Synthesis and in vitro activity of novel 2-(benzylthio)-4-chloro-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide derivatives

Kamil Brożewicz · Jarosław Sławiński

Abstract Two series of novel 4-chloro-2-(benzylthio)-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamides and their N-arylated derivatives have been synthesized and evaluated for in vitro anticancer activity against the full NCI-60 cell line panel. Most of the compounds exhibited antiproliferative activity. Among them a compound bearing an N-(thien-2-ylcarbonyl) moiety showed broad-spectrum activity with 50% growth inhibition (GI50) values in the range of 2.02–7.82 μM over 50 cell lines.

Keywords Acylsulfonamides · 2-Mercaptobenzenesulfonamides · Antitumor agents · Phase-transfer catalysis · Heterocycles

Introduction

Aryl- and heteroaryl sulfonamides are an important class of therapeutic agents in current medicinal science [1]. Various arylsulfonamides have been reported to possess anticancer [2–6] and/or anti-human immunodeficiency virus (HIV) properties [6, 7]. Our systematic studies on the synthesis of 1,4,2-benzodithiazine 1,1-dioxides and their subsequent transformations into 2-mercaptobenzenesulfonamide (MBSA) derivatives (Fig. 1) having a variety of heterocyclic ring systems or acyclic polynitrogen moieties at the sulfonamide functionality resulted in promising anticancer [8–13], HIV antiviral [14–16], or antibacterial agents [17] as well as potent inhibitors of transmembrane cancer-associated carbonic anhydrase isozymes hCAIX and hCAXII [18, 19].

A number of structurally novel N-acylbenzenesulfonamides have recently been reported either as potent antitumor agents against a broad spectrum of human tumor xenografts (colon, lung, breast, ovary, and prostate) in nude mice [22] (Fig. 2) or clinically investigated drug candidates with cytostatic activity against malignant tumors such as Eli Lilly’s tasisulam sodium [23] or Abbott’s WO-2002024636, ABT-737 [24], and ABT-263 [25] (Fig. 3).

This led us to an assumption that expansion of the series of 2-mercapto-N-acylbenzenesulfonamide potential anticancer agents, in which groups of varying size and electronic properties are placed at positions 2, 5, and N- of the benzenesulfonamide ring, may shed light on the structural features contributing to the biological activities.

Results and discussion

Chemistry

Several methods for synthesis of 2-mercaptobenzenesulfonamides are known. The simplest and most efficient method employs the ring-opening reaction of preformed 3-mercapto-1,1-dioxo-1,4,2-benzodithiazine derivatives under alkaline conditions [27]. Alternatively, access to 2-mercaptobenzenesulfonamides is provided by direct reaction of 2-halogenobenzenesulfonamides with sodium polysulfide (Na2Sx) [28] or conversion of 2-aminobenzenesulfonamides via diazonium salt decomposition utilizing disodium sulfide (Na2S) or potassium ethyl xanthate [28–30]. Herein, we report a direct synthetic route to novel 4-chloro-2-benzylthiobenzenesulfonamides and their N-acylated derivatives. Due to our ongoing research in the field of biologically active 2-mercaptobenzenesulfonamides with
five-membered rings incorporated in 5-position of the MBSA scaffold [9], we choose 1,3,4-oxadiazole as our model heterocyclic residue.

The expected 1,3,4-oxadiazoles 1a, 1b were conveniently prepared in good yields by the reaction of 2,4-dichloro-5-sulfamoylbenzhydrazide [31] with orthoesters in refluxing glacial acetic acid (Scheme 1).

We found that 2,4-dichloro-5-(1,3,4-oxadiazol-2-yl) benzenesulfonamide (1a) under standard conditions (BnSH/K$_2$CO$_3$/DMF ($N,N$-dimethylformamide)/RT) undergoes a selective S$_N$Ar addition–elimination reaction in 2-position. Moderate yields (14–58%, Table 1, entries 1–4, 6, and 8) of this reaction led us to optimize the conditions. Higher yields were observed when tetrabutylammonium bromide (TBAB) was used as a phase-transfer catalyst, especially in acetonitrile/water (300:1, v/v) reaction environment (Table 1, entry 9). Slight decrease of substrate conversion was observed in the absence of argon atmosphere (Scheme 1).

The desired $N$-acylsulfonamides 4a–4j (Scheme 2) were prepared by carbodiimide-mediated coupling of aromatic carboxylic acids with sulfonamides [32–34] promoted by 4-($N,N$-dimethylamino)pyridine (DMAP) in the appropriate

![Fig. 1 MBSA scaffold [20, 21]](image1)

![Fig. 2 Acyl sulfonamide antiproliferative (ASAP) scaffold [26]](image2)

![Fig. 3 Tasisulam sodium (LY573636-sodium): clinically evaluated (phase II/III in metastatic melanoma) antitumor $N$-acylsulfonamide; pan-Bcl family inhibitors targeting Bcl-2, Bcl-w, and Bcl-x$_L$: WO-2002024636, ABT-737, and ABT-263 [23–25]](image3)
Compound 2a was isolated and characterized, which by treatment with 10% (w/v) ethanolic solvent. In some cases crystalline 4-((N,N-dimethylamino)pyridinium N-heteroaroylsulfonamidates (3a–3c) were isolated and characterized, which by treatment with 10% (w/v) ethanolic p-toluensulfonic acid (p-TSA) solution were converted to the desired N-acylsulfonamides 4a–4c.

**In vitro biological activity**

Compounds 2a–2d and 4a–4j submitted to National Cancer Institute (NCI) were evaluated for their in vitro anticancer activity. Sulfonamides 2a and 2c showed significant selectivity toward leukemia cell line CCRF-CEM (Fig. 4), whereas 2d appears to be substantially inactive.

HOP-92, non-small cell lung cancer, and renal cancer A498 cell lines reveal some insight into structure–activity relationship (SAR). Cytostatic activity of 2a–2c toward those cell lines increases when CLogP and calculated molar refractivity (CMR) of the compound increase (Table 2).

Over a series of N-(thien-2-ylcarbonyl)benzenesulfonamide derivatives (4c–4g), substitution on the heterocyclic (4e, 4f: R1 = Me) or benzylthio (4d, 4f: R2 = Cl) moiety decreases activity significantly. It seems interesting that closely related six-membered N-heteroaroyl derivatives (4a, 4b, and 4h) showed no activity, which renders 4e as a lead for further optimization.

**Table 1 Reaction of 2,4-dichloro-5-(1,3,4-oxadiazol-2-yl)benzene-sulfonamide (1a) with benzyl mercaptan and optimization of the reaction conditions**

| Entry | Solvent     | BnSH/mmol | K2CO3/mmol | Yield a/% |
|-------|-------------|-----------|------------|-----------|
| 1     | EtOH        | 1.0       | 1.2        | Trace     |
| 2     | DMF         | 1.0       | 1.2        | 32        |
| 3     | DMF         | 2.0       | 2.2        | 27        |
| 4     | DMF         | 1.0       | 2.2        | 41        |
| 5     | DMF/H2O     | 1.0       | 2.2 (cat.) | 55        |
| 6     | DMSO        | 1.0       | 2.2        | 14        |
| 7     | DMSO/H2O    | 1.0       | 2.2 (cat.) | 33        |
| 8     | MeCN        | 1.0       | 2.2        | 58        |
| 9     | MeCN/H2O    | 1.0       | 2.2 (cat.) | 81        |

Reaction conditions: 5 cm³ solvent at room temperature (ca. 25 °C) under argon atmosphere

DMSO dimethylsulfoxide

a Isolated yield of 2a

b (n-Bu₄ N)⁺ Br⁻ (0.01 mmol)

solvent. In some cases crystalline 4-((N,N-dimethylamino)pyridinium N-heteroaroylsulfonamidates (3a–3c) were isolated and characterized, which by treatment with 10% (w/v) ethanolic p-toluensulfonic acid (p-TSA) solution were converted to the desired N-acylsulfonamides 4a–4c.

**Scheme 2**

![Scheme 2](image-url)
COMPARE [38, 39] analysis at the NCI of compound 4c showed moderate Pearson correlation coefficient (PCC = 0.446–0.549) with DNA interfering agents such as actinomycin D, echinomycin, bruceantin, chromomycin A3, or didemnin B (Table 4).

**Table 2** CLogP and CMR molecular descriptors of 2a–2d

| Compd. | Growth (%) | CLogP$^a$ | CMR$^a$ |
|--------|------------|-----------|---------|
|        | HOP-92    | A498      |         |
| 2a     | 62.58      | 94.43     | 1.86852 | 9.5054  |
| 2b     | 57.35      | 59.49     | 2.13752 | 9.9692  |
| 2c     | 48.61      | 56.18     | 2.58152 | 9.9968  |
| 2d     | 84.54      | 91.46     | 2.85052 | 10.4606 |

SAR based on HOP-92 and A498 cell line screen at 10 μM concentration of the test agent

$^a$ Molecular descriptors calculated using BioByte software package [35]

**Conclusions**

We designed a new and efficient method of obtaining substituted 2-mercaptobenzensulfonamides from readily available 2,4-dichlorobenzensulfonamides under optimized mild phase-transfer catalysis conditions. This approach offers easy and quick isolation of the products and preparative-scale synthesis. Novel 2-mercaptobenzensulfonamides and their structurally diverse N-(hetero)aroyl derivatives were evaluated for in vitro antiproliferative activity. The discovered $N$-acylbenzenesulfonamide 4c shows promising anticancer activity toward 50 human cancer cell lines and could be considered as a lead for further optimization.

**Experimental**

Melting points were determined with a Boëtius apparatus. Infrared (IR) spectra were taken using a Thermo Mattson Satellite FTIR spectrophotometer, $^1$H and $^{13}$C nuclear magnetic resonance (NMR) were taken with a Varian Gemini 200 MHz or Varian Unity Plus 500 MHz spectrometer. Chemical shifts are reported in ppm (δ). The results of elemental analyses for C, H, and N were in agreement with the calculated values within ±0.4% range. Column chromatography was carried out on silica gel Fluka Silica gel 60 (0.035–0.070 mm). The starting 2,4-dichloro-5-sulfamoylbenzhydrazide was obtained from commercially available 2,4-dichloro-5-sulfamoylbenzoic acid according to methods described previously [31].

**General procedure for the synthesis of 1a, 1b**

A mixture of 2.84 g 2,4-dichloro-5-sulfamoylbenzhydrazide (10 mmol) and the appropriate orthoester (60 mmol) in 30 cm$^3$ glacial AcOH was refluxed for 7–12 h. After cooling to room temperature, stirring was continued overnight. The precipitate was filtered off, washed with cold EtOH and petroleum ether, and purified by crystallization from EtOH.
| Subpanel                  | Cell line | GI$_{50}$/µM | TGI/µM | LC$_{50}$/µM |
|--------------------------|-----------|-------------|--------|-------------|
|                          | Conc.     | Subpanel MID$^b$ | Subpanel SSR$^d$ | TGI-MID$^e$ | LC$_{50}$-MID$^f$ |
|                          | per cell line |            |        |             |
| **Leukemia**             |           |             |        |             |
|                          | CCRLF-CEM | 3.08        | 0.51   | 83.57       | $^{a}$ |
|                          |         |             |        |             |
|                          | HL-60(TB) | 12.9        | 29.3   | $^{a}$      | $^{a}$ |
|                          |         |             |        |             |
|                          | K-562    | 3.19        | 29.3   | $^{a}$      | $^{a}$ |
|                          |         |             |        |             |
|                          | MOLT-4   | 3.69        | 72.1   | $^{a}$      | $^{a}$ |
|                          |         |             |        |             |
|                          | RPMI-8226| 23.4        | 83.57  | $^{a}$      | $^{a}$ |
|                          |         |             |        |             |
|                          | SR       | 3.33        | 83.57  | $^{a}$      | $^{a}$ |
| **Non-small cell lung cancer** |       |             |        |             |
|                          | A549/ATCC | 2.04        | 4.82   | 13.7        |
|                          | EKVX     | 6.01        | 36.2   | $^{a}$      |
|                          | HOP-62   | 3.19        | 9.48   | 36.2        |
|                          | HOP-92   | 3.14        | 9.48   | 36.2        |
|                          | NCI-H226 | 5.07        | 25.0   | $^{a}$      |
|                          | NCI-H23  | 7.25        | 90.8   | $^{a}$      |
|                          | NCI-H32M | 7.82        | 90.8   | $^{a}$      |
|                          | NCI-H460 | 2.29        | 54.2   | 20.7        |
|                          | NCI-H522 | 3.90        | 17.1   | $^{a}$      |
| **Colon**                |           |             |        |             |
|                          | COLO 205 | 2.61        | 6.84   | 38.3        |
|                          | HCC-2998 | 21.1        | $^{a}$ | $^{a}$      |
|                          | HCT-116  | 4.01        | 16.1   | 71.1        |
|                          | HCT-15   | 10.5        | 69.8   | $^{a}$      |
|                          | HT29     | 3.11        | 8.97   | 35.7        |
|                          | KM12     | 3.69        | 51.6   | $^{a}$      |
|                          | SW-620   | 1.88        | 3.88   | 7.99        |
| **CNS cancer**           |           |             |        |             |
|                          | SF-268   | 2.07        | 5.32   | 23.4        |
|                          | SF-295   | 3.40        | 14.6   | 51.4        |
|                          | SF-539   | 3.40        | 3.95   | 27.1        |
|                          | SNB-19   | 6.75        | 44.3   | $^{a}$      |
|                          | SNB-75   | 1.96        | 4.25   | 9.18        |
|                          | U251     | 1.85        | 3.67   | 7.29        |
| **Melanoma**             |           |             |        |             |
|                          | LOX IMVI | 3.09        | $^{a}$ | $^{a}$      |
|                          | MALME-3 M| 6.79        | 25.1   | $^{a}$      |
|                          | M14      | 4.51        | $^{a}$ | $^{a}$      |
|                          | MDA-MB-435| 2.90       | 10.8   | 75.0        |
|                          | SK-MEL-2 | 2.64        | 8.73   | 49.2        |
|                          | SK-MEL-28| 5.50        | 21.4   | 69.9        |
|                          | SK-MEL-5 | 2.91        | 10.3   | $^{a}$      |

Table 3 continued

| Subpanel | Cell line | GI$_{50}$/µM | TGI/µM | LC$_{50}$/µM |
|----------|-----------|-------------|--------|-------------|
| Conc.    | Subpanel MID$^b$ | Subpanel SSR$^d$ | TGI-MID$^e$ | LC$_{50}$-MID$^f$ |
|          | per cell line |            |        |             |
| **Ovarian cancer** |       |             |        |             |
|          | IGROV1     | 10.2       | 39.0   | $^{a}$      |
|          | OVCAR-3    | 2.29       | 4.37   | 8.34        |
|          | OVCAR-4    | 2.10       | 3.86   | 7.08        |
|          | OVCAR-5    | 16.4       | 46.6   | $^{a}$      |
|          | OVCAR-8    | 2.55       | 7.74   | 36.6        |
|          | NCI/ADR-RES| $^{a}$     | $^{a}$ | $^{a}$      |
|          | SK-OV-3    | 3.65       | 15.6   | 68.6        |
| **Renal cancer** |       |             |        |             |
|          | 786-0      | 2.64       | 6.47   | $^{a}$      |
|          | A498       | 3.18       | $^{a}$ | $^{a}$      |
|          | ACHN       | 10.9       | $^{a}$ | $^{a}$      |
|          | CAKI-1     | 3.65       | 18.3   | 80.4        |
|          | RXF 393    | 2.39       | 5.14   | 16.4        |
|          | SN12C      | 3.57       | 15.5   | 75.3        |
|          | TK-10      | 2.68       | 5.77   | 34.6        |
|          | UO-31      | 3.68       | 16.3   | 74.0        |
| **Prostate cancer** |       |             |        |             |
|          | PC-3       | 7.54       | $^{a}$ | $^{a}$      |
|          | DU-145     | 3.33       | 12.3   | 68.1        |
| **Breast cancer** |       |             |        |             |
|          | 2.93       | 1.46       | 25.0   | 83.77       |
|          | MCF7       | 3.22       | 17.7   | $^{a}$      |
|          | MDA-MB-231/ATCC | 2.02   | 6.10   | 54.0        |
|          | HS 578T    | 2.26       | 7.04   | $^{a}$      |
|          | BT-549     | 4.02       | $^{a}$ | $^{a}$      |
|          | T-47D      | 2.80       | 7.02   | $^{a}$      |
|          | MDA-MB-468 | 3.30       | 12.4   | 48.6        |
|          | MG-MID$^e$ | 4.27       | 21.38  | 58.88       |

* Parameter not determined in five-dose assay, thus assumed 100 µM for the purpose of midpoint calculations
* Subpanel GI$_{50}$ midpoint = average sensitivity of subpanel cell lines toward the test agent
* Mean-graph GI$_{50}$, TGI, and LC$_{50}$ midpoints = average sensitivity of all cell lines toward the test agent
* Subpanel selectivity ratio = subpanel MID:MG-MID
* Subpanel TGI midpoint
* Subpanel LC$_{50}$ midpoint
2,4-Dichloro-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide (1a, C₇H₆Cl₂N₃O₃S₂)
Starting from 8.89 g triethyl orthoformate. Yield: 2.42 g (82%); m.p.: 195–197 °C; R₁ = 0.59 (benzene/EtOH = 4:1); IR (KBr): ν = 3,323, 3,229, 3,165, 3,100, 1,359, 1,340, 1,168 cm⁻¹; ¹H NMR (200 MHz, DMSO-d₆): δ = 7.79 (s, 2H, SO₂NH₂), 8.20 (s, 1H, H-3), 8.56 (s, 1H, H-6), 9.54 (s, 1H, Ar-H) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 121.84, 131.12, 134.09, 134.56, 136.02, 140.83, 155.47, 160.89 ppm.

2,4-Dichloro-5-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide (1b, C₈H₇Cl₂N₃O₃S₂)
Starting from 9.73 g triethyl orthoformate. Yield: 2.13 g (69%); m.p.: 217–219 °C; R₁ = 0.61 (benzene/EtOH = 4:1); IR (KBr): ν = 3,305, 3,205, 3,094, 1,579, 1,542, 1,460, 1,354, 1,174 cm⁻¹; ¹H NMR (200 MHz, DMSO-d₆): δ = 2.64 (s, 3H, CH₃), 7.95 (s, 2H, SO₂NH₂), 8.18 (s, 1H, H-3), 8.51 (s, 1H, H-6) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 10.93, 122.02, 130.75, 134.08, 134.21, 135.74, 140.77, 161.04, 165.08 ppm.

General procedure for the synthesis of 2a–2d
To a suspension of the appropriate 2,4-dichlorobenzenesulfonyl chloride 1a, 1b (5 mmol) in 30 cm³ MeCN and 0.1 cm³ water, 1.52 g K₂CO₃ (11 mmol) and 0.016 g TBAB (0.05 mmol) were added. The obtained reaction mixture was vigorously stirred under an argon atmosphere, and slowly the appropriate mercaptan (5 mmol) was added dropwise. After 24 h of stirring at room temperature, the reaction mixture was concentrated under reduced pressure to dryness, and 15 cm³ EtOH was added. The precipitate was filtered off and suspended in 30 cm³ water, stirred for 30 min, and filtered off. The crude product was purified by crystallization from EtOH.

2-Benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide (2a, C₁₅H₁₂ClN₃O₃S₂)
Starting from 1.47 g 1a and 0.62 g benzyl mercaptan. Yield: 1.55 g (81%); m.p.: 153–154 °C; R₁ = 0.64 (benzene/EtOH = 4:1); IR (KBr): ν = 3,435, 3,332, 3,142, 2,926, 1,590, 1,532, 1,495, 1,450, 1,350, 1,161 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ = 4.54 (s, 2H, SCH₂), 7.29–7.32 (m, 1H, Ar-H), 7.36–7.39 (m, 2H, Ar-H), 7.52–7.54 (m, 2H, Ar-H), 7.73 (s, 2H, SO₂NH₂), 7.84 (s, 1H, H-3), 8.42 (s, 1H, H-6), 9.48 (s, 1H, Ar-H) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 36.16, 118.23, 127.88, 128.89, 129.12, 129.61, 130.29, 135.19, 135.57, 139.55, 143.08, 155.18, 161.33 ppm.

2-Benzylthio-4-chloro-5-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide (2b, C₁₆H₁₄ClN₃O₃S₂)
Starting from 1.54 g 1b and 0.62 g benzyl mercaptan. Yield: 1.54 g (78%); m.p.: 208–210 °C; R₁ = 0.67 (benzene/EtOH = 4:1); IR (KBr): ν = 3,429, 3,246, 2,924, 2,854, 1,624, 1,591, 1,577, 1,558, 1,525, 1,495, 1,347, 1,161 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ = 2.58 (s, 3H, CH₃), 4.50 (2H, SCH₂), 7.27–7.30 (m, 1H, Ar-H), 7.34–7.37 (m, 2H, Ar-H), 7.44–7.46 (m, 2H, Ar-H), 7.74 (s, 2H, SO₂NH₂), 7.78 (s, 1H, H-3), 8.35 (s, 1H, H-6) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 10.87, 36.23, 118.89, 127.87, 128.70, 128.96, 129.14, 129.26, 129.33, 129.47, 129.60, 133.49, 135.70, 137.31, 145.04, 161.70, 164.32 ppm.

4-Chloro-2-(4-chlorobenzthio)-5-(1,3,4-oxadiazol-2-yl)benzenesulfonamide (2c, C₁₅H₁₄Cl₂N₃O₃S₂)
Starting from 1.47 g 1a and 0.79 g 4-chlorobenzyl mercaptan. Yield: 1.58 g (76%); m.p.: 185–187 °C; R₁ = 0.63 (benzene/EtOH = 4:1); IR (KBr): ν = 3,248, 3,156, 3,087, 2,918, 2,858, 1,859, 1,530, 1,490, 1,440, 1,350, 1,333, 1,162 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ = 4.55 (s, 2H, SCH₂), 7.42–7.44 (m, 2H, Ar-H), 7.55–7.57 (m, 2H, Ar-H), 7.73 (s, 2H, SO₂NH₂), 7.84 (s, 1H, H-3), 8.42 (s, 1H, H-6), 9.48 (s, 1H, Ar-H) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 35.29, 118.41, 128.84, 129.32, 130.29, 131.42, 132.49, 134.86, 135.20, 139.74, 142.55, 155.19, 161.29 ppm.

4-Chloro-2-(4-chlorobenzothio)-5-(5-methyl-1,3,4-oxadiazol-2-yl)benzenesulfonamide (2d, C₁₆H₁₄Cl₂N₃O₃S₂)
Starting from 1.54 g 1b and 0.79 g 4-chlorobenzyl mercaptan. Yield: 1.79 g (83%); m.p.: 250–252 °C; R₁ = 0.68 (benzene/EtOH = 4:1); IR (KBr): ν = 3,363, 3,239, 2,925, 2,853, 1,636, 1,587, 1,574, 1,559, 1,520, 1,493, 1,456, 1,349, 1,167 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ = 2.59 (s, 3H, CH₃), 4.51 (s, 2H, SCH₂), 7.41–7.43 (m, 2H, Ar-H), 7.47–7.49 (m, 2H, Ar-H), 7.76–7.77 (m, 3H, H-3 and SO₂NH₂), 8.35 (s, 1H, H-6) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 10.87, 35.36, 119.03, 128.78,

For definitions and methods of calculation of the correlation coefficient from the COMPARE analysis, see Ref. [39]
4-(N,N-Dimethylamino)pyridinium 2-benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)-N-(thien-2-ylcarbonyl)benzenesulfonamidate (3c, C$_2$H$_2$ClN$_6$O$_4$S$_2$)

Starting from 0.141 g thiophene-2-carboxylic acid. Yield: 0.295 g (48%); m.p.: 201–202 °C; $R_f = 0.16$ (benzene/ EtOH = 4:1); IR (KBr): $\tilde{\nu} = 3,318, 3,294, 1,730, 1,647, 1,590, 1,530, 1,496, 1,450, 1,347$ cm$^{-1}$; $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta = 3.17$ (s, 6H, N(CH$_3$)$_2$), $3.17$ (s, 6H, N(CH$_3$)$_2$), $3.17$ (s, 6H, N(CH$_3$)$_2$), $3.17$ (s, 6H, N(CH$_3$)$_2$), $3.17$ (s, 6H, N(CH$_3$)$_2$) ppm; $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta = 35.76, 107.14, 117.12, 127.52, 127.67, 128.64, 129.25, 132.10, 133.50, 136.12, 139.89, 141.52, 143.23, 151.73, 161.75, 167.83 ppm.

General procedure for the synthesis of N-acylbenzenesulfonamides 4a–4c

To a suspension of the appropriate pyridinium salt 3a–3c (0.5 mmol) in 5 cm$^3$ EtOH, 2 cm$^3$ 10% p-TSA solution in EtOH was added and stirred at room temperature for 1 h. The precipitate was filtered off and washed with EtOH and water.

2-Benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)-N-(pyrazine-2-carbonyl)benzenesulfonamide (4a, C$_{20}$H$_{16}$ClN$_3$O$_2$S$_2$)

Yield: 0.242 g (99%); m.p.: 294–296 °C; $R_f = 0.10$ (benzene/EtOH = 4:1); IR (KBr): $\tilde{\nu} = 3,485, 3,364, 3,298, 3,203, 2,871, 1,612, 1,585, 1,549, 1,492, 1,450, 1,362, 1,159$ cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta = 4.51$ (s, 2H, SCH$_2$), $7.08–7.17$ (m, 3H, Ar–H), $7.29–7.31$ (m, 2H, Ar–H), $7.89$ (s, 1H, H-3), $7.85$ (s, 1H, H-6), $8.81$ (s, 1H, Ar–H), $8.94$ (s, 1H, Ar–H), $9.47$ (s, 1H, Ar–H) ppm; $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta = 35.98, 118.54, 127.81, 128.61, 129.27, 129.74, 133.91, 135.58, 136.99, 143.88, 144.09, 144.87, 148.89, 155.25, 161.03, 163.39, 163.44 ppm.

2-Benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)-N-(pyridine-2-carbonyl)benzenesulfonamide (4b, C$_{20}$H$_{16}$ClN$_3$O$_2$S$_2$)

Yield: 0.241 g (99%); m.p.: 173–175 °C; $R_f = 0.40$ (benzene/EtOH = 4:1); IR (KBr): $\tilde{\nu} = 3,138, 2,924, 2,854, 1,730, 1,647, 1,590, 1,530, 1,496, 1,450, 1,347, 1,174$ cm$^{-1}$; $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta = 4.47$ (s, 2H, SCH$_2$), $7.02–7.19$ (m, 3H, Ar–H), $7.26–7.30$ (m, 2H, Ar–H), $7.82$ (s, 1H, H-3), $7.88–7.95$ (m, 1H, Ar–H), $8.12–8.16$ (m, 1H, Ar–H), $8.26–8.35$ (m, 1H, Ar–H), $8.57$ (s, 1H, H-6), $8.76–8.78$ (m, 1H, Ar–H), $9.47$ (s, 1H, Ar–H) ppm; $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta = 35.82, 118.20,$
2-Benzthiazole-4-chloro-5-(1,3,4-oxadiazol-2-yl)-N-(thien-2-ylcarbonyl)benzenesulfonamide (4c, C₂₀H₁₄ClN₃O₄S₃)

To a solution of 0.164 g 5-chlorothiophene-2-carboxylic acid (1 mmol) in 3 cm³ dry MeCN, 0.192 g EDCI (1 mmol) was added and stirred at room temperature for 5 min. 2c (0.416 g, 1 mmol) and 0.184 g DMAP (1.5 mmol) were added and stirred at room temperature for 18 h. The reaction mixture was acidified with 2 cm³ 10% p-TSA/MeCN and concentrated under reduced pressure, and the residue was chromatographed with C₂₀H₁₃ClMeOH/ACOH (97:1:2) on silica gel column giving pure 4d. Yield: 0.248 g (47%); Rf = 0.16 (benzene/EtOH = 4:1); m.p.: 205–207 °C; IR (KBr): 2,925, 2,854, 1,658, 1,591, 1,577, 1,525, 1,495, 1,453, 1,361, 1,176 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ = 2.62 (s, 3H, CH₃), 4.50 (s, 2H, SCH₂), 7.21–7.23 (m, 1H, Ar–H), 7.28–7.31 (m, 1H, Ar–H), 7.34–7.37 (m, 2H, Ar–H), 7.44–7.46 (m, 2H, Ar–H), 7.79 (s, 1H, H-3), 7.97–7.98 (m, 1H, Ar–H), 8.15–8.16 (m, 1H, Ar–H), 8.50 (s, 1H, H-6) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 10.87, 36.39, 118.83, 127.98, 128.56, 129.02, 129.56, 132.56, 132.64, 133.06, 133.48, 135.35, 135.48, 136.27, 147.66, 160.11, 161.39, 164.43 ppm.

4-Chloro-2-(4-chlorobenzthiazole)-5-(5-methyl-1,3,4-oxadiazol-2-yl)-N-(thien-2-ylcarbonyl)benzenesulfonamide (4f, C₂₁H₁₅Cl₂N₃O₄S₃)

To a solution of 0.128 g thiophene-2-carboxylic acid (1 mmol) in 5 cm³ dry tetrahydrofuran (THF), 0.206 g 1,3-dicyclohexylcarbodiimide (DCC, 1 mmol) was added and stirred for 5 min at room temperature. 2d (0.430 g, 1 mmol) and 0.184 g DMAP (1.5 mmol) were added and stirred at room temperature for 48 h. The products were filtered off and washed thoroughly with THF. The filtrate was acidified with 2 cm³ 10% p-TSA/EtOH and concentrated under reduced pressure, and the resulting oily residue was chromatographed with AcOEt/petroleum ether (1:1) on silica gel column giving pure 4f. Yield: 0.135 g (25%); m.p.: 134–136 °C; Rf = 0.22 (benzene/EtOH = 4:1); IR (KBr): 2,925, 2,853, 1,601, 1,549, 1,332, 1,318, 118.90, 119.01, 126.03, 127.87, 128.46, 128.72, 128.96, 129.25, 129.50, 132.64, 135.75, 137.16, 137.65, 144.38, 155.74, 159.68 ppm.

2-Benzthiazole-4-chloro-N-(5-chlorothien-2-ylcarbonyl)-1,3,4-oxadiazol-2-yl)benzenesulfonamide (4g, C₂₁H₁₅Cl₂N₃O₄S₃)

To a solution of 0.164 g 5-chlorothiophene-2-carboxylic acid (1 mmol) in 5 cm³ dry MeCN, 0.192 g EDCI (1 mmol) was added and stirred at room temperature for 5 min. 2a (0.382 g, 1 mmol) and 0.184 g DMAP (1.5 mmol) were added and stirred at room temperature for 12 h. The obtained solution was acidified with 2 cm³ 10% p-TSA/MeCN and stirred under cooling (ice bath) for 2 h. The precipitated white solid was filtered off and purified by crystallization from MeCN. Yield: 0.268 g (51%); m.p.: 254–255 °C; Rf = 0.12 (benzene/EtOH ≈ 4:1); IR (KBr): 2,925, 2,854, 1,658, 1,591, 1,577, 1,525, 1,495, 1,453, 1,361, 1,176 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ = 2.62 (s, 3H, CH₃), 4.50 (s, 2H, SCH₂), 7.21–7.23 (m, 1H, Ar–H), 7.28–7.31 (m, 1H, Ar–H), 7.34–7.37 (m, 2H, Ar–H), 7.44–7.46 (m, 2H, Ar–H), 7.79 (s, 1H, H-3), 7.97–7.98 (m, 1H, Ar–H), 8.15–8.16 (m, 1H, Ar–H), 8.50 (s, 1H, H-6) ppm; ¹³C NMR (50 MHz, DMSO-d₆): δ = 10.87, 36.39, 118.83, 127.98, 128.56, 129.02, 129.56, 132.56, 132.64, 133.06, 133.48, 135.35, 135.48, 136.27, 147.66, 160.11, 161.39, 164.43 ppm.
s, 1H, Ar–H), 8.49 (s, 1H, H-6), 9.46 (s, 1H, Ar–H) ppm; 
$^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta = 35.64, 118.22, 127.52, 128.39, 128.81, 129.01, 129.37, 132.56, 133.56, 134.98, 135.25, 135.75, 136.51, 136.62, 143.19, 154.91, 159.11, 160.73 ppm.

2-Benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)-N-(pyridine-3-carbonyl)benzenesulfonamide (4a, C$_{21}$H$_{20}$ClN$_4$O$_4$S$_2$)
To a suspension of 0.135 g pyridine-3-carboxylic acid (1.1 mmol) in 5 cm$^3$ dry MeCN, 0.227 g DCC (1.1 mmol) was added and stirred at room temperature for 5 min. The precipitate was filtered off, washed with EtOH, and purified by crystallization from EtOH. Yield: 0.29 (benzene/MeCN = 4:1); IR (KBr): $\nu = 3,436, 3,096, 3,060, 2,926, 1,633, 1,589, 1,565, 1,520, 1,495, 1,355, 1,135$ cm$^{-1}$; $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta = 4.47$ (s, 2H, S=CH$_2$), 7.20–7.33 (m, 5H, Ar–H), 7.73–7.78 (m, 2H, H-3 and Ar–H), 8.49–8.55 (m, 2H, H-6 and Ar–H), 8.85–8.87 (m, 1H, Ar–H), 8.09 (s, 1H, Ar–H), 8.47 (s, 1H, Ar–H) ppm; $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta = 35.88, 117.98, 125.11, 127.74, 127.96, 128.74, 129.29, 131.77, 133.19, 135.59, 135.76, 137.50, 139.95, 143.52, 146.76, 149.56, 151.14, 161.32, 164.82 ppm.

2-Benzylthio-4-chloro-5-(1,3,4-oxadiazol-2-yl)-N-(3,4,5-trimethoxybenzoyl)benzenesulfonamide (4j, C$_{25}$H$_{22}$ClN$_3$O$_7$S$_2$)
To a suspension of 0.36 g 3,4,5-trimethoxybenzoic acid (1.1 mmol) in 5 cm$^3$ dry MeCN, 0.227 g DCC (1.1 mmol) was added and stirred at room temperature for 5 min. The precipitate was filtered off, washed with EtOH, and purified by crystallization from EtOH. Yield: 0.30 (benzene/MeCN = 4:1); IR (KBr): $\nu = 3,442, 3,158, 3,092, 2,962, 1,716–1,736$ (m, 2H, Ar–H), 7.90 (s, 1H, Ar–H), 8.59 (s, 1H, Ar–H) ppm; $^{13}$C NMR (50 MHz, DMSO-$d_6$): $\delta = 36.01, 56.38, 60.45, 106.49, 118.49, 125.99, 127.87, 128.74, 129.39, 134.16, 134.79, 135.32, 136.94, 142.05, 143.73, 152.93, 155.25, 161.05, 164.81 ppm.

NCl in vitro anticancer screen
As of early 2007 all compounds submitted to the NCI-60 cell screen are tested initially at a single high dose (10 $\mu$M) in the full NCI-60 cell panel representing human leukemia, melanoma and lung, colon, brain, breast, ovary, kidney, and prostate cancers. Briefly, the compounds were solubilized in DMSO and added at a single concentration, and the cell culture was incubated for 48 h at 37 °C, 5% CO$_2$, 95% air, and 100% relative humidity. End points were determined by colorimetric sulforhodamine B (SRB) assay [40]. Results for each compound were reported as a mean-graph of the percent growth of the treated cells relative to the no-drug control, and relative 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) [41].
test agent, TGI signifies a cytostatic effect, and LC₅₀ signifies a cytotoxic effect.

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