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A diaryl sulfide, sulfoxide, and sulfone bearing structural similarities to combretastatin A-4

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Abstract

Studies examining various spacer groups that link the two aromatic rings of combretastatin A-4 (CA4) have shown that the biological activity of analogs does not require the *cis*-stilbene configuration of CA4. Oxygen or nitrogen, carbonyl, methylene and ethylene spacers, for example, are present in CA4 analogs that show good activity. Up to now sulfur was not tested for this purpose. In this article we describe the synthesis of sulfide, sulfoxide and sulfone spacers between two aromatic rings comparable to those of CA4. We also compared them with CA4 for inhibitory effects on cell growth, tubulin polymerization, and the binding of [^{3}H]colchicine to tubulin. We found that the sulfide is highly active and may be a lead compound for the preparation of antitumor compounds.

Keywords

Diaryl sulfide; Diaryl sulfoxide; Diaryl sulfone; Combretastatin A-4; Cytotoxicity; Tubulin polymerization

1. Introduction

Microtubules play an important role in cell division and structure. Their critical role in the mitotic spindle is exploited in the use of antitubulin agents as chemotherapeutic drugs. One of the most successful of these compounds clinically is taxol [1], and the colchicine site compound combretastatin A-4 (CA4) [2] (Fig. 1) has attracted a great deal of experimental attention. While agents that bind at the taxoid site of tubulin promote increased microtubule assembly [1], colchicine site compounds cause microtubule depolymerization. The *cis*-stilbene CA4 was discovered by Pettit and co-workers as the most potent of numerous related compounds produced by the African plant *Combretum caffrum*. CA4, in its high affinity for the colchicine site of tubulin, causes significant cytotoxicity and, moreover, *in vivo* shows marked inhibitory effects on angiogenesis [3].

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Because of the structural simplicity of CA4, numerous analogs have been synthesized, and many of these analogs exhibit similar biological activity. The literature demonstrates the possibilities for structural variability in analogs in this class [4]. SAR studies have shown that the two phenyl rings can be separated by cyclic and non-cyclic linkers. Phenstatin, for example, is highly active and has a carbonyl moiety rather than the 2-carbon stilbene bridge of CA4 between the aromatic rings [5]. Oxygen and nitrogen linkers were also tested. Some ether analogs are comparable to CA4 as inhibitors of tubulin polymerization, but they are much less cytotoxic. Amines are significantly less active than their ether counterparts [6]. Double bond reduction of CA4 yields compounds with moderately reduced activity, as does a methylene spacer group [7–9]. Moreover, if the ring B hydroxyl group is missing in the *cis*-stilbene structure, activity is only modestly reduced relative to the activity of CA4 [7]. In this communication, we report our exploration of sulfur bridges as biosteric replacements for other active atoms between the two phenyl rings of combretastatin A-4. We found high activity with a sulfide bridge, while compounds with a sulfone or a sulfoxide bridge were inactive.

2. Results and discussion

2.1. Chemistry

Since the potential activity of CA4 analogs with a sulfur atom linker has not yet been explored, we devised a synthetic route to the sulfide **1**, which was then oxidized to the sulfoxide **2** and sulfone **3** (Scheme 1).

Compound **1** was obtained in high yield by an efficient coupling reaction in which an iodobenzene and a thiophenol were mixed and refluxed in an oxygen free environment with neocuproine–Cu^{+} catalyst and the base Na-*tert*-butoxide [10]. Sulfoxide **2** and sulfone **3** were obtained in high yield reactions using *m*-CPBA, one equivalent resulting in sulfoxide **2** and two equivalents in sulfone **3** [11]. The success of the coupling and oxidation reactions was confirmed by spectral analysis (see Material and methods).

2.2. Bioevaluations

As shown in Table 1, we examined compounds **1–3** for activity as inhibitors of the growth of MCF-7 human breast cancer cells, the binding of colchicine to tubulin, and tubulin assembly, in comparison with CA4, phenstatin, and other available, closely related analogs [2,5,7–9] (we thank Drs. G.R. Pettit of Arizona State University and M.S. Cushman of Purdue University for supplying most of these compounds). Only the sulfide **1** had substantial activity. The relative antiproliferative activities of compounds **1**–**3** obtained in the MCF-7 cells were also observed in four additional human cancer cell lines (Table 2).

In comparison with CA4, **1** was similar as an inhibitor of tubulin assembly and over half as active as an inhibitor of colchicine binding, but it was about 10-fold less active as an inhibitor of MCF-7 cell growth. Comparing **1** only with the tetramethoxy series summarized in Table 1, it was more active in all assays as compared with the compounds with dihydrostilbene, carbonyl, and methylene spacers, but less active than the analog with the *cis*-stilbene linker.

Sulfur oxidized compounds **2** and **3** displayed greatly reduced activity when compared with sulfide **1**. This could derive from conformational issues. Crystallographic structures show that planes formed by rings in diphenyl sulfoxides and sulfones tend to be almost orthogonal [12, 13], unlike diphenyl sulfides [14], *Z*-diphenylethenes [15], and benzophenones [5]. Unfortunately, however, we have not yet been able to crystallize compound **1** to confirm this hypothesis.

3. Conclusions

Three new compounds with structural similarity to CA4 were prepared using reported methodologies. Surveying the data presented in Table 1, one can conclude that the hydroxyl group in the B ring generally enhanced the activity of this family of diaryl compounds, making it worthwhile to consider synthesis of a hydroxyl analog of compound **1**, which may exhibit even better biological activity than **1**. However, activity was not entirely predictable. The additional hydroxyl group in the B ring seemed to disproportionately enhance activity with a carbonyl spacer, as in phenstatin, while having only negligible effect with a *cis*-stilbene linker. The conformational status of the sulfoxide (**2**) and sulfone (**3**) probably results in a poor interaction with key amino acids residues in the colchicine site, and this in turn results in poor cytotoxicity.

4. Material and methods

4.1. Chemistry

All melting points were determined using Uniscience of Brazil Mod. 498 equipment. Absorption FT-IR spectra were obtained using the KBr pellet method or in chloroform solution performed with a Perkin Elmer Mod. 783-FT spectrometer. NMR spectra were recorded on Bruker DPX-300 spectrometer, the chemical shifts were presented in parts per million (δ) relative to TMS ($\delta = 0.0$), and $CDCl_3$ was employed as a solvent. High resolution electrospray ionization–mass spectrometry (ESI–MS) analyses were performed using an UltroTOF-Q – Electrospray Quadrupole Time-of-Flight Mass Spectrometer with electronspray ionizer APOLLO as the interface in positive ion mode. Internal patterns: coumarin (147 and 169) and monensin (693).

The solvents employed in the reactions and silica gel column chromatography were previously purified and dried according to procedures found in the literature [16]. Thin-layer chromatography (TLC) was carried out on silica gel plates with a fluorescence indicator of F_{254} (0.2 mm, E. Merck); the spots were visualized in UV light and by spraying with a 1% ethanol solution of vanillin or by using a charring reagent. Purification of compounds was performed by column chromatography, the stationary phase was silica gel 60 (80–230 mesh) from ACROS (Brazil), silica gel 60 (230–400 mesh) from Merck and celite. All reagents used in the present study were of analytical grade.

4.1.1. General procedure for the ipso iodination of 3,4,5–trimethoxyaniline: synthesis of 5-iodo-1,2,3-trimethoxybenzene (i)—A solution of 3,4,5-trimethoxyaniline (Aldrich Chemical Co., 1.0 g, 2.3 mmol) in 2.2 mL of a 1:1 mixture of conc. HCl and H_2O was added dropwise into a solution of sodium nitrite (0.70 g, 2.3 mmol) over 15 min, taking care to keep the mixture below 5 °C. Sodium iodine was then added very slowly, keeping the solution temperature below 0 °C. After being stirred overnight at room temperature, the mixture was extracted with EtOAc (3 × 50 mL). The organic extracts were combined and dried over $MgSO_4$. The purification was carried out by flash chromatography, yielding an amorphous solid (85% yield). M.p. = 83–85 °C. The product has been well described in the literature [17].

4.1.2. Synthesis of 1,2,3-trimethoxy-5-[(4-methoxyphenyl)thio]benzene (1)—Into a round-bottomed flask, stirred magnetically, were placed 0.186 g (1.94 mmol) of sodium *tert*-butoxide and 0.025 g of copper iodide (10 mol% of 5-iodo-1,2,3-trimethoxybenzene). After the reaction vessel was sealed, 0.17 mL (1.33 mmol) of 4-methoxybenzenethiol and 0.38 g (1.29 mmol) of 5-iodo-1,2,3-trimethoxybenzene in 6.0 mL of toluene were injected through the septum. The reaction mixture was heated for 24 h at 110 °C. Purification was performed by flash chromatography, and an amorphous solid was obtained (92% yield). M.p. = 119–120

°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 6H), δ 3.81 (s, 3H), δ 3.82 (s, 3H), δ 6.47 (s, 2H), δ 6.98 (dj = 8.85 Hz, 2H), δ 7.39 (dj = 8.85 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 55.35, δ 56.11, δ 60.87, δ 106.61, δ 114.88, δ 125.18, δ 134.64, δ 134.37, δ 136.84, δ 153.61, δ 159.61. HRMS [ESI (+) − MS]: $C_{16}H_{18}O_4S[M + H]^+$ m/z, calc. 307.1004, found 307.1097.

4.1.3. Synthesis of 1,2,3-trimethoxy-5[(4-methoxyphenyl)sulfinyl]benzene (2)—

To a solution of 30 mg (9.2 mmol) of compound **1** and 5 mL of dichloromethane was added very slowly 1 equiv. of *m*-CPBA over 3 h. Sulfoxide formation was monitored by thin-layer chromatography. Purification was performed with a flash chromatographic column, and an amorphous powder was obtained (87% yield). M.p. = 107.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H), δ 3.86 (s, 3H), δ 3.89 (s, 6H), δ 6.98 (d,*J* = 9.04 Hz, 2H), 7.14 (s, 2H), 7.87 (d, *J* = 9.04 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 55.62, δ 56.45, δ 60.89, δ 104.73, δ 114.5, δ 128.27, δ 129.6, δ 133.72, δ 136.84, δ 153.46, δ 163.29. HRMS [ESI (+) − MS]: $C_{16}H_{18}O_5S[M + H]^+$ m/z, calc. 323.0953, found 323.0976.

4.1.4. Synthesis of 1,2,3-trimethoxy-5[(4-methoxyphenyl)sulfonyl]benzene (3)—

To a solution of 30 mg (9.2 mmol) of compound **1** and 5 mL of dichloromethane was added very slowly 2 equiv. of *m*-CPBA over 3 h. The reaction was monitored by thin-layer chromatography. Purification was performed by flash chromatography, and an amorphous powder was obtained (90% yield). M.p. = 111–112 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 3H), δ 3.86 (s, 3H), δ 3.89 (s, 6H), δ 6.98 (d, *J* = 9.04 Hz, 2H), 7.14 (s, 2H), 7.87 (d,*J* = 9.04 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 55.62, δ 56.45, δ 60.89, δ 104.73, δ 114.5, δ 128.27, δ 129.6, δ 133.72, δ 136.84, δ 153.46, δ 163.29. HRMS [ESI (+) − MS]: $\mathbf{C}_{16}H_{18}O_6S[M + H]^+$ *m/z*, calc. 339.0902, found 339.0993.

4.2. Antiproliferative and antitubulin activities

MCF-7 human breast cancer cells were obtained from the National Cancer Institute drug screening program. They were grown in monolayer culture in RPMI 1640 medium supplemented with 5% fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. Compounds, dissolved in dimethyl sulfoxide, were added at varying concentrations, with a final dimethyl sulfoxide concentration of 1% (v/v). Compounds were added to cells that had been seeded in 96-well plates 24 h earlier, and incubation continued for 48 h. Cells were fixed with 5% (w/v) trichloroacetic acid, and cell protein was stained with sulforhodamine B [18]. The plates were read in a Molecular Devices plate reader (Spectra Max 340). The IC_{50} was the compound concentration that reduced the increase in cell protein after 48 h by 50%.

Cytotoxicity assay in other cell lines: All compounds (0.0009-5 μg/mL) were tested for cytotoxic activity against four additional human cell lines during 72 h of incubation: MDA/MB-435 (breast), HCT-8 (colon), SF-295 (glyoblastoma) and HL-60 (leukemia). Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dymethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann [19].

The tubulin assembly and [^{3}H]colchicine binding assays were performed with electrophoretically homogenous bovine brain tubulin [20]. The assembly assay [21] was performed with 10 μM(1.0 mg/mL) tubulin in 0.8 M monosodium glutamate (pH 6.6 with HCl in a 2.0 M stock solution), 0.4 mM GTP, and 4% (v/v) dimethyl sulfoxide (as compound solvent). Tubulin and varying compound concentrations were preincubated without GTP for 15 min at 30 °C, samples were placed on ice, and GTP was added. The samples were transferred to 0 °C cuvettes in Beckman DU7400 and DU7500 recording spectrophotometers equipped with electronic temperature controllers. After baselines were established at 350 nm, the temperature was jumped to 30 °C (less than 1 min), and sample turbidity was followed for 20

min. The IC_{50} was the compound concentration that reduced the turbidity reading at 20 min by 50% relative to a control reaction mixture without compound.

The colchicine binding assay was described in detail by Verdier-Pinard et al. [22]. The reaction mixtures contained a tubulin stabilizing buffer system [22] and 1.0 μM (0.1 mg/mL) tubulin, 5.0 μM [^{3}H]colchicine (from Perkin–Elmer), compound at 1.0, 5.0, or 50 μM in a final dimethyl sulfoxide concentration of 5% (v/v). The 0.1 mL reaction mixtures were incubated for 10 min at 37 °C, at which time the reaction is about 40–50% complete in the absence of compound. The reactions were stopped with 3 mL of ice water, and each diluted reaction mixture was filtered through a stack of two DEAE-cellulose filters from Whatman. The amount of radiolabeled colchicine bound to the tubulin adsorbed to the filters was determined in a scintillation counter.

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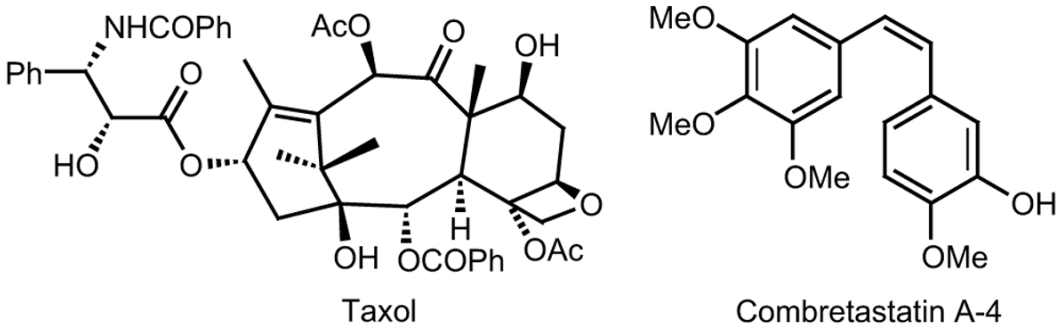
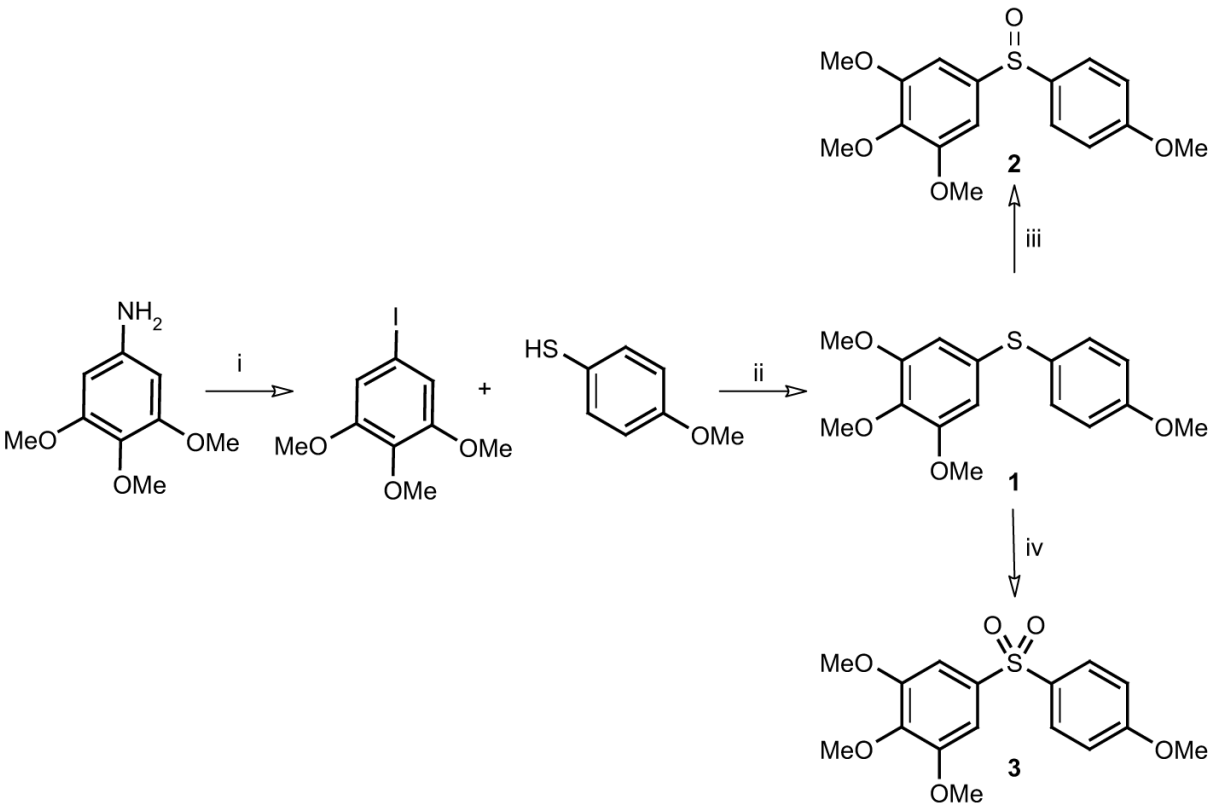


Fig. 1.
Structures of of taxol and CA4.

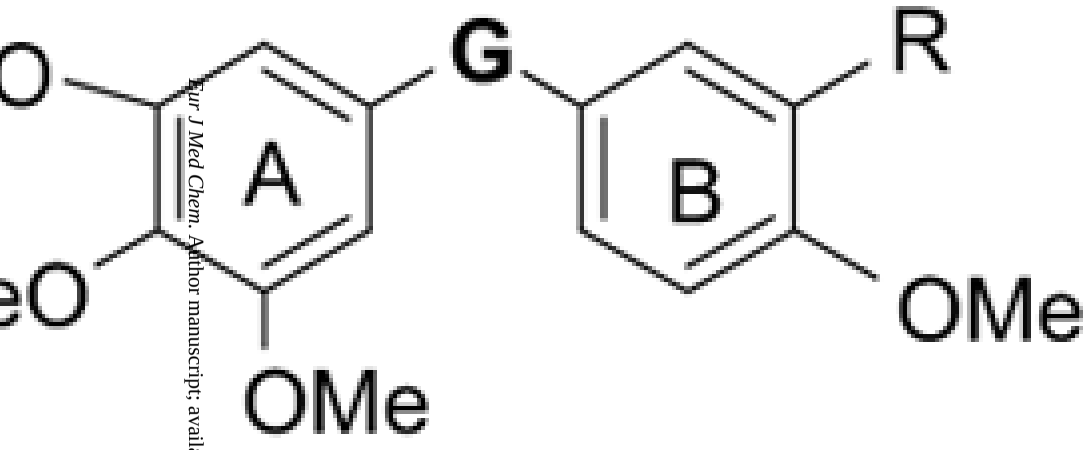


Scheme 1.

Reagents and conditions (i) $NaNO_2$, 1:1 cone. HCl/H_2O, 5 °C, KI (85%); (ii) neocuproine and CuI (10 mol%), 1.5 equiv. Na-*t*-butoxide, toluene, 110 °C (98%); (iii) **1** equiv. *m*-CPBA, CH_2Cl_2 (96%); (iv) 2 equiv. *m*-CPBA, $CHCl_3$ (100%).

Table 1

Inhibitory effects of compounds **1**–**3**, CA4, and structurally related analogs on tubulin assembly, colchicine binding to tubulin, and the growth of MCF-7 human breast cancer cells.[a]



Tubulin polymerization IC_{50} (μM) ± SD	Colchicine binding % inhibition ± SD			MCF-7 cell growth IC_{50} (nM) ± SD
	1 μM Drug	5 μM Drug	50 μM Drug	
1.2 ± 0.1	56 ± 0.7	89 ± 1	-	16 ± 5
31 ± 3	-	16 ± 7	41 ± 6	2200 ± 500
>40	-	18 ± 7	53 ± 9	2600 ± 700
1.6 ± 0.03	49 ± 3	67 ± 5	-	13 ±3
2.6 ± 0.3	-	50 ± 6	89 ± 0.1	78 ± 10
0.73 ± 0.02	70 ± 9	78 ± 4	-	34 ± 20
2.2 ± 0.03	-	50 ± 6	95 ± 6	160 ± 70
2.9 ± 0.04	-	44 ± 3	91 ± 9	59 ±30
4.0 ± 0.3	-	36 ± 5	83 ± 3	130 ± 40
1.1 ± 0.1	85 ± 5	99 ± 0.5	-	1.4 ± 0.1
1.1 ± 0.2	74 ± 5	97 ± 2	-	4.8 ± 1

	Tubulin polymerization IC_{50} (μM) ± SD	Colchicine binding % inhibition ± SD			MCF-7 cell growth IC_{50} (nM) ± SD
		1 μM Drug	5 μM Drug	50 μM Drug	

re obtained in contemporaneous experiments.

Table 2

Cytotoxic activity of the compounds **1–3** observed in four additional human tumor cell lines.

Drug	IC_{50}[Mg/mL(μM)][a]			
	MDA/MB-435	**HCT-8**	**SF-295**	**HL-60**
1	0.001 (0.003) 0.001–0.004	0.01 (0.04) 0.01–0.02	0.01 (0.03)0.01–0.02	0.001 (0.003) 0.001–0.002
2	0.09 (0.28) 0.07–0.12	0.15(0.47)0.10–0.26	0.47 (1.46)0.28–0.80	0.11 (0.34) 0.09–0.15
3	0.75 (2.32) 0.64–0.89	3.95(12.25) 1.13–3.71	2.61 (8.10) 0.62–0.94	1.53 (4.75) 1.03–2.25

[a] Data are presented as IC_{50} values and 95% confidence intervals obtained by non-linear regression for leukemia (HL-60), breast (MDA/MB-435), colon (HCT-8) and glyoblastoma (SF-295) cells from two independent experiments.