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NOVEL HALOGENATED SULFONAMIDES INHIBIT THE GROWTH OF MULTIDRUG RESISTANT MCF-7/ADR CANCER CELLS

Julio C. Medina,* Daniel Roche, Bei Shan, R. Marc Learned, Walter P. Frankmoelle, David L. Clark, Terry Rosen and Juan C. Jaen

Tularik Inc., Two Corporate Drive, South San Francisco, CA 94080, U.S.A.

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Abstract: In this report, we describe the synthesis of halogenated benzenesulfonamide compounds and their ability to inhibit the growth of HeLa, MCF-7 and MCF-7/ADR tumor cells *in vitro*. The multidrug resistance (MDR) phenotype of certain cells does not affect their sensitivity to these compounds. These agents belong to a family of compounds previously shown to bind irreversibly to cysteine-239 of β–tubulin. Consistent with this mechanism of action, the cytotoxicities of these compounds appear to correlate with their ability to undergo nucleophilic aromatic substitution.

Introduction

Recent reports describe a novel class of pentafluorobenzenesulfonamide compounds with broad cytotoxicity against a variety of cancer cells, independent of the MDR phenotype of the cells.[1,2] These compounds undergo nucleophilic aromatic substitution (NAS) by cysteine-239 of β–tubulin, resulting in the disruption of cellular microtubules.[2] The irreversible nature of this interaction may be invoked to explain their insensitivity to the drug efflux pumps (Pgp, MRP-1) often expressed by MDR cells.[1,2]

In this report, we describe the synthesis and biological activity of several novel analogs of the previously described pentafluorobenzenesulfonamides. In these compounds, the fluorine atoms of the reactive pentafluorophenyl moiety have been systematically replaced by hydrogen or other halogen atoms. Evaluation of these compounds yields useful information about the presumed site of NAS attack and advances our understanding of how the electrophilic phenyl ring affects their reaction with β–tubulin. Further evidence is provided that this mechanism of action is not affected by the MDR phenotype of cells that have lost their sensitivity to paclitaxel and other cytotoxic agents.

Chemistry[3]

Treatment of compounds **1a**-**4a** with fuming sulfuric acid (20% SO_3) at 110 °C, followed by PCl_5 at room temperature, produced the corresponding arylsulfonyl chlorides,[4] which were dissolved in methanol and treated with two equivalents of *p*-anisidine to afford compounds **1b**-**4b**, respectively. Compounds **1c**, **2c** and **3c** were prepared by catalytic hydrogenation of **1b**, **2b** and **3b**, respectively. Treatment of compound **5** with chlorosulfonic acid[5] produced a 1:2 mixture of isomeric sulfonyl chlorides. This mixture was treated with *p*-anisidine to afford compounds **6a** and **7a**, which were separated by silica gel chromatography. Catalytic hydrogenation of these compounds yielded compounds **6b** and **7b**, respectively. Compounds **8b** and **9b** were synthesized from **8a** and **9a**, respectively, using a procedure similar to the one used for the synthesis of **6a**. The synthesis of compound **10** has been reported previously.[1]

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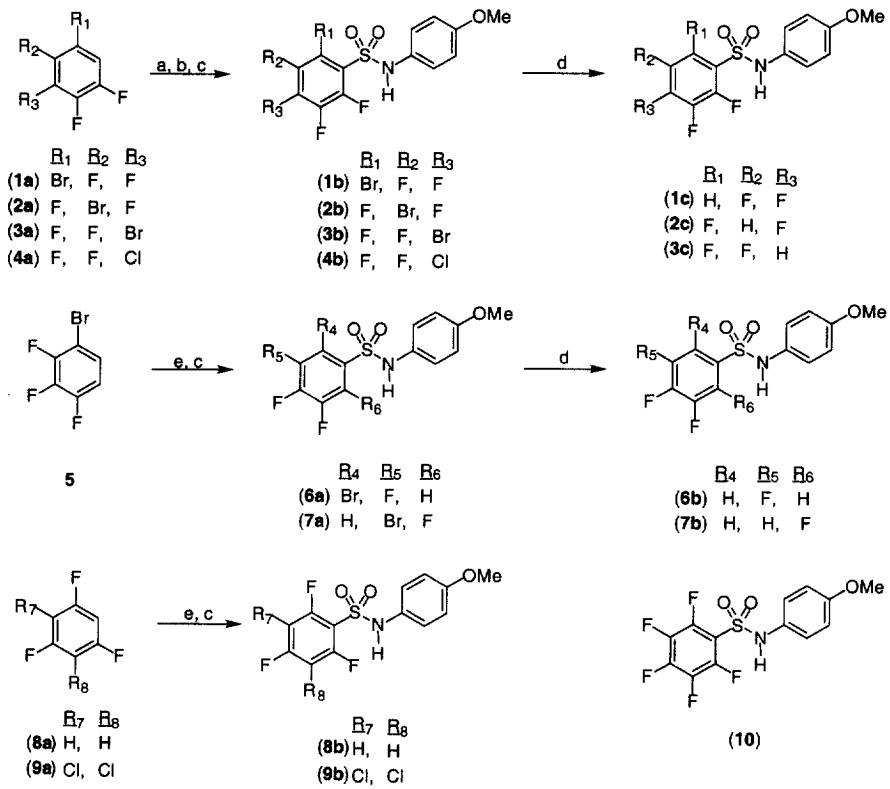
Scheme 1



Reagents and conditions: (a) 20% SO_3, H_2SO_4 110 °C; (b) PCl_5; (c) *p*-anisidine, MeOH; (d) H_2, 10% Pd/charcoal, MeOH; (e) $ClSO_3H$.

Biology

The ability of test compounds to arrest the growth of tumor cells in culture was evaluated using HeLa, MCF-7 and MCF-7/ADR cells.[6] The MCF-7/ADR cell line is derived from MCF-7 cells and displays resistance to several chemotherapeutic agents, including paclitaxel, vinblastine and doxorubicin.[6]

The cellular growth rate was calculated from the culture's metabolic activity (Alamar Blue assay) after 72 hours of drug treatment.[7] The concentration resulting in 50% growth inhibition (GI_{50}) was calculated using a curve fitting program, and the results are shown in Table 1.

Results and Discussion

We have previously reported that a series of novel compounds, exemplified by **10**, possess antimitotic activity as a result of their covalent binding to cysteine-239 of β–tubulin.[1,2] The goals of the present study were to elucidate the role that the halogenated phenyl ring plays in the reactivity/cytotoxicity of these

compounds and to investigate whether the cytotoxicity of other analogs remains independent of the MDR phenotype of the cells.

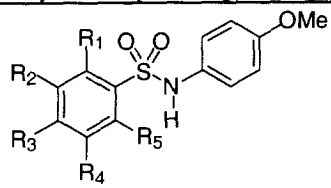
The reactivity of the original compounds involves nucleophilic aromatic substitution of an aromatic fluorine atom by the thiol group of a cysteine residue.[2] Therefore, it was anticipated that replacement of such fluorine with either chlorine or bromine (both less effective as leaving groups than fluorine)[8] would have a more substantial effect on biological activity than similar replacements of the non-reactive fluorine atoms. Comparison of the GI_{50} values for compounds **1b**, **2b** and **9b** with the parent compound **10** confirms that substitution of fluorine by other halogen atoms at positions *ortho* or *meta* to the sulfonamide have little or no influence on the biological activity of these compounds. However, substitution of the fluorine atom *para* to the sulfonamide group with either bromine (**3b**) or chlorine (**4b**) results in about 100-fold loss of potency, suggesting that the *para*-position is the site of NAS attack by β-tubulin. A consistent interpretation may be draw from results with compounds **1c**, **2c** and **3c**, in which one fluorine atom at each position of the pentafluorobenzene ring has been systematically replaced with hydrogen. When the fluorine atom *para* to the sulfonamide is missing, the molecule (**3c**) is no longer cytotoxic. These data all confirm that the *para*-position is the site of nucleophilic aromatic substitution, consistent with our own *in vitro* experiments with various sulfur nucleophiles (data not shown) and literature reports on similar aromatic systems.[9]

It is well established that electron-withdrawing groups at positions *ortho* and *para* to the leaving group increase the rate of nucleophilic aromatic substitution more substantially than similar substituents *meta* to the leaving group.[8] With this in mind, compounds **6b**, **7b** and **8b** were synthesized. Compound **6b**, which should be the most reactive of this group, is the only one that shows any cytotoxicity at the concentrations tested. A similar observation was made with compounds **1c** and **2c**. Thus, there is a good correlation between cytotoxicity and expected reactivity at the *para* -position of the phenylsulfonyl moiety.

In order to study the effects of the new compounds on MDR tumor cells, they were evaluated against MCF-7/ADR cells. Resistance by these cells to antimitotic agents is due, in part, to the overexpression of Pgp.[10] This membrane transport protein exports structurally unrelated chemotherapeutic agents from the cytosol to the extracellular medium. With the possible exception of **1b**, all sulfonamide compounds display similar effects towards MCF-7 and MCF-7/ADR cells, suggesting that they are not affected by the Pgp pump or other mechanisms responsible for the MDR phenotype of MCF-7/ADR cells. Paclitaxel, on the other hand, is quite innocuous towards MCF-7/ADR cells (greater than 50,000-fold loss of potency).

In summary, we have explored the structure-activity relationship of a novel series of compounds that inhibit the growth of human tumor cells. The cytotoxicity of these agents is not affected by the MDR phenotype of MCF-7/ADR cells, which may in part be due to their evasion of the Pgp drug efflux mechanism by irreversibly binding to β-tubulin. We have demonstrated that replacement of the *para*-fluorine atom with other halogens results in a substantial decrease in potency. Furthermore, when this position is occupied by a hydrogen atom, the compound is no longer cytotoxic. These observations strongly suggest that the site of NAS reaction is the *para*-position of the benzenesulfonamide ring. Finally, the anticipated NAS reactivity of this position is shown to be a good predictor of the cytotoxicity of this type of compound.

Table 1. Cytotoxicity of Target Compounds.



							Cell Type		
							HeLa	MCF-7	MCF-7/ ADR
No.	R_1	R_2	R_3	R_4	R_5	MP (°C)	GI_{50} (μM)	GI_{50} (μM)	GI_{50} (μM)
1b	Br	F	F	F	F	104-106	0.16 ± 0.07	0.039 ± 0.06	0.16 ± 0.08
2b	F	Br	F	F	F	74-75	0.22 ± 0.1	0.27 ± 0.3	0.19 ± 0.09
3b	F	F	Br	F	F	147-148	17 ± 8	36 ± 7	21 ± 9
4b	F	F	Cl	F	H	141-143	21 ± 10	44 ± 4	22 ± 9
1c	H	F	F	F	F	65-66	0.37 ± 0.2	0.12 ± 0.1	0.40 ± 0.3
2c	F	H	F	F	F	67-68	5.8 ± 3	4.5 ± 4.5	4.5 ± 3.3
3c	F	F	H	F	F	145-146	> 50	> 50	> 50
6b	H	F	F	F	H	85-86	3.9 ± 2.3	2.0 ± 0.9	3.4 ± 1
7b	H	H	F	F	F	88-89	> 50	> 50	> 50
8b	F	H	F	H	F	107-108	> 50	> 50	> 50
9b	F	Cl	F	Cl	F	128-130	0.096 ± 0.05	0.037 ± 0.04	0.058 ± 0.03
10	F	F	F	F	F	108-109	0.099 ± 0.06	0.029 ± 0.04	0.058 ± 0.05
paclitaxel							0.007 ± 0.001	0.0011 ± 0.0002	> 50

References and Notes

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