Arylthioindoles, Potent Inhibitors of Tubulin Polymerization

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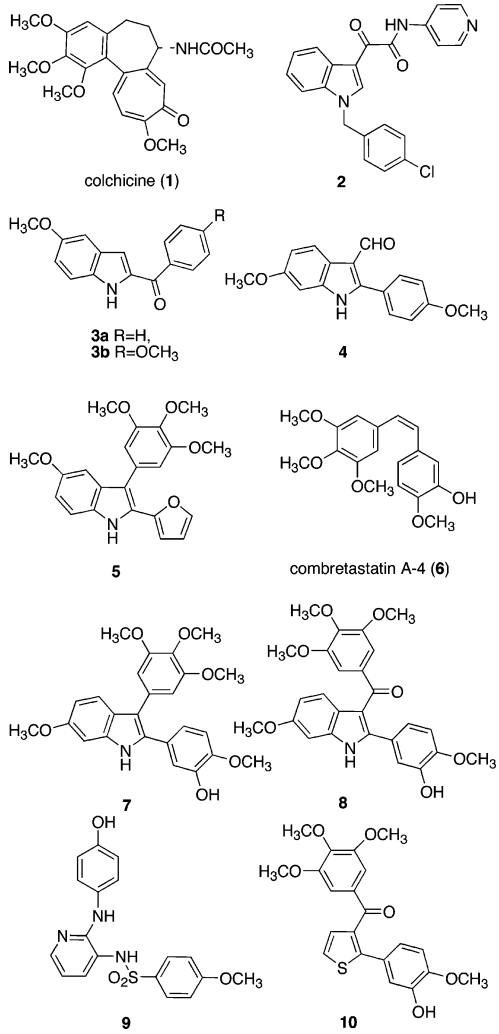
Abstract: Several arylthioindoles had excellent activity as inhibitors both of tubulin polymerization and of the growth of MCF-7 human breast carcinoma cells. Methyl 3-[(3,4,5-trimethoxyphenyl)thio]-5-methoxy-1*H*-indole-2-carboxylate (**21**), the most potent derivative, showed IC_{50} = 2.0 μM, 1.6 times more active than colchicine and about as active as combretastatin A-4 (CSA4). Compound **21** inhibited the growth of the MCF-7 cells at IC_{50} = 13 nM. Colchicine and CSA4 had 13 nM and 17 nM IC_{50} values, respectively, with these cells.

Microtubules are involved in a wide number of cellular functions, such as motility, division, shape maintenance, and intracellular transport. The major protein component found in microtubules is tubulin. Interference with microtubule assembly, either by inhibition of tubulin polymerization or by blocking microtubule disassembly, leads to an increase in the number of cells in metaphase arrest. Inhibition of microtubule function using tubulin targeting agents is a validated approach to anticancer therapy.[1−5]

Many well-described antimitotic agents are derived from natural sources. Colchicine (**1**) and *Vinca* alkaloids, the first tubulin binding agents discovered, cause destabilization of microtubules and, as a consequence, induce the cell to undergo apoptosis.[6] On the other hand paclitaxel, a standard antitumor agent originally extracted from *Taxus brevifolia,* binds to a different site within tubulin leading to the stabilization of microtubules.[7] Unfortunately, clinical use of these compounds is restricted by toxicity, drug resistance, complex formulations, and limited bioavailability.

Various synthetic molecules have also been reported as tubulin inhibitors. The majority of them inhibit the binding of colchicine to tubulin and thereby act as destabilizing agents. In recent years about 30 natural, semisynthetic, or synthetic tubulin binding agents, acting by either stabilizing or destabilizing mechanisms, have been under preclinical/clinical investigation.[2]

Chart 1



The indole nucleus is the core structure of a great number of tubulin polymerization inhibitors.[2,8] The indolyl-3-glyoxamide D-24851 (**2**) and the 2-aroylindoles D-64131 (**3a**) and D-68144 (**3b**) were discovered by Baxter Oncology. Compounds **3** are highly active against various tumors including those resistant to paclitaxel[9] (Chart 1).

Several 2-phenylindoles were designed by von Angerer as simple analogues of 12-formyl-5,6-dihydroindolo[2,1-*a*]isoquinoline. Among them, indole **4** completely blocked microtubule assemby at a concentration of 40 μM.[10] On the basis of the structure of the natural product combretastatin A-4 (CSA4, **6**), some 2,3-diarylindoles, known as heterocombretastatins, were prepared (e.g., **5**) by Medarde.[11] Flynn et al. reported the tubulin polymerization inhibitory activity of 2,3-diarylindole **7** and 2-aryl-3-arylcarbonylindole **8**.[12]

Sulfur containing compounds, such as sulfonamide E-7010 (**9**),[13] thiophene **10**,[14] and benzothiophene[12] derivatives, proved effective inhibitors of tubulin polymerization. To our knowledge there have been no reports on the inhibition of tubulin polymerization by arylthio/sulfonylindoles.

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Therefore, here we describe the synthesis of some novel 3-arylthio/sulfonylindole-2-carboxylates (**12**−**21**). Several were found to be highly potent inhibitors of tubulin polymerization.

3-Phenylthio-1*H*-indole (**11**) was synthesized by reacting indole (**22**) with 1,1′-diphenyldisulfide (**29**) in the presence of NaH in anhydrous DMF, following the procedure reported by Atkinson.[15] By this method was prepared in poor yield methyl 3-phenylthio-1*H*-indole-2-carboxylate (**12**) starting from methyl indole-2-carboxylate (**23**).

To improve the yield, compound **12** was synthesized by reacting ester **23** with *N*-phenyl-thiosuccinimide (**30**) in the presence of boron trifluoride diethyl etherate, as we previously reported for compound **15** starting from **24**.[16]

By the same procedure were prepared methyl 3-[(4-methoxyphenyl)thio]-5-chloro-1*H*-indole-2-carboxylate (**17**) starting from methyl 5-chloro-1*H*-indole-2-carboxylate (**25**) and *N*-[(4-methoxyphenyl)thio]succinimide (**31**), and methyl 3-(phenylthio)-5-methoxy-1*H*-indole-2-carboxylate (**20**) starting from methyl 5-methoxyindole-2-carboxylate (**26**) and **30**. Methyl 3-[(3,4,5-trimethoxyphenyl)thio]-1*H*-indole-2-carboxylate (**14**) and methyl 3-[(3,4,5-trimethoxyphenyl)thio]-5-chloro-1*H*-indole-2-carboxylate (**18**) were prepared by heating at 50 °C indole-2-carboxylic acid (**27**) or its 5-chloro derivative **28** with the 1,1′-(3,4,5-trimethoxyphenyl)disulfide (**32**) in the presence of NaH. The crude acids were then transformed into the corresponding methyl esters by reaction with trimethylsilyldiazomethane (TMSDM) at room temperature for 30 min. 5-Methoxyindole **21** was prepared following a two step procedure involving the addition of 3,4,5-trimethoxylthiophenol (**33**) to a solution of *N*-chlorosuccinimide (NCS) at −78 °C, and then this mixture was treated with acid **26** while the reaction temperature was warmed to 0 °C within 1 h. Oxidation of sulfur derivatives **12**, **15**, and **18** with 3-chloroperoxybenzoic acid (MCPBA) furnished the corresponding sulfones **13**, **16**, and **19**, respectively (Scheme 1).

Inhibition of tubulin polymerization, colchicine binding,[17] and the growth of MCF-7 human breast carcinoma cells[18] by the novel indoles **11**−**21** in comparison with the effects of the reference compounds colchicine (**1**) and CSA4 (**6**) are shown in Table 2.

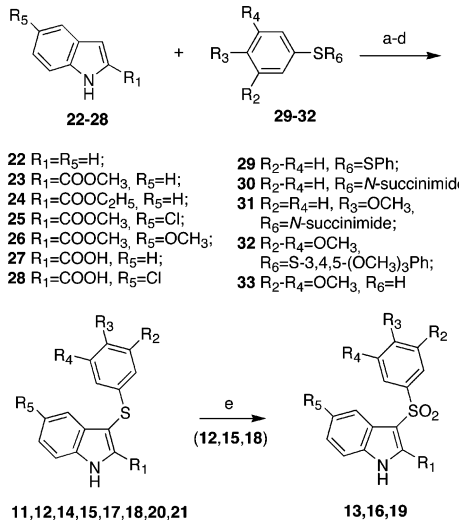
The compound we initially evaluated was 3-phenylthio-1*H*-indole (**11**), which inhibited tubulin polymerization with an IC_{50} value of 15 μM, ca. 5 and 7 times inferior to the reference compounds **1** and **6**, respectively.

We therefore attempted the introduction of a methoxycarbonyl function at position 2 of the indole ring. This chemical modification produced methyl 3-(phenylthio)-1*H*-indole-2-carboxylate (**12**), which was about twice as potent as **11** (IC_{50} = 8.2 μM).

A further step was the oxidation of the sulfur atom of **12** to the sulfone to produce methyl 3-(phenylsulfonyl)-1*H*-indole-2-carboxylate (**13**). This compound was found to be inactive at the highest concentration tested.

However, a different behavior was observed for the analogous pair **15**/**16**. Replacement of the 2-methoxycarbonyl group of **12** with an ethoxycarbonyl group (**15**) doubled the inhibitory potency of the parent compound, and the inhibitory activity of **15** was retained by the sulfone **16** (compare **12** with **15** and **16**). Despite appreciable inhibition of tubulin polymerization displayed by indoles **11**, **12**, **15**, and **16**, these compounds were unable to inhibit the growth of MCF-7 human breast carcinoma cells.

Scheme 1[a]



[a] Reagents and reaction conditions: (a, **11**) ArSSAr, NaH, anhydrous DMF, rt, 2 h; (b, **12**, **15**, **17**, **20**) *N*-(ArS)succinimide, $BF_3{\cdot}Et_2O$, anhydrous CH_2Cl_2, rt, 1.5 h, then 45 °C, 2 h; (c, **14**, **18**) (i) ArSSAr, NaH, anhydrous DMF, 50 °C, overnight, anhydrous nitrogen stream; (ii) TMSDM, CH_3OH/CH_2Cl_2, rt, 30 min; (d, **21**) (i) ArSH, NCS, CH_2Cl_2, −78 °C; (ii) **26**, −78 to 0 °C, 1 h; (e, **13**, **16**, **19**) MCPBA (2.5 equiv), $CHCl_3$, rt, 1 h.

Table 1. Structure of Compounds **11**−**21**

compd	R_1	R_2	R_3	R_4	R_5	S/SO_2
11	H	H	H	H	H	S
12	$COOCH_3$	H	H	H	H	S
13	$COOCH_3$	H	H	H	H	SO_2
14	$COOCH_3$	OCH_3	OCH_3	OCH_3	H	S
15	$COOC_2H_5$	H	H	H	H	S
16	$COOC_2H_5$	H	H	H	H	SO_2
17	$COOCH_3$	H	OCH_3	H	Cl	S
18	$COOCH_3$	OCH_3	OCH_3	OCH_3	Cl	S
19	$COOCH_3$	OCH_3	OCH_3	OCH_3	Cl	SO_2
20	$COOCH_3$	H	H	H	OCH_3	S
21	$COOCH_3$	OCH_3	OCH_3	OCH_3	OCH_3	S

At this point we decided to introduce a trimethoxy substitution pattern, a common structural feature of colchicine, CSA4, and other tubulin inhibitors (see Chart 1). Replacement of the phenylthio of **12** with the 3,4,5-trimethoxyphenylthio moiety furnished methyl 3-[(3,4,5-trimethoxyphenyl)thio]-1*H*-indole-2-carboxylate (**14**), which showed IC_{50} = 2.9 μM, 2.8 times superior to that of **12** and comparable with those of colchicine (IC_{50} = 3.2 μM) and CSA4 (IC_{50} = 2.2 μM). Most importantly, this compound inhibited the growth of the MCF-7 cells by 50% at 25 nM, a concentration only 2 or 1.4 times higher than observed with colchicine (IC_{50} = 13 nM) or CSA4 (IC_{50} = 17 nM), respectively.

We also evaluated the effects of substituents at position 5 of the indole ring. To this end we chose two substituents with opposite properties, the electron withdrawing chlorine atom and the electron donating methoxy group. Introduction of a chlorine atom at position 5 of the indole of **14** gave methyl 3-[(3,4,5-trimethoxyphenyl)thio]-5-chloro-1*H*-indole-2-carboxylate (**18**), which only marginally increased potency as an

Table 2. Inhibition of Tubulin Polymerization, Colchicine Binding and Growth of MCF-7 Human Breast Carcinoma Cells by Compounds **11**−**21**

compd	tubulin[a] $IC_{50} \pm SD$ (μM)	colchicine binding[b] (% ± SD)	MCF-7[c] $IC_{50} \pm SD$ (nM)
11	15 ± 0.7	13 ± 5	>2500
12	8.3 ± 0.6	21± 7	>2500
13	>40	5.1 ± 1	>2500
14	2.9 ± 0.1	81 ± 1	25 ± 1
15	4.4 ± 0.3	19 ± 7	>1250
16	4.4 ± 0.2	33 ± 0.6	>1000
17	>40	3.6 ± 8	>2500
18	2.5 ± 0.3	76 ± 0.2	42 ±10
19	>40	1.6 ± 2	>2500
20	6.2 ± 0.3	31 ± 8	>2500
21	2.0 ± 0.2	93 ± 0.8 (68 ± 3)[d]	13 ± 3
1 (colchicine)	3.2 ± 0.4		13 ± 3
4[e]	nd[g]	nd	160
6 (CSA4)	2.2 ± 0.2	100 ± 0.9 (88 ± 0.4)[d]	17 ± 10
7[f]	4.1 ± 0.6	28 ± 8	370 ± 2
8[f]	1.6	54 ± 0.7	45 ± 5

[a] Inhibition of tubulin polymerization. Tubulin was at 10 μM.[13] [b] Inhibition of [^{3}H]colchicine binding. Tubulin was at 1 μM; both [^{3}H]colchicine and inhibitor were at 5 μM.[14] [c] Inhibition of growth of MCF-7 human breast carcinoma cells.[14] [d] Inhibition of [^{3}H]colchicine binding. [^{3}H]Colchicine was at 5 μM; both tubulin and inhibitor were at 1 μM. [e] Reference 6. [f] Reference 8. [g] No data.

inhibitor of tubulin polymerization (IC_{50} = 2.5 μM), whereas inhibition of the growth of MCF-7 cells (IC_{50} = 42 nM) was decreased almost 2-fold. In contrast, the presence of a methoxy group at position 5 of the indole, giving methyl 3-[(3,4,5-trimethoxyphenyl)thio]-5-methoxy-1*H*-indole-2-carboxylate (**21**), enhanced inhibitory potency in both the biochemical and cytological assays. Compound **21** (IC_{50} = 2.0 μM) was 1.6 times more active than colchicine and about as active as CSA4 as an inhibitor of tubulin polymerization, while as an inhibitor of the growth of MCF-7 cells (IC_{50} = 13 nM) it was as potent as colchicine and CSA4. Among the derivatives tested, this compound was the most active.

It is interesting to note that, independently of the presence/nature of the substituent at position 5 of the indole, the 3,4,5-trimethoxyphenylthio moiety was crucial for effective inhibition of the growth of MCF-7 cells (compare **12** with **14**, **17** with **18**, and **20** with **21**).

In contrast, inhibition of tubulin polymerization was less affected by the substituent at position 5 (compare **12** with **14** and **20** with **21**). Surprisingly, methyl 3-[(4-methoxyphenyl)thio]-5-chloro-1*H*-indole-2-carboxylate (**17**) was quite inactive in both assays.

To investigate the possible binding mode of these novel compounds, we carried out docking studies of the arylthioindole **21** in the colchicine binding site of tubulin, which was obtained from the recently reported 3D structure of tubulin cocrystallized with a colchicine analogue: *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (DAMA-colchicine).[19]

Compound **21** was docked using a modified version of the docking tool of MOE,[20] which implements a genetic algorithm based search method.[21] The resulting protein/ligand complex was then minimized, revealing a very interesting and efficient binding model for the inhibitor to tubulin.

Figure 1 clearly shows how the trimethoxy ring is well situated in proximity to Cys241 (residue numbers as in ref 19), adopting an orientation very similar to that of the corresponding ring in the DAMA-colchicine of the crystallized structure (see also Figure 2 in the Supporting Information). The methoxy substituent of the indole is also very close to the corresponding group on ring C of colchicine, leading to a very similar general binding of the two inhibitors. Furthermore, the indole moiety establishes one hydrogen bond between the NH and the backbone of Thr179 (shown in blue). Several other hydrophobic contacts (not shown for the sake of clarity) stabilize further the binding of **21** to the protein. These observations are consistent with the highly efficient inhibition of [^{3}H]colchicine binding that occurs with compound **21** (Table 2).

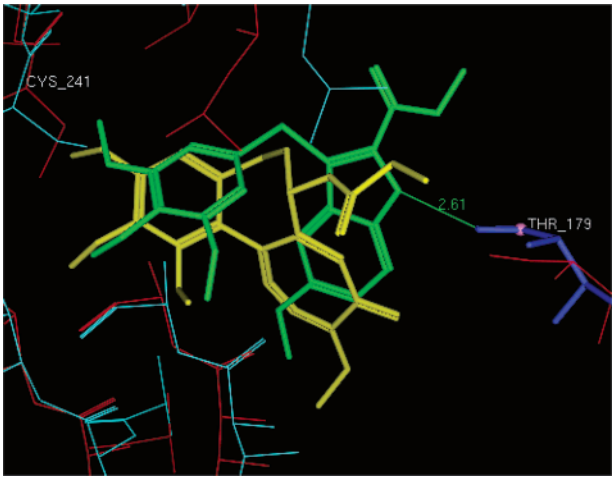


Figure 1. Proposed binding of **21**. DAMA-colchicine is represented in yellow, compound **21** in green. In red are represented the residues of the X-ray structure of tubulin, in cyan the same residues after minimization of tubulin with bound **21**. Residue numbers are those used by Ravelli et al.[19]

Further studies are currently underway to verify if this model can give a rational justification of the SAR obtained from the biological data.

In conclusion, we synthesized arylthioindoles, a novel class of inhibitors of tubulin polymerization. Some compounds had excellent activity in both inhibition of tubulin polymerization and inhibition of the growth of MCF-7 human breast carcinoma cells. Methyl 3-[(3,4,5-trimethoxyphenyl)thio]-5-methoxy-1*H*-indole-2-carboxylate (**21**), the most potent derivative, showed IC_{50} = 2.0 μM, 1.6 times more active than colchicine and about as active as CSA4 as an inhibitor of tubulin assembly.

This compound inhibited the growth of MCF-7 human breast carcinoma cells with IC_{50} = 13 nM, essentially equivalent to the values obtained with colchicine and CSA4.

A SAR study led to identification of several crucial structural requirements needed to enhance the effectiveness of the arylthioindoles. In particular, we found required determinants to be (i) the presence of a methoxy/ethoxycarbonyl group at position 2 of the indole nucleus; (ii) the 3,4,5-trimethoxyphenylthio group at position 3 of the indole; (iii) the sulfur atom in the sulfide oxidation state; (iv) the substituent at position 5 of the indole.

The last seems especially important for inhibition of the growth of MCF-7 cells. Furthermore, initial molecular modeling studies have revealed a possible binding mode for these novel inhibitors that could support the

observed biological data. These findings have led us to continue our studies on novel arylthioindoles in order to investigate further SAR rules and mechanism of action and to evaluate their activity against a wider range of tumor cell lines.

Supporting Information Available: Experimental procedures for synthesis and biological evaluation of **11**−**21** and for molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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