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# Novel diarylsulfonylurea derivatives as potent antimitotic agents

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**Abstract**—A novel series of diarylsulfonylurea derivatives were synthesized and evaluated for interaction with tubulin and for cytotoxicity against human cancer cell lines. These derivatives demonstrated good inhibitory activity against tubulin polymerization, which was well correlated with promising antiproliferative activity as well as G2/M phase cell cycle arrest. Furthermore, several compounds were also efficacious against multidrug-resistant cancer cells, which are resistant to many other known microtubule inhibitors.


Tubulin, the major protein component of microtubules, is the target of numerous antimitotic drugs.[1–3] Microtubules play an important role in a variety of cellular processes, including mitosis and cell division.[4–6] Various antimitotic agents interfering with the natural dynamics of tubulin polymerization and depolymerization inhibit cancer cell proliferation.[2,3,7]

Antimitotic agents largely fall into three major classes. The taxanes such as paclitaxel and docetaxel stabilize microtubules by preventing the depolymerization of tubulin.[1,2] The Vinca alkaloids (e.g., vincristine, vinblastine, and vinorelbine) and colchicine inhibit the polymerization of tubulin.[1,2] Disruption of tubulin dynamics leads to cell cycle arrest in the G2/M phase and induction of apoptosis.[5,8] Although antimitotic compounds such as paclitaxel and vinblastine have been used clinically in the treatment of different cancers, a major drawback of taxanes and Vinca alkaloids in clinical application is the development of drug resistance through the expression of efflux pumps, including P-glycoprotein (Pgp) and multidrug resistance-associated protein MRP.[9,10] Therefore, there is a high medical demand to find and develop small molecule tubulin inhibitors that are effective in treating multidrug-resistant (MDR) tumors.

Diarylsulfonylureas represent a new class of antitumor agents with a broad spectrum of activity against rodent and human models of cancer in vivo.[11–13] The precise mechanism of antitumor action has not been elucidated. Some prototypic compounds, such as sulofenur and LY295501 (Fig. 1) have been studied in clinical trials. However, the development of sulofenur was precluded by dose-limiting toxicities including methemoglobinemia and hemolytic anemia,[14,15] whereas LY295501 recently showed improved side effects with a specific pattern of myelotoxicity and paucity of nonhematological toxicity.[16] Further modification of diarylsulfonylureas is continuing as promising anticancer activity and some clinical benefit have made the development of structural analogues the focus of much research. Hwang et al.[17] have reported a novel derivative of diarylsulfonylurea, DW2282 (Fig. 1), which strongly

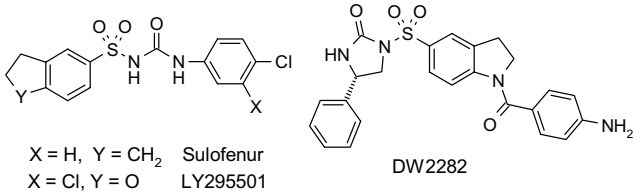


**Figure 1.** Structures of diarylsulfonylureas.

*Keywords*: Diarylsulfonylurea; Antimitotic; Tubulin inhibitor; Antitumor; MDR.
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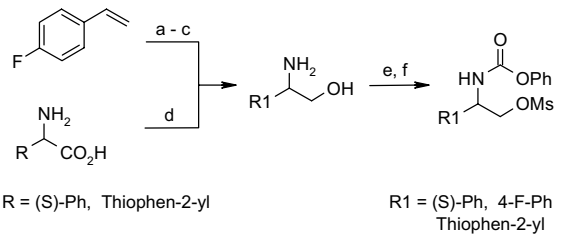
suppressed the growth of human tumors in vitro and in vivo.[18] The precise mode of action of DW2282 remains unclear, however.

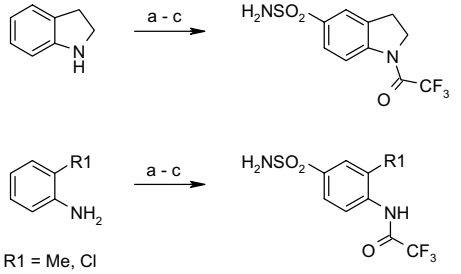
Here, we report a new series of diarylsulfonylurea derivatives as new potent antimitotic agents. Numerous derivatives of DW2282 were synthesized and evaluated for interaction with tubulin and for cytotoxicity against human cancer cell lines. These new derivatives demonstrated good inhibitory activity against tubulin polymerization, which was well correlated with in vitro antiproliferative activity as well as G2/M phase cell cycle arrest. Furthermore, several of the new derivatives reported here maintain activity against multidrug-resistant tumor cell lines, which indicates that they are not substrates for P-glycoprotein mediated transport. These findings indicate that these newly synthesized diarylsulfonylurea derivatives represent promising new antimitotic agents that inhibit tubulin polymerization with efficacy against multidrug-resistant tumor cells.

Novel derivatives of diarylsulfonylurea DW2282 were synthesized as shown in Schemes 1–3 (Table 1). All these compounds were characterized by physical and spectral analysis data that confirmed the assigned structures. These new derivatives were evaluated for inhibition of tubulin polymerization and antiproliferative activity against human cancer cell lines. The in vitro tubulin polymerization reaction was evaluated turbidimetrically.[19] As shown in Table 1, compounds **1e**, **h**, and **j** showed little inhibitory activity in tubulin polymerization assay and compounds **1b** and **m** displayed weak activity. However, compounds **1f**, **k**, **2a**, and **b** had moderate activity, whereas compounds **1c** and **l** had modest activity. Compounds **1d**, **g**, **i** as well as DW2282 displayed strong inhibitory activity against tubulin polymerization. Compound **1a** proved to be the most potent tubulin polymerization inhibitor, comparable to vincristine. The antiproliferative activity of these new synthetics was assessed using human colon carcinoma (HCT116), and human non-small cell lung cancer cell lines (A549, and NCI-H460). After 48 h continuous drug exposure, the concentration required for 50% growth inhibition ($GI_{50}$) was determined by the sulforhodamine B (SRB) colorimetric assay.[20] These derivatives displayed promising in vitro antiproliferative activity with $GI_{50}$ values in the low micromolar to nanomolar concentration range (Table 1). In general, a good correlation was found between inhibition of tubulin polymerization and cytotoxicity. Among these derivatives, compound **1d** acted as a very potent cell growth inhibitor with $GI_{50}$s similar to those of DW2282. Compound **1a**, showing the most potent tubulin polymerization inhibitory activity, displayed the most significant antiproliferative effect (average $GI_{50} = 7$ nM), comparable to those of paclitaxel and vincristine.

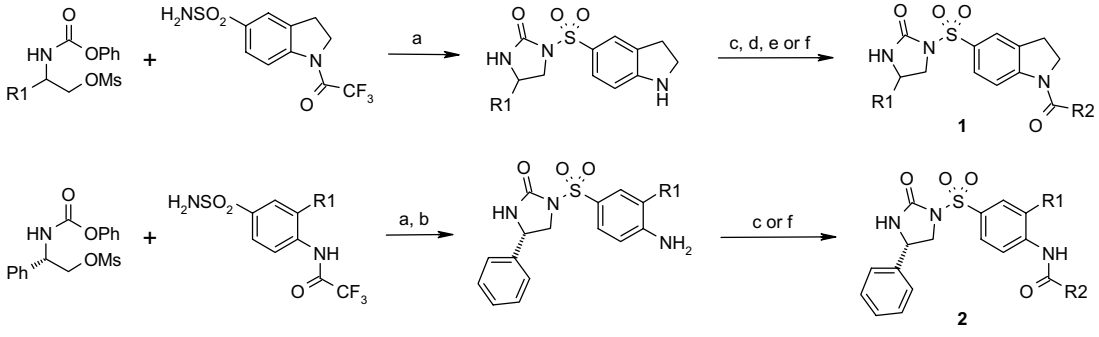
The effect on cell cycle progression of selected compounds (**1b**–**d**, paclitaxel) was also examined using flow cytometry.[21] MCF7 cells were exposed to different concentrations of the compounds for 16 h. At 1 μM




**Scheme 1.** Synthesis of methanesulfonates. Reagents and conditions: (a) m-CPBA, $CH_2Cl_2$, rt; (b) $NaN_3$, $NH_4Cl$, aq MeOH, 60 °C; (c) 10% Pd/C, MeOH, rt; (d) $LiAlH_4$, THF, reflux; (e) PhOCOCl, $NaHCO_3$, aq THF, rt; (f) MsCl, $Et_3N$, $CH_2Cl_2$, rt.

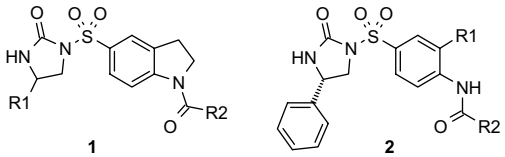



**Scheme 2.** Synthesis of arylsulfonamides. Reagents and conditions: (a) $(CF_3CO)_2O$, Py., $CH_2Cl_2$, 0 °C; (b) $ClSO_3H$, 60 °C; (c) $NH_3$, $CH_2Cl_2$, rt.




**Scheme 3.** Synthesis of diarylsulfonylurea derivatives. Reagents and conditions: (a) NaOH, DMF, 0 °C ~ rt; (b) $NaBH_4$, EtOH, 0 °C; (c) $R_2COCl$, Py., $CH_2Cl_2$, rt; (d) $BrCOCH_2Br$, Py., $CH_2Cl_2$, rt; $PhNH_2$, $K_2CO_3$, DMF, rt; (e) triphosgene, $Et_3N$, $CH_2Cl_2$, 0 °C; c-HexylNH$_2$, 2-(morpholin-4-yl)ethylamine or 2-(4-methyl-piperazin-1-yl)ethanol, rt; (f) p-$NO_2$-BzCl, Py., $CH_2Cl_2$, rt; Raney-Ni, MeOH, rt.

**Table 1.** Inhibition of tubulin polymerization and cellular proliferation for new diarylsulfonylurea derivatives, DW2282, vincristine, and paclitaxel




1 2

| Compound | R1 | R2 | ITP[a] $IC_{50}$ (μM) | Cell line, $GI_{50}$ (μM)[b] | | |
|---|---|---|---|---|---|---|
| | | | | HCT116 | A549 | NCI-H460 |
| **1a** | (S)-Ph | $CH_2$-thiophen-2-yl | <1 (82)[c] | 0.006 | 0.007 | 0.007 |
| **1b** | (*S*)-Ph | 2,6-Dichloropyridin-4-yl | 22 | 0.386 | 0.386 | 0.7 |
| **1c** | (*S*)-Ph | Pyridin-4-yl | 10.3 | 0.089 | 0.133 | 0.09 |
| **1d** | (*S*)-Ph | Thiophen-2-yl | 1.6 | 0.019 | 0.028 | 0.022 |
| **1e** | (*S*)-Ph | 5-Nitrofuran-2-yl | 40 | 0.27 | 0.95 | 0.95 |
| **1f** | (*S*)-Ph | $CH_2NHPh$ | 3.5 | 0.055 | 0.08 | 0.15 |
| **1g** | (*S*)-Ph | NH-cyclohexyl | 2 | 0.05 | 0.065 | 0.08 |
| **1h** | (*S*)-Ph | $NH(CH_2)_2$-morpholin-4-yl | 30 | 1.2 | 2.8 | 1.0 |
| **1i** | (*S*)-Ph | OEt | 1.5 | 0.08 | 0.08 | 0.08 |
| **1j** | (*S*)-Ph | $O(CH_2)_2$-4-methylpiperazin-1-yl | 30 | 1.0 | 1.0 | 0.9 |
| **1k** | Thiophen-2-yl | Furan-2-yl | 4 | 0.075 | 0.062 | 0.064 |
| **1l** | Thiophen-2-yl | 4-Aminophenyl | 10.5 | 0.3 | 0.18 | 0.25 |
| **1m** | 4-Fluorophenyl | 4-Aminophenyl | 25 | 1.1 | 1.2 | 0.9 |
| **2a** | Me | OEt | 6 | 0.07 | 0.048 | 0.07 |
| **2b** | Cl | 4-Aminophenyl | 5.5 | 0.06 | 0.06 | 0.06 |
| DW2282 | | | 1.5 | 0.012 | 0.02 | 0.022 |
| Vincristine | | | <1 (83)[c] | 0.001 | 0.027 | 0.003 |
| Paclitaxel | | | <1[d] (80)[c] | <0.001 | 0.013 | 0.007 |

[a] ITP = inhibition of tubulin polymerization, evaluated as described in Ref. 19. A minimum of two independent determinations were performed with each compound. The $IC_{50}$ value represents the concentration that inhibits the extent of assembly by 50% after 30 min at 37 °C.

[b] All experiments were performed at least in triplicate using the SRB assay as described in Ref. 20, and $GI_{50}$ data were calculated from dose–response curves by nonlinear regression analysis.

[c] Numbers in parentheses represent the percentage of inhibition or induction of polymerization at 1 μM.

[d] Concentration that induces tubulin polymerization by 50% in the absence of GTP after 30 min at 37 °C.

concentration of each compound, a clear shift from G1 to G2/M phase was observed (Fig. 2A). Thus, compounds **1b–d** induce an accumulation of MCF7 cells specifically in the G2/M phase of the cell cycle, similar to the known G2/M cell cycle inhibitor paclitaxel. When the percentage of cells arrested in the G2/M phase was plotted against different concentrations of the compounds, compounds **1b–d** arrested the cell cycle in a concentration-dependent manner with $IC_{50}$ values of 0.3, 0.1, and 0.03 μM, respectively (Fig. 2B). G2/M arrest of these compounds increased in parallel with both tubulin polymerization inhibition and antiproliferative activity. This consistency indicates that the G2/M phase arrest of the compounds is probably induced by inhibition of tubulin polymerization, which is also correlated with cell growth inhibition. Taken together, these results demonstrate that this series is a potent antimitotic agent inhibiting tubulin polymerization with promising antiproliferative activity.

In order to investigate the effects of the derivatives on MDR tumor cell lines, several compounds were evaluated against MCF7, MCF7/ADR, HCT-15, A498, and NCI-H226 cancer cell lines (Table 2). The MCF7/ADR cell line is derived from MCF7 cell line and exhibits the MDR phenotype.[22] HCT-15, A498, and NCI-H226 are all Pgp-expressing MDR tumor cell lines.[23] To quantitatively express the effect of MDR phenotype on the cytotoxicity of each compound, resistance factor (rF) was calculated by dividing the $GI_{50}$ value observed with resistant cell line MCF7/ADR with that observed with parental cell line MCF7. As shown in Table 2, compounds **1f**, **2a**, and **2b** strongly maintained their activity against the multidrug-resistant MCF7/ADR cell_line ($rF \sim 1$). They were also similarly active against three other MDR tumor cell lines. In contrast, DW2282 (rF = 133) and compound **1g** (rF = 8.7) were less active against MCF7/ADR than against the parent cell line. The A498 cell line was also relatively resistant to DW2282 and compound **1g**. Paclitaxel was markedly less toxic toward the MCF7/ADR cell line relative to the parent cell line (rF = 727) and not highly active against other MDR tumor cell lines. This observation indicates that some of these derivatives are efficacious against multidrug-resistant tumor cell lines and are not substrates of Pgp-mediated transport.

In summary, we have synthesized novel derivatives of diarylsulfonylurea and have found these compounds to be potent antimitotic agents. These compounds have potent in vitro tumor growth inhibitory activity correlated with inhibition of tubulin polymerization and are also efficacious against multidrug-resistant cancer cells. Thus, this series represents a new class of antimitotic

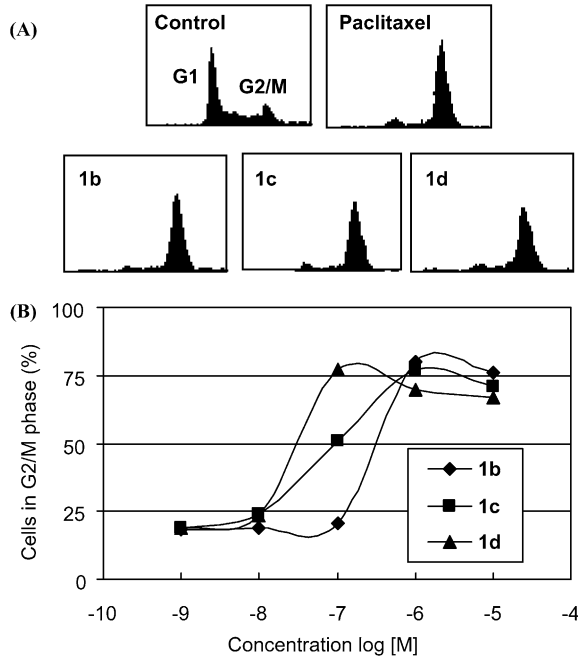



**Figure 2.** Effect on cell cycle progression. MCF7 cells were treated with different concentrations of the compounds for 16 h and DNA content of the cells was analyzed by FACS. (**A**) Treatment with 1 μM of the compounds. The horizontal axis represents relative DNA content and the level of the vertical direction corresponds to the number of cells. (**B**) Percentage of cells in G2/M phase is plotted against the concentrations of the compounds.

**Table 2.** Effect on growth of MDR tumor cell lines in vitro

| Compound | Cell line, $GI_{50}$ (μM)[a] | | | | |
|---|---|---|---|---|---|
| | MCF7[b] | MCF7/ ADR[c] (rF[d]) | HCT-15[c] | A498[c] | NCI-H226[c] |
| **1f** | 0.15 | 0.18 (1.2) | 0.1 | 0.15 | 0.15 |
| **1g** | 0.15 | 1.3 (8.7) | 0.12 | 0.7 | 0.08 |
| **2a** | 0.15 | 0.15 (1.0) | 0.1 | 0.13 | 0.45 |
| **2b** | 0.15 | 0.15 (1.0) | 0.11 | 0.06 | 0.15 |
| DW2282 | 0.015 | 2 (133) | 0.12 | 1.2 | 0.1 |
| Paclitaxel | 0.011 | 8 (727) | 0.3 | 3.5 | 20 |

[a] All experiments were performed at least in triplicate using the SRB assay as described in Ref 20.
[b] MDR (−), not multidrug resistant.
[c] MDR (+), multidrug resistant.
[d] rF = resistance factor, calculated from the ratio of $GI_{50}$ for MCF7/ADR and that for MCF7.

agents distinct from previous diarylsulfonylurea compounds. Further investigation of the SAR of this series is ongoing and will be reported in due course.

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