates.

Table 2: Anti-proliferative activity of **4j** in human cancer cell lines.

analysed by flow cytometry. As shown in Figure 1, the treatment with E7010 resulted in a substantial accumulation of MIA PaCa-2 cells at the G2/M phase (54.7 and 55.7% at 2.5 and 5 µM, respectively) compared to the untreated cells (28.7% in the G2/M). This is consistent with the tubulin targeting mechanism of E7010 [36-39]. Similarly, 4j increased in the population of the G2/M cells (~ 42%) at concentrations of 10 (5 × GI$_{50}$ µM) and 20 µM (10 × GI$_{50}$ µM), suggesting a similar but weaker cellular mechanism compared to E7010.

To further assess the apoptotic effect of 4j, MIA PaCa-2 cells were treated with 4j (or E7010) for 24 hours, and the cells were stained with dual annexin V-FITC and propidium iodide (annexin V-FITC/PI), and analysed by flow cytometry. As shown in Figure 2, the apoptotic cells, as indicated by annexin V$^+$/PI$^-$ and annexin V$^+$/PI$^+$, increased at least 6% upon treatment with 4j (or E7010) at the concentration of 5x or 10 × GI$_{50}$ µM when compared to the untreated cells.

Conclusion

We have identified a new series of sulphonamides. The anti-proliferative activity of these compounds was evaluated against A2780 and HCT-116 tumour cell lines, and the structure-activity relationship was analysed. The lead compound 4j exhibited a high potency against human pancreatic cancer cell line MIA PaCa-2. Cellular mechanistic investigation suggested that the anti-tumour activity of 4j was a consequence of the G2/M cell cycle effects and induction of apoptosis. Although further investigation is needed in order to elucidate the exact molecular targeting mechanism, this work suggests that the *N*-(4-sulphamoylphenyl)benzamide is a highly valuable scaffold to develop anti-cancer agents.
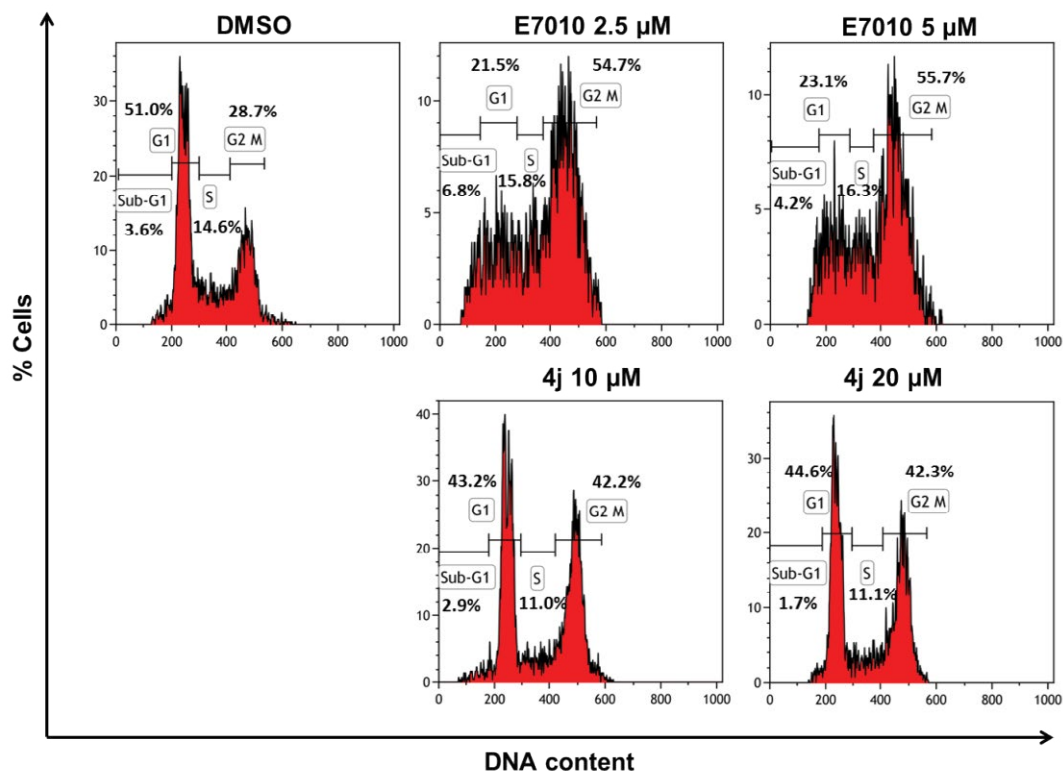
Acknowledgements

Figure 1: Cell cycle analysis of MIA PaCa-2 cells after treatment with **4j** or E7010 for 24 h.
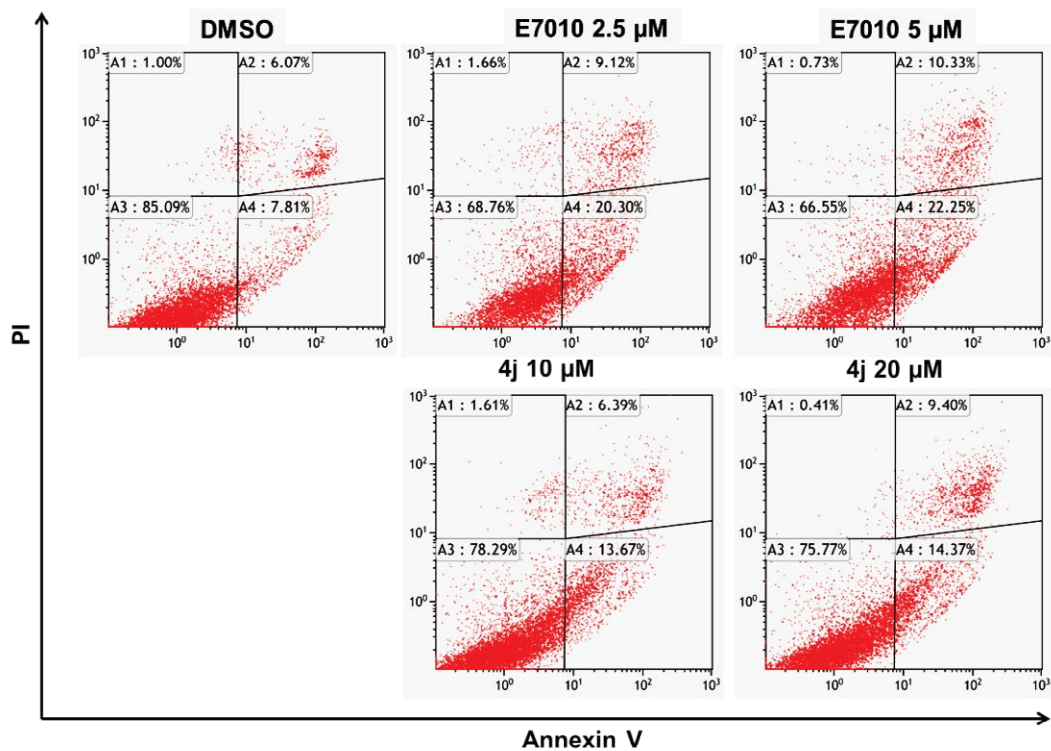

Figure 2: Cell cycle analysis of MIA PaCa-2 cells after treatment with **4j** or E7010 for 24 h.

References

1. Drews J (2000) Drug discovery: a historical perspective. Science 287: 1960-1964.

2. Supuran CT, Conroy CW, Maren TH (1996) Carbonic anhydrase inhibitors: Synthesis and inhibitory properties of ,3,4-thiadiazole-2,5-bissulphonamide. Eur J Med Chem 3: 843-846.

3. Boyd AE (1988) Sulfonylurea receptors, ion channels, and fruit flies. Diabetes 37: 847-850.

4. Wouters J, Michaux C, Durant F, Dogne JM, Delarge J, et al. (2000) Isosterism among analogues of torasemide: conformational, electronic and lipophilic properties. Eur J Med Chem 35: 923-929.

5. Supuran CT, Scozzafava A (2001) Carbonic anhydrase inhibitors, Current Medicinal Chemistry-Immunology. Endocrine & Metabolic Agents 61-97.

6. Supuran CT, Scozzafava A, Casini A (2003) Carbonic anhydrase inhibitors. Med Res Rev 23: 146-189.

7. Supuran CT, Casini A, Scozzafava A (2003) Protease inhibitors of the sulfonamide type: anticancer, antiinflammatory, and antiviral agents. Med Res Rev 23: 535-558.

8. Yoshino H, Ueda N, Niijima J, Sugumi H, Kotake Y, et al. (1992) Novel sulfonamides as potential, systemically active antitumor agents. J Med Chem 35: 2496-2497.

9. Banerjee M, Poddar A, Mitra G, Surolia A, Owa T, et al. (2005) Sulphonamide drugs binding to the colchicine site of tubulin: thermodynamic analysis of the drug-tubulin interactions by isothermal titration calorimetry. J Med Chem 48: 547-555.

10. Mohan R, Banerjee M, Ray A, Manna T, Wilson L, et al. (2006) Antimitotic sulphonamides inhibit microtubule assembly dynamics and cancer cell proliferation. Biochemistry 45: 5440-5449.

11. Skiles JW, Gonnella NC, Jeng AY (2004) The design, structure, and clinical update of small molecular weight matrix metalloproteinase inhibitors. Curr Med Chem 1: 2911-2977.

12. Hu L, Li ZR, Wang YM, Wu Y, Jiang JD, et al. (2007) Novel pyridinyl and pyrimidinylcarbazole sulfonamides as antiproliferative agents. Bioorg Med Chem Lett 17: 1193-1196.

13. Abbate F, Casini A, Owa T, Scozzafava A, Supuran CT (2004) Carbonic anhydrase inhibitors: E7070, a sulphonamide anticancer agent, potently inhibits cytosolic isozymes I and II, and transmembrane, tumor-associated isozyme IX. Bioorg Med Chem Lett 14: 217-223.

14. Carta F, Scozzafava A, Supuran CT (2012) Sulfonamides: a patent review (2008 - 2012). Expert Opin Ther Pat 22: 747-758.

15. Bukowski RM, Yasothan U, Kirkpatrick P (2010) Pazopanib. Nat Rev Drug Discov 9: 17-18.

16. Ajeet A, Tripathi L, Kumar A (2014) Synthesis, Spectral Characterization, Docking Studies and QSAR Screening of 4-amino-benzenesulphonamides/N-acetyl 4-amino-benzenesulphonamide Derivatives as Antimicrobial Agents. Journal of Pharmacy and Nutrition Sciences 4: 135-153.

17. Nielsen FE, Jacobsen P, Worsaae A, Arkhammar PO, Wahl P, et al. (2004) 2-(4-Methoxyphenoxy)-5-nitro-N-(4-sulfamoylphenyl)benzamide activates Kir6.2/SUR1 K(ATP) channels. Bioorg Med Chem Lett 14: 5727-5730.

18. Khanfar MA, Quinti L, Wang H, Choi SH, Kazantsev AG, et al. (2014) Development and characterization of 3-(benzylsulfonamido)benzamides as potent and selective SIRT2 inhibitors. Eur J Med Chem 76: 414-426.

19. Wang P, Liu C, Sanches T, Zhong Y, Liu B, et al. (2009) Design and synthesis of novel nitrogen-containing polyhydroxylated aromatics as HIV-1 integrase inhibitors from caffeic acid phenethyl ester. Bioorg Med Chem Lett 19: 4574-4578.

20. Domagala JM, Bader JP, Gogliotti RD, Sanchez JP, Stier MA, et al. (1997) A new class of anti-HIV-1 agents targeted toward the nucleocapsid protein NCp7: the 2,2'-dithiobisbenzamides. Bioorg Med Chem 5: 569-579.

21. Singh J, Shaik B, Singh S, Agrawal VK, Khadikar PV, et al. (2008) Comparative QSAR study on para-substituted aromatic sulphonamides as CAII inhibitors: information versus topological (distance-based and connectivity) indices. Chem Biol Drug Des 7: 244-259.

22. Brands M, Erguden JK, Hashimoto K, Heimbach D, Krahn T, et al. (2006) Selective indole-based ECE inhibitors: synthesis and pharmacological evaluation. Chem Med Chem 1: 96-105.

23. Melagraki G, Afantitis A, Sarimveis H, Igglessi-Markopoulou O, Supuran CT (2006) QSAR study on para-substituted aromatic sulphonamides as carbonic anhydrase II inhibitors using topological information indices. Bioorg Med Chem 14: 1108-1114.

24. Al-Said MS, Ghorab MM, Al-Qasoumi SI, El-Hossary EM, Noaman E (2010) Synthesis and in vitro anticancer screening of some novel 4-[2-amino-3-cyano-4-substituted-5,6,7,8-tetrahydroquinolin-1-(4H)-yl]benzenesulfonamides. Eur J Med Chem 45: 3011-3018.

25. Diab S, Teo T, Kumarasiri M, Li P, Yu M, et al. (2014) Discovery of 5-(2-(phenylamino)pyrimidin-4-yl)thiazol-2(3H)-one derivatives as potent Mnk2 inhibitors: synthesis, SAR analysis and biological evaluation. Chem Med Chem 9: 962-972.

26. Wang S, Meades C, Wood G, Osnowski A, Anderson S, et al. (2004) 2-Anilino-4-(thiazol-5-yl)pyrimidine CDK inhibitors: synthesis, SAR analysis, X-ray crystallography, and biological activity. J Med Chem 47: 1662-1675.

27. Lu T, Laughton CA, Wang S, Bradshaw TD (2015) In vitro antitumor mechanism of (E)-N-(2-methoxy-5-(((2,4,6-trimethoxystyryl)sulphonyl)methyl)pyridin-3-yl) methane sulphonamide. Mol Pharmacol 87: 18-30.

28. Liu X, Lam F, Shi S, Fischer PM, Wang S (2012) In vitro antitumor mechanism of a novel cyclin-dependent kinase inhibitor CDKI-83. Invest New Drugs 30: 889-897.

29. Abbassi N, Chicha H, Rakib el M, Hannioui A, Alaoui M, et al. (2012) Synthesis, antiproliferative and apoptotic activities of N-(6(4)-indazolyl)-benzenesulphonamide derivatives as potential anticancer agents. Eur J Med Chem 57: 240-249.

30. Seo JH, Jung KH, Son MK, Yan HH, Ryu YL, et al. (2013) Anti-cancer effect of HS-345, a new tropomyosin-related kinase A inhibitor, on human pancreatic cancer. Cancer Lett 338: 271-281.

31. Wang W, Ao L, Rayburn ER, Xu H, Zhang X, et al. (2012) KCN1, a novel synthetic sulphonamide anticancer agent: in vitro and in vivo anti-pancreatic cancer activities and preclinical pharmacology. PLoS One 7: e44883.

32. Liu ZL, Tian W, Wang Y, Kuang S, Luo XM, et al. (2012) A novel sulphonamide agent, MPSP-00, exhibits potent activity against human cancer cells in vitro through disruption of microtubule. Acta Pharmacol Sin 33: 261-270.

33. Tercel M, Atwell GJ, Yang S, Ashoorzadeh A, Stevenson RJ, et al. (2011) Selective treatment of hypoxic tumor cells in vivo: phosphate pre-prodrugs of nitro analogues of the duocarmicins. Angew Chem Int Ed Engl 50: 2606-2609.

34. Haritunians T, Gueller S, O'Kelly J, Ilaria R, Koeffler HP (2008) Novel acyl sulphonamide LY573636-sodium: effect on hematopoietic malignant cells. Oncol Rep 20: 1237-1242.

35. Supek F, Kralj M, Marjanovic M, Suman L, Smuc T, et al. (2008) Atypical cytostatic mechanism of N-1-sulfonylcytosine derivatives determined by in vitro screening and computational analysis. Invest New Drugs 26: 97-110.

36. Owa T, Okauchi T, Yoshimatsu K, Sugi NH, Ozawa Y, et al. (2000) A focused compound library of novel N-(7-indolyl)benzenesulfonamides for the discovery of potent cell cycle inhibitors. Bioorg Med Chem Lett 10: 1223-1226.

37. Kamal A, Subba AVR, Vishnuvardhan MV, Srinivas Reddy T, Swapna K, et al. (2015) Synthesis of 2-anilinopyridyl-triazole conjugates as antimitotic agents. Org Biomol Chem 13: 4879-4895.

38. Kim ND, Park ES, Kim YH, Moon SK, Lee SS, et al. (2010) Structure-based virtual screening of novel tubulin inhibitors and their characterization as anti-mitotic agents. Bioorg Med Chem 18: 7092-7100.

39. Yokoi A, Kuromitsu J, Kawai T, Nagasu T, Sugi NH, et al. (2002) Profiling novel sulfonamide antitumor agents with cell-based phenotypic screens and array-based gene expression analysis. Mol Cancer Ther 1: 275-286.