Sulfonate Derivatives of Naphtho[2,3-*b*]thiophen-4(9*H*)-one and 9(10*H*)-Anthracenone as Highly Active Antimicrotubule Agents. Synthesis, Antiproliferative Activity, and Inhibition of Tubulin Polymerization

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Benzenesulfonate derivatives of naphtho[2,3-*b*]thiophen-4(9*H*)-one and 9(10*H*)-anthracenone were prepared and found to inhibit microtubule formation by an in vitro tubulin polymerization assay. Several analogues showed potent cytotoxic activity in an assay based on K562 leukemia cells with IC_{50} values of <100 nM. The methylamino analogue **14i** was the most active compound in this assay (**14i**, IC_{50} K562: 0.05 μM). Antiproliferative activities of selected compounds were additionally evaluated against a panel of 12 tumor cell lines, including multi-drug-resistant phenotypes. All resistant cell lines were sensitive to these compounds. Concentration-dependent flow cytometric studies showed that KB/HeLa cells treated with selected compounds were arrested in the G2/M phases of the cell cycle. In competition experiments, these compounds strongly displaced radiolabeled colchicine from its binding site in the tubulin, showing IC_{50} values lower than that of colchicine. The results demonstrate that the antiproliferative activity is related to the inhibition of tubulin polymerization.

Introduction

The mitotic spindle, constituted by microtubules generated by polymerization of tubulin α-,β-dimers, remains an attractive target for the development of compounds useful in anticancer chemotherapy.[1,2] A large number of antimitotic drugs displaying a wide structural diversity have been identified to interfere with tubulin.[3,4] The Vinca alkaloids vinblastine (**1**, Chart 1) and vincristine as well as the taxanes, such as taxol (INN[a]: paclitaxel) and taxotere (INN: docetaxel) are widely used clinically as important chemotherapeutic agents for the treatment of many malignancies. It was mainly the discovery of these compounds that has stimulated intense research aimed at additional microtubule-targeting drugs. Colchicine (**2**) has limited medicinal utility due to its narrow therapeutic window but played an important role in the elucidation of the properties and functions of tubulin and microtubules. Many natural products, such as combretastatin A-4[5] (**3**) or the epothilones[6] as well as some synthetic molecules including sulfonamide E-7010[7] (**4**), thienopyrrolizinone[8] (**5**), acridinyl-9-carboxamide D-82318[9] (**6**), indolinone A-432411[10] (**7**), or propenenitrile CC-5079[11] (**8**) (Chart 1), to cite just a few, are known to mediate cytotoxic activities through a binding interaction with tubulin. In recent years, substantial efforts have been made to develop small molecular colchicine-site binders derived from natural sources or by screening compound libraries in combination with traditional medicinal chemistry.[4,12]

We previously reported the potent in vitro antitumor activity of 10-[(3-hydroxy-4-methoxybenzylidene)]-9(10*H*)-anthracenone (**10**) and 9-[(4-hydroxy-3,5-dimethoxybenzylidene]naphtho[2,3-*b*]thiophen-4(9*H*)-one (**12,** Chart 2).[13,14] These compounds strongly inhibited tumor cell growth as well as tubulin polymerization and caused significant arrest of mitosis. As part of our search for novel antimitotic agents, we describe the synthesis and biological evaluation of sulfonate derivatives related to 9(10*H*)-anthracenone (**9**) and naphtho[2,3-*b*]thiophen-4(9*H*)-one (**11**) as tubulin polymerization inhibitors. Several compounds strongly inhibited the growth of various tumor cell lines, acted in a cell cycle dependent manner, and were found to be potent inhibitors of tubulin polymerization. Antitubulin activities of the most active compounds are comparable to those of the reference compounds, such as nocodazole, podophyllotoxin, and colchicine.

Chemistry

Sulfur-containing compounds, such as sulfonamide E-7010 (**4**),[7] thienopyrrolizinones,[8] and benzothiophene derivatives,[8,15,16] proved effective inhibitors of tubulin polymerization. Previous investigations by Gwaltney et al.[17,18] have demonstrated the potent antimitotic activities of sulfonate analogues of combretastatin A4 and the sulfonamide E-7010 and have revealed that the sulfonate can effectively replace the *cis* olefin of CA-4 (**3**). These findings prompted us to synthesize sulfonates related to **9** and **11** in order to study their cytotoxic activity and their inhibitory effect on tubulin polymerization. On the basis of our previous chemical studies,[13,14] it was expected that reaction of **9** and **11** with benzenesulfonyl chlorides would lead to sulfonate derivatives. Keto−enol tautomerism of anthracenones as well as in the thiophene analogues of anthrone has been described,[19] and the formation of the sulfonates is suggested to be due to an

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[a] Abbreviations: ADR, adriamycin; DMAP, 4-dimethylaminopyridine; G, Gap; M, mitosis; INN, international nonproprietary name; ITP, inhibition of tubulin polymerization; Kip, kinase inhibitor protein; MDR, multi-drug-resistant; MTP, microtubule protein; ND, not defined; TBAB, tetra-n-butylammonium bromide; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide; VCR, vincristine.

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Chart 1. Examples of Tubulin Interacting Agents

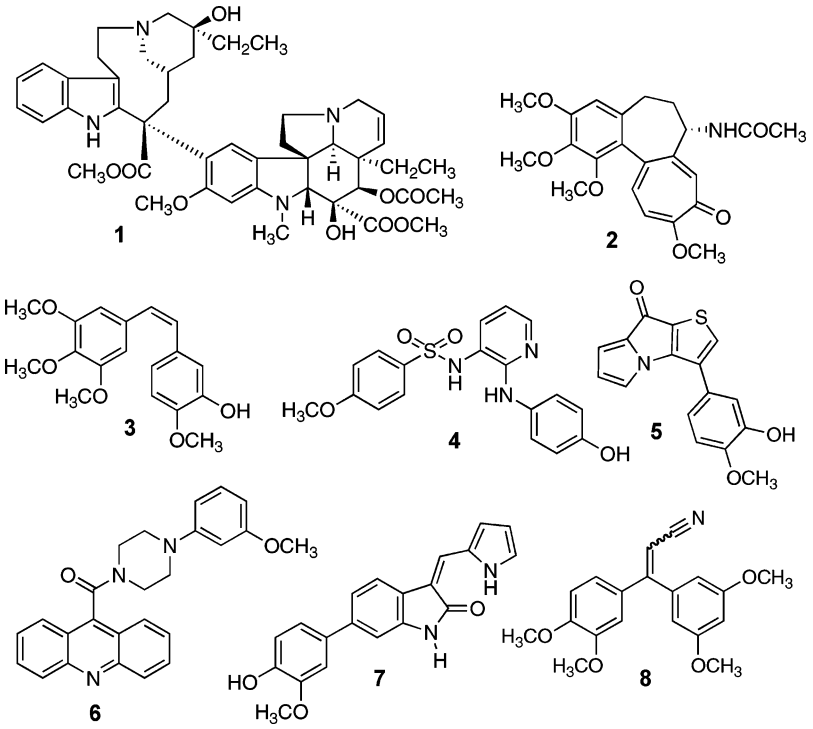
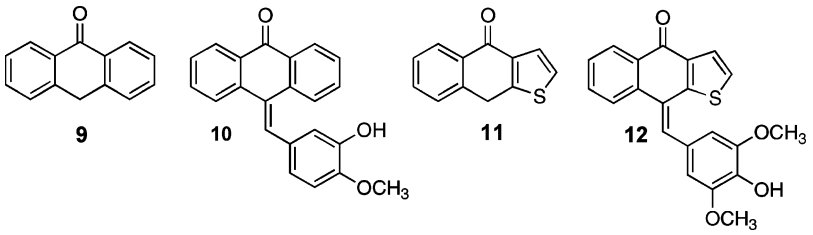


Chart 2. Structure of **9**, **11**, and Related Benzylidene Derivatives **10** and **12**

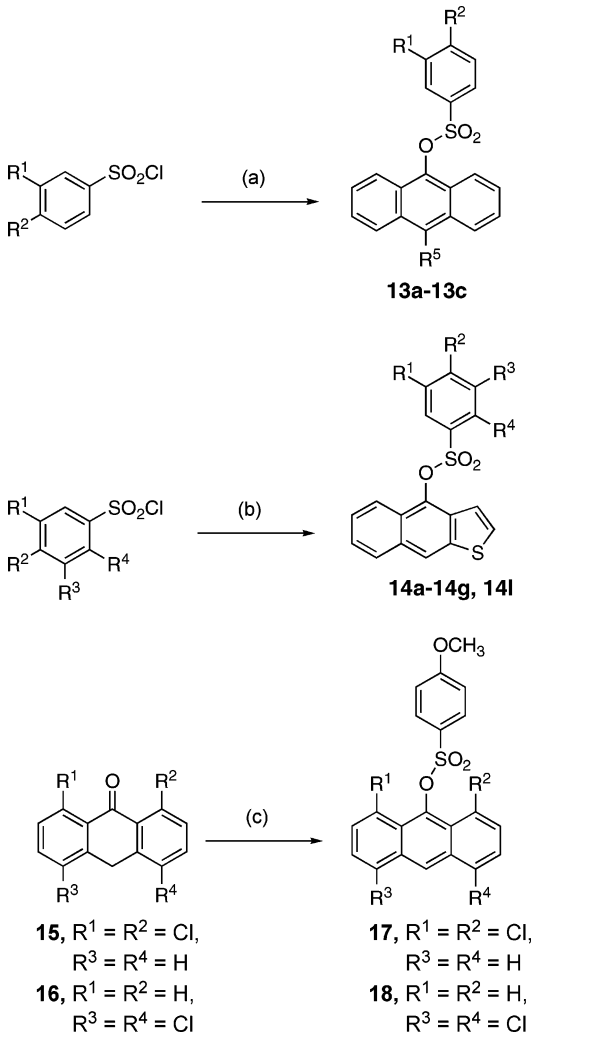


O-sulfonylation of the enol tautomeric form of the starting **9** and **11** under basic conditions. The general method for the synthesis of the sulfonates is outlined in Scheme 1. Most of the benzenesulfonyl chlorides as well as **9** were commercially available, and **11** was prepared as previously described.[14] Additional modifications concerned substitution in the tricyclic ring system. Accordingly, 1,8-dichloro- and 4,5-dichloro-9(10*H*)-anthracenone were prepared as described.[20,21] The desired sulfonates **13a**−**c**, **14a**−**g**, **14l**, **17**, and **18** were obtained by a sulfonylation reaction of **9**, **11**, **15**, and **16** with appropriately substituted benzenesulfonyl chlorides under standard conditions in the presence of sodium hydroxide or pyridine/DMAP in the case of **11**. Interestingly, as a side product of the sulfonylation reaction of **9**, we isolated the chloro-substituted compound **13b**. Reduction of the nitro group of **14g** provided the amino compound **14h**, which was subsequently N-mono- and bimethylated to obtain target compounds **14i** and **14j** (Scheme 2). The 4-hydroxyaminobenzensulfonic acid derivative **14k** was prepared using a Pd/C-catalyzed hydrogenation of **14g**.[22−24] Furthermore, we explored the effect of bioisosteric replacement of the 10-carbon atom in **9** with nitrogen. To this end, the O-sulfonylation of commercially available 9(10*H*)-acridone (**19**) with 4-methoxybenzenesulfonyl chloride and NaOH in the presence of tetra-n-butylammonium bromide (TBAB) as a phase transfer catalyst[25] was studied. However, no satisfactory analytical data were obtained for acridone-derived **21**, since traces of **19** could not be separated. By contrast, 1,2,3,4-tetrahydro-9-acridanone (**20**) smoothly yielded sulfonate **22** (Scheme 3) under identical conditions.

Biological Results and Discussion

In Vitro Cell Growth Inhibition Assay. In an initial screen, the compounds were evaluated for antiproliferative activity against the human chronic myelogenous leukemia cell line K562[26] which is widely used for potential antitumor compounds. Cell proliferation was determined directly by counting the cells with a hemocytometer after 48 h of treatment. The structures and the growth inhibitory data of the synthesized sulfonates are listed in Table 1. With the exception of the nitro compounds **14g** and **14l** most compounds showed IC_{50} values in the submicromolar (lower than 1 μM) range. In the benzenesulfonic acid naphtho[2,3-*b*]thiophen-4-yl-ester series, three compounds (**14b**, **14i**, and **14j**) displayed strong antiproliferative activity with IC_{50} values of lower than 0.1 μM. A similar activity was found for the 4-methoxybenzenesulfonic acid anthracen-9-yl ester (**13a**) (IC_{50} K562: 0.09 μM). Replacement of the 4-methoxy group of **14b** (IC_{50} K562: 0.06 μM) with a 4-methyl group (**14e**) led to a loss of potency. A similar loss of potency was observed for the isomeric **14c** versus **14f**, respectively. In addition, our findings indicate that a methoxy group located at the para-position to the sulfonyl part resulted in strong antiproliferative activity, while shifting it to the meta-position decreased the activity (**13a** versus **13c**, **14b** versus **14c**). Noteworthy, potent inhibition of tubulin polymerization was found for **13c** and **14c** (vide infra). Chloro-substituted compound

Scheme 1[a,b]



[a] R[1]−R[5] are defined in Table 1. [b]Reagents and conditions: (a) 9(10*H*)-anthracenone, 2 N NaOH/THF, rt; (b) naphtho[2,3-*b*]thiophen-4(9*H*)-one, CH_2Cl_2, DMAP, pyridine, N_2, 0−5 °C to rt; (c) 4-(MeO)$PhSO_2Cl$, 2 N NaOH/THF, rt.

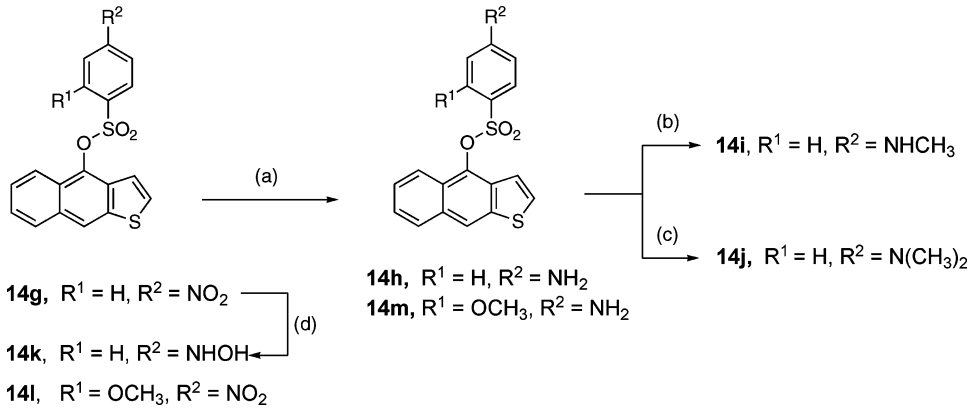
13b was determined to be clearly less potent than **13a** (**13a**, 0.09 μM vs 0.35 μM for **13b**). The replacement of the methoxy group of **14b** with a dimethylamino (**14j**) or a methylamino (**14i**) group in the benzenesulfonyl part retained strong activity with IC_{50} values in the range of 0.05−0.08 μM, indicating that the methoxy and the methylamino groups are obviously bioequivalent at the C4-position of the benzenesulfonyl part. Activities were comparable to that of **13a** which is a bioisostere of **14b**. The methylamino analogue **14i** was the most active compound in this assay (IC_{50} K562: 0.05 μM), being slightly less potent than adriamycin (IC_{50} K562: 0.01 μM) and colchicine (IC_{50} K562 0.02 μM). The amino-substituted derivative **14h** had an IC_{50} of 0.13 μM, thus being slightly less active than its methylated congeners **14i** and **14j**. However, a substantial loss of activity was found for the 4-hydroxyamino-substituted compound **14k**. The 1,8- and 4,5-dichloro-9(10*H*)-anthracenone-derived compounds **17** and **18** showed only moderate cytotoxic effects. Moreover, naphtho[2,3-*b*]thiophen-4(9*H*)-one (**11**) displayed only weak activity (data not shown), documenting that the antiproliferative activity is closely related to the sulfonate structure.

Effect on Growth of Different Tumor Cell Lines. To further characterize the cytotoxicity profile of these compounds, the effect of the highly active methoxy analogues **13a** and **14b** against a panel of five tumor cell lines derived from solid tumors was measured by cellular metabolic activity using the XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) assay.[27] Generally, compound **14b** was found to be slightly more potent than **13a** and showed activities with IC_{50} values in the range of 0.2 μM toward several proliferating cell lines (Table 2). Both compounds provide a good example of the classical bioisosteric equivalence between benzene and thiophene, with both compounds exhibiting nearly the same biological activity. However, the thiophene derivative **14b** was found to be three-fold more active against the NCI-H460 cell line than **13a**. Interestingly, **13a** and **14b** were not active against RKO cells (human colon adenocarcinoma) with ectopic inducible expression of cyclin-dependent kinase inhibitor $p27^{kip1}$.[28] By contrast, growth of proliferating RKO cells was strongly inhibited by **13a** and **14b** with IC_{50} values of 0.28 and 0.18 μM, respectively, indicating activity toward cycling cells. A major obstacle to the treatment of many human cancers is the development of multiple drug resistance (MDR) in patients and a loss of efficacy over time, meaning that cancer cells do not respond to treatment or develop a broad spectrum resistance to several anticancer drugs, among them antitubulin agents.[29,30] Multiple drug resistance is mediated, among other factors, by overexpression of transmembrane cellular pumps, such as the 170 kDa P-glycoprotein (pgp),[31] encoded by the *mdr*1 gene and the 180 kDa MDR protein (MRP).[32] Important aspects concerning the key mechanisms of antimicrotubule drug resistance have recently been reviewed.[33] The antiproliferative activity of **13a** and **14b** against tumor cell lines with different resistance phenotypes was evaluated in an XTT-based assay. On the whole, as documented by the IC_{50} data (Table 3), **13a** and **14b** were effective against the cell lines tested and retained activity in cell lines with various multiple drug resistance phenotypes. This feature was distinct from paclitaxel and vindesine because LT12MDR, L1210VCR, and P388ADR cell lines were more resistant to these chemotherapeutics than the nonresistant cell-lines. Therefore, the sulfonate analogues **13a** and **14b** are inhibitors of tumor cell proliferation and are not subject to resistance mediated by drug efflux activity by overexpression of pgp170.

Effect on Cell-Cycle Progression. To gain further insight into the mode of action, representative compounds **13a** and **14b** were assayed for their effects on cell cycle using an established KB/HeLa (human cervical epitheloid carcinoma) cell-based assay system. For a thorough comparison of **13a** and **14b** with known G2/M cell cycle inhibitors, subconfluent KB/HeLa cells were exposed to test compounds, and the percentage of cells in G2/M phase after 24 h was plotted against different concentrations of the compounds. The concentration for 50% cells arrested in G2/M phase by **13a** and **14b** was found to be in the range of 0.13 μM (Table 4), thus being somewhat less active than nocodazole (EC_{50} 0.09 μM). For comparison, the recently described anthracenone derivative **10** had an EC_{50} value of 0.2 μM in this assay. In summary, the effect of compounds **13a** and **14b** on cell cycle progression correlated well with their strong antiproliferative and antitubulin activities and is similar to that observed for the majority of antimitotic agents. However, the reference compounds were more potent than any of the new compounds examined in this assay.

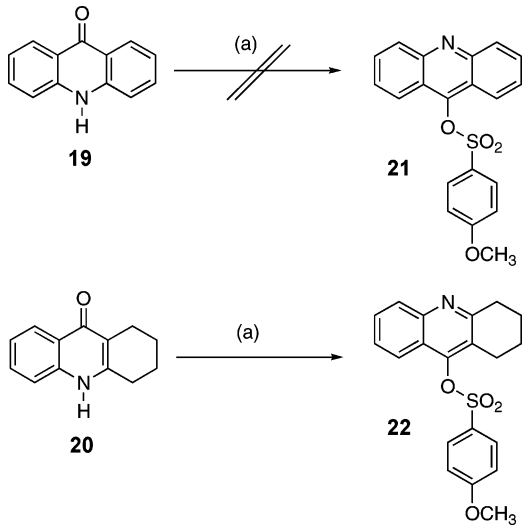
In Vitro Tubulin Polymerization Assays. To investigate whether the antiproliferative activities were related to an interaction with tubulin, 15 analogues were assayed for their inhibitory effects on tubulin polymerization using assay conditions as described previously.[14] Depending on the temperature-

Scheme 2[a]



[a] Reagents and conditions: (a) $SnCl_2 \cdot 2H_2O$, C_2H_5OH, N_2, reflux, 18 h; (b) $(CH_3O)_2SO_2$, acetone, K_2CO_3, 50 °C, 24 h; (c) CH_3I, DMF, K_2CO_3, 80 °C, 24 h; (d) Pd/C, C_2H_5OH, H_2, rt, 6 h.

Scheme 3[a]

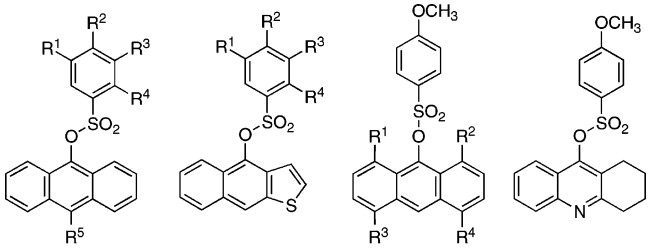


[a] Reagents and conditions: (a) 4-(MeO)$PhSO_2Cl$, NaOH 50%, THF/H_2O, TBAB.

dependent equilibrium between α/β-tubulin dimers and microtubules, physiological temperature (37 °C) favors polymerization of tubulin dimers into microtubules in the presence of GTP and some cofactors, which is detected by an increase in turbidity. Depolymerization is induced by shifting the temperature to 2 °C. If a tubulin polymerization inhibitor binds to or interferes with tubulin, the assembly steady-state level is decreased, as exemplified for **13a** (Figure 1). The results obtained with the test agents are summarized in Table 1. For comparison, the data of the potent antimitotic compounds colchicine, podophyllotoxin, nocodazole, and vinblastine are also presented. With the exception of compounds **14a**, **14g**, **17**, **18**, and **22**, all compounds tested were found to inhibit tubulin polymerization. When comparing the inhibition of tubulin polymerization versus the growth inhibitory effect, we found a good correlation for most of the active compounds, but not for all of them. A noticeable finding is the high potency of compound **13c** as an inhibitor of tubulin polymerization (IC_{50} 0.39 μM). A similar observation was found for **14c,** possibly indicating the significance of a 3-methoxy group, which is a well-defined pharmacophore for the inhibition of tubulin polymerization.[9] The strong decrease in potency on K562 cells in the case of **13c** and **14c** can possibly be rationalized by a limited penetration into the cell or any other mechanism limiting the accessibility of these molecules to the cellular tubulin. It has to be considered that the tumor cell growth assay and the tubulin polymerization assay differ in a number of further important issues, such as tubulin concentration present within the cells and during microtubule formation outside a cell, the presence of different types of microtubule-associated proteins, and the possible effects of regulatory proteins expressed in the cell but being absent in the tubulin assay. The 4-methoxy-substituted compound **13a** (IC_{50} 1.20 μM) was as good an inhibitor of tubulin polymerization as colchicine. Furthermore, compounds **14b**, **14i**, and **14j** proved to be exceptionally strong inhibitors of tubulin polymerization ($IC_{50} \leq 1.0$ μM), as did the reference drugs with the exception of colchicine (1.4 μM). For these four compounds, the order of inhibitory action on tubulin polymerization was **14i** > **14b** > **14j** > **13a**, which was consistent with the results from the K562 cell growth inhibition assay. Again, this finding indicates that a methoxy group plays an essential role to exhibit strong antitubulin and antiproliferative activities within this series of compounds. The nitro compound **14g** did not show any appreciable activity as an inhibitor of tubulin polymerization. The introduction of chloro substituents into the anthracenoid core caused a complete loss of activity as seen with **17** and **18**, suggesting that electronic or lipophilic factors account for the loss of activity observed with these two compounds. Moreover, compound **22** proved inactive as an inhibitor of tubulin polymerization, indicating that steric factors such as planarity of the tricyclic ring system may contribute to a potent tubulin inhibitory activity. Moderate inhibitors of tumor cell growth showed only poor inhibitory effects on tubulin polymerization (IC_{50} values ranging from 2.8 to >10 μM).

Microtubule-destabilizing drugs often reveal specific tubulin binding sites. Therefore, we assessed the capability of **13a** and **14b** to compete with either colchicine or paclitaxel for binding to tubulin using a competitive scintillation proximity assay.[34] Both **13a** and **14b** competitively inhibited [^{3}H]colchicine binding to biotinylated tubulin (Figure 2) with exceptionally low IC_{50} values of 0.56 μM (**13a**) and 0.51 μM (**14b**) as compared to colchicine (1.05 μM). The results were consistent with their growth inhibitory and tubulin polymerization inhibitory activities. However, the compounds did not compete with [^{3}H]-paclitaxel. No stabilization of the colchicine binding was observed, as it is documented for Vinca site binders.[35,36] Therefore, we conclude that binding to tubulin at the colchicine-binding site is highly probable and that the capacity to interact with the mitotic spindle contributes to the antiproliferative activity of the compounds.

Table 1. Antiproliferative Activity of **13a**−**c**, **14a**−**m**, **17**, **18**, and **22** against K562 Cells and Antitubulin Activities



compd	R^1	R^2	R^3	R^4	R^5	K562 IC_{50}[a] [μM]	ITP IC_{50}[b] [μM]
13a	H	OCH_3	H	H	H	0.09	1.20
13b	H	OCH_3	H	H	Cl	0.35	
13c	OCH_3	H	H	H	H	0.48	0.39
14a	H	H	H	H		0.80	>14
14b	H	OCH_3	H	H		0.06	0.91
14c	OCH_3	H	H	H		0.25	0.29
14d	OCH_3	OCH_3	H	H		0.17	1.27
14e	H	CH_3	H	H		0.44	1.05
14f	CH_3	H	H	H		0.53	0.74
14g	H	NO_2	H	H		2.3	>14
14h	H	NH_2	H	H		0.13	0.71
14i	H	$NHCH_3$	H	H		0.05	0.29
14j	H	$N(CH_3)_2$	H	H		0.08	0.94
14k	H	NHOH	H	H		0.40	ND
14l	H	NO_2	H	OCH_3		1.4	ND
14m	H	NH_2	H	OCH_3		0.28	ND
17	Cl	Cl	H	H		10	>10
18	H	H	Cl	Cl		1.7	>10
22						0.83	>10
colchicine						0.02	1.4
nocodazole						ND	0.76
podophyllotoxin						ND	0.35
vinblastine sulfate						0.001	0.13
adriamycin						0.01	ND

[a] IC_{50}, concentration of drug required for 50% inhibition of cell growth (K562). Cells were treated with drugs for 2 days. IC_{50} values are the means of at least three independent determinations (SD < 10%). [b] ITP = inhibition of tubulin polymerization; IC_{50} values were determined after 20 min at 37 °C and represent the concentration for 50% inhibition of the maximum tubulin assembly rate.

Table 2. Cytotoxic Activity of **13a** and **14b** against Different Tumor Cell Lines

	IC_{50} [μM][a]					
					RKOp27^{kip1} (human colon adenocarcinoma)	
compd	KB/HeLa (cervix)	SKOV3 (ovary)	SF268 (glioma)	NCI-H460 (lung)	not induced	induced
13a	0.37	0.25	0.34	0.64	0.28	>9
14b	0.18	0.21	0.26	0.21	0.18	>9
paclitaxel	0.01	0.01	0.01	0.01	0.01	>3
nocodazole	0.14	0.17	0.30	0.15	0.11	>14
colchicine	0.03	0.05	0.05	0.07	0.02	>14

[a] IC_{50} values were determined from XTT proliferation assays after incubation with test compound for 48 h. All experiments were performed at least in two replicates ($n = 2$), and IC_{50} data were calculated from dose−response curves by nonlinear regression analysis.

Conclusion

A novel series of sulfonate derivatives of 9(10*H*)-anthracenone and naphtho[2,3-*b*]thiophen−4(9*H*)-one was synthesized and we have identified several representatives of this series to be highly potent antiproliferative agents and inhibitors of tubulin polymerization. Similar to many other inhibitors of tubulin polymerization, selected compounds **13a** and **14b** were efficacious in inhibiting tumor cell proliferation with IC_{50} values at the nanomolar level. Our studies revealed that a 4-methoxy or a 4-methylamino substitution pattern in the terminal phenyl ring is critical for strong inhibition of tumor cell proliferation and inhibition of tubulin polymerization. Sulfonates **13a** and **14b** are potent inhibitors of the colchicine binding to tubulin and interact most likely with tubulin at the colchicine site. As with other microtubule-interacting agents, the induction of G2/M arrest was demonstrated for these compounds. Notably, no growth inhibitory effect was found in cell cycle arrested cells. Whereas the effectiveness of numerous clinically useful drugs is limited by the fact that they are substrates for the efflux pumps Pgp170 and MRP, compounds **13a** and **14b** were found to be active toward parental tumor cell lines and multi-drug-resistant cell lines, with **14b** being slightly more potent. This feature is distinct from those of paclitaxel and vindesine, because three cell lines were more resistant to these agents than the nonresistant cell lines. As pointed out, it has previously been shown that the sulfonate group is an effective replacement for the *cis* olefin of CA-4.[17] Therefore, in the figurative sense, the sulfonates described herein can be considered as anthracenoid analogues of combretastatin A4. As low aqueous solubilities and short iv half-lives have been documented for sulfonate

Table 3. Antiproliferative Activity of **13a** and **14b**, Paclitaxel, Nocodazole, and Vindesine against Tumor Cell Lines with Different Resistance Phenotypes (XTT Assay)

	IC_{50} [μM][a]					
compd	LT12 (blood)	LT12 MDR (blood)	L1210 (blood)	L1210 VCR-resistant (blood)	P388 (blood)	P388 ADR-resistant (blood)
13a	0.44	0.36	0.54	0.46	0.38	0.27
14b	0.17	0.19	0.19	0.20	0.18	0.18
paclitaxel	0.006	0.40	0.06	>5	0.04	>5
nocodazole	0.30	0.05	0.06	0.07	0.07	0.05
vindesine	0.001	0.26	0.02	>5	0.01	1.14

[a] IC_{50} values were determined from XTT proliferation assays after incubation with test compound for 48 h. All experiments were performed at least in two replicates ($n = 2$), and IC_{50} data were calculated from dose−response curves by nonlinear regression analysis.

Table 4. Cell Cycle Analysis of KB/HeLa Cells Treated with **13a** and **14b** and Reference Compounds Vincristine, Colchicine, Paclitaxel, and Nocodazole

	13a	**14b**	colchicine	nocodazole	paclitaxel	vincristine
EC_{50} [nM][a]	135	125	14	91	49	2.4

[a] EC_{50} values were determined from dose−response cell cycle analysis experiments and represent the concentration for 50% cells arrested in G2/M phase after 24 h. All experiments were performed at least in two replicates ($n = 2$), and EC_{50} data were calculated from dose−response curves by nonlinear regression analysis (GraphPad Prism).

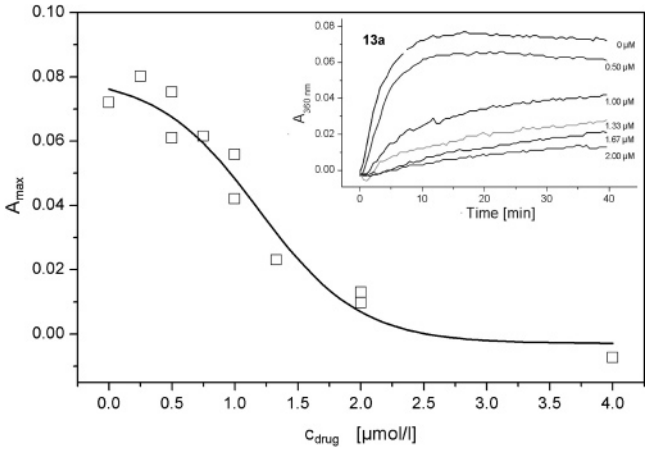


Figure 1. Inhibition of in vitro tubulin polymerization (microtubule formation) at 37 °C by various concentrations of **13a**. Turbidity was recorded at 360 nm (MTP 1 mg/mL). Tubulin assembly in the absence of inhibitor gave the control readings. IC_{50} values were determined after 20 min and represent the concentration for 50% inhibition of the maximum tubulin polymerization level.

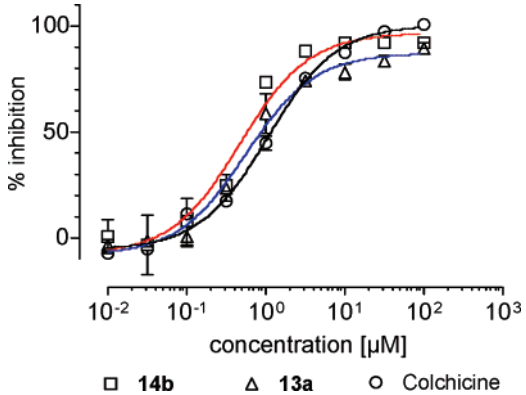


Figure 2. [^{3}H]Colchicine competition binding assay of **13a**, **14b**, and colchicine. Radiolabeled colchicine, unlabeled compound, and biotin-labeled tubulin were incubated together for 2 h at 37 °C.

analogues of CA-4, resulting in a poor pharmacokinetic behavior, this may also account for the sulfonates presented herein. The pharmacokinetic profile of our compounds has not been studied yet. Nevertheless, due to their attractive in vitro antitumor activities, we believe that compounds of this structural class are attractive for further structural modifications and that our findings may provide useful information for the design of novel antitumor agents. Investigations on the role of the anthracene and naphthothiophene moiety for antimitotic activity are in progress, and results from other modifications will be reported in due course.

Experimental Section

Melting points were determined with a Kofler melting point apparatus and are uncorrected. Spectra were obtained as follows: ^{1}H NMR spectra were recorded with a Varian Mercury 400 plus (400 MHz) spectrometer, using tetramethylsilane as an internal standard. Fourier-transform IR spectra were recorded on a Bio-Rad laboratories Typ FTS 135 spectrometer and analysis was performed with WIN-IR Foundation software. Elemental analyses were performed at the Münster microanalysis laboratory, using a vario EL III CHNOS elemental analyzer (Elementar Analysensysteme GmbH), and all values were within ±0.4% of the calculated composition. Mass spectra were recorded in the EI mode using a MAT GCQ Finnigan instrument. All organic solvents were appropriately dried or purified prior to use. Benzenesulfonyl chlorides were obtained from commercial sources. Analytical TLC was done on Merck silica 60 F_{254} alumina coated plates (E. Merck, Darmstadt). Silica gel column chromatography was performed using Acros 60−200 mesh silica gel. All new compounds displayed ^{1}H NMR, FTIR, and MS spectra consistent with the assigned structure. Yields have not been optimized. Chromatography solvent (vol %): EE = ethyl acetate; H = hexane; M = methanol; MC = methylene chloride. Elemental analyses were within ±0.4% of calculated values, except where stated otherwise.

4-Methoxybenzenesulfonic Acid Anthracen-9-yl Ester (13a). 9(10*H*)-anthracenone (**9**) (0.97 g, 5 mmol) and 4-methoxybenzenesulfonyl chloride (1.03 g, 5 mmol) were dissolved in a mixture of 2 N NaOH (20 mL) and THF (10 mL) at room temperature under N_2. The mixture became orange and was thereafter stirred until the reaction was complete (TLC control). Then, the reaction mixture was poured into water (250 mL) and HCl (50 mL) and extracted with CH_2Cl_2 (3 × 75 mL). The organic phase was dried over Na_2SO_4, washed with water, and then evaporated. Purification by silica gel chromatography (MC) afforded **13a** as a pale-yellow powder (0.37 g, 20% yield): mp 162−163 °C.

4-Methoxybenzenesulfonic Acid 10-Chloroanthracen-9-yl ester (13b). The title compound was obtained from (**9**) (0.97 g, 5 mmol) and 4-methoxybenzenesulfonyl chloride (1.03 g, 5 mmol), as described for **13a**. Purification by silica gel chromatography (MC) afforded **13b** as a yellow crystalline powder (54 mg, 3% yield): mp 158−159 °C.

3-Methoxybenzenesulfonic Acid Anthracen-9-yl ester (13c). The title compound was obtained from (**9**) (0.97 g, 5 mmol) and 3-methoxybenzenesulfonyl chloride (1.03 g, 5 mmol), as described for **13a**. Purification by silica gel chromatography (MC/H, 8:2) afforded **13c** as fine yellow crystals (0.13 g, 7% yield): mp 130 °C.

Benzenesulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14a). According to a literature method,[17,37] to a solution of naphtho[2,3-

b]thiophen-4-one (**11**) (0.7 g, 3.5 mmol) in absolute CH_2Cl_2 (20 mL) were added DMAP (0.15 g) and pyridine (5 mL) under N_2 at 0 °C. Then, a solution of benzenesulfonyl chloride (0.62 g, 0.45 mL, 3.5 mmol) in absolute CH_2Cl_2 was added dropwise. The mixture was stirred until the reaction was complete (TLC control). Thereafter, the reaction mixture was poured into water (250 mL) and extracted with CH_2Cl_2 (3 × 75 mL). The organic phase was dried over Na_2SO_4, washed with water, and then evaporated. Purification by silica gel chromatography (MC/H 1:1) afforded **14a** as white crystals (0.49 g, 38% yield): mp 152−154 °C.

4-Methoxybenzenesulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14b). The title compound was prepared from **11** (0.5 g, 2.5 mmol) and 4-methoxybenzenesulfonyl chloride (0.52 g, 2.5 mmol) as described for **14a**. Purification by silica gel chromatography (MC/H 8:2) afforded **14b** as fine, white crystals (0.44 g, 47% yield): mp 155−156 °C.

3-Methoxybenzenesulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14c). The title compound was prepared from **11** (0.8 g, 4 mmol) and 3-methoxybenzenesulfonyl chloride (0.82 g, 0.56 mL, 4 mmol) as described for **14a**. Purification by silica gel chromatography (MC/H 8:2) afforded **14c** as a white powder (0.55 g, 38%): mp 110−112 °C.

3,4-Dimethoxybenzenesulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14d). The title compound was prepared from **11** (0.85 g, 4.23 mmol) and 3,4-dimethoxybenzenesulfonyl chloride (1 g, 4.23 mmol) as described for **14a**. Purification by silica gel chromatography (H/PE 1:1) afforded **14d** as white crystals (0.43 g, 25%) yield): mp 163−165 °C.

4-Methylbenzensulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14e). The title compound was prepared from **11** (0.6 g, 3 mmol) and 4-methylbenzenesulfonyl chloride (0.57 g, 3 mmol) as described for **14a**. Purification by silica gel chromatography (MC/H 1:1) afforded **14e** as a fine white powder (0.47 g, 44%) yield): mp 164−165 °C.

3-Methylbenzenesulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14f). The title compound was prepared from **11** (0.8 g, 4 mmol) and 3-methylbenzenesulfonyl chloride (0.76 g, 0.57 mL, 4 mmol) as described for **14a**. Purification by silica gel chromatography (MC/H 1:1) afforded **14f** as colorless crystals (0.64 g, 45%): mp 122−124 °C.

4-Nitrobenzenesulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14g). The title compound was prepared from **11** (0.8 g, 4 mmol) and 4-nitrobenzenesulfonyl chloride (0.89 g, 4 mmol) as described for **14a**. Purification by silica gel chromatography (MC/H 1:1) afforded **14g** as yellow crystals (0.14 g, 9%) yield): mp 199−201 °C.

4-Aminobenzenesulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14h).[38] **14g** (0.39 g, 1 mmol) was suspended in absolute ethanol (50 mL). Then, $SnCl_2 \cdot 2H_2O$ (3.89 mmol) was added, and the mixture was refluxed for 18 h under N_2 until the reaction was complete. Thereafter, the mixture was cooled and the solvent evaporated. Purification by silica gel chromatography (MC) afforded **14h** as a fine white powder (0.27 g, 76% yield): mp 187−189 °C.

4-Methylaminobenzensulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14i). 14h (0.15 g, 0.42 mmol) and dry K_2CO_3 (0.58 g, 4.2 mmol) were suspended in absolute acetone (50 mL) under N_2. Dimethyl sulfate (0.39 g, 0.3 mL, 3.15 mmol) was added dropwise, and the mixture was heated at 50 °C under nitrogen until the reaction was completed (TLC control). The reaction mixture was then cooled and filtered with suction. The filtrate was then poured into water (200 mL) and extracted with CH_2Cl_2 (4 × 25 mL). The combined CH_2Cl_2 extracts were washed, dried over Na_2SO_4, and then evaporated. The residue was purified by chromatography (MC/H 8:2) and afforded **14i** as white crystals (0.03 g, 20%): mp 178−180 °C.

4-Dimethylaminobenzensulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14j). Following a literature method,[39,40] **14h** (0.11 g, 0.31 mmol) and dry K_2CO_3 (0.09 g, 0.62 mmol) were suspended in absolute DMF (20 mL) under N_2. Methyl iodide (1.6 g, 0.7 mL, 0.01 mol) was added at room temperature, and the mixture was heated at 80 °C. After 24 h the reaction mixture was cooled and filtered with suction. The filtrate was then poured into water (100 mL) and extracted with CH_2Cl_2 (4 × 30 mL). The combined organic extracts were washed with water (3 × 25 mL), dried over Na_2SO_4, and then evaporated. Purification by silica gel chromatography (MC/H 8:2) afforded **14j** as white crystals (0.05 g, 43% yield): mp 221−224 °C.

4-Hydroxyaminobenzensulfonic Acid Naphtho[2,3-*b*]thiophen-4-yl Ester (14k). According to literature methods,[22−24] **14g** (0.8 g, 2.10 mmol) was suspended in absolute ethanol (50 mL) and hydrogenated over 10% Pd/C (0.5 g) at room temperature and atmospheric pressure. The mixture was stirred until the reaction was complete. Then, the catalyst was removed by filtration, and the solution was evaporated. Purification by silica gel chromatography (MC) afforded **14k** as a pale-yellow powder (0.39 g, 38%): mp 168−171 °C.

2-Methoxy-4-nitrobenzenesulfonic Acid Naphtho[2,3-*b*]-thiophen-4-yl Ester (14l). The title compound was obtained from **11** (0.8 g, 4 mmol) and 2-methoxy-4-nitrobenzenesulfonyl chloride (1 g, 4 mmol) as described for **14a.** Purification by silica gel chromatography (MC/H 1:1) afforded **14l** as yellow crystals (0.61 g, 37% yield): mp 171−173 °C.

4-Amino-2-methoxybenzenesulfonic Acid Naphtho[2,3-*b*]-thiophen-4-yl Ester (14m). The title compound was obtained from **14l** (0.5 g, 1.2 mmol) as described for **14h**.

Purification by silica gel chromatography (MC/H 8:2) afforded **14m** as a white powder (0.04 g, 8% yield): mp 218−220 °C.

4-Methoxybenzenesulfonic Acid 1,8-Dichloroanthracen-9-yl Ester (17). The title compound was obtained from 1,8-dichloro-9(10*H*)-anthracenone (**15**, 1.32 g, 5 mmol) and 4-methoxybenzenesulfonyl chloride (1.03 g, 5 mmol) as described for **13a**. Purification by silica gel chromatography (MC) afforded **17** as fine yellow crystals (0.79 g, 37% yield): mp 177 °C.

4-Methoxybenzenesulfonic Acid 4,5-Dichloroanthracen-9-yl Ester (18). The title compound was obtained from 4,5-dichloro-9(10*H*)-anthracenone (**16**, 1.32 g, 5 mmol) and 4-methoxybenzenesulfonyl chloride (1.03 g, 5 mmol) as described for **13a**. Purification by silica gel chromatography (MC) afforded **18** as fine yellow crystals (0.44 g, 20% yield): mp 176−177 °C.

4-Methoxybenzenesulfonic Acid 1,2,3,4-Tetrahydroacridin-9-yl Ester (22). A suspension of 1,2,3,4-tetrahydro-9-acridanone (**20**, 1.25 g, 6.25 mmol), TBAB (0.3 g, 1.03 mmol), and 50% sodium hydroxide (10 mL) in THF (20 mL) and water (5 mL) was stirred vigorously for 20 min. A solution of 4-methoxybenzenesulfonyl chloride (2.58 g, 12.5 mmol) in THF 5 mL was added dropwise to the reaction mixture. The mixture was stirred until the reaction was complete (TLC control), poured into water (250 mL) and HCl (50 mL), and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, washed with water, and then concentrated under vacuum.

Purification by silica gel chromatography (MC/MeOH, 9.97:0.03) afforded **22** as white crystalline powder (1.44 g, 62% yield): mp 172−173 °C.

Biological Assay Methods. These were described previously in full detail.[14]

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Supporting Information Available: IR, ^{1}H NMR, and MS data of new compounds **13a**−**c**, **14a**−**m**, **17**, **18**, and **22**; cell cycle analysis of KB/HeLa cells treated with **13a, 14b**, and reference compounds; table of elemental analysis results of all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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