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Novel Sulfonate Analogues of Combretastatin A-4: Potent Antimitotic Agents

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Abstract—Sulfonate analogues of combretastatin A-4 have been prepared. These compounds compete with colchicine and combretastatin A-4 for the colchicine binding site on tubulin and are potent inhibitors of tubulin polymerization and cell proliferation. Importantly, these compounds also inhibit the proliferation of P-glycoprotein positive (+) cancer cells, which are resistant to many other antitumor agents. © 2001 Elsevier Science Ltd. All rights reserved.

Combretastatin A-4 (CA-4, Fig. 1) is a naturally occurring stilbene that inhibits tubulin polymerization by binding to tubulin at the colchicine binding site.[1] This phenomenon leads to potent antitumor activity against a wide variety of cell lines, including multidrug resistant lines.[2] However, the limited water solubility and poor oral bioavailability of this compound have limited its in vivo efficacy,[3] and efforts to develop more efficacious analogues and prodrugs are ongoing.[4]

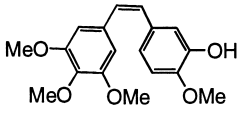
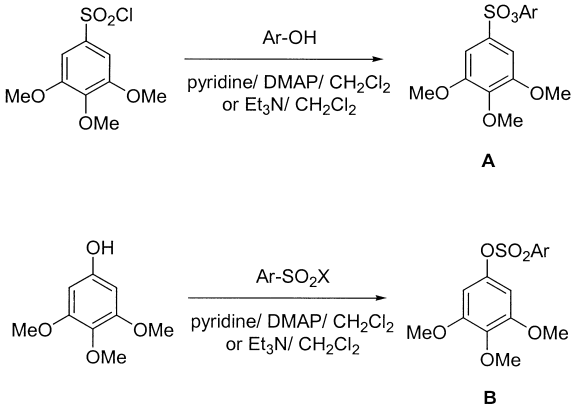


Figure 1. Combretastatin A-4.

As part of our program to identify novel antimitotic agents, we have synthesized a series of sulfonate analogues of CA-4 as shown in Scheme 1. 3,4,5-Trimethoxyphenylsulfonyl chloride[5] was treated with phenols under standard conditions[6] to provide sulfonates **A**. Alternatively, the reversed sulfonates **B** could be prepared by treatment of 3,4,5-trimethoxyphenol with aryl sulfonyl halides.



Scheme 1. Synthesis of sulfonate analogues of combretastatin A-4.

Most of the phenols and sulfonyl chlorides used were commercially available. The others were synthesized by literature methods[7] or as shown in Scheme 2.

The structures of the diarylsulfonates synthesized are shown in Scheme 3. The yields for the sulfonylation reactions are shown in parentheses. In some cases, further modification of the initial sulfonate was performed to provide others. Compound **12** was prepared by treating 4-methoxymetanilyl fluoride with 3,4,5-trimethoxyphenol and triethylamine in methylene chloride.

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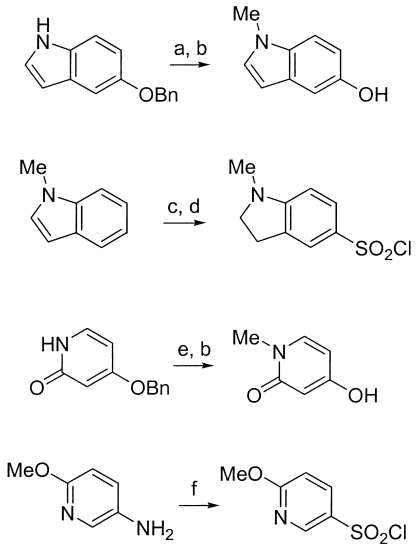


Scheme 2. Synthesis of phenols and sulfonyl chlorides. Reagents: (a) NaH, MeI, 55%; (b) H_2, Pd–C, 67–91%; (c) $NaCNBH_3$, AcOH, 38%; (d) $ClSO_3H$, 27%; (e) Me_2SO_4, NaOH, 77%; (f) $NaNO_2$; SO_2, $CuCl_2$, 41%.

To evaluate the properties of the sulfonates prepared above, they were compared to several *Z*-olefins. Combretastatin A-4 was prepared as in the literature.[1] Using the same method, we prepared olefins **16** and **17** (Fig. 2) which share substituents with sulfonates **4**, **7**, **9**, and **11**.

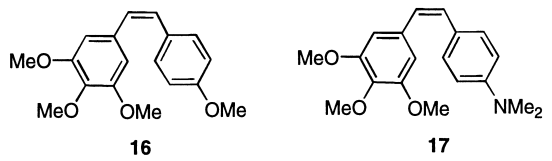
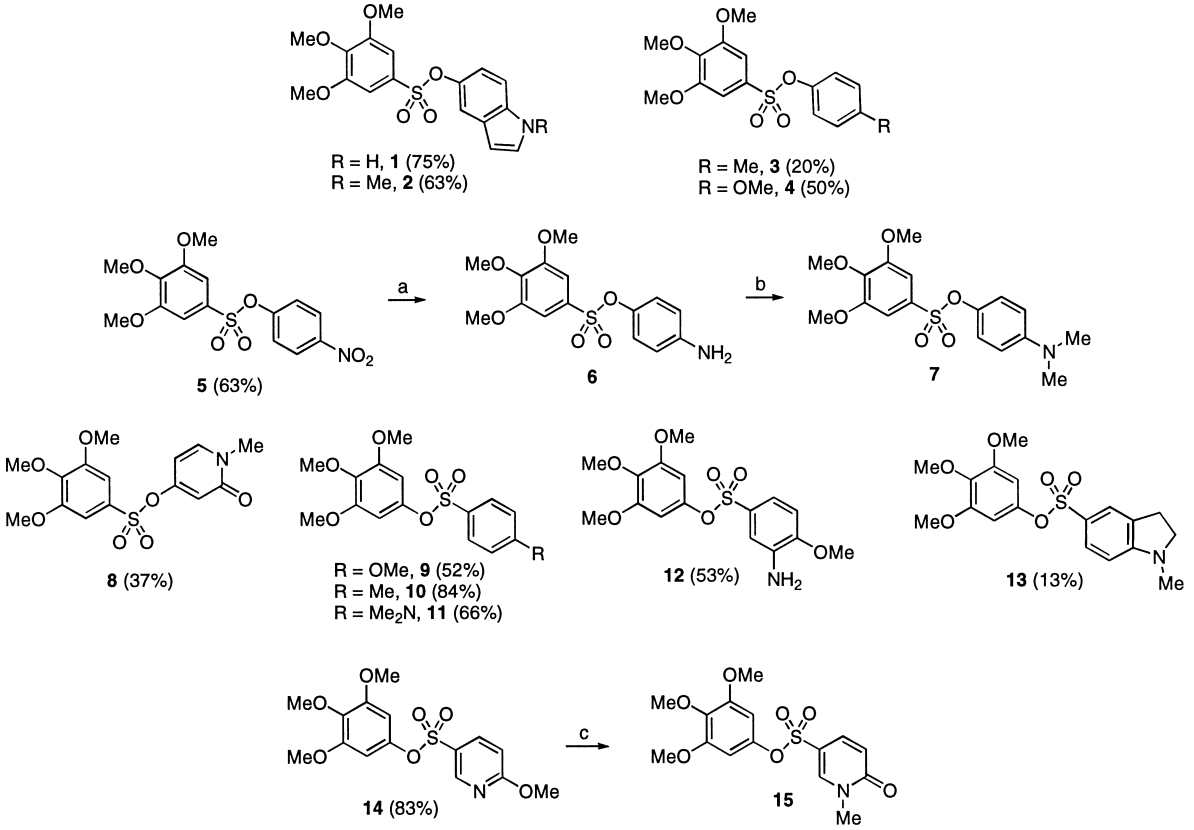


Figure 2. Olefins **16** and **17**.

The compounds above were tested for their ability to inhibit the proliferation of two cancer cell lines (Table 1).[8] NCI-H460 is a human lung carcinoma that does not express P-glycoprotein, while HCT-15 is a human colon carcinoma that expresses a high level of this protein. HCT-15 therefore shows multidrug resistance. This fact can be appreciated by examining the inhibition data for a P-glycoprotein substrate such as paclitaxel against the two cell lines.

Many of the sulfonate analogues show excellent antiproliferative activity. In fact, the potency of compounds **2** and **12** rivals that of CA-4 itself. Importantly, the potent sulfonates in Table 1 maintain their activity against the multidrug resistant HCT-15 cell line. The orientation of the sulfonate group relative to the two aryl rings makes little difference in the antiproliferative activity of the compounds, as demonstrated by compound pairs **3**/**10**, **4**/**9**, and **7**/**11**. That the sulfonate is an effective replacement for the *cis* olefin of CA-4 can be appreciated by comparing the activities of compounds



Scheme 3. Sulfonate analogues of combretastatin A-4. Structures, sulfonylation yields, and synthetic modifications. Reagents: (a) H_2, Pd–C, 36%; (b) $(CH_2O)_n$, $NaCNBH_3$, 49%; (c) MeI, 135 °C, 45%.

Table 1. Inhibition of cellular proliferation (IC_{50}) for sulfonate analogues of CA-4, olefins **16** and **17**, CA-4, and paclitaxel

Compounds	IC_{50} (nM), HCT-15[a]	IC_{50} (nM), NCI-H460[b]
1	35 (±1.8)	47 (±14)
2	3.3 (±2.8)	3.1 (±1.6)
3	71 (±0.85)	620 (±71)
4	36 (±9.9)	67 (±4.0)
5	>10,000	>10,000
6	700 (±81)	740 (±13)
7	17 (±20)	29 (±22)
8	580 (±53)	550 (±55)
9	56 (±22)	140 (±160)
10	250 (±2.8)	540 (±120)
11	30 (±8.8)	41 (±12)
12	4.1 (±0.064)	2.7 (±1.5)
13	440 (±200)	480 (±250)
14	670 (±270)	900 (±510)
15	>10,000	>10,000
16	8.7 (±1.2)	60 (±4.5)
17	74 (±3.1)	140 (±140)
CA-4	1.7 (±1.1)	3 (±0.66)
Paclitaxel	450 (±190)	15 (±6.0)

[a]MDR (+), multidrug resistant.
[b]MDR (−), not multidrug resistant.

4/**9** with that of **16**, and comparing the activities of compounds **7**/**11** with that of **17**.

Our efforts to replace the 3-hydroxy-4-methoxyphenyl ring of CA-4 with other groups in our sulfonates led to several findings. The 5-substituted indole nucleus (as in compound **1**), and especially *N*-methyl indole (as in compound **2**), are good replacements for the 3-hydroxy-4-methoxyphenyl ring of CA-4. Conversion of the indole to an indoline as in **13** leads to significant loss of potency. It has been previously established that the 3-amino-4-methoxyphenyl moiety is also a good replacement for the 3-hydroxy-4-methoxyphenyl ring of CA-4.[4d,j] This is supported by the potency reported here for **12**. Pyridones have been incorporated into CA-4 analogues and these compounds have shown good antitumor activity.[4g] With this in mind, compounds **8** and **15** were designed to improve water solubility; however, these compounds suffered substantial losses in potency.

Selected compounds that showed significant antiproliferative effects were also tested for their ability to inhibit the polymerization of tubulin[9] (Table 2) and

Table 2. Inhibition of tubulin polymerization (IC_{50}) for sulfonate analogues of CA-4, olefins **16** and **17**, CA-4, and paclitaxel

Compounds	IC_{50} (μM)
1	6.6 (±1.3)
2	1.4 (±0.15)
9	8.2 (±2.5)
10	>100
11	16 (±1.3)
12	6.7 (±3.7)
16	1.3 (±0.89)
17	1.7 (±0.042)
CA-4	1.2 (±0.76)
Paclitaxel	1.3 (±0.50)

Table 3. Percent inhibition of colchicine (5 μM) binding to tubulin (10 μM) for compounds **2**, **9**, and CA-4 at 5 μM and 50 μM

Compounds	% Inhibition	
	5 μM	50 μM
2	63	95
9	43	80
CA-4	53	93

their ability to compete with colchicine for its binding site on tubulin[10] (Table 3).

The data shown above demonstrate that these sulfonate analogues of CA-4 inhibit the polymerization of tubulin and compete effectively for the colchicine binding site on tubulin. Of the sulfonates in Tables 2 and 3, compound **2** proved to be the best antimitotic agent, inhibiting tubulin polymerization with an IC_{50} similar to that of CA-4 and competing more effectively than CA-4 for the colchicine binding site.

The pharmacokinetic behavior of several of the more active analogues shown above was examined. These compounds are characterized by low aqueous solubility, short iv half-lives, and low oral bioavailablity (F). This problem can be ameliorated in some cases through the preparation of prodrugs. For example, in rats, compound **12** has an iv half-life of 0.56 h, and F = 0%; however, prodrug **18** (Fig. 3) provided improved plasma levels of **12** after a 10 mg/kg oral dose ($t_{1/2}$ = 1.82 h, $AUC_{(0-6\,h)}$ = 0.8 mcg·h/mL, F∼100%).

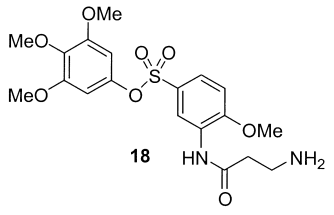


Figure 3. Prodrug **18**.

Compound **18** was tested in vivo against M5076, a murine cell line. Mice were dosed once daily on days 1–5 of the trial with either 200, 150, or 50 mg/kg. Tumor growth was measured and compared to tumor growth in untreated animals. Compound **18** did not show appreciable efficacy in this model.

In conclusion, we have synthesized sulfonate analogues of combretastatin A-4 and have found many of these compounds to be potent in vitro inhibitors of the proliferation of two cancer cell lines. These compounds inhibit the polymerization of tubulin and compete effectively for the colchicine binding site on tubulin. Examination of the SAR in this series of compounds reveals that the sulfonate replacement of the *cis* olefin and various replacements for the 3-hydroxy-4-methoxyphenyl ring of CA-4 are well tolerated.

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